INFLUENCE OF ESTABLISHMENT PERIODS, AERATION, AND SILVER NANOPARTICLES ON THE FUNCTION OF VERTICAL FLOW CONSTRUCTED WETLANDS

INFLUENCE DE LA PÉRIODE D'ÉTABLISSEMENT, DE L'AÉRATION ET DES NANOPARTICULES D'ARGENT SUR LA FONCTION DES MARAIS FILTRANTS VERTICAUX

A Thesis Submitted to the Division of Graduate Studies

of the Royal Military College of Canada

by

Laura Jean Ogilvie, B. Sc. in Chemistry

In Partial Fulfillment of the Requirements for the Degree of Masters of Science

April 2017

Acknowledgements

First, I would like to thank my supervisor, Dr. Kela Weber, for his guidance, encouragement, and support throughout my Master of Science journey. With Dr. Weber's assistance, I have grown both personally and professionally over the past two years and feel confident and prepared to start a career in the environmental sector.

I would like to thank Dr. Vincent Gagnon and Dr. Mark Button. Both Vincent and Mark have provided me with invaluable advice and academic support throughout this project. I greatly appreciated their help with data analysis and forming my results into a presentable thesis. Thank you so very much!

I would like to thank Dr. Denis O'Carroll for his expertise, input and advice.

Thank you to past and current members of the Environmental Sciences Research Group: Antoine Hnain, Richard Stanton, Jesse Thomas, Dr. Michelle Nearing and Dr. Iris Koch for your guidance and assistance with my research.

To my friends, Carolina Dahmer, Devon Gray and Anbareen Farooq, thank you for making the good times shine and being there to support me in the lab, in class and in my personal life over the past two years.

I would like to thank my grandparents, Bob and Bernice Ogilvie and Jean Sutcliffe, for being my inspiration and for the support to continue to pursue my academic career.

Finally, I would like to thank my parents, Penelope Sutcliffe and Kevin Ogilvie. Your love and unconditional support has allowed me to grow and to succeed in my many endeavors. I would not be where I am today without your belief in me. Thank you for teaching me to be an environmentally conscious person from day one.

Abstract

Constructed wetlands (CWs) are a self-adaptive, passive, energy efficient and cost-effective substitute to conventional wastewater treatment methods. The microbial communities in CWs are responsible for much of the complex wastewater treatment potential. CWs require a start-up period after initialization for the ecosystem (microbial, plant) to establish before wastewater treatment processing is optimized. To improve wastewater treatment capacity CWs may use certain intensification designs, such as artificial aeration. Emerging contaminants may enter CWs from wastewater streams after leaching from medial, consumer and personal care products. These emerging contaminants, such as nanoparticles, may affect CW ecology and wastewater treatment performance. The overall objective of this thesis is to evaluate the effects of various perturbations to wetland steady-state (initialization, planting and antimicrobial exposure). First, the development period for twelve planted CW mesocosms was evaluated over a three-month period. The effects of artificial aeration were investigated on water chemistry, wastewater treatment ability, nutrient cycling, microbial activity/function, and plant health. Secondly, *Phalaris arundinacea* was seeded in a different set of twelve CWs, which had developed unplanted for 4 months prior to seeding. The effects of plant addition and establishment were evaluated for the same metrics as previous. Finally, the effects of antimicrobial silver nanoparticles (Ag NPs) (differing concentrations and types) on CW microbial communities were evaluated using an *ex-situ* technique involving community level physiological profiling. Microbial communities from both aerated and non-aerated CWs were evaluated as the presence of oxygen alters the environmental conditions within the wetland and therefore may alter both the inherent microbial communities, and the fate/transformation of Ag NPs.

During the planted wetland development period stabilization of water chemistry parameters occurred readily and differentiation was evident between aerated and non-aerated mesocosms. The addition of aeration did not enhance the start-up efficiency for the CW mesocosms. Microbial stabilization occurred after approximately 60 to 75 days in both aerated and non-aerated mesocosms. The addition of *Phalaris arundinacea* to a set of well-developed CW mesocosms exerted a stabilizing effect on the microbial community, starting to bridge the microbial catabolic functionality between the two system types (aerated and non-aerated). TOC and TN mass removal increased as a result of planting in non-aerated systems. In aerated systems, TOC removal was high (>95%) independent of plant addition, TN removal fluctuated more after plant establishment. The effect of plants on the mesocosms was more defined in non-aerated systems than in aerated systems. The exposure of microbial communities from both aerated and non-aerated CW mesocosms, to Ag NPs showed aerated microbial communities to be much more susceptible to toxicity from silver. This may be a result of fundamental differences in water chemistry between wetland types and the nature of aqueous chemistry for silver. Artificially sulphidized Ag NPs were not toxic to the microbial communities tested. The wash water from Ag NP containing socks catabolically inhibited the microbial communities at a concentration that is environmentally relevant for the release of silver from wastewater treatment plants (0.1 mg/L). Ag NPs preferentially reduced the microbial utilization of carbohydrates and carboxylic and acetic acids, while the utilization of amines and amides was less hindered.

The characterization methods used in this thesis to holistically evaluate CW mesocosms elucidated the subtle effects of wetland development and plant addition on the overall wetland dynamics. Complex interactions between plants, water chemistry and microbial communities lead to changes in pollutant removal in the aerated and non-aerated systems. This thesis validates the importance of rigorous characterization of wetland health and function. Studying wetlands using

several lines of evidence informs research and industry on subtle interactions between microbial communities, plants and wastewater components. Further research should continue to examine CWs from a variety of perspectives (physical, chemical, microbial, vegetation, water treatment potential), as well as utilizing the most environmentally relevant concentrations and forms of emerging contaminants available for ecotoxicity studies.

Résumé

Le marais filtrant artificiel (MFA) est une technologie extensive, économique et nécessitant peu énergie comparativement aux méthodes classiques de traitement des eaux usées. Les communautés microbiennes dans les MFA sont responsables d'une grande partie du traitement des eaux usées. Les MFA nécessitent une période de démarrage afin que l'écosystème (microbien, plante) s'établisse et que le traitement des eaux usées soit optimisé. Pour améliorer la capacité de traitement des eaux usées, les MFA peuvent utiliser des processus d'intensification, tels que l'aération artificielle. Les contaminants émergents dans les eaux usées proviennent de la lixiviation de certains produits de consommations et peuvent entrer dans les MFA. Ces contaminants émergents, tels que les nanoparticules, peuvent avoir une incidence sur l'écologie et le traitement des eaux usées. L'objectif général de cette étude est d'évaluer les effets de diverses perturbations sur la stabilité des marais filtrants artificiels (initialisation, plantation et exposition aux agents antimicrobiens). La période de développement a d'abord été évaluée sur douze mésocosmes plantés sur une période de trois mois. Les effets de l'aération artificielle sur la chimie de l'eau, la capacité de traitement des eaux usées, le cycle des nutriments, l'activité / la fonction microbienne et la santé des plantes. Ont été étudiés la deuxièmement partie de l'expérience consistait de mesurer l'effet de l'addition et de l'établissement des plantes sur les MFA selon les mêmes paramètres que l'expérience précédente. Pour ce faire, douze nouveaux mésocosmes ont été alimentés avec des eaux usées pendant 4 mois et ont été par la suite ensemencés de *Phalaris arundinacea*. Finalement, les effets antimicrobiens des nanoparticules d'argent (NP-Ag), de différentes concentrations et types, sur les communautés microbiennes ont été évalués à l'aide de la mesure du profil microbien. Les communautés microbiennes des MFA aérés et non aérés ont été évaluées, car la présence d'oxygène modifie les conditions environnementales des MFA et peut donc modifier à la fois les communautés microbiennes inhérentes et le devenir / la transformation des NP-Ag.

Au cours de la période de développement des marais filtrants artificiels, la stabilisation des paramètres de la chimie de l'eau s'est avérée facile et la différenciation était évidente entre les mésocosmes aérés et non aérés. L'ajout d'aération n'a pas influencé la période de démarrage des mésocosmes. La stabilisation microbienne s'est produite après environ 60 à 75 jours dans les mésocosmes aérés et non aérés. L'ajout de *Phalaris arundinacea* aux mésocosmes bien établies a exercé un effet stabilisant sur la communauté microbienne, en réduisant l'écart entre la fonctionnalité microbienne catabolique des deux types de système (aérés et non aérés). L'élimination du carbone organique total (COT) et de l'azote total (AT) a augmenté dans les systèmes plantés non aérés. Dans les systèmes aérés, l'élimination des COT était élevée (> 95%) indépendamment de l'addition de la plante, l'élimination de AT a fluctué davantage après l'établissement des plantes. L'effet des plantes sur les mésocosmes était plus défini dans les systèmes non aérés que dans les systèmes aérés. L'exposition des communautés microbiennes des mésocosmes aérés et non aérés aux Ag NP a montré que les communautés microbiennes de mésocosmes aérés étaient beaucoup plus susceptibles. Cela peut résulter de différences fondamentales dans la chimie de l'eau entre les types MFA ainsi que la chimie de l'argent. Les NP-Ag sulfuré artificiellement n'étaient pas toxiques pour les communautés microbiennes testées. L'eau de lavage contenant NP-Ag a inhibé cataboliquement les communautés microbiennes à des concentrations possiblement présentes dans l'environnement lors de la libération d'Ag par les stations de traitement des eaux usées (0,1 mg/L). Les NP-Ag ont préférentiellement réduit l'utilisation microbienne des glucides et des acides carboxylique et acétique, tandis que l'utilisation d'amines et d'amides était moins affectée.

Les méthodes de caractérisation utilisées dans cette thèse pour évaluer globalement les marais filtrants artificiels ont permis d'élucider les effets subtils du développement des MFA et l'addition de plantes sur la dynamique des MFA. Les interactions complexes entre les plantes, la chimie de l'eau et les communautés microbiennes entraînent des changements dans l'élimination des polluants dans les systèmes aérés et non aérés. Cette thèse confirme l'importance d'une caractérisation rigoureuse de la santé et de la fonction des MFA. Étudier les MFA en utilisant plusieurs techniques informe la recherche et l'industrie sur les interactions subtiles entre les communautés microbiennes, les plantes et les composantes des eaux usées. Das recherches subséquentes devraient permettre d'examiner les MFA sous diverses perspectives (physique, chimique, microbienne, végétale, potentiel de traitement de l'eau), ainsi que l'utilisation des concentrations et des formes de contaminants émergents représentant ce qui est susceptible d’être libérés dans l'environnement et causé de l'écotoxicité.

Table of Contents

List of Tables

List of Figures

Abbreviations and Acronyms

Acronym or Abbreviation	Definition
Ag NP	Silver nanoparticles
ANOVA	Analysis of variance
AWCD	Average well colour development
BOD	Biochemical oxygen demand
BPEI	Branched polyethylenimine
CLPP	Community Level Physiological Profiling
CMC	Carboxymethyl cellulose
CBOD	Carbonaceous biochemical oxygen demand
COD	Chemical oxygen demand
CSUP	Carbon source utilization pattern
CW	Constructed wetland
df	Degrees of freedom
DGGE	Denaturing gradient gel electrophoresis
dH_2O	Deionized water
DO	Dissolved oxygen
EC50	Effective concentration of 50 percent
EPS	Extracellular polymeric substances
ER	Evapotranspiration
FDA	Fluorescein diacetate
FL	Fluorescein
FWS	Free water surface
GA	Gum arabic
HRT	Hydraulic retention time
HSSF	Horizontal subsurface flow
ICP-MS	Inductively coupled plasma – mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
mg/L	Milligram per litre
NADH	Nicotinamide adenine dinucleotide
ng/L	Nanogram per litre
ORP	Oxidative-reductive (redox) potential
PCA	Principal component analysis
PPCP	Pharmaceuticals and personal care products
PVC	Polyvinyl chloride
PVP	Polyvinyl pyrrolidone
ROS	Reactive oxygen species
SP-ICP-MS	Single particle inductively coupled plasma – mass spectrometry
TEM	Transmission electron microscopy
TIS	Tanks-in-series
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TOC	Total organic carbon

TSS	Total suspended solids
µg/L	Microgram per litre
VF	Vertical subsurface flow

1 CHAPTER 1: THESIS INTRODUCTION

1.1 Constructed Wetlands Background

1.1.1 Modern Wastewater Treatment

Population growth results in a corresponding increase in the amount of anthropogenic waste generated. The main intent of wastewater treatment is to remove or reduce the concentration of many common contaminants. Many wastewater treatment processes work for a wide variety of treatment schemes and pollutant types. Different types of wastewater provide different challenges based on their constituents. Domestic wastewater contains organic matter, nitrogen, phosphorus, suspended solids and remnants of consumer and household products which can include antimicrobial agents, pharmaceuticals, personal care products and nanomaterials, among others (CCME, 2006). Agricultural wastewaters are typically high in suspended solids, organic matter, phosphorus, ammonia nitrogen and pesticides (Kadlec and Wallace 2008). Industrial wastewaters can vary widely based on the industry but commonly contain high amounts of organic matter, nutrients and metals (Wu et al. 2015). Storm water largely consists of suspended solids, nutrients and metals (Kadlec and Wallace 2008).

Conventional wastewater treatment plants (centralized, secondary, biological treatment) are capable of handling large volumes of wastewater and are the major method of wastewater treatment in Canada (Canada 2011). The disadvantages of centralized wastewater treatment plants are the large space requirements, cost and high energy input required for operation. The principle of wastewater treatment is to convert soluble pollutants to solid matter (biomass, flocculated phosphates, etc.) that can settle over time and be removed. A large part of the matter is also transformed to various gases (CO_2, N_2, CH_4, etc.) and thus volatilized from the water.

Wastewater treatment plants use physical, chemical and biological processes to remove pollutants from water before discharge to natural systems (Metcalf and Eddy 2003; EPA 2004). Physical processes involve the removal of solids and debris through screening and sedimentation via gravity. Chemicals such as alum, lime or iron salts can be added as flocculating agents to cause specific pollutants, such as phosphorus, to agglomerate. The heavier mass of the larger flocs is removed more readily by physical processes such as sedimentation (Metcalf and Eddy 2003; EPA 2004). Biological processes include the consumption of organic matter and other nutrients in sewage by microorganisms. These biological processes rely on large microbial communities ("activated sludge", rotating biological contactors, trickling filters) to break down pollutants as part of their metabolic processes (Metcalf and Eddy 2003; EPA 2004). A by-product of microbial degradation of pollutants is the production of residual solids, which must be reused, burned, buried or disposed of in a manner with low environmental implications. The residuals have value as they contain nutrients like organic matter, phosphorus and nitrogen. The residual solids are still approximately 98% water after separation from the wastewater stream. These solids are stabilized to control odors and pathogenic microorganisms and dewatered before their final use. The final product, called "biosolids", is often applied to land as a soil conditioner and fertilizer (Metcalf and Eddy 2003; EPA 2004; Kadlec and Wallace 2008).

1.1.2 Natural Wetlands in Canada

Natural wetlands are a prominent feature of the Canadian landscape covering 14% of Canada's total land area (Kennedy and Mayer 2002). They are an extremely important ecological

feature, providing a transitional landscape between terrestrial and aquatic environments. Wetlands occupy a pivotal role in the environment: they cycle nutrients, provide protection for coastal regions, buffer and absorb water during hydrological events, provide food and habitat for wildlife and facilitate large scale drinking water purification for Canada (Kennedy and Mayer 2002; Kadlec and Wallace 2008). In addition to being an essential part of the water cycle, wetlands also provide socioeconomic benefits such as areas for recreation, such as hunting, fishing and hiking, and areas for resource development (Kennedy and Mayer 2002; Kadlec and Wallace 2008). Wetlands are described by Kadlec and Wallace (2008) as "land areas that are wet during part or all of the year because of their location in the landscape" with "the unifying principle that wetlands are wet long enough to exclude plant species that cannot grow in saturated soils and to alter soil properties because of the chemical, physical, and biological changes that occur during flooding". Wetlands are some of the most biologically productive ecosystems on the planet with complex interactions between plant, animal and microbial populations which drive water polishing, contaminant removal and nutrient cycling (Kadlec and Wallace 2008).

Natural wetlands have been used for wastewater discharge and treatment for a long time. Wastewater discharge to natural wetlands started out of convenience rather than for the purposes of wastewater treatment (Vymazal 2010). One historical Canadian example is the Cootes Paradise natural wetland near Hamilton, Ontario which started receiving wastewater in 1919 (Kadlec and Knight 1996; Kadlec and Wallace 2008). The uncontrolled discharge of wastewater has led to the irreversible degradation of many natural wetland areas. Growing knowledge about wetland functions and value caused a change in attitude towards natural wetlands in the 1950s (Vymazal 2010). The use of natural wetlands for wastewater discharge has decreased in some parts of the world and the use of constructed wetlands (CWs) is now preferred.

1.1.3 History of Constructed Wetlands

Constructed wetlands (CWs), also known as treatment wetlands, are man-made systems designed to emphasize specific characteristics of wetland ecosystems for improved water treatment capacity (Kadlec and Knight 1996; Kadlec and Wallace 2008). Perhaps the first recorded "constructed wetland" was a vertical flow system with a similar design to a planted vertical flow wetland or a vegetated trickling filter presented in a 1901 US Patent by Cleophas Monjeau (Monjeau 1901).

Later, in the 1950s, Dr. Käthe Seidel started experimenting with wetland plants for the purposes of wastewater treatment in Germany. The first full-scale CW systems were put into operation in the late 1960s (Vymazal 2010). Seidel's work mainly focused on subsurface flow technology with the use of plants for the treatment of various wastewaters including phenol, dairy and livestock. Other work in Europe led to free water surface CW technology research and implementation to protect water quality in lakes (Vymazal 2010).

Ecological engineering of natural wetlands in the 1970s in North America led to the use of free water surface technologies for wastewater treatment. Also in the 1970s the first subsurface flow CWs were built to treat municipal wastewaters (Fetter et al. 1976). The use of CWs spread slowly in North America, as there were concerns over treatment limitations in cold climates as well as in densely populated areas.

In the 1980s and 1990s, the exchange of information between professionals and countries intensified with many international conferences and publications (Vymazal 2010). Interest in

subsurface flow wetland technologies continued to increase in Europe and hybrid systems like the ones created by Dr. Käthe Seidel were implemented in France (Boutin 1987). In North America, free water surface CW technology spread quickly and many systems were built for the tertiary treatment of municipal wastewaters (Vymazal 2010). Subsurface CW technology spread more slowly to North America but in 1988 the U.S. Environmental Protection Agency issued a design manual on CWs and aquatic plant systems for municipal wastewater treatment (EPA 1988).

The end of the twentieth century saw the spread of CW technology to all inhabited continents and further applications of the technologies to other types of wastewater including: refinery, pulp and paper, explosives, mining, chemical industry, textile industry, landfill leachate, pesticides and herbicides, pig farms, fish pond effluent, abattoir facility, cheese and dairy, food processing, highway runoff, airport runoff, greenhouse runoff, urban runoff, and hydrocarbons (Vymazal 2010). Still the majority of CWs were designed to treat domestic and municipal wastewaters. Strict discharge guidelines for nitrogen in many European countries resulted in the increased use of vertical subsurface flow and hybrid CWs (Vymazal 2010).

Today, CWs are thought of as a viable alternative in modern wastewater treatment. CWs have been used in many applications in all parts of the world, with new applications growing. CWs are increasingly being used to service waters from industry and additionally municipal wastewater containing a variety of emerging contaminants such as pharmaceuticals, personal care products and nanoparticles (Matamoros and Bayona 2006; Weber et al. 2011; Ávila et al. 2014; Button et al. 2016).

1.2 Constructed Wetlands for Wastewater Treatment

1.2.1 Constructed Wetlands for Wastewater Treatment

Constructed wetlands are a self-adaptive, passive, energy efficient, and cost effective substitute to conventional wastewater treatment methods (Jenssen et al. 1993; Kivaisi 2001; Vymazal 2010). CWs have been used to polish many types of wastewater including those from residential, agricultural, industrial and storm water sources (Jenssen et al. 1993; Crites et al. 1997; Kadlec and Wallace 2008; Vymazal 2010; Vymazal and Březinová 2015; Wu et al. 2015). CWs have been used to treat wastewater from acid mine drainage; landfill leachate; livestock, manure and feeding operation run-off; agricultural field run-off; potato, wine, olive oil, sugar, seafood, starch, alcohol and meat processing; contaminated groundwater; urban storm water; pulp and paper mills; oil field and refinery; and coke production (Kadlec and Wallace 2008; Vymazal 2010; Vymazal and Březinová 2015; Wu et al. 2015).

A CW utilizes the pollutant removal processes innate to naturally-occurring wetlands such as sedimentation, filtration, chemical precipitation, microbial degradation, plant uptake and adsorption to soil particles (Kadlec and Wallace 2008). CWs are often designed with specific water quality parameters in mind: biochemical oxygen demand (BOD) or chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH_4-N), total suspended solids (TSS), total phosphorus (TP) and pathogens. Specific outlet concentration requirements can be met with optimal hydraulic and spatial design considerations (Kadlec and Wallace 2008). An overview of selected removal mechanisms available in CWs is listed in Table 1.1.

Table 1.1: Selection of mechanisms for pollutant removal in constructed wetlands (adapted from Kennedy and Mayer, 2002).

	Pollutant
Process	
Filtration and sedimentation	Suspended solids, particulate organic C, N and P
Chemical	
Adsorption	Dissolved organic compounds, anions (PO_4^{3-}) and cations (metals)
Precipitation	Inorganic P, sulphides and metals
Volatilization	Ammonia (NH_3) and volatile organic compounds
Biological	
Microbial	
Respiration	BOD, O_2, NO^{3-}, SO_4^{2-}, HCO^{3-} and volatile fatty acids
Nitrification	NH_4-N
Denitrification	NO_3^- and NO_2^-
Mineralization	Organic N and P
Assimilation	Nutrients (N and P)
Plants	
Growth and uptake	Nutrients (N and P)
Gas transport	O_2 and related reactions

1.2.2 Role of Microbial Communities in Constructed Wetlands

Pollutants are removed in CWs by sedimentation, filtration, precipitation, volatilization, adsorption and plant uptake but primarily due to microbial activity (Reddy and D'angelo 1997; Kennedy and Mayer 2002; Kadlec and Wallace 2008). The microbial communities living in CWs provide wastewater treatment services invariably through metabolic processes which result in the general degradation of wastewater components (Weber 2016). The microbial community utilizes wastewater components to survive, for either cellular mass and reproduction (anabolism) or energy (catabolism).

The microbial communities in CWs are both structurally and functionally diverse (Truu et al. 2009). They are found throughout CWs, but are most commonly identified in three compartments: attached or closely associated with plant roots (rhizospheric region), associated with biofilm encompassing the bulk wetland media or suspended within the interstitial water (Weber 2016). Microbial communities excrete extracellular polymeric substances (EPS) to anchor themselves in an area to create a more stable and suitable environment where they can thrive (Weber and Gagnon 2014).

As the microbial community establishes itself by creating microbial mass and biofilm by utilizing wastewater constituents (anabolism) it grows within the pore space of the subsurface flow wetland (see Section 1.2.3). As the biofilm develops within the wetland pore space, hydrology and flow path are impacted at a local-scale. This will drive the wastewater (and nutrients) through a slightly different flow path providing additional areas with increased nutrients and limiting the nutrients to areas where biofilm growth is more robust (Weber 2016). Over time, a subsurface

biofilm can develop to the point where it is close to homogeneous throughout the wetland. At the same time as the biofilm is developing, biofilm detachment also takes place due to matrix destabilization (from cell death) or local velocity changes exerting a shear stress on the biofilm (Samsó and Garcia 2013).

Pollutants are broken down by microorganisms in CWs via a range of catabolic processes. Different microenvironments exist within the greater CW which can select for different catabolic pathways available to the microbial community (Weber 2016). Processes such as respiration and fermentation transform complex organic pollutants into simple substances such as carbon dioxide and water. These reactions proceed by an oxidation-reduction (redox) electron transfer, with the microorganisms using the energy differential for the purposes of their growth and reproduction. A redox reaction involves the transfer of electrons from one compound to another, where electrons are transferred from a donor compound of a higher energy state to an electron acceptor compound at a lower energy state (Faulwetter et al. 2009).

The microbial community will induce these redox electron transfers based on the path of least resistance. Therefore, the wastewater constituents available and the physical conditions (dissolved oxygen content, pH, ORP, etc.) of the wastewater drive the functional ability of the microbial community in a predictable sequence (Table 1.2). A high redox potential is characteristic of an oxidized environment and promotes aerobic processes, such as respiration, while a low redox potential is associated with reducing conditions which favour anaerobic processes, such as denitrification, sulfate reduction and methanogenesis (Faulwetter et al. 2009).

Table 1.2: Selected types of microbial oxidation-reduction reactions (adapted from Faulwetter et al. 2009).

Process	Electron acceptor (EA)	End products	Moles of e^- per mole of EA	ΔG° (kJ/mole of electron)	Redox potential (mV)
Aerobic respiration	O_2	H_2O	4	-125.1	300 to 700
Nitrate reduction	NO_3	N_2, NOx	5	-118.8	100 to 350
Manganese reduction	Mn^{4+}	Mn^{2+}	2	-94.5	-100 to 300
Iron reduction	Fe^{3+}	Fe^{2+}	1	-24.3	-100 to 200
Sulfate reduction	SO_4^{2-}	S^{2-}	8	-25.4	-200 to -100
Methanogenesis	CO_2	CH_4, CO_2	8	-23.2	-350 to -100

The majority of wastewater treatment provided by the microbial community in a CW will come from catabolism (Weber 2016); however anabolism should not be ignored. For instance, in a CW start-up phase as the microbial community is growing and developing anabolic processes will contribute a percentage of pollutant removal. The majority of pollutant removal mechanisms attributed to microbial communities throughout this thesis will discuss only the catabolic removal pathways in CWs, unless otherwise noted.

1.2.3 Types of Constructed Wetlands

A variety of CW designs exist which can influence the efficiency of pollutant removal. Differences in design characteristics associated with free water surface (FWS) CWs, horizontal

subsurface flow (HSSF) CWs and vertical subsurface flow (VF) CWs allow for the treatment of wastewaters with varying influent pollutant profiles. Selection of a CW design depends on the nature of pollutants to be treated, climate of the region and the land area available for construction. In addition to the three main types of CWs, there are numerous variations, including hybrid systems, intensified CWs and systems designed for specific wastewater types. A short description of the main CW technologies is presented here.

1.2.3.1 Free Water Surface Constructed Wetlands

Free water surface (FWS) CWs have the largest land footprint to treatment ratio and appear most like natural wetlands. FWS CWs often have open water zones and a variety of wetland plants and animals (Kadlec and Wallace 2008). They differ from natural wetlands due to the addition of berms, dikes and liners to control the flow of water within the wetland as well as infiltration to and from the wetland. Natural wetland vegetation, typically both emergent and floating vegetation, can be added or allowed to establish naturally. The water flows at the surface of the wetland where oxygen diffusion can occur (Brix 1994). The surface water of FWS CWs is considered aerobic because of this oxygen diffusion, however oxygen rapidly decreases with depth as it is used by the microbial community for biological processes (Kadlec and Wallace 2008). This allows for some anaerobic processes to occur lower in the water column and within the sediment. Basic elements of FWS CWs are depicted in Figure 1.1.

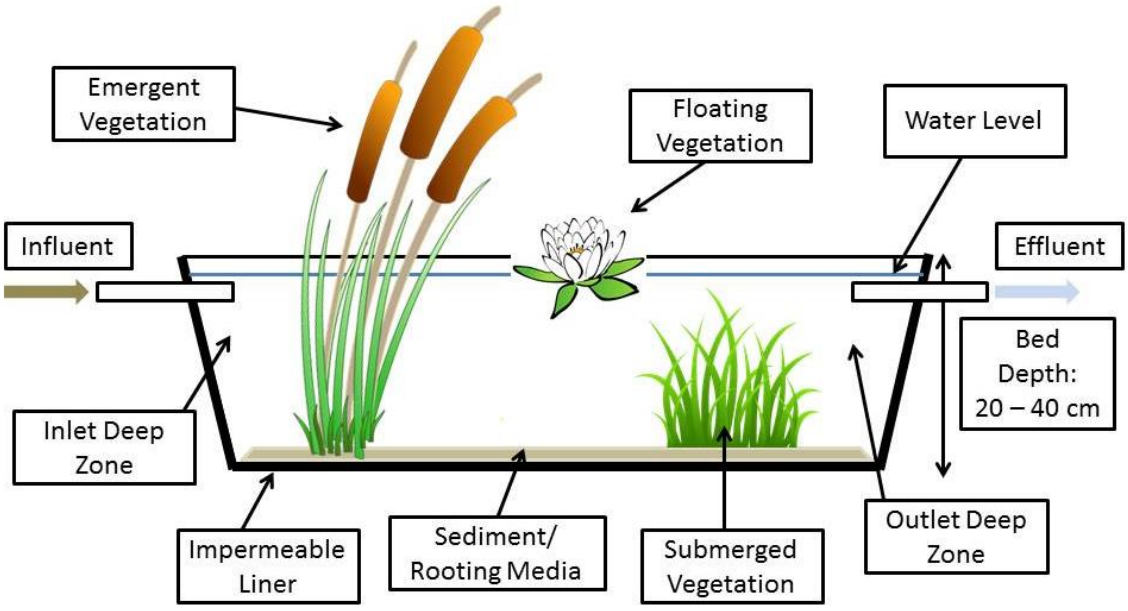

Figure 1.1: Basic elements of a free water surface wetland constructed wetland (adapted from Kadlec and Wallace 2008).

1.2.3.2 Horizontal Subsurface Flow Constructed Wetlands

Horizontal subsurface flow (HSSF) CWs are typically gravel filled beds in which water flows horizontally from inlet to outlet below the surface (Figure 1.2). These systems are water saturated and typically planted with emergent wetland vegetation (Kadlec and Wallace 2008). HSSF CWs are designed to treat secondary or tertiary effluent beneath the surface of the bed media

where wastewater is forced to flow within plant rhizosphere (root zone). Minimal oxygen exchange occurs with the atmosphere as the water level stays below the surface of the bed media, thus these systems are largely anaerobic (Brix 1994; Kadlec and Wallace 2008).

In the direct vicinity of root structures, an increase in oxygen is noted in HSSF systems. Plants exude oxygen from their roots resulting in localized increases of dissolved oxygen near plant roots structures, also known as the rhizosphere (Bais et al. 2006). This anecdote allows limited aerobic processes to occur in HSSF CWs. HSSF CWs can be used in cold climates as the water is buffered from the atmosphere which can help to reduce hydraulic shortages from ice formation and subsequent clogging (Werker et al. 2002).

Primary solids settling is required to remove most of the suspended solids in the water prior to introduction to HSSF CWs. This pre-treatment step is taken so the subsurface matrix (typically gravel or sand) does not clog with solids. Even when pre-treatment is performed clogging can still occur. This stems from the development of biofilm on the subsurface media in the wetland. With nutrient influx into the wetland the biofilm can grow in density to near homogeneity throughout the wetland. If webs which bridge across the pore spaces in the wetland subsurface media develop this may induce solids accumulation. The accumulation of solids in the subsurface pore spaces can lead to clogging and hydraulic issues in the wetland (Knowles et al. 2011).

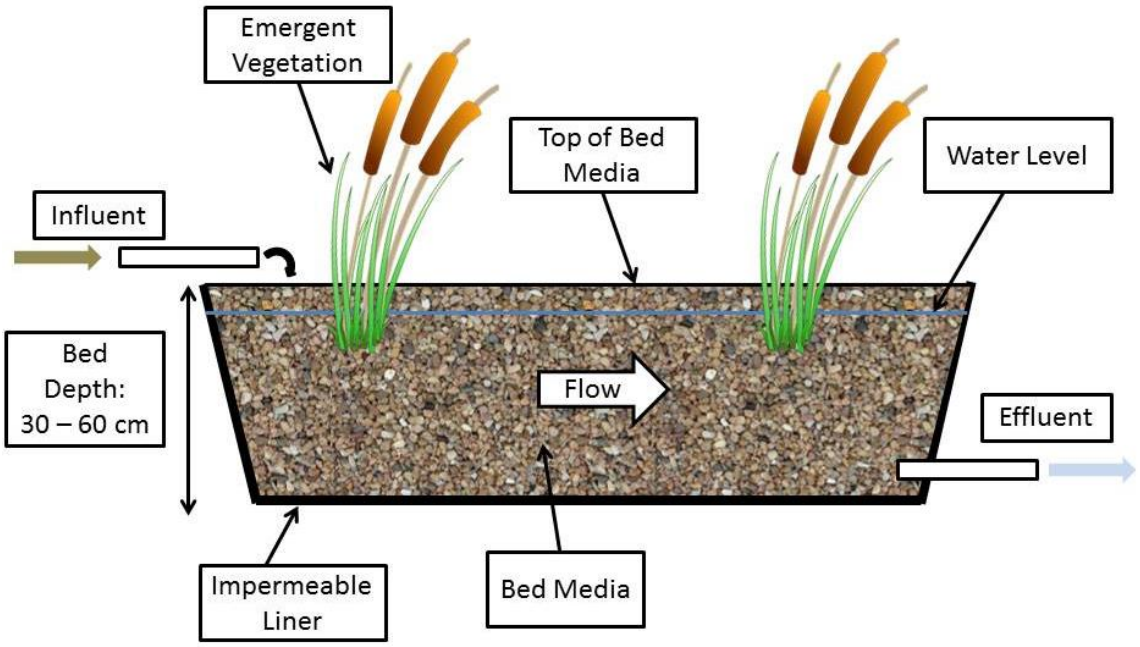

Figure 1.2: Horizontal subsurface flow constructed wetland schematic (adapted from Kadlec and Wallace 2008).

1.2.3.3 Vertical Subsurface Flow Constructed Wetlands

Vertical subsurface flow (VF) CWs are typically unsaturated gravel and/or sand filled systems where wastewater flows vertically from the surface to the bottom of the bed (Figure 1.3). Wastewater is treated as it passes through the root zone of the planted vegetation and the

subsequent wetland bed media (Kadlec and Wallace 2008). This mode of CW is largely aerobic due to oxygen diffusion during wastewater introduction (often ponded on top of bed media) as well as the downward convection of air associated with the venturi effect (Armstrong et al. 1992; Kadlec and Wallace 2008). The venturi effect occurs within VF CWs when the surface water is drawn vertically down through pore spaces in the bed media. This action causes an increase in velocity and a decrease in pressure whereby air is drawn into the wetland system (Armstrong et al. 1992). VF CWs can run in an alternative mode where the system is kept saturated and with water leaving the system by an overflow outlet. This mode may be used in situations where an anaerobic wastewater treatment is preferred (Kadlec and Wallace 2008).

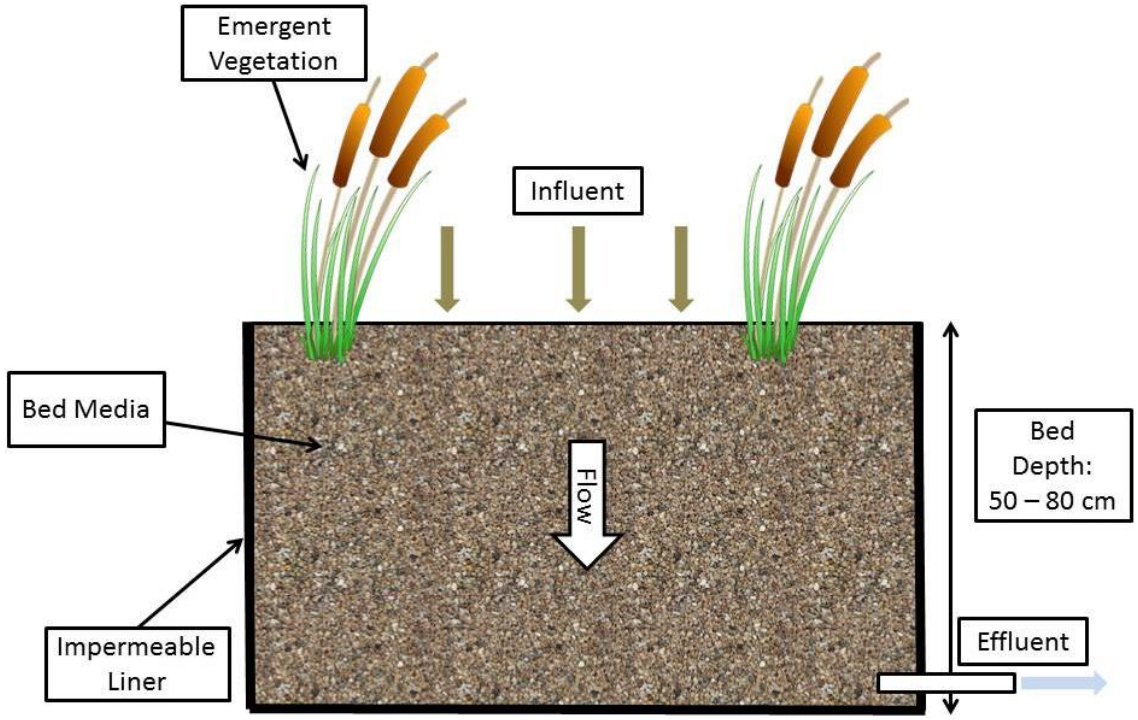

Figure 1.3: Vertical subsurface flow constructed wetland schematic (adapted from Kadlec and Wallace 2008).

1.2.3.1 Hybrid Constructed Wetlands and Intensification Designs

CWs may be further intensified to increase pollutant removal using a variety of methods including hybrid CW designs, manipulation of flow mode, the addition of artificial aeration and the use of novel substrate materials. Newer CW systems are now employing more than one type of CW in succession to attain desired wastewater treatment. This is known as a hybrid system (Figure 1.4) (Kadlec and Wallace 2008). VF CWs may be operated with different flow modes (saturated, unsaturated, tidal), based on the nature of the incoming wastewater and the desired treatment effect of the wetland (Stein et al. 2003; Cooper 2005; Kadlec and Wallace 2008; Sklarz et al. 2009; Vymazal 2010).

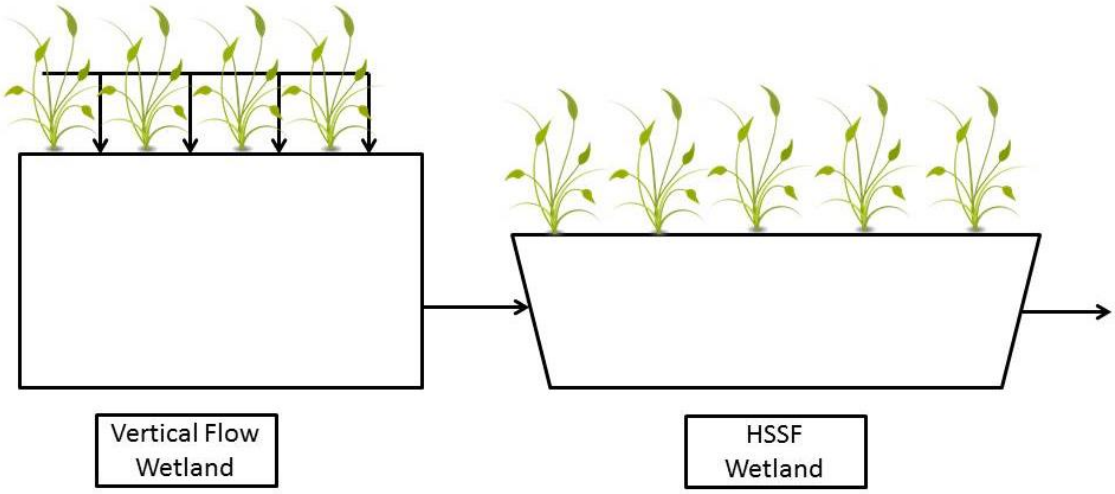

Figure 1.4: Example hybrid constructed wetland scheme (adapted from Kadlec and Wallace, 2008).

In addition to flow mode, changes to the physical properties of the CW may be used for intensification to increase pollutant removal. For instance, novel substrate medias such as bauxite, shale, limestone, zeolite, light expanded clay aggregate and fly ash (Sakadevan and Bavor 1998; Drizo et al. 1999; Vohla et al. 2005) have been tested in an attempt to improve phosphorus removal capacity in subsurface flow CWs. Artificial aeration may be employed in CWs to improve the removal of organic matter and increase the availability of certain nitrogen transformations (Murphy et al. 2016). Artificial aeration can be implemented in oxygen-limited subsurface wetlands to assist inherent processes limited by the availability of oxygen. Implementation involves a mechanical blower and distribution system which often comes at a high operational cost (Kadlec and Wallace 2008). A study using a HSSF CW to treat landfill leachate reported improved treatment performance with the addition of aeration. Biochemical oxygen demand (BOD) removal efficiency improved from between 75-81% to 88-97% with aeration (data represents all four seasons). In the same wetland, ammonium removal efficiency improved from between 14-44% to 93-98% with supplemental aeration (data represents all four seasons) (Nivala et al. 2007). Artificial aeration has also been reported to stimulate biofilm development and reduce solids accumulation in planted CWs (Chazarenc et al. 2009).

1.2.4 Pollutant Removal in Constructed Wetlands

Carbon, nitrogen and phosphorus are the major nutrients cycled within natural and CW systems (Kennedy and Mayer 2002; Kadlec and Wallace 2008; Faulwetter et al. 2009; Garcia et al. 2010). These nutrients are also considered pollutants when concentrated in wastewater. In excess quantities they can lead to rapid algae and microbial growth which in turn may severely decrease dissolved oxygen in receiving waters, impacting a variety of aquatic life. CWs transform and remove these pollutants via physical, chemical and biochemical processes (Kennedy and Mayer 2002). These processes involve many types of microorganisms (and to some extent plants) but are limited by available oxygen and redox conditions within the wastewater (Garcia et al. 2010).

Carbon is present within incoming wastewater in various forms and is added to wastewater via decomposing wetland vegetation. Different wastewater sources will have varying levels of

carbon in different forms (organic, mineral, solids, dissolved). Carbon is assimilated within CWs by microorganisms (Kadlec and Wallace 2008; Faulwetter et al. 2009). Carbon is an essential nutrient in several biological reactions of both aerobic and anaerobic nature – respiration, fermentation, denitrification, iron reduction, sulfate removal and methanogenesis (Faulwetter et al. 2009). Therefore, the rate of carbon utilization and removal in CWs is based on the redox conditions within the wetland (Table 1.2). In large scale CWs there may be a lack of carbon containing compounds further along the treatment path. This lack of carbon has implications for microbial biological treatment processes which require carbon compounds to transform many other pollutants, such as nitrate to nitrogen gas in denitrification (Kadlec and Wallace 2008).

Nitrogen is present in wastewater as organic nitrogen, ammonium, nitrate and nitrite (Halling-Sørensen and Jorgensen 1993). Microbial biological processes dominate the nitrogen transformations within CWs (Garcia et al. 2010). The first step is ammonification, where organic nitrogen is transformed to ammonia in aerobic and anaerobic microbial processes. Secondly, nitrification takes place. Ammonia must be converted to nitrite and nitrate by nitrifying bacteria in the presence of oxygen, see reactions (a) and (b). Finally, nitrate is converted to nitrogen gas by microbial processes (denitrification) in the absence of oxygen, see reaction (c).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \quad \text{(a)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad \text{(b)}$$

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O \quad \text{(c)}$$

Denitrification is anaerobic in nature and typically the rate limiting step in nitrogen removal in CWs (Faulwetter et al. 2009; Garcia et al. 2010). Within CWs there is also the possibility for large amounts of organic nitrogen to be assimilated by vegetation at specific times during the growing season (Kadlec and Wallace 2008). In HSSF CWs nitrification occurs largely within the rhizosphere (root zone); it is here that small microenvironments of aerobic conditions exist within the greater anaerobic environment which dominates the wetland (Bais et al. 2006; Kadlec and Wallace 2008). Denitrification can occur elsewhere where anaerobic conditions dominate. VF CWs are generally well aerated so nitrification can occur throughout the wetland, however denitrification is challenging due to a lack of anaerobic microenvironments.

Phosphorus is present in wastewater in both organic and inorganic forms (Vymazal 2007; Garcia et al. 2010). Most of the phosphorus removal mechanisms available in wetlands are reversible and there are no microbial pathways for the removal of phosphorus (Garcia et al. 2010). Additionally, plant uptake of phosphorus is relatively low (Kadlec and Knight 1996; Vymazal 2007; Garcia et al. 2010). Sedimentation and chemical precipitation (via reaction with metals to form insoluble compounds) are important removal processes for phosphorus in CWs (Vymazal 2007; Garcia et al. 2010). Phosphorus may also absorb to the wetland substrate/bed media within a subsurface flow wetland technology. However, gravel which is the most common wetland bed media (Kadlec and Wallace 2008), has a low phosphorus sorption capacity. New media substrates with increased phosphorus sorption potential, such as bauxite, shale, limestone, zeolite, light expanded clay aggregate and fly ash (Sakadevan and Bavor 1998; Drizo et al. 1999; Vohla et al. 2005) have been tested in an attempt to improve phosphorus removal capacity in subsurface flow CWs.

1.2.5 Constructed Wetland Vegetation

Wetland vegetation plays an important role in the ecology of CWs and can have an indirect and positive influence on pollutant removal. Plants have a wide variety of functions in CWs including: storing and releasing relevant nutrients in seasonal cycles; chemical uptake and sequestration (N, P and metals); providing surfaces for microbial attachment and biofilm development; increasing oxygen supply and nutrients through root exudation within the rhizosphere (root zone); providing structural stability, blocking wind, decreasing re-suspension of suspended solids and providing shade to decrease algal growth (Brix 1997; IWA 2000; Kadlec and Wallace 2008).

The majority of studies comparing planted and unplanted systems show that the presence of plants has a positive effect on nutrient removal in CWs (IWA 2000; Allen et al. 2002; Jing et al. 2002). The presence of plants in CWs correlated with an increase in microbial community diversity (Zhang et al. 2010), density (Gagnon et al. 2007) and overall microbial activity, with microbial communities from planted wetland mesocosms displaying up to ten times the activity of similar unplanted systems (Weber and Legge 2013). In addition, plants are known to exude enzymes, chemicals and nutrients from their roots which can be beneficial for nearby microbial communities and consequently increase water treatment performance (Bais et al. 2006). Removal efficiencies differ between plant species depending on the pollutant, therefore macrophyte species selection is an important part of CW design (Brisson and Chazarenc 2009).

Wetland vegetation can be classified as emergent, floating, submerged and woody based on morphology and physiology (Wetzel 2001; Kadlec and Wallace 2008; Vymazal 2013). Emergent vegetation grows on water-saturated or submersed soils and extends above the water surface. Typical emergent vegetation species found in wetlands are common reed (*Phragmites australis*), reed canary grass (*Phalaris arundinacea*) and cattail (*Typha latifolia*) (Vymazal 2013). Floating vegetation may be rooted or not rooted but can float on the water surface. Typical floating plant species found in wetlands include water lily (*Nymphaea alba*) and water hyacinth (*Eichhornia crassipes*) (Kadlec and Wallace 2008). Submerged wetland vegetation occurs at all depths within the water column. Submerged aquatic vegetation which have been used to treat wastewater include: waterweed (*Elodea* spp.) and naiads (*Najas* spp.) (Kadlec and Wallace 2008). Woody species include trees and shrubs, most notably willows (*Salix* spp.) (Gregersen and Brix 2001; Brix and Arias 2005). It is important to note when selecting vegetation for CWs that some wetland plant species are invasive. This factor should be considered when planning experimental work and the execution of full-scale CWs (Brisson and Chazarenc 2009).

1.2.6 Constructed Wetland Modeling

Contaminant removal in CWs can be predicted with mathematical models (Kadlec and Wallace 2008). The removal of contaminants depends on many factors such as local contaminant concentration, mechanisms and pathways available for removal. Generally, contaminant removal in CWs can be described using zero order, first order or second order reactions. The reaction rate model used will depend on local concentrations of said contaminants and the mechanisms or removal pathways available in the wetland (Kadlec and Wallace 2008). The use of first order rate kinetics in CW modeling is typically suitable:

$$k = q \cdot \ln\left(\frac{C_i}{C_O}\right) \quad (1)$$

k = first order aerial reaction rate constant (m/day)

q = hydraulic loading rate (m/day)

C_i = incoming concentration (mg/L)

C_O = outflow concentration (mg/L)

For many pollutants in wetlands, there is a background concentration which either remains resistant to degradation or has returned to the water from the static compartments of the ecosystem (plant biomass and sediment) creating a negative contribution which limits the total removal of the pollutant (Kadlec and Wallace 2008). Another first order model, the k-C* model, considers the background concentration (C*) of the pollutant and may be more suitable for use in wetland pollutant removal modeling:

$$k = q \cdot \ln\left(\frac{C_i - C^*}{C_O - C^*}\right) \quad (2)$$

C_O = outflow concentration (mg/L)

C* = background concentration (mg/L)

C_i = incoming concentration (mg/L)

k = first order aerial reaction rate constant (m/day)

q = hydraulic loading rate (m/day)

The first order removal rate models described have a few shortcomings. Mainly, the reaction rate (k) is estimated over the entire surface area of the CW (Kadlec and Wallace 2008). This does not consider wetland depth or the imperfect interactions of the pollutants with microbial communities which grow in a biofilm that may not develop uniformly across the wetland. More sophisticated CW models which incorporate biofilm development and kinetics exist (Rajabzadeh et al. 2015). However, due to the ease of use associated with first order models, they are still favoured for use in industry (Kadlec and Wallace 2008).

1.3 Constructed Wetland Research

1.3.1 Constructed Wetlands as an Ecosystem

Constructed wetlands, like natural wetlands, are complex, living ecosystems. Natural wetlands are some of the most productive ecosystems in the world and this directly translates to the wastewater treatment ability of CWs (Kadlec and Wallace 2008). The multifaceted wastewater treatment ability of CWs results from the biological interactions of the ecosystem (plants, microbial community) with the chemical and physical aspects of the wastewater and wetland substrate. Additionally, this provides CWs a unique advantage over other wastewater treatment technologies as microenvironments of a wide range of redox potentials and dissolved oxygen concentrations are

possible in the wetland biofilm and rhizosphere. Natural variability in treatment performance occurs as a result of this like in any other biological wastewater treatment process. CWs, as an ecosystem, are generally resilient and are designed to be efficient at wastewater treatment even when changes to wastewater composition, season (temperature, rainfall), biofilm development and plant growth occur. This resilience stems from the interconnectivity of the CW ecosystem which can adapt to fluctuations and changes within a steady-state equilibrium. It is important to recognize that CWs are a fluid, ecosystem which will adapt to changes over time, based on the on robust connections between the physical, chemical and biological regimes within. Therefore, it is very important to acknowledge the interconnectedness of the ecosystem when planning experiments and monitoring full-scale systems. Gathering as much information about CWs as feasible can help to highlight trends and fluctuations which can be applied to changes in wastewater treatment performance.

1.3.2 Constructed Wetland Start-Up

When CWs are initially established typically time is reserved for a "start-up" or development period before full functional operation and wastewater treatment is expected. This time is allotted to allow sufficient plant growth and microbial establishment. Metrics of pollutant removal and microbial activity or function are not often tracked over this phase of development. A few studies have been performed regarding microbial community establishment during wetland start-up (Ragusa et al. 2004; Weber and Legge 2011; Ramond et al. 2012; Oopkaup et al. 2016). Ragusa et al. (2004) used a variety of metrics to measure biofilm growth and activity in wetland microcosms and found that biomass can take up to 100 days to stabilize. Weber and Legge (2011) employed Community Level Physiological Profiling (CLPP) to determine microbial community function and found stabilization of the microbial community between 75 to 100 days. This correlated with hydrological parameters, porosity and dispersivity, which alluded to the increase of microbial biomass over the same time period. Ramond et al. (2012) used denaturing gradient gel electrophoresis (DGGE) to track microbial community structure in a start-up situation and found community convergence between 89 to 100 days. Oopkaup et al. (2016) found the abundance of the bacterial community of a subsurface flow wetland treating municipal wastewater reached a maximum after 60 days of operation. Despite using a variety of different methods to characterize the microbial community from different types of CWs, all studies concluded that microbial community stabilization takes between 60 to 100 days. The increase in the study of microbial community temporal dynamics over the wetland development period correlates to the increase in research focusing on the study of bacterial communities in CWs over the last ten years (Weber 2016). The effect of wetland intensification designs, such as aeration, on wetland start-up has not been explored. The initial establishment of the ecosystem within a CW is of interest as a more robust biological system (microbial and plant community) will provide increased wastewater treatment services. It is important to know the length of time required before full-scale water treatment services are available and whether technology additions, such as aeration, will increase the speed of ecological stabilization. Additionally, correlations between microbial community development (structure, function, activity) and wastewater treatment performance during start-up should be explored further.

From an ecological stand point a "start-up period" does not have to be utilized only in terms of a "wetland initialization". Start-up period data sets can be called upon when there is a regression in wetland performance from an external environmental factor and the wetland is no longer under steady-state conditions. This may be a change of season (temperature, plant growth), after plant

harvesting or replanting, natural disaster, clogging event, cleaning event (to remove clogging), after a maintenance window or other unplanned shut-down (i.e. pump failure), or if antimicrobial inputs (nanoparticles, pharmaceuticals) set off the balance of the microbial community. Start-up period data is essentially analyzing a point at which wetland characteristics settle into a steady-state. Any time a wetland is set off from its steady state value by an external perturbation (as listed previously) these data sets can inform timelines for returning to the wetland steady-state. The more all-encompassing these data sets are (hydrology, ecology, water treatment), the more useful to engineers and policy makers who have to create/adhere to guidelines for water regulation and surface water protection. External factors can cause perturbations to CWs at any time. More information regarding the after effects and return to full-scale treatment is needed to increase the ease of use and accessibility of CW technology. A limited number of studies have evaluated perturbations and after effects on CWs which are discussed in the section to follow.

1.3.3 Constructed Wetland Perturbations

As CWs are a wastewater treatment technology, studies often focus on pollutant removal mechanisms in reference to system design and wastewater loading. This may be for typical wastewater pollutants or those which are of emerging concern. Often the short-term and long-term effects of emerging pollutants on CWs in terms of plant and microbial health, system performance and hydrology are somewhat ignored. It is important to evaluate the capacity of CWs to remove new pollutants but it also important to acknowledge potential effects on the CW ecosystem from these pollutants. A rapid increase of a certain pollutant may cause a perturbation in an element of the wetland dynamic which may permeate to all aspects of the living ecosystem and change how the CW is able to handle pollutants in wastewater.

Two early papers which evaluated perturbations on wetland microbial communities assessed changes in the microbial community of CW mesocosms in response to acid mine drainage (AMD) (Weber et al. 2008) and an antibiotic (ciprofloxacin) (Weber et al. 2011). The first study explored changes to the microbial community over a 22 day period after an acute exposure to simulated AMD. Weber et al. (2008) reported a disturbance to the microbial community after AMD exposure citing a detachment of fixed biofilm from the subsurface media. Additionally, microbial communities from mesocosms which were planted with *Phragmites australis* did not experience as large of an effect to the microbial ecology as those from unplanted mesocosms. The second study investigated the effect of ciprofloxacin on the development, function and stability of CW microbial communities by tracking the microbial community over the course of 22 weeks after wetland initialization and subsequent dosing with ciprofloxacin for 5 days (after 1 week of development) (Weber et al. 2011). Weber et al. (2011) reported that the microbial community was initially adversely affected by the dose of ciprofloxacin but recovered after 2-5 weeks in activity and catabolic function to values of those found in control systems. Additionally, wetland plants in the ciprofloxacin dosed wetlands did not adapt to the antimicrobial exposure and a die-off was noted.

Since these two studies a number of studies have successfully evaluated the removal of pharmaceuticals (Vymazal et al. 2017), antimicrobials and personal care products (Ávila et al. 2015) and pesticides (Lv et al. 2016) within CWs. These studies were performed in the short-term and did not evaluate effects to plant health, microbial activity or structure, or whether the introduction of these contaminants influenced other wetland processes. This type of extended analysis can be challenging for systems receiving domestic wastewater (Vymazal et al. 2017; Ávila et al. 2015) but other studies have provided information regarding the changes a wetland may

undergo after the introduction of new pollutants. In a follow up to their pesticide removal study based on treatment performance, Lv et al. (2016) discuss microbial activity and richness after dosing with pesticides (imazalil and tebuconazole). Overall, microbial activity and richness were not differentiated by the presence of pesticides. Lv et al. (2016) cited plant species and season as major drivers of microbial activity and richness trends rather than the introduction of pesticides. Other longer-term studies (1.5 and 5 years) reported efficient removal of antibiotics in CWs (Berglund et al. 2014) and temporal removal trends for pharmaceuticals and personal care products (PPCPs) (Reyes-Contreras et al. 2012). Berglund et al. (2014) also evaluated the risk of antibiotic resistance gene formation with the added antimicrobial inflow and did not notice a significant affect to antibiotic resistance gene concentration with short-term treatment of environmentally relevant concentrations of antibiotics. Reyes-Contreras et al. (2012) evaluated the medium term (3-5 years) removal trends of PPCPs in CWs. They found seasonality (summer versus winter), presence of vegetation and age of the CW to influence the removal of PPCPs. A wetland microcosm study by Button et al. (2016) examined the effects of an antimicrobial exposure of silver nanoparticles to the wetland microbial catabolic function and genetic fingerprinting. Changes in microbial community function and structure were monitored in wetland microcosms over a period of 4 weeks following an *in-situ* exposure of silver nanoparticles. Low doses of Ag did not appear to exert significant toxic effects on the microbial community in the short term (1 month) when dosed *in-situ.* There was evidence of microbial resistance to toxicity when microbial communities had been previously exposed to a low dose of Ag. Experiments such as the ones listed provide information regarding a perturbation to a CW system and subsequent monitoring of standard wetland characteristics.

Other scenarios, such as a pump failure or cleaning of a clogged wetland, may also create perturbations to the wetland steady-state. Only a few studies have evaluated the potential effects of these scenarios on CW systems. Murphy et al. (2016) completed a field trial to understand the effects of an aeration pump failure on nitrification in an aerated wetland. Dissolved oxygen disappeared from the water after 12 hours, while nitrate was observed in the wastewater for over 48 hours after the pump had been turned off. After two weeks, the aeration resumed and pre-perturbation nitrification levels (around 80%) returned within 48 hours. In contrast, the start-up nitrification of an aerated system required 23 days to reach greater than 80%. Nivala and Rousseau (2009) completed field trials to manage wetland clogging with hydrogen peroxide (H_2O_2) application. Typically, clogging is remediated by removing gravel and replacing with new gravel or washing and replacing the old gravel. This can prove to be very costly; therefore, a new method of *in-situ* remediation with hydrogen peroxide was evaluated. The aggressive oxidation of the organic matter by the hydrogen peroxide reduced sludge build-up and water ponding with minimal effects to wastewater treatment performance (N, P, carbon, TSS). The idea behind these two studies (perturb and monitor) is extremely important for the field of CWs. It can inform policy markers and regulating bodies and allow security in the use of newer intensification designs, such as artificial aeration. The use of these intensification designs can increase wastewater treatment performance, decrease wetland size and allow new applications for the use of CWs.

1.3.4 Artificial Aeration in Constructed Wetlands

Artificial aeration was identified as an intensification system for CW design in Section 1.2.3.1. Aeration is an emerging intensification technology used in CWs which are oxygen-limited (sub-surface wetlands). The addition of aeration can assist processes which are oxygen-limited by increasing the availability of dissolved oxygen. Artificial aeration may be employed in CWs to

improve the removal of organic matter and increase the availability of certain nitrogen transformations (ammonification and nitrification) (Murphy et al. 2016). It may also be used in cold climates to increase oxygen availability and reduce the seasonal effects associated with pollutant removal in winter months (Ouellet-Plamondon et al. 2006). Artificial aeration can significantly improve total Kjeldahl nitrogen removal (TKN; organic nitrogen and ammonium nitrogen) as it provides the increased dissolved oxygen necessary for their microbial transformations to proceed (Maltais-Landry et al. 2009). Aerated CWs have been reported to handle shock loads of organic pollutants better than non-aerated CWs and reach certain levels of pollutant removal 6 hours faster than the non-aerated control wetland (Zhu et al. 2013). The addition of aeration can also broaden wastewater types for which CWs can provide treatment, for instance concentrated hospital wastewater containing many pharmaceuticals (Auvinen et al. 2017).

On the other hand, there are some drawbacks to the addition of aeration to a CW. Artificial aeration can be detrimental to the growth of some wetland plants (Butterworth et al. 2016). The implementation of artificial aeration can be costly if it is not offset by a reduction in the size of the wetland and associated capital costs (Kadlec and Wallace 2008). Aeration also has positive and negative effects on solids accumulation in wetlands. Water agitation reduces the settling of suspended solids so they can be better removed from the system, but aeration has been associated with increased microbial mass (Chazarenc et al. 2009). Therefore, this may have implications for wetland clogging in the long-term.

Continuing the study of artificial aeration in CWs is important to advance the field of CWs for wastewater treatment. Understanding fundamental differences (hydrology, microbial community, water quality) and pollutant removal mechanisms between aerated and non-aerated CWs is important for the widespread implementation of aeration in wetlands and increasing the applications available to CWs.

1.4 Emerging Contaminants

Emerging contaminants are chemicals or microorganisms which have recently been shown to occur widely in the environment. Emerging contaminants are identified as being a potential environmental or public health risk, but adequate data does not exist to quantify their risk in the environment (Jörg and Laurence 2001). These substances can enter the environment from municipal, industrial and storm water waste streams (Figure 1.5). Industrial chemicals, pesticides, pharmaceuticals and personal care products are known to release new emerging contaminants into the environment (Richardson and Ternes 2011). Emerging contaminants to note include: ionic liquids, sucralose and other artificial sweeteners, nanomaterials, perfluorinated compounds, pharmaceuticals, hormones, drinking water disinfection by products, sunscreens and UV filters, brominated flame retardants, benzotriazoles, naphthenic acids, antimony, siloxanes, musks, algal toxins, perchlorate, dioxane, pesticide transformation products and microorganisms (Richardson and Ternes 2011). Research for most of these emerging contaminants continues to focus on their removal from water and the transformation products that can occur when they are not effectively removed.

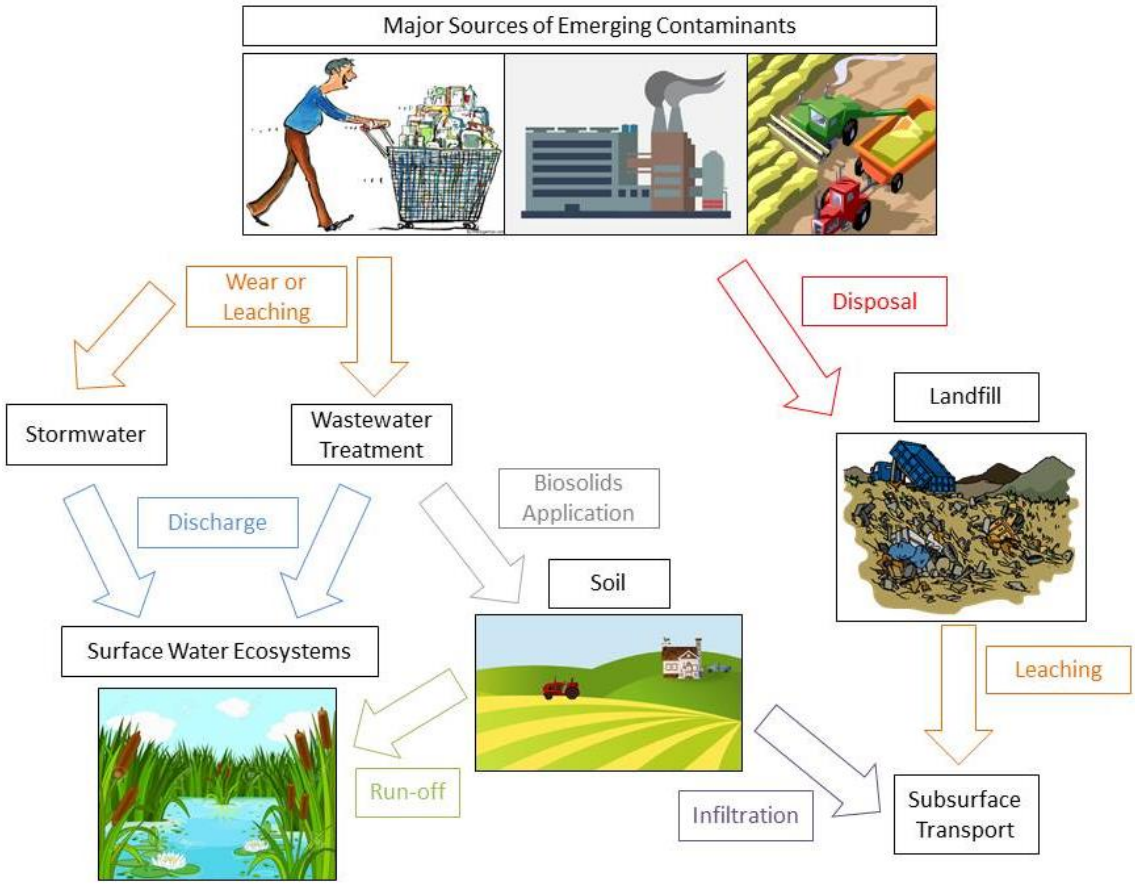

Figure 1.5: Major sources of emerging contaminants and their potential release pathways to the natural environment. Environmental acceptor compartments include soil and surface water ecosystems, such as farmland, lakes and wetlands.

Data regarding the ecological effects of emerging contaminants in the environment is lacking. Studies have emerged in recent years to define the impacts of some of these emerging contaminants on indictor aquatic and terrestrial organisms, such as titanium dioxide nanoparticles on developing zebra fish (Bar-Ilan et al. 2012), silver nanoparticles on green algae (*Chlamydomonas reinhardtii*) (Navarro et al. 2008), silver nanoparticles on zebra fish (Asharani et al. 2008), silver nanoparticles on *Daphnia magna* (Zhao and Wang 2011), ethinylestradiol on early life stages of mink frogs and green frogs (Park and Kidd 2005), poly brominated diphenyl ethers and polychlorinated biphenyls on various wild aquatic species (Wu et al. 2008), and ciprofloxacin on wetland microbial populations (Weber et al. 2014). The persistence of these emerging contaminants in the environment may be a threat and the effects on aquatic and terrestrial ecosystems are mostly unknown.

1.4.1 Effect of Emerging Contaminants on Constructed Wetlands

Emerging contaminants are expected to reach the natural environment (soil, air and water) through landfills, industrial waste streams, and waste water treatment plants. Emerging contaminants are therefore expected to reach wetlands via landfill leachate, wastewater treatment

plants, runoff from agricultural biosolids spreading and human recreation (Figure 1.5). The effect of emerging contaminants on CW ecology and wastewater treatment performance is relatively unknown. A few studies have found that emerging contaminants can have a dramatic effect on the wetland microbial and plant communities (Colman et al. 2009; Weber et al. 2011; Helt et al. 2012; Lowry et al. 2012; Colman et al. 2014; Cosway 2014; Weber et al. 2014). Exposures of antimicrobial agents in the ng/L to µg/L range has caused the reduction of wetland microbial and plant populations (Lowry et al. 2012) as well as a decrease microbial activity and function (Weber et al. 2011; Button et al. 2016). Knowledge of the effects of emerging contaminants on wetlands and CWs for the purposes of wastewater treatment are limited. Decreases in microbial and plant activity may prove to be detrimental to the treatment performance of CWs. This can have implications for the safety of surface waters treated by CWs. To date the effects of only a handful of emerging contaminants have been tested on CWs mostly pharmaceuticals (100+ papers) and very recently nanoparticles (3 papers). Continued research is required to assess the effects of emerging contaminants, especially those which are antimicrobial in nature, to the health and productivity of both natural and constructed wetlands.

1.5 Introduction to Nanoparticles

1.5.1 Nanoparticles

The International Union of Pure and Applied Chemistry (IUPAC) defines a nanoparticle as a "particle of any shape with dimensions in the 1 nm to 100 nm range" (Vert et al. 2012). Under 100 nm, novel properties and characteristics develop which differentiate the nanoparticle from the equivalent bulk material (Vert et al. 2012). When approaching the nanoscale, the percent of atoms at the surface of the material becomes significant and the surface chemistry begins to dominate in lieu of the bulk properties. Nearly all properties of the material change when transitioning from the bulk material into the nanoscale, including hardness, strength, ductility, elasticity, melting point, density, thermal conductivity, thermal expansion coefficient and diffusivity (Murty et al. 2013). The increase in surface area to mass ratio at the nanoscale allows the interactions of many more atoms on the surface of the material, which causes enhanced chemical reactivity and increased biological activity (Frimmel and Delay 2010; Murty et al. 2013).

The physical and chemical property distinction of nanoparticles from bulk materials have led to the incorporation of nanoparticles in an ever increasing number of consumer products and industrial processes (Vance et al. 2015). Nanoparticles have made their way into a wide range of consumer products including: batteries; household appliances; heating and cooling devices; automobiles; lubricants; coatings; audio visual equipment; computers and mobile devices; televisions; food and beverages; food and beverage storage materials; children's toys; clothing; cosmetics; personal care items; sporting goods; sunscreen; health and fitness supplements; construction materials; cleaning materials; home furnishings; luggage; paint and pet products (Vance et al. 2015). Nanoparticles perform a variety of functions in consumer products and industry. Major growth of nanoparticle applications has come in the medical, health and fitness industries as well as in personal care products. Silver nanoparticle applications have grown immensely in part due to their antimicrobial properties. Silicon dioxide and titanium dioxide nanoparticles still make up a large part of the nanoparticle market (Keller et al. 2013). Silver nanoparticles were selected as a priority nanoparticle for ecotoxicity modeling and investigations due to the known antimicrobial properties of silver (Maynard 2006).

1.6 Silver Nanoparticles

1.6.1 Background, Applications and Release of Silver Nanoparticles

Silver nanoparticles (Ag NPs) are the fastest growing sector of the commercial engineered nanomaterial market (Vance et al. 2015). The most notable applications of Ag NPs are related to the antimicrobial properties of silver (Richards 1980; Ratte 1999). Ag NPs possess a large surface area to volume ratio relative to bulk silver which increases contact with microorganisms. This unique feature of Ag NPs may increase their applicability as a broad spectrum antimicrobial agent as toxicity pathways may differ between Ag NPs and ionic silver (Morones et al. 2005; Kim et al. 2007; YeonáLee et al. 2007; Marambio-Jones and Hoek 2010). New applications for nanosilver technology have increased its abundance in a wide range of consumer products and medical equipment (Reidy et al. 2013). The number of Ag NP containing products has increased from 30 in 2006 to over 300 at the beginning of 2011. As of 2015, the Project on Emerging Nanotechnologies at the Woodrow Wilson International Center for Scholars had compiled a list of more than 1824 consumer products that claim to include some form of engineered nanomaterial (Vance et al. 2015). Of these products about 25% contain Ag NPs. Socks, paints, bandages and other medical equipment, children's toys, fitness equipment, cosmetics and food containers incorporate Ag NPs to exploit the natural antimicrobial properties of silver (Benn and Westerhoff 2008; El Badawy et al. 2010). For example, in clothing such as socks, Ag NPs are incorporated into fabrics in an attempt to restrict the growth of odor-causing bacteria (Morones et al. 2005; Durán et al. 2007; Dobias et al. 2011).

The market for Ag NPs in consumer products is on the rise which increases the likelihood of their release into the environment at all stages of development (Keller and Lazareva 2014). An estimated 452 metric tons of Ag NPs were produced in 2010 and are expected to be released to the environment via the intended use of nanoparticle-containing products, wastewater treatment plant effluent and waste incineration plant emissions (Keller et al. 2013). Another estimate reported current production and industrial use of Ag NPs at about 320 tons per year (Nowack et al. 2012). In the United States, specifically, a production estimate of Ag NPs between 2.8 and 20 tons per year was calculated by Hendren et al. (2011). Piccinno et al. (2012) estimated the production rate of Ag NPs in Europe is estimated to be between 0.6-55 tonnes/year. The total use of Ag NPs until 2015 is estimated at 1120 tons (Stensberg et al. 2011). Keller et al. (2013) estimated global materials flows for Ag NPs in metric tons per year and found that Ag NPs would reach the natural environment in estimated amounts of 150 tons to soil, 63 tons to water and 11 tons to air. Current models predict that the environmental concentrations of Ag NPs are in the ng/L range (Fabrega et al. 2011; Gottschalk and Nowack 2011).

Silver or Ag NPs may be released into the environment as a result of their integration into many different consumer products. Silver has been shown to leach from paints containing Ag NPs when exposed to ambient weather conditions (Kaegi et al. 2010). Ag NP release has also been documented for a wide variety of consumer products including socks (Benn and Westerhoff 2008; Geranio et al. 2009); children toys, personal care products, medical supplies and textiles (Benn et al. 2010; Cleveland et al. 2012)); a washing machine (Farkas et al. 2011); other various textiles (Kulthong et al. 2010) and clothing (Lorenz et al. 2012).

In some cases significant amounts of silver are released from Ag NP impregnated textiles within the first few washing cycles, in both particulate and ionic forms (Benn and Westerhoff 2008; Benn et al. 2010; Quadros et al. 2013; von Goetz et al. 2013). Benn and Westerhoff (2008) reported

releases of 1.5 – 650 µg of Ag from a variety of socks when washing in 500 mL of distilled water. After 4 cumulative washes the amount of silver released equated to between 1% and 99% of the total silver in the various socks. Benn et al. (2010) reported the following releases of silver from consumer products after washing for 1 hour with tap water: medical cloth (46 µg Ag/g product), toothpaste (18 µg Ag/g product), athletic shirt (0.56 µg Ag/g product) and medical mask (11 µg Ag/g product). The effluent from a commercially available washing machine (containing nanosilver) was reported to contain an average of 11 µg/L of silver (Farkas et al. 2011). Recent calculations estimate laundry wastewater (from clothing containing X-STATIC™ or AgKilBact™ silver treated fabrics) could realistically contain 1.5 mg Ag/L (Button et al. 2016). The inputs of Ag NPs from different consumer products and goods may combine to reach a quantity that has negative effects for aquatic environments.

1.6.2 Toxicity Mechanisms of Silver Nanoparticles

Organism toxicity and ecosystem effect studies with Ag NPs have not kept pace with the rapid industrialization and commercialization of Ag NPs in recent years. Single organism toxicity testing in a laboratory setting has been performed in detail (Morones et al. 2005), with research now advancing to studies regarding the transformations of Ag NPs in different release environments as well as the effects of Ag NPs in aquatic environments in a more holistic ecosystem approach (Lowry and Casman 2009; Lowry et al. 2010; Das et al. 2012; Das et al. 2012; Lowry et al. 2012; Dale et al. 2013; Colman et al. 2014; Yang et al. 2014; Dale et al. 2015).

The mode of toxicity of Ag NPs is debated in the literature. Some studies show evidence that the mode of toxicity involves the release of Ag^+ ions from Ag NPs (Navarro et al. 2008; Miao et al. 2009; Xiu et al. 2012) while others demonstrate that the toxicity of Ag NPs is a result of particle size and size specific interactions (Choi and Hu 2008; Fabrega et al. 2009; Kawata et al. 2009; Laban et al. 2010; Jiang et al. 2012). Ag^+ ions interact strongly with thiol groups, which can inactivate important enzymes, including those involved with the electron-transport chain, which in turn affects cellular oxidation, RNA translation and DNA replication (Morones et al. 2005; Kim et al. 2007; Gordon et al. 2010; Massarsky et al. 2014). Navarro *et al.* (2008) concluded that Ag NPs alone have minimal toxicity and serve mostly as a source of Ag^+ ions. Another study came to a similar conclusion, that the dissolution of silver ions from the nanoparticle dictate toxicity (Miao et al. 2009). The dissolution of Ag^+ ions from Ag NPs requires an aerobic, oxidizing environment. A study by Xiu *et al*. (2012) showed that Ag^+ is the definitive toxicant to bacteria when Ag NPs synthesized and tested under strictly anaerobic conditions lacked toxicity. Anaerobic conditions ($NaHCO_3$ buffer) prevented Ag(0) oxidation and Ag^+ release, and without the release of Ag^+, the Ag NPs were not toxic to *E. coli* (Xiu et al. 2012). In contrast, Fabrega et al. (2009) concluded that the effect of released Ag^+ ions is not significant, therefore the dominating factor of toxicity is bacterial contact with the nanoparticles themselves. Additional studies also agreed that the toxicity induced by nanosilver cannot be attributed solely to the released Ag+ ions but rather to the size of Ag NPs (Choi and Hu 2008; Jiang et al. 2008; Kawata et al. 2009; Laban et al. 2010).

In many studies, the level of toxicity of Ag NPs is attributed to the size of the particles (Jiang et al. 2008). Smaller nanoparticles were found to be most toxic, often explained by easier uptake (Choi and Hu 2008) and larger surface area per mass of silver (Johnston et al. 2010), which can facilitate faster dissolution and release of silver ions. Different particle sizes can be obtained by employing different methods of synthesis (El Badawy et al. 2010). There is evidence that the fraction of the smallest particles (<5 nm) may be responsible for the toxic effects of the nanoparticles on nitrifying bacteria (Choi and Hu 2008). Silva *et al.* (2014) compared three types

of Ag NPs (polyvinylpyrrolidone coated, citrate stabilized, and branched polyethyleneimine coated) whose sizes ranged from 72 nm to 56 nm to 28 nm, respectively. They found the smaller branched polyethyleneimine coated Ag NPs to be the most toxic, attributable to its smaller particle size and the greater charge difference between the nanoparticle surface and the biological surface (Silva et al. 2014).

Another theory on the mode of toxicity of Ag NPs involves the generation of reactive oxygen species. Ag^{+}-mediated generation of reactive oxygen species has been reported in the literature (Kim et al. 2007; Carlson et al. 2008; Choi and Hu 2008; Foldbjerg et al. 2009; Gordon et al. 2010; Piao et al. 2011; Zhang et al. 2013; Jiang et al. 2014; Massarsky et al. 2014). The ability of Ag NPs to generate reactive oxygen species originates from enhanced surface area to volume ratio at particles sizes below 30 nm (Carlson et al. 2008; Auffan et al. 2009). One method of reactive oxygen species generation via Ag NPs is by surface plasmon enhancement. This occurs when the frequency of the free electrons within the metal nanoparticle oscillate at the same frequency as incident light photons, resulting in a phenonomen called localized surface plasmon resonance. This leads to the formation of superoxide radical ($O_2^{\cdot -}$) (He et al. 2014). While the ability of Ag NPs to produce reactive oxygen species is evident in the literature, the interactions between Ag NPs and reactive oxygen species at the cellular level is unknown. Ag NPs can generate reactive oxygen species externally and induce oxidative damage at the cell membrane (Massarsky et al. 2014).

Additionally, physiochemical factors, including the size, shape, composition, and surface coating, are expected to affect the toxicity of Ag NPs. Coatings can be applied to nanoparticles to increase their stability and dispersion in solution (Silva et al. 2014). Citrate and polyvinylpyrrolidone (PVP) are commonly employed coating and stabilizing agents (Tolaymat et al. 2010). Different mechanisms of stabilization are imparted by different coating materials – electrostatic, steric and electrosteric forces (Silva et al. 2014). Different surface charges can also result from different coatings, which distinctly affect nanoparticle toxicity. El Badawy *et al.* (2010) investigated four Ag NPs, with surface charges ranging from highly negative to highly positive (citrate, H_2, PVP, branched polyethyleneimine coated (BPEI)). The more negative nanoparticles studied were the least toxic while the most positively charged nanoparticles were the most toxic to Gram-positive *bacillus* species (El Badawy et al. 2010). The physical interactions between Ag NPs and bacteria are important in determining the level of toxicity. These interactions are governed by the surface charge of both the Ag NPs (positive or negative) and cellular membranes of the bacteria, which are usually negatively charged (El Badawy et al. 2010).

1.6.3 Ecotoxicity of Silver Nanoparticles

Most studies performed to quantify the effects of Ag NPs on organisms to date have focussed on single species of microorganisms, small aquatic organisms, and plants in a laboratory setting. These studies have shown that silver nanoparticle exposure leads to membrane damage, oxidative stress and significant mortality in many different species. A brief summary of silver nanoparticle toxicity studies will follow.

Initial toxicity testing with Ag NPs was performed on individual bacterial species in laboratory conditions because of the known bactericidal effects of silver. Many studies performed toxicity testing with *E. coli* (Sondi and Salopek-Sondi 2004; Raffi et al. 2008) or tried to decipher a difference in the response of Gram negative and Gram positive bacterial strains (Vertelov et al. 2008; Sintubin et al. 2011; Jagtap and Bapat 2013). Kim et al. (2007) studied the antimicrobial

activity of Ag NPs against yeast, *Escherichia coli*, and *Staphylococcus aureus*. The growth of yeast and *E. coli* were inhibited at low concentrations (0.7 µg/L) of Ag nanoparticles, whereas the growth-inhibitory effects on *S. aureus* were mild. Another study found complete growth inhibition of *E. coli* at 60 mg/L with 16 nm Ag NPs, where TEM images showed Ag NPs adhered to the bacteria and penetrated into the bacterial cells (Raffi et al. 2008). Another study noted significant growth inhibition of the Gram-negative and Gram-positive species of bacteria, *Escherichia coli* and *Staphylococcus aureus*, at concentrations exceeding 5 mg/L and 10 mg/L, respectively (Cho et al. 2005). Conversely, Sondi *et al.* (2004) noted complete bacterial inhibition of *E. coli* at a much higher dosing, 50 mg/L of 12 nm Ag NPs. Nitrifying bacteria are slow growing and often sensitive to environmental stressors, and for this reason Choi and Hu (2008) targeted them for nanoparticle toxicity studies. They reported a 50% inhibition of nitrifying cultures at an effective concentration of 0.140 mg/L Ag. The inhibition was correlated with the fraction of Ag NPs less than 5 nm in size (Choi and Hu 2008).

Toxicity studies have also focused on groups of microorganisms and bacteria, such as those occurring in biofilms. For a laboratory grown biofilm containing exclusively *Pseudomonas putida*, a decrease in the biofilm volume was observed when exposed to uncoated Ag NPs at 0.02 – 2 mg/L (Fabrega et al. 2009). Another study looked at interactions of Ag NPs with *E. coli* cells in planktonic and biofilm cultures. The minimum bactericidal concentrations of Ag NPs, defined as the lowest concentration that kills at least 99.9% of a population, were 38 and 10 mg/L Ag for particles sized 15 to 21 nm, respectively. Planktonic and biofilm bacteria were more strongly affected by silver ions than Ag NPs. Ag NPs aggregated in the presence of planktonic and biofilms cells, causing an increase of the average particle size. The authors suggested that the biofilm resistance to Ag NPs could be partially a result of nanoparticle aggregation (Choi et al. 2010).

An ecotoxicity study of the effects of Ag NPs on interstitial water microbial communities from natural wetlands reported differing effects based on nanoparticle type. Uncoated, PVP-coated and carboxymethyl cellulose (CMC)-coated nanoparticles (20-30 nm) were evaluated between 0.1 and 10 mg/L. Ionic silver ($Ag+$, from $AgNO_3$) was evaluated alongside the nanoparticles as a positive control. The study found that ionic silver and CMC-coated Ag NPs displayed similar ecotoxicity with complete inhibition of microbial activity at 1 mg/L. The uncoated and PVP-coated Ag NPs displayed a lower toxicity with partial or complete inhibition not occurring until 10 mg/L of Ag (Schneider 2015).

There is evidence that plants may also be impacted by Ag NPs. Plant uptake of silver was positively correlated with Ag NP exposure concentrations (Jiang et al. 2012; Yin et al. 2012). Ag NPs and ionic silver negatively impacted plant biomass, causing disintegration and root growth inhibition for some species (Jiang et al. 2012; Mirzajani et al. 2013). For example, Ag NPs inhibited seedling growth in *Lolium multiflorum* (Yin et al. 2012). On the other hand, uptake of Ag NPs has been reported to have no effect on seed germination, while ionic silver exhibited a noticeable delay in seed germination at high concentrations relative to Ag NPs and control (Krishnaraj et al. 2012).

Ag NP toxicity is influenced by intrinsic nanoparticle features like size, shape, chemistry, and capping agents, but also by the aquatic chemistry through such factors as solution pH, redox state, ionic strength, and ionic composition (Dale et al. 2015). Most of the toxicity data presented in the literature has been obtained in relatively simple media like distilled water or cell culture media with pristine, manufactured nanoparticles. These situations do not reflect the conditions in the natural environment. Hence, the surface chemistry, reactivity and state of dispersion achieved with nanoparticles in the laboratory may not be relevant for assessing behavior in natural systems.

In natural waters, the wide variation of pH, ionic strength, ionic composition, and natural organic matter may change the aggregation state and bioavailability of Ag NPs, thus resulting in a divergence from expected antimicrobial activities and toxicities based on laboratory experiments. Additionally, the use of pristine or manufactured types of Ag NPs for toxicity testing may not be entirely representative of the form or composition that reaches the environment. Ag NPs are typically embedded into or coating consumer products, finding relevant "leached" or "weathered" nanoparticles which come from consumer products is important to elucidate the most likely scenario in the environment. In the process of leaching or weathering from the consumer product the Ag NPs are likely to change chemical form, size, shape and bioavailability. As we become aware of the potential release pathways of Ag NPs to the environment it becomes even more important to take the most relevant total ecosystem approach while assessing toxicity.

1.6.4 Silver Nanoparticles in Surface Waters

Environmental introduction of Ag NPs into surface waters can occur at different stages of the lifecycle of a Ag NP: during synthesis, manufacturing, incorporation into goods, intended use of nanoparticle containing products, post consumption recycling or disposal of the goods, and disposal of raw Ag NP materials from industry (Fabrega et al. 2011). A 2008 study examining cumulative risk from silver exposure estimated that 15% of the total silver released into water in the European Union by 2010 would come from nano-functionalized plastics and textiles (Blaser et al. 2008). Ag NPs are known to partition to sewage sludge during secondary wastewater treatment processes (Kaegi et al. 2013; Johnson et al. 2014; Yang et al. 2014). If wastewater treatment is not an option, or if it is inadequate, the potential exists for the release of Ag NPs to receiving waters at concentrations that may pose a threat to aquatic organisms (Blaser et al. 2008). Dissolution of Ag NPs to Ag^{+} in natural waters is recognized as a significant environmental fate process (Levard et al. 2012). Once released into the aquatic environment transformations of Ag NPs are affected by physiochemical parameters such as: concentration and type of organic matter in solution, pH and ionic strength of water, redox environment, and the presence of inorganic ligands (Choi et al. 2009; Gao et al. 2009). Environmental fate processes which will affect Ag NPs include abrasion, oxidation, dissolution, precipitation, adsorption, desorption, sedimentation and microbial transformation (Nowack et al. 2012).

Predicting the behaviour of Ag NPs in solution is difficult because of the complex chemistry of silver in aqueous solutions. Ag NPs undergo many environmental transformations including changes in aggregation state, changes in oxidation state, precipitation, and sorption to natural organic matter and inorganic species (Levard et al. 2012). Metallic silver is thermodynamically unstable under most environmental conditions and will oxidize or react with natural organic matter and inorganic ligands (Liu and Hurt 2010; Xiu et al. 2011). Many silver complexes involve Ag in an oxidation state of +1. Therefore, the metallic silver core (Ag^{0}) in Ag NPs requires oxidation prior to complexation with inorganic and organic compounds (Levard et al. 2012). The oxidation of silver is thermodynamically favourable at room temperature; therefore, this process should occur readily in the environment. Inorganic ligands such as sulphide (Levard et al. 2011) and chloride (Fabrega et al. 2011) are known to react strongly with silver. In freshwater, silver is likely to complex with sulphur to form solid Ag_2S (Fabrega et al. 2011; Levard et al. 2012). Organic matter dissolved in water and occurring in sediments is also a likely source of silver complexation in freshwater, especially organic matter containing sulphur groups. The formation of solid AgCl is also likely based on the availability of chloride in natural waters (Fabrega et al. 2011; Levard et al. 2012). Ag NPs show increased reactivity therefore transformations in the environment are expected

to be faster than for bulk silver (Levard et al. 2012). These environmental transformations of silver will affect the surface chemistry of Ag NPs and therefore their transport, reactivity and toxicity in the environment.

1.6.5 Silver Nanoparticles in Natural Wetlands

Ag NPs are expected to reach wetlands via landfill leachate, wastewater treatment plants, spills, run-off from agricultural biosolids spreading and human recreation. To date limited research has evaluated the effects of Ag NPs on natural wetland ecosystems. Little is known regarding the transformations and fate of Ag NPs in natural wetlands and potential effects to wetland ecology. Wetlands could be at risk from Ag NPs as the efficacy of their self-sustaining water polishing ability relies heavily on microbial communities.

Three different studies have investigated the effects of Ag NPs on natural wetlands and wetland biota. Yin et al. (2012) looked at the effects of various types of Ag NPs on eleven common wetland plant species, representing taxonomically and functionally diverse species. With direct exposure to Ag NPs by soaking seeds in nanoparticle solutions (40 mg/L, 6 nm size), seed germination rates for multiple plant species were inhibited. Additionally, plant growth was affected by the Ag NPs, with root growth more strongly inhibited than leaf growth, for multiple plant species.

Lowry et al. (2012) performed the first natural wetland mesocosm experiments assessing the transformation and final fate of Ag NPs. Mesocosms were built to simulate an emergent wetland environment. Wetland mesocosms were planted with *Juncus effuses*, *Carex lurida*, *Panicum viragtum* and *Lobelia cardinalis*. All plants were allowed to develop prior to dosing with PVP-coated Ag NPs. The mesocosms contained a terrestrial component and an emergent wetland water column. Ag NP dispersions were added to either the terrestrial compartment or the water column of the mesocosms. The fate of the Ag NPs was assessed 18 months after dosing. The majority of added silver remained in the compartment in which it was dosed (wetland water column vs. terrestrial soil), associated mostly with soils and sediments. More movement was observed from terrestrial soil compartment to sediment compartment of the mesocosm, therefore runoff may be a potential pathway for Ag NPs to enter surface waters. Plant uptake was another pathway for Ag movement in the mesocosms. The tissue concentrations of Ag in dosed mesocosms were well above background levels. Eighteen months after dosing, the Ag NPs added to the wetland mesocosms were partially oxidized and sulphidized. Nanoparticles residing in the aquatic compartment sediments were sulphidized to a greater degree than those present in the terrestrial compartment. The authors attributed this to the drier and more oxic conditions in the terrestrial compartment (Lowry et al. 2012).

Colman et al. (2014) studied the effects of two Ag NPs and ionic silver (as $AgNO_3$) on large outdoor wetland mesocosms. Size and coating effects of Ag NPs to wetland mesocosms were assessed by dosing with 12 nm gum arabic (GA)-coated and 49 nm polyvinylpyrrolidone (PVP)-coated Ag NPs at 2.5 mg/L. The wetland mesocosms were left undisturbed after silver treatments were applied. Silver concentrations declined rapidly from the water column after dosing. Widespread leaf loss and browning of submersed and floating aquatic plants was observed in all mesocosms dosed with silver. Dissolved organic carbon and chloride concentrations initially spiked in the wetland mesocosms after dosing with silver, with increases being similar for GA-AgNPs and $AgNO_3$, but less pronounced for PVP-AgNPs. At the same time, dissolved methane

concentrations increased forty-fold relative to the controls in all three mesocosms. Depletion of dissolved oxygen was also seen in dosed mesocosms.

1.6.6 Silver Nanoparticles in Wastewater Treatment Plants

Ag NPs are expected to reach wastewater treatment plants through the intended use of Ag NP-containing consumer products as they are washed from the products into sewers and transported for water treatment. Recent estimates show concentrations of Ag NP in water entering wastewater treatment plants between 0.06 and 1.5 µg/L (Li et al. 2013). Transformations of Ag NPs may occur during wastewater treatment processes, which can have implications for toxicity mechanisms associated with the Ag NPs. Any changes in their coating, size, shape, or stability will impact the bioavailability of Ag NPs and therefore toxicity in natural systems. Fate and environmental transformations of Ag NPs during wastewater treatment have been studied (Brar et al. 2010; Kim et al. 2010; Kaegi et al. 2011; García et al. 2012; Kaegi et al. 2013; Lombi et al. 2013; Westerhoff et al. 2013; Ma et al. 2014; Brunetti et al. 2015) and their effects on the systems have been assessed (Sheng and Liu 2011; Garcia et al. 2012; Yang et al. 2014). Due to the bactericidal effects of Ag NPs there may be long-term issues with the biological treatment mechanisms used for wastewater treatment.

Studies of transformations in sewage networks show Ag NPs are effectively transported by the sewer system to the wastewater treatment plant with minimal loss to the sewer biofilms. Sulphidation of the particles occurred down the line with smaller nanoparticles (10 nm) being sulphidized more quickly and in larger abundance than the larger 100 nm particles (Kaegi et al. 2013). Another study confirmed that Ag NPs undergo fast and complete transformations during their transport through the sewage network. Ag NPs formed reduced sulphur species as suspected but also sorbed to chloride and organic matter containing sulphur groups such as cysteine and histidine. Ionic silver and Ag NPs formed secondary silver sulphide nanoparticles, which were revealed by TEM analysis (Brunetti et al. 2015).

Ag NPs have been shown to partition (to some degree) into activated sludge during wastewater treatment (Kaegi et al. 2013; Johnson et al. 2014). Ag NPs may thus be in sewage sludge applied to land as biosolids. An estimated 1.8 to 105 µg/L of silver in wastewater/sewage is entering wastewater treatment facilities (Shafer et al. 1998). A reported 2 to 195 mg/L of silver is discarded from wastewater treatment plants in biosolids, indicating that the activated sludge in wastewater treatment plants may be a sink for Ag NPs (Radniecki et al. 2011). The dominant type of silver particles found in activated sludge plants are sulphidized Ag NPs (Ag_2S) (Kim et al. 2010), which is formed when Ag NPs or Ag^+ react with sulphides in sewage collection systems or other anaerobic environments within the wastewater treatment process.

Impellitteri *et al.* (2013) assessed the chemical transformation of Ag NPs in fresh, aged, and incinerated biosolids. The results show that AgNPs are converted to Ag-sulphur species (sulphide and sulfhydryl) in both fresh and aged biosolids. A significant proportion of the silver (30-50%) is converted to elemental Ag in the incineration process while the presence of additional Ag-S complexes such as Ag_2SO_4 (up to 25%), and silver associated with sulfhydryl groups (26-50%) are also found in the incinerated biosolids (Impellitteri et al. 2013). Colman *et al.* (2013) applied a single dose of Ag NPs (0.14 mg Ag/kg soil) in a terrestrial mesocosm field experiment via sewage biosolids application. Aboveground plant biomass of one plant species, Japanese stiltgrass (*Microstegium vimeneum*), in the dosed system, was 32% less compared with control plants. A significantly different bacterial community composition was seen between areas applied with Ag

NP biosolids and control biosolids. Nitrous oxide (N_2O) gas flux also increased 4.5 fold when Ag NPs were applied with biosolids (Colman et al. 2013).

The biofilms in wastewater treatment plants, which contain a large and diverse microbial community, have been found to be tolerant to the biocidal effects of Ag NPs. Sheng *et al.* (2011) applied 200 mg Ag/L to wastewater biofilms and no significant change in the viability of bacteria was observed. However, the accumulated effect of Ag NPs in wastewater biofilms may impact the microbial activity in the long term (Sheng and Liu 2011). A study by Kaegi *et al*. (2011) in a pilot wastewater treatment plant (containing a non-aerated tank, aerated tank and a secondary clarifier) confirmed the sorption of Ag NPs onto the wastewater biosolids, with transmission electron microscopy (TEM) images. X-ray absorption measurements indicated that most Ag in the sludge and in the effluent was present as Ag_2S. Ag NP transformations to Ag_2S occurred in the non-aerated tank within less than 2 hours (Kaegi et al. 2011). Another group studied the impacts of Ag NPs and Ag^+ on the microbial community structure of activated sludge. They found that Ag NPs (40 mg/L of 35 nm Ag NPs) decreased the abundance of nitrifying bacteria and damaged the activated sludge floc structure (Yang et al. 2014). These findings have implications for nitrogen removal in the wastewater treatment process as well as sludge clarification and recycling.

In summary, the release of Ag NPs in consumer products and subsequent transport to wastewater treatment plants leads to incorporation of Ag NPs in biosolids (Blaser et al. 2008; Gottschalk et al. 2009). Ag NPs are expected to concentrate in biofilm and activated sludge within wastewater treatment plants (Kaegi et al. 2011; Kaegi et al. 2013; Yang et al. 2014; Brunetti et al. 2015). Ag NPs may adversely affect wastewater treatment processes and farmland to which biosolids are applied as a soil fertilizer. When treatment of wastewater is inadequate there is the potential risk for the release of Ag NPs directly into receiving waters at concentrations which may pose a threat to aquatic organisms (Blaser et al. 2008).

1.6.7 Silver Nanoparticles in Constructed Wetlands

Knowledge of the effects of Ag NPs on CWs for the purposes of wastewater treatment is limited. A reduction in function from microbial and plant populations may prove detrimental to the treatment performance of CWs. CWs are commonly used to treat wastewater prior to discharge into surface waters which therefore may be at risk if pollutant removal requirements are not met. Research is limited regarding the effects of Ag NPs on the activity and overall function of CW microbial communities. More research is required as the discharge of Ag NPs to wastewater streams is increasing as they are incorporated into more consumer products every year.

Research to date regarding the effects of Ag NPs on CWs has looked into the capability of plant matter in CWs to adsorb Ag NPs as part of wastewater treatment (Sharif et al. 2013) and the fate of Ag NPs in CWs for wastewater treatment (Sepúlveda 2014). Sharif *et al*. (2013) investigated the natural attenuation of Ag NPs by wetland plants in laboratory microcosms and found that Ag NPs aggregated in the wetland environment increasing particle size from 20 nm to between 50 to 100 nm. They found that Ag NPs remained in the wetland likely adsorbing to plant matter. Adsorption of Ag NP by the constructed wetland was affected by the organic matter content and size of the substrate. Sepúlveda (2014) also reported Ag NPs adsorption to wetland substrate materials (sand, zeolites, gravel). Substrates with higher organic content and smaller particle sizes adsorbed more silver. Biofilms accounted for a 350% increase in Ag NP removal relative control samples without biofilm. Therefore, microbial uptake/adsorption could account for Ag NP removal. Initially, plants (*Phalaris arundinacea*) provided an important sink for Ag NPs via

adsorption/uptake into roots. However, translocation to aboveground plant tissue was negligible (Sepúlveda 2014). Based on these findings, CWs receiving wastewater containing nanoparticles may provide a sink for Ag NPs.

The most recent work, by Button et al. (2016) reported the impact of different Ag NPs and dissolved silver on microbial community catabolic function (community level physiological profiling) and genetic fingerprinting (denaturing gradient gel electrophoresis) of species in microcosm CWs. Microbial community samples were taken from CW microcosms, those associated with both biofilm and interstitial waters. Changes in microbial community function and structure were monitored in wetland microcosms over a period of 4 weeks following an *in-situ* exposure of 100 µg/L Ag NPs. Ag NPs included PVP-coated, citrate-stabilized and those produced via biogenic synthesis by bacteria. $AgNO_3$ was used as a positive control. Low doses of Ag did not appear to exert significant toxic effects in the short term (1 month) whether dissolved or in nanoparticle form, when dosed *in-situ.* This may suggest some natural ability to adapt to the stress from the addition of silver. However, higher doses (>500 µg/L Ag) of silver significantly reduced microbial community catabolic activity in *ex-situ* tests in the case of citrate-coated, biogenic NPs and ionic silver. There was evidence of microbial resistance to toxicity when microbial communities had been previously exposed to a lower dose of Ag.

1.7 Knowledge Gaps

Data regarding constructed wetland development and function, in terms of the effects of plants and artificial aeration is limited at this time. Additionally, information regarding the potential effect of silver nanoparticles on constructed wetland microbial communities is very limited. Identified knowledge gaps are listed to follow:

- Differences between constructed wetland development and conditions (plant health, microbial community activity, wastewater treatment potential) for aerated and non-aerated systems
- Are start-up and development time scales different for aerated and non-aerated constructed wetlands?
- The effects of the addition of plants to a developed wetland and impacts on plant health, microbial community activity, wastewater treatment potential
- Impact of silver nanoparticles on the microbial community activity, function and structure in constructed wetlands
- Difference of effects between pristine and weathered/leached silver nanoparticles

1.8 Research Objectives and Milestones

1.8.1 Research Objectives

The overall objective of this thesis is to observe and quantify constructed wetland mesocosm dynamics during development periods, and the anti-microbial effects of silver nanoparticles.

A. Characterize the development (start-up) period of aerated and non-aerated constructed wetland mesocosms planted with *Phalaris arundinacea*.

B. Characterize the plant initialization and establishment of *Phalaris arundinacea* in unplanted, well-developed aerated and non-aerated constructed wetland mesocosms.

C. Quantify the effects of various types of silver nanoparticles (pristine and weathered) on interstitial microbial communities from constructed wetlands.

1.8.2 Novelty of Research

A rigorous developmental monitoring of aerated constructed wetland mesocosms has not been previously studied. The development of aerated constructed wetland mesocosms has also not been compared alongside that of non-aerated systems.

Evaluating the potential changes and effects of the addition of plants to well-developed aerated and non-aerated constructed wetland mesocosms has not been previously studied.

An additional novel aspect of this research is the use of silver nanoparticles which have been weathered from consumer products, rather than solely pristine types, for ecotoxicity testing with wetland microbial communities.

1.8.3 Research Timeline and Milestones

Figure 1.6 outlines a timeline for project and experiment work associated with this Master's thesis from Winter 2015 to Fall 2016.

Figure 1.6: Research Timeline

1.9 Thesis Organization

This thesis consists of six chapters, as listed below.

Chapter 1 introduces wastewater treatment, relevant background information on constructed wetlands for wastewater treatment and discusses silver nanoparticles as an emerging contaminant.

Chapter 2 introduces relevant background information about the methodologies which were included in this thesis.

Chapter 3 provides a description of quantification methods for water chemistry, wastewater treatment performance, plant dynamics and microbial community characteristics as well as a summary of results from the wetland development study of aerated and non-aerated wetland mesocosms.

Chapter 4 includes a summary of the water chemistry, wastewater treatment performance, plant dynamics and microbial community characteristics after the addition of plants to developed aerated and non-aerated wetland mesocosms.

Chapter 5 describes exposures of constructed wetland microbial communities from aerated and non-aerated mesocosms to various types of pristine and weathered silver nanoparticles.

Chapter 6 summarizes the principal outcomes of the study. Future work and recommendations for further research are presented in this chapter.

2 CHAPTER 2: METHODS INTRODUCTION

The following chapter has been included to frame the usage of methods within this thesis. It will detail why methodologies were chosen to be included in this thesis, the theory behind the methods and briefly the limitations of each method.

2.1 Water Quality

2.1.1 Water Chemistry

Water chemistry is monitored in constructed wetland research to understand the chemical environment within the system and therefore understand what water treatment mechanisms and microbial processes are available. In this thesis a variety of common water chemistry parameters (pH, oxidative-reductive potential, ammonia, nitrate, dissolved oxygen, conductivity and temperature) were monitored with YSI Professional Plus Probes to evaluate the general environment within the constructed wetland mesocosms.

YSI Professional Plus Probes are multi-channeled electrode probes which are suitable for field applications. They are frequently used in industry and in the field for a variety of water applications. The multi-channel capabilities of the probe allow for rapid determination of up to four water chemistry values within a few minutes; however, some accuracy is sacrificed with this method. The YSI Professional Plus Probes were used here to allow the determination of a number of water chemistry parameters with ease.

2.1.2 Water Treatment

Water treatment ability of the constructed wetland mesocosms was analyzed in this thesis based on Total Organic Carbon (TOC) and Total Nitrogen (TN) removal. In constructed wetlands research water treatment is also commonly analyzed based on a combination of biochemical oxygen demand (BOD), carbonaceous biochemical oxygen demand (CBOD), chemical oxygen demand (COD), ammonium (NH_4^+), and total Kjeldahl nitrogen (TKN) (Kadlec and Wallace 2008). TOC and TN removal were selected for analysis of water treatment in this thesis based on the availability of a high throughput TOC/TN analyzer (Analytik Jena, TOC/TNb: multi N/C® Series, Germany). With 72 to 96 samples created weekly depending on the sampling regime in the study, this method was the most realistic option to gain insight into the constructed wetland's ability to remove nutrients from the simulated wastewater.

TOC and TN removal from the wetland systems was analyzed using a modified first order rate kinetics model (k-C*) (Section 1.2.6) as described in Kadlec and Wallace (2008). The use of first order rate kinetics in constructed wetland modeling is typically suitable and worked as a good approximation for the data within this thesis. The k-C* modification of typical first order rate kinetics is more suitable for constructed wetlands as there is often a background concentration which either remains recalcitrant to degradation or has returned to the water from the static compartments of the ecosystem (plant biomass and sediment) creating a negative contribution which limits the total removal of the pollutant (Kadlec and Wallace 2008). The k-C* model, considers the background concentration (C*) of the pollutant and is more suitable for use in wetland pollutant removal modeling.

The downside to this method is time points have to be selected carefully to capture the removal of TOC or TN. Additionally, this method does not consider wetland depth or the imperfect interactions of the pollutants with microbial communities which grow in a biofilm that may not develop uniformly across the wetland (Kadlec and Wallace 2008). Percent mass removal was also used to calculate the removal of TOC and TN from the constructed wetlands within this thesis. This is also a very common way of presenting removal data in constructed wetland literature as it requires less frequent sampling (Kadlec and Wallace 2008).

2.2 Hydrological Measurements

2.2.1 Evapotranspiration

Evapotranspiration (ET) is a combination of water loss to the atmosphere from the surface of a wetland and through the transpiration of wetland vegetation (Kadlec and Wallace 2008). With the mesocosms used in this thesis, this should be the only water loss from the systems each day (except on days where water sampling occurred) as no infiltration can take place as this is a laboratory scale experiment. It is important to know the water loss from a constructed wetland system as this will have implications for nutrient and contaminant concentrations in outlet wastewater if significant amounts of water are lost along the wetland flow path.

The measurement of ET can also be used to supplement plant growth statistics as ET is related to above ground plant biomass. Additionally, the measurement of ET for the mesocosms in this thesis involved adding a measured water volume to the mesocosm until an overflow volume was reached. This regulation of water volume in the mesocosms allowed other parameters (water chemistry, water treatment) to be evaluated without a concentration bias from ET.

2.2.2 Porosity

Porosity is a fraction of the void spaces in a material relative to the total volume. In the case of this thesis it is the fraction of the mesocosm which can be filled with water versus the total volume of the bed medium which both the gravel and water occupy. In this thesis, the volume of the pore space is calculated from the fillable volume of water. The downside to this method is air bubbles may be present within the pore spaces and not accounted for with this sampling method. Additionally, the volume of biofilm may shrink if the mesocosm is left empty for significant time as biofilms are predominantly water and are therefore subject to evaporation. A more accurate method would have weighed the water which was drained from the mesocosm systems to capture a volume with less bias.

The measurement of porosity can also be used to supplement microbial activity and function statistics as changes in porosity can be related to the development of biofilm (and microbial biomass) within the pore spaces of the gravel media in the wetland mesocosms.

2.2.3 Dispersivity

The removal of pollutants in CWs occurs as a function of many diverse processes and interactions between the wastewater and biological and physical media of the CW. The dynamics of water movement through the wetland has a significant influence on the efficiency and extent of these interactions (Kadlec and Wallace 2008). The internal water hydraulics of CWs can be quantified using inert, soluble chemical tracers (Kadlec and Wallace 2008).

Typically interpretation of data from constructed wetland tracer tests involves the use of models which combine two idealized flow elements: perfectly mixed units and plug flow sections (Kadlec and Wallace 2008). Constructed wetlands are neither plug flow nor well mixed systems. The tanks-in-series (TIS) model is flexible enough to describe both mixing and preferential flow paths for a wide range of hydraulic efficiencies (Kadlec and Wallace 2008). Another model which superimposes a dispersion process on a plug flow model is also frequently used to model CW flow (Kadlec and Wallace 2008). In this model mixing is presumed to follow an advection-dispersion equation in 1D space. More sophisticated models of constructed wetland hydraulics exist which take into account biofilm growth and accumulation of inert solids (Samsó and Garcia 2013) as well as organic pollutant degradation as a function of microbial growth and additionally shear stress on local biofilm detachment (Rajabzadeh et al. 2015). However, their use and development can be time consuming and costly, as well as requiring specialized expertise and significant knowledge of higher mathematics. Therefore, in this thesis the 1D advection-dispersion equation was used to model wetland hydraulics.

To perform the tracer tests, sodium bromide (NaBr) was selected since it is conservative, readily soluble in water and relatively inert. Data from the tracer tests was fit to a 1D advection-dispersion equation using Aquasim v.1.0.0.1 (Eawag Institute, Switzerland, 1995). The 1D advection-dispersion equation, as outlined in Weber and Legge (2011):

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} \tag{3}$$

where C is concentration (mg/mL), t is time (minutes), v is velocity (cm/minute), x is distance (cm) and D is the dispersion coefficient (cm^2/minute). This model and software was selected as a previous study (Weber and Legge 2011) had utilized this model for a similar mesocosm system so it was readily adaptable for use in this thesis. The calculation of a dispersion coefficient allows a discussion of wetland hydraulics which may complement information provided by porosity, plant growth and microbial activity/function in terms of biofilm and plant growth and their influence on water flow and treatment performance.

2.3 Plant Growth

Plant growth (health) was assessed in this thesis based on plant height, stem count and a qualitative measure of plant colour. Photos were taken weekly to document plant growth. These measures were selected as they are simple and could be performed non-destructively on a weekly basis with ease. They may not provide the most comprehensive analysis of plant growth/health but they provide a good qualitative comparison of plant growth over time which was suitable for this thesis.

2.4 Microbial Community Analysis

2.4.1 Microbial Activity

Total microbial activity is a good indicator of organic matter processing in natural environments as more than 90% of energy flows through microbial decomposers (Schnürer and Rosswall 1982). A suitable technique for measuring microbial activity should be nonspecific but sensitive and requiring only a short incubation period (Schnürer and Rosswall 1982).

In the context of this thesis, microbial activity was assessed indirectly based on a measurement of enzymatic activity associated with the hydrolysis of fluorescein-diacetate (FDA) to fluorescein (FL). FDA contains a FL molecule quenched by two acetate groups. The acetate groups can be enzymatically cleaved by a number of enzymes including proteases, lipases and esterases, revealing the molecule fluorescein (Schnürer and Rosswall 1982) (Figure 2.1). The production of FL can be monitored photometrically at 490 nm.

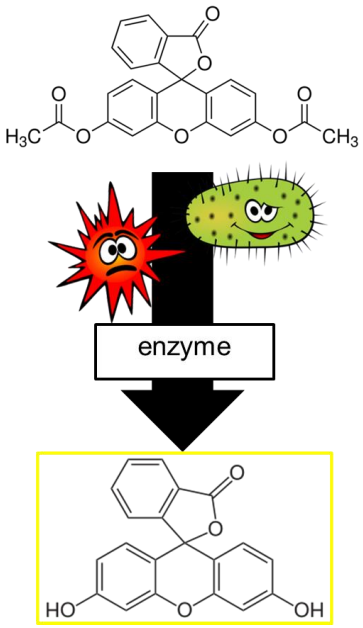

Figure 2.1: Illustration of the process involved with the enzymatic hydrolysis of fluorescein-diacetate to fluorescein.

Microbial activity was included in the microbial community analysis within this thesis as a supplementary method to provide additional information and back up findings provided from Community Level Physiological Profiling (CLPP). The hydrolysis of FDA to FL was chosen for the assessment of microbial activity as it could be done rapidly without interfering with other measurements and has been tested on a variety of natural microbial communities previously with success (Schnürer and Rosswall 1982; Battin 1997; Adam and Duncan 2001).

2.4.2 Microbial Function

Community Level Physiological Profiling (CLPP) is a technique which can be used to compare microbial community function based on sole carbon source utilization patterns (CSUPs) (Weber and Legge 2010). CLPP can be used to evaluate the metabolic characteristics and overall stability of a specific microbial community over time and space. The term CLPP is currently synonymous with the use of BIOLOG™ microplates, which were initially designed to characterize soil microbial communities (Garland and Mills 1991) but have since been adopted for use with water based microbial communities. BIOLOG™ microplates, specifically BIOLOG EcoPlates™, were used in this thesis as they contain 96 wells, within which are triplicate replications of 31 carbon sources, which allows for statistical analysis. Within each well, in addition to the carbon source, is a redox dye indicator (tetrazolium violet) which changes from colourless to purple as a

result of carbon utilization. CLPP has advantages over other microbial techniques as it does not require specialized expertise and can be used easily, without the isolation of the microbial community from the sample matrix. Limitations of CLPP include complex data analysis, bias towards rapidly growing bacteria, long incubation times, the need to reduce time between sampling and inoculation of the microplates, and the need to ensure similar sample sizes in each well (Preston-Mafham et al. 2002; Weber and Legge 2010).

The analysis of data from BIOLOG EcoPlates™ can involve large datasets. To deal with this, multivariate techniques can be used, such as principal component analysis (PCA). Weber and Legge (2010) list the steps required before a multivariate analysis technique, such as PCA, can be used: (1) Selection of a metric for analysis, (2) standardization of the data, (3) assessment of heterogeneity and normality of the data, and (4) performing a data transformation (if required).

First, the metric used for data analysis should be selected. Frequently, an absorbance value for each well for a specific incubation time point is selected. The time point selection is important as variance between well absorbance values will increase with respect to time and therefore increase the information provided by the method. However, this must be balanced with the absorbance saturation value (of 2, indicating the value is no longer in the linear range) which may become important at later time points. In this thesis, time point selection was based on a time where the absorbance values provided the greatest variance (represented as standard deviation) and where the minimal number of well absorbance values were greater than 2 (Weber and Legge 2010).

Second, data should be standardized to reduce bias occurring from differences in inoculum density when studying different mixed microbial communities in space or over time (Weber and Legge 2010). Standardization of the data involves correcting each absorbance value by its corresponding blank and then dividing by the average well colour development for that time point. The standardized absorbance for well *k* can be calculated as:

$$\bar{A}_k = \frac{A_k - A_0}{\frac{1}{31}\sum_{i=1}^{31}(A_i - A_0)} \tag{4}$$

where A_i represents the absorbance reading of well *i* and A_0 is the absorbance reading of the blank well (inoculated, but without a carbon source) (Weber and Legge 2010).

Third, the suitability of the data for multivariate analysis must be evaluated. Many multivariate analysis techniques assume two fundamental properties of a data set: normality and homoscedasticity (homogeneity of variance). The normality of the data was evaluated through a two-tailed statistical test of the kurtosis and skewness of the variables for the null hypothesis that the data is normally distributed and the alternative hypothesis that it is not normally distributed. The z values for kurtosis and skewness can be calculated as:

$$z_{kurtosis} = \frac{kurtosis}{SE_{kurtosis}} \tag{5}$$

$$z_{skewness} = \frac{skewness}{SE_{skewness}} \tag{6}$$

where $SE_{kurtosis}$ and $SE_{skewness}$ are the standard errors for kurtosis and skewness, respectively. $SE_{kurtosis}$ and $SE_{skewness}$ can be calculated from:

$$SE_{kurtosis} = \sqrt{\frac{24}{n}} \tag{7}$$

$$SE_{skewness} = \sqrt{\frac{6}{n}} \tag{8}$$

where n is the number of observations. In this thesis, the null hypothesis was rejected with 95 % confidence if |z| > 1.96 (Weber and Legge 2010).

Homoscedasticity is the homogeneity of the variances for all variables. A lower variance ratio should indicate a higher degree of homogeneity between the variances of the variables. This variance was evaluated qualitatively as:

$$variance\ ratio = \frac{highest\ variance}{lowest\ variance} \tag{9}$$

Fourth, data transformations can be applied if the BIOLOG EcoPlate™ data does not meet the above normality and homoscedasticity assumptions to increase the suitability of the data for multivariate analysis. Two commonly used transformations for ecological data are the Taylor power law transformation and the logarithmic transformation (Weber and Legge 2010). The Taylor power law assumes that:

$$S^2 = a\bar{y}^2 \tag{10}$$

where *S* is the standard deviation of a variable, *a* is the sampling factor and $\bar{y}$ is the mean of a variable. Linearization of equation (10) leads to:

$$\log S^2 = \log a + b \log \bar{y}^2 \tag{11}$$

where *b* is the slope of the linear equation, which can be calculated by linear regression of the data for all variables. The transformation of the variables was performed using:

$$y_i^{`} = y_i^{(1-b/2)} \tag{12}$$

where $y_i^{`}$ is the value of the Taylor transformed variable.

If instead the logarithmic transformation was used, the following equation details the transformation of data:

$$A^{`} = \ln(\bar{A}_k + 1) \tag{13}$$

where $A^{`}$ is the value of the logarithmic transformed variable (Weber and Legge 2010).

PCA was the multivariate technique used to analyze CLPP data in this thesis. It allows the visualization of high dimensional space (31 dimensions in this case) on a two-dimensional plane

while preserving the maximum amount of variance possible within the data set. For PCA each plate (where p is the number of plates) is considered as an object with n variables (where n is the number of different carbon sources – 31). This gives a matrix with p rows and n columns. The dimensionality of the obtained data is reduced by extracting an orthogonal set of principal components made up of linear subsets of the original ordinates. The maximum amount of variance is concentrated in the first principal component, and the next largest variance is concentrated in the second principal component and so on. This technique allows the understanding of linear correlations between different carbon source utilization patterns for different microbial communities (Weber and Legge 2010).

CLPP was elected for analysis of the microbial communities from the CWs in this thesis as it gives an overall look into the microbial community function/activity. The analysis can be furthered to elucidate trends in carbon source utilization. The ability of the microbial community to break down carbon sources from similar guilds, based on chemical structure can be used to further apply the data to the wastewater treatment potential of the microbial community.

3 CHAPTER 3: WETLAND START-UP MONITORING OF AERATED AND NON-AERATED MESOCOSM CONSTRUCTED WETLANDS

3.1 Introduction

Constructed wetlands (CWs) are a robust, economical and environmentally friendly option for wastewater treatment. CWs utilize the pollutant removal processes innate to naturally-occurring wetlands such as filtration, microbial degradation, and plant assimilation to provide wastewater treatment (Kadlec and Wallace 2008). The presence of plants in CWs may increase pollutant removal by increasing microbial activity and dissolved oxygen concentrations associated with the plant rhizosphere (root zone) (Weber and Legge 2011). The physiochemical parameters (such as temperature, pH, dissolved oxygen, conductivity and oxidative-reductive potential) of the influent wastewater will also affect pollutant removal mechanisms (Imfeld et al. 2009). The composition of the incoming wastewater and design of the CW influence the internal environmental conditions, which dictate the pollutant removal processes available to the plants and microbial community. Microbial degradation of pollutants from wastewater is a result of microbial metabolic processes which result in either cellular mass and reproduction (anabolism) or energy (catabolism) for the microbial community (Weber 2016). Pollutants are broken down by microorganisms in CWs by a predicted sequence based on physiochemical parameters and composition of wastewater (Section 0).

CWs are typically efficient in the removal of organic compounds, but may have difficulty with the removal of total nitrogen (Kadlec and Wallace 2008). The inability to remove total nitrogen within CWs results from a lack of oxygen associated with many subsurface CW designs (Kadlec and Wallace 2008). A subsurface flow CW receives limited oxygen from exchange at the air/water interface and through oxygen diffusion from plants in the rhizosphere. Nitrogen is present in wastewater as organic nitrogen, ammonium and nitrate (Halling-Sørensen and Jorgensen 1993). To remove total nitrogen, two main reactions must take place – first, the nitrification process converts ammonia to nitrate in the presence of oxygen, and secondly, the denitrification process converts nitrate to nitrogen gas in the absence of oxygen (Section 0). These reactions are difficult to achieve in succession in subsurface flow wetlands due to a lack of oxygen diffusion. The removal of nitrogen in wetlands has to compete with other processes for the use of oxygen, such as organic carbon degradation. As a way to increase the conversion of ammonia to nitrate, artificial aeration has been added to subsurface wetland beds (Cottingham et al. 1999; Wallace 2001). This addition of aeration can also increase the degradation of organic carbon-containing compounds in the wetland. Carbon is present in wastewater in both simple and complex forms and can be represented as total organic carbon. Carbon is broken down in CWs by microorganisms in both aerobic and anaerobic catabolic processes (Faulwetter et al. 2009).

When constructed wetlands are initially established typically time is reserved for a "start-up" or development period before full functional operation and wastewater treatment is expected. This time is allotted to allow sufficient microbial establishment. Metrics of pollutant removal and microbial activity or function are not always tracked over this phase of development. A few studies characterized the microbial community establishment during the wetland start-up phase (Ragusa et al. 2004; Song et al. 2011; Weber and Legge 2011; Ramond et al. 2012; Oopkaup et al. 2016). Ragusa et al. (2004) analyzed biofilm growth and activity in wetland microcosms and found that biomass can take up to 100 days to stabilize. Weber and Legge (2011) employed Community Level

Physiological Profiling (CLPP) to determine microbial community function and found stabilization of the microbial community between 75 to 100 days. The microbial community stabilization also correlated with changes to hydrological parameters, porosity and dispersivity, which alluded to the increase of microbial biomass over the same time period. Ramond et al. (2012) tracked the evolution of the microbial community structure in a start-up experiment using a microbial fingerprinting technique, denaturing gradient gel electrophoresis (DGGE). They found community convergence between 89 to 100 days. Another study reported a maximum bacterial community abundance after 60 days of operation for a subsurface flow wetland treating municipal wastewater (Oopkaup et al. 2016). These studies all concluded that microbial community stabilization requires between 60 and 100 days despite using a variety of different methods to characterize the microbial community. The increase in the study of microbial community temporal dynamics over the wetland development period correlates with the increase in research focusing on the study of bacterial communities in CWs over the last ten years (Weber 2016). Increasing awareness of microbial processes and their role in constructed wetlands comes after years of research inattention in this area, assuming a "black box" philosophy to treatment results based solely on inlet and outlet concentrations (Stottmeister et al. 2003). Suitable testing methods had not yet been developed or configured for use in the environmentally complex system of a CW. Even with the current increase in research on microbial communities in CWs much can still be learned including the effects of wetland intensification, like the addition of artificial aeration, on the growth and stabilization of microbial communities in CWs. Also, why it takes microbial communities in CWs 60 to 100 days to develop and start to affect wastewater treatment processes, and whether this length of time can be decreased can be explored further.

To the knowledge of the author, the effects of the addition of artificial aeration to a CW have not been characterized in terms of microbial community activity and function. The objective of this study is to investigate the effects of aeration on the water chemistry, hydrology and microbial parameters of the start-up period of recirculating, saturated vertical flow CWs. Six replicates of aerated and non-aerated CWs, seeded with *Phalaris arundinacea*, were fed with a simulated wastewater solution once a week for twelve weeks and rigorously monitored for a variety of water chemistry, water treatment, hydrological and microbial parameters.

3.2 Materials and Methods

3.2.1 Experimental Design

For this study, twelve CW mesocosms were used; six artificially aerated and six non-aerated, all seeded with *Phalaris arundinacea* (Figure 3.1). The twelve mesocosms were allowed to naturally develop for 12 weeks after seeding while water chemistry, system hydrology, pollutant removal and microbial community metrics were characterized as outlined to follow. The length of the development period was based on previous work, which indicated ecological stabilization of CW mesocosm systems, in terms of both hydrological and microbial parameters, after ninety days (Weber and Legge 2011). Mesocosm size CWs were used in this study as they are an effective tool for investigating CW fundamentals. Shorter development "start-up" period, ease of replication, adaptability, as well as environmental and experimental control options allow targeted experiments on underlying principles of wetland development and function. The use of mesocosm replicates allows for a rigorous and holistic approach to wetland monitoring, which may not be achievable with a pilot or full-scale CW. Mesocosms allow effective examination of a wide range of characteristics which can reveal trends and further the fundamental understanding of CW

mechanisms and performance. CW mesocosms do not entirely represent the environment and hydraulics of full-scale CWs, but they can be used to study physical and ecological properties which may be applied to larger scale systems. The specific design of smaller, recirculating wetland mesocosms was used to capture microbial information related to the biofilm. The subsurface biofilm in a CW is where the majority of the microbial communities are located and where wastewater treatment occurs. By sampling the interstitial water which flows through the wetland pore space, small amounts of biofilm which have sheared from the greater community can be analyzed (Weber and Legge 2013).

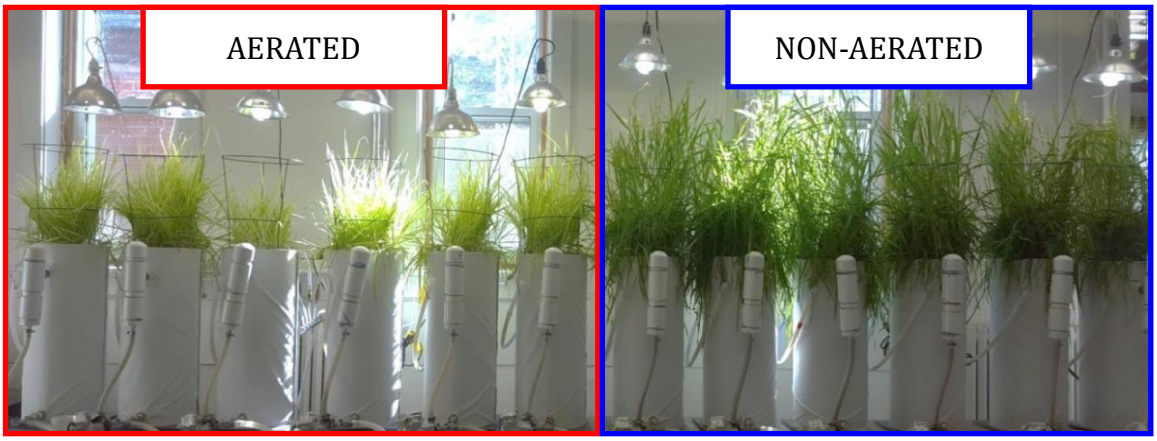

Figure 3.1: Mesocosm experimental design depicting the use of six aerated and six non-aerated, planted mesocosms.

3.2.2 Mesocosm Set-Up and Maintenance

Twelve CW mesocosms (Figure 3.2; C) were built using clear PVC pipes (60.96 cm height x 25.45 cm diameter). A sampling port was constructed of 2" white PVC pipe and connected to the side of each mesocosm for accessibility. Mesocosms were filled to ~55 cm with pea gravel (approximately 5 to 20 mm) and had a starting void volume of 9.3 to 10.0 L. Mesocosms also had an over flow outlet, which was situated just under the gravel fill line. The over flow outlet is used to allow consistent measurements of porosity and evapotranspiration. The twelve mesocosms were split into two groups of six, with one group of six artificially aerated and the other group a non-aerated control. To achieve exceptionally aerobic conditions, the aerated mesocosms were fitted with an aeration stone (15 cm) at the bottom of the mesocosm which was externally linked to an EcoPlus Air 3 air pump (Atlantic Pond Supply; Moncton, NB) via ½" Nalgene tubing (Figure 3.3). Water was continuously recirculated through the mesocosms using a magnetic drive pump (Little Giant Pump Company, 1-AA-MD) and distributed below the surface of the gravel with a distribution apparatus made from ½" clear PVC tubing (Figure 3.2; B).

The mesocosms were seeded with activated sludge (500 mL/mesocosm) from a local wastewater treatment plant (Cataraqui Bay Wastewater Treatment Plant, Kingston, ON). The activated sludge was applied in three layers (at 15, 30 and 45 cm) during the gravel filling stage. All mesocosms were seeded with red canary grass (*Phalaris arundinacea*) using a planting ratio of 100 mg of seeds/mesocosm (Figure 3.5). Mesocosms were seeded on day one after filling the mesocosms with simulated wastewater.

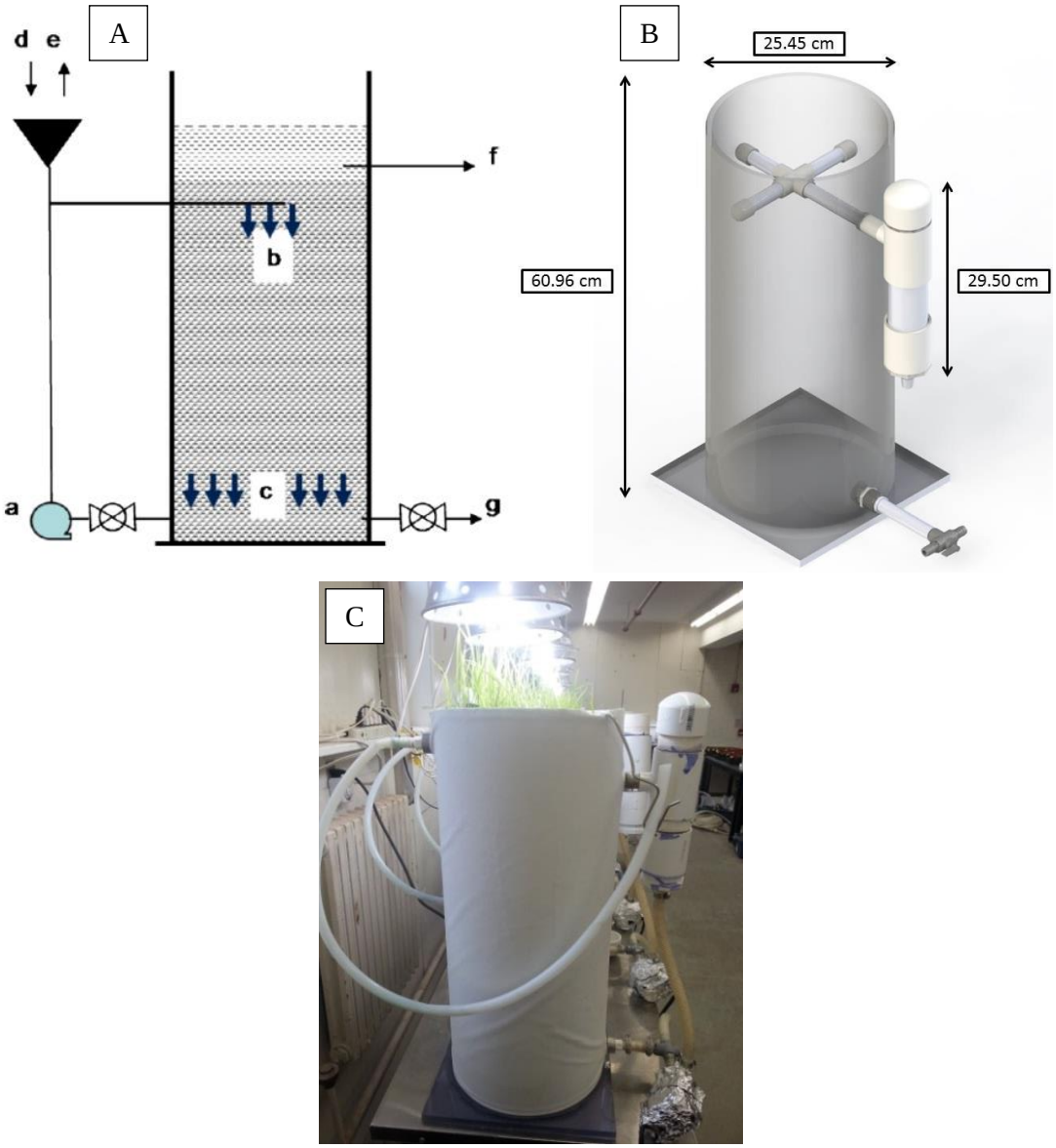

Figure 3.2: (A) Mesocosm schematic: Water is continuously circulated by the pump (a) and distributed into the wetland (b) water flows vertically through the mesocosm and is collected at the bottom (c). A port has been affixed to the mesocosm near the water inlet. This is used for injection of tracers (d) and sampling (e). An outlet is positioned at (f) to ensure consistent filling, and as well at (g) for convenient draining. (B) A render of the mesocosm was constructed with Solid Works which details the build without the tubing and pump. (C) A sample photo of a mesocosm set up in the laboratory.

Figure 3.3: Image depicting the placement of an aeration stone within a mesocosm**.** The blue corrugated tubing surrounding the Nalgene tubing was used to prevent collapse from the weight of the substrate media.

The mesocosms were maintained under laboratory conditions throughout the start-up period and artificial illumination with a 12-hour photoperiod (Figure 3.5) (OttLite, Natural Light Supplement, 20W Plant Bulb, 950 lumens; OttLite Technologies, Inc., Tampa, USA) was added after plant growth stalled and mesocosms were reseeded at week 10 (with a seeding ratio of 500 mg of seeds/mesocosm). Mesocosms were completely drained once a week. Draining occurred by opening the bottom valve pictured in Figure 3.3 (B) and allowing the water to flow out naturally via gravity.

Following draining the mesocosms were refilled with a simulated wastewater solution described in Weber and Legge (2011). The simulated wastewater solution was prepared using chlorinated tap water with additives to simulate the complexity of wastewater solutions. Additional essential plant nutrients were added to ensure adequate plant growth. The simulated wastewater solution contains 1 g/L molasses, 28.75 mg/L $NH_4H_2PO_4$, 151.5 mg/L KNO_3, 236 mg/L, $Ca(NO_3)_2$-$4H_2O$, 123.25 m/L $MgSO_4$-$7H_2O$, 9.175 mg/L FeNaEDTA, 0.715 mg/L H_3BO_3, 0.4525 mg/L $MnCl_2$-$4H_2O$; 0.055 mg/L $ZnSO_4$-$7H_2O$; 0.0125 mg/L $CuSO_4$ and 0.005 mg/L $(NH_4)_6Mo_7O_{24}$-$4H_2O$. The molasses contributes approximately 500 mg/L of chemical oxygen demand (COD) giving the simulated wastewater a COD:N:P ratio of 100:5:1.

The mesocosms were drained and subsequently refilled with a fresh batch of simulated wastewater each week. Therefore, the water in the mesocosms was constantly recycled for 1 week. This is similar to the hydraulic retention time (HRT) of 7 days which is frequently reported for full-scale CWs (Kadlec and Wallace 2008). This concept is illustrated in Figure 3.4, where 7 mesocosm systems are overlaid with a sample HSSF CW design. As the number of days the water

remains in the system increases, the amount of nutrients available in the water will decrease accordingly. This aligns with the decrease in nutrients observed along the flow path in a HSSF CW as time and distance from influent holding increases. For the mesocosms used in this study, the flow rate is admittedly high at approximately 10 L/minute (total system volume between 8.5 to 9.5 L), which may not entirely represent realistic flow rates in a full-scale CW, however the concept stands. The fact that water is flowing within these systems increases the suitability to relate to full scale CW systems versus a mesocosm system in which there is no flow. The vertical direction of the water flow path of the mesocosms in this study was a result of increased suitability for a bench top laboratory set-up. Additionally, operating the drain/feed cycle in this way allows for hydrological parameters such as porosity and evapotranspiration to be assessed directly (see Section 3.2.4).

The continuous recirculation of water throughout the mesocosm system causes some amount of the microbial population associated with the rhizosphere and gravel media biofilm to shear off. Since the mesocosms were completely drained weekly it can be assumed that the microbial population within the interstitial water largely reflects the microbes that detached from the biofilm communities in that week. This is a result of the constant recirculation of the water within the mesocosms. Detached biofilm may become integrated within the interstitial water and remain there to be captured in sampling campaigns until a system is drained the following week. Throughout this experiment the mesocosms were not disassembled to keep the biofilm established within the gravel medium and rhizosphere intact.

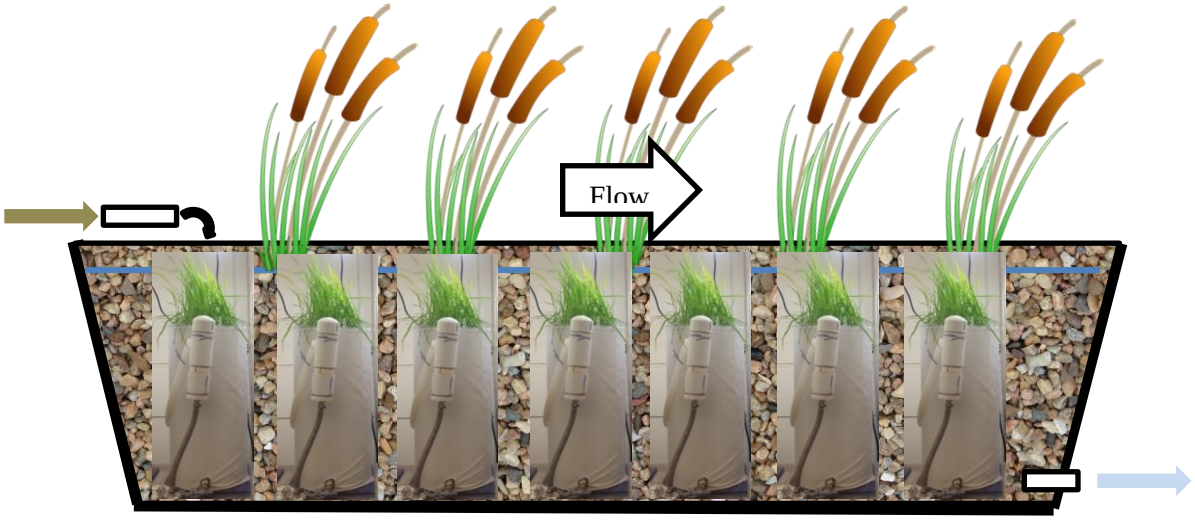

Figure 3.4: Illustration of mesocosm systems drain and refill cycle aligning with a 7-day HRT of a HSSF CW.

Figure 3.5: Laboratory mesocosm set up. The six aerated mesocosms are on the left with the six non-aerated mesocosms on the right. Plant lights were affixed approximately 6-12 inches above the maximum stem height of the plants and moved accordingly as the plants grew. This is a sample picture taken a few months after the start-up study experimental period.

Mesocosms were sampled frequently for twelve weeks to monitor their development. Chemical, hydrological and microbial parameters were monitored over this time and pollutant removal was characterized. The parameters monitored during the wetland development period and their frequencies are listed in Table 3.1. Water chemistry was typically analyzed daily and data was analyzed so that the entire week was compared for aerated and non-aerated replicates, unless otherwise specified. Water treatment was analyzed for TOC and TN removal rates on a weekly basis between aerated and non-aerated replicates. Sampling times are detailed in Section 3.2.3.2 which were used to calculate the rates of removals. Porosity was determined from a single weekly measurement and compared between aerated and non-aerated replicates. Evapotranspiration was measured 5 times a week and combined to determine a weekly average over all six replicates of the aerated and non-aerated systems. Dispersivity, microbial activity and microbial function were evaluated on a bi-weekly basis with a single measurement which was evaluated for six replicates of the aerated and non-aerated systems.

Table 3.1: Sampling Frequency during Wetland Development Period

Parameter Measured	Method	Frequency
Water Chemistry (NH_4^+, NO_3^-, pH, DO, ORP, temperature)	YSI Professional Plus field probe	5x per week
Water Treatment (TOC/TN)	Analytik Jena TOC/TN analyzer	Weekly cycle (5x in a week)
Evapotranspiration	Water loss from mesocosm/day	5x per week
Plant Health	Growth & colour	1x per week
Porosity	Drainable volume	1x per week
Dispersivity	NaBr tracer test	Bi-weekly
Microbial Community Activity	1.Community Level Physiological Profiling 2.FDA hydrolysis	Bi-weekly

3.2.3 **Water Quality**

3.2.3.1 Water Chemistry

YSI Professional Plus probes (YSI Inc., Yellow Springs, OH) were used to collect daily measurements of ammonium (NH_4^+, mg/L), nitrate (NO_3^-, mg/L), conductivity (µS/cm), dissolved oxygen (mg/L), redox potential (mV), pH, and water temperature (°C). The YSI Professional Plus probes were inserted into the mesocosms via the sampling port where wetland pore water is constantly recirculated throughout. The probes were completely submerged in the water and readings were made once the variables had stabilized, typically within 2 to 5 minutes.

3.2.3.2 Total Organic Carbon and Total Nitrogen Removal

Total Organic Carbon (TOC) and Total Nitrogen (TN) concentrations in simulated wastewater and mesocosm interstitial water were monitored to assess the water treatment capacity of the wetland mesocosms. Water samples were collected from the simulated wastewater solution before introduction into mesocosms (time 0) and from the interstitial water at time points of 1, 3, 5, 24 and ~96 hours post mesocosm refill to assess TOC and TN degradation within the wetland mesocosms. Water samples were analyzed using a TOC/TN analyzer (Analytik Jena, TOC/TNb: multi N/C® Series, Germany).

TOC and TN removal from the wetland system was analyzed using the k-C* first order rate kinetics model (Kadlec and Wallace 2008):

$$k = \ln\left(\frac{C_i - C^*}{C_O - C^*}\right) \quad (2)$$

C_O = outflow concentration (mg/L)

C* = background concentration (mg/L)

C_i = incoming concentration (mg/L)

k = first order aerial reaction rate constant (m/day)

For this analysis, 96 hour measurements of TOC and TN were used as the background concentrations (C*).

3.2.4 Hydrological Measurements

3.2.4.1 Evapotranspiration

Evapotranspiration is a measure of water loss to the atmosphere from the surface of the wetland and through the transpiration of wetland vegetation (Kadlec and Wallace 2008). Each mesocosm has an overflow tube just below the gravel surface and water was added daily to reach this point. The volume added each day represents the water loss from the previous day and is used as evapotranspiration (L/day).

3.2.4.2 Porosity

Porosity is calculated from the total volume of the bed medium and the volume of the pore space. The volume of the bed medium includes the dimensions which both the gravel and water occupy. The volume of the pore space is represented as the fillable volume of water from the wetland. The mesocosms are drained weekly prior to feeding with new simulated wastewater and the volume of water added back to the system will be used to calculate porosity.

To calculate porosity the mesocosms were drained from the port at the bottom of the mesocosm body by gravity until no more water could be evacuated. The drained water was then discarded and the mesocosms were filled with a known quantity of new simulated wastewater. The mesocosms were filled until water began to evacuate from the overflow tube. The mesocosm water pump was turned on (without aeration, where applicable) to ensure an accurate measurement of volume and the evacuation of most air bubbles. The volume of water added to the mesocosm was recorded (as the volume of the pore space) and used with the equation (14) to calculate porosity.

$$\phi = \frac{volume\ of\ pore\ space\ (volume\ added)}{volume\ of\ bed\ media} \tag{14}$$

3.2.4.3 Dispersivity

The internal hydraulics of CWs can be quantified using inert, soluble chemical tracers (Kadlec and Wallace 2008). Sodium bromide (NaBr) was selected for use in this study since it is conservative, readily soluble in water and relatively inert. NaBr tracer tests were conducted on the mesocosms bi-weekly over the study period. Two mL aliquots of a 200 g/L NaBr stock solution were injected into the mesocosms through the sampling port and a handheld conductivity probe (YSI Professional Plus, YSI Inc., Yellow Springs, OH) was then inserted into the sampling port to measure the conductivity of the recirculating water. Conductivity readings were taken every second until stable values were reached (typically 15 to 20 minutes). Data was then fit to a 1D advection-dispersion equation using Aquasim v.1.0.0.1 (Eawag Institute, Switzerland, 1995). The 1D advection-dispersion equation, as outlined in Weber and Legge (2011):

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} \tag{3}$$

where C is concentration (mg/mL), t is time (minutes), v is velocity (cm/minute), x is distance (cm) and D is the dispersion coefficient (cm^2/minute). Average flow rate and apparent cross sectional area were manually entered based on porosity and flow rates measured before starting the tracer test. The dispersion coefficient (D) was then determined using the parameter estimation function and peak fitting. A sample plot of the measured data versus the simulated output from Aquasim can be found in Appendix A, Figure A.1.

3.2.5 Plant Growth

Photos of plants were taken periodically over time to qualitatively monitor changes in growth and colour. A count of the number of plant stems in each mesocosm was performed weekly.

3.2.6 Microbial Community Analysis

3.2.6.1 Microbial Activity

Microbial activity was assessed indirectly based on a measurement of enzymatic activity associated with the hydrolysis of fluorescein-diacetate (FDA) to fluorescein (FL). FDA contains a FL molecule quenched by two acetate groups. When the acetate groups are enzymatically cleaved by the microbial community within the wetland the remaining molecule fluoresces. This fluorescence was monitored photometrically. One mL of a 5 mM FDA solution was injected into the mesocosm via the sampling port. Water samples were taken every minute after injection of FDA for 30 minutes. Samples were read using a handheld fluorometer (Turner Designs, Picofluor™) using an excitation wavelength of 490 nm and an emission wavelength of 520 nm. The FDA utilization rate was determined for time increments between 8 and 15 min. The final FDA utilization rate was calculated as the average of these incremental slopes. The results from this test are treated as *in-situ* microbial activity. Measurements were performed bi-weekly.

3.2.6.2 Microbial Function

Community Level Physiological Profiling (CLPP) was developed for the characterization of soil microbial communities (Garland and Mills 1991) but is increasingly used for the characterization of mixed microbial communities from CWs. CLPP is a way of characterizing the metabolic function of a mixed microbial community based on carbon source utilization patterns (CSUPs). The method uses BIOLOG EcoPlates™, with multiple sole carbon sources, to accurately and rapidly determine differences in microbial community function, carbon utilization intensity and overall catabolic capability (Weber and Legge 2010). This method has advantages over other techniques since it does not require specialized expertise and can be used easily, without the isolation of the microbial community from the sample matrix. For recirculating wetland mesocosms, interstitial water samples work as a non-destructive method to relate to the greater biofilm community housed within the wetland subsurface media (Weber and Legge 2013). Another additional benefit is the interstitial water samples do not require any sample pre-treatment step with the CLPP method and can be directly plated without dilution in most cases (Button et al. 2016).

The BIOLOG EcoPlate™ (Biolog Inc., Hayward CA., USA) is a 96-well plate which contains 31 carbon sources and a blank, in triplicate. Along with the carbon source, each well contains a redox dye indicator, tetrazolium violet. When a mixed microbial community sample is inoculated into the well and starts to utilize the carbon source, the production of NADH via cell respiration reduces the tetrazolium dye to formazan. The development of formazan induces a

change in colour from clear to purple which can be monitored over time to evaluate the microbial community activity in each well. This colour development is evaluated photometrically by absorbance with a spectrophotometer (Eon microplate reader, BioTek Instruments, Inc., Winooski, Vermont, United States) at 590 nm.

Three days after the wetland renewal/feeding was performed wetland interstitial water (50 mL), containing a mixed microbial community, was collected from the sampling port of each wetland mesocosm. Samples were then inoculated onto the BIOLOG EcoPlates™ using aseptic techniques inside a clean hood that was washed with a 70% ethanol/water solution prior to use. After gentle agitation of the sample and with the use of sterile pipette tips, 100 µL of the interstitial sample was inoculated into each well on the BIOLOG EcoPlate™. New pipette tips were used for each sample to avoid cross contamination. As well, each wetland water sample received its own BIOLOG EcoPlate™. Once all twelve BIOLOG EcoPlates™ were inoculated they were incubated in the dark at room temperature. Microplates were read photometrically at defined time intervals (every 4 hours for 96 hours) using a Eon microplate reader equipped with a Biostack |3 microplate stacker and Gen5 All-in-One Microplate Reader Software (version 2.05.5) (all from BioTek Instruments, Inc., Winooski, Vermont, United States). The microplates were read individually at 590 nm following a 3 second shake at medium setting to ensure each well was well mixed. CLPP evaluations were performed bi-weekly.

3.3 Data Analysis

3.3.1 Community Level Physiological Profiling

Analysis of CLPP data was performed as described in Weber et al. (2007) and Weber and Legge (2010). One plate was used for each time point (6 time points) for each system. From each plate, 3 replicates of carbon source utilization patterns are collected for a total of 216 data "sets" for analysis. Each of these data sets represents the 31 variables (carbon sources) giving a total of 6696 data points. To manage the quantity of data and analyze temporal microbial community trends over the course of the wetland development period, a single time point was selected for analysis. The selection was based on the greatest variance between well responses as well as the least number of absorbance values over 2.0 (values over 2.0 are considered outside the linear absorbance range) (Button et al. 2016). Based on these two factors a time point of 48 hours was chosen for the calculation of the average well colour development (AWCD) and richness.

3.3.1.1 Average well color colour development:

AWCD represents the average catabolic activity of carbon sources over all wells and is calculated as:

$$\frac{1}{31}\sum_{i=1}^{31}(A_i - A_0) \tag{15}$$

where A_i represents the absorbance reading of well *i* and A_0 is the absorbance reading of blank well. This overall catabolic activity metric for the microbial community to utilize a variety of carbon sources can be thought of as microbial function.

3.3.1.2 Substrate richness

Substrate richness constitutes the number of different substrates which are utilized by a microbial population. Previous work has used an absorbance value of 0.25 as a cut-off value to determine whether or not a microbial community has effectively utilized said carbon source (Button et al. 2016). Substrate richness was defined as the number of wells where $(A_i - A_0) \geq 0.25$. A_i represents the absorbance reading of well *i* and A_0 is the absorbance reading of the blank well.

3.3.1.3 Carbon Source Guild

Zak et al. (1994) suggested grouping the 31 individual carbon sources from the BIOLOG EcoPlate™ according to their chemical structure as carbohydrates, polymers, carboxylic acids, amino acids, and amines/amides to decrease the complexity of data analysis. Grouping the carbon sources together as such decreases the complexity of the analysis from 31 dimensions to 5 dimensions. The groupings were slightly modified later by Weber and Legge (2009) and their classification of carbon sources will be used in this analysis (Table 3.2).

Table 3.2: Carbon source guild groupings for Community Level Physiological Profiling using BIOLOG EcoPlates™.

Well #	Carbon source	Guild
1	water (blank)	N/A
2	pyruvic acid methyl ester	carbohydrates
3	Tween 40	polymers
4	Tween 80	
5	α-cyclodextrin	
6	glycogen	
7	D-cellobiose	carbohydrates
8	α-D-lactose	
9	β-methyl-D-glucoside	
10	D-xylose	
11	i-erythritol	
12	D-mannitol	
13	N-acetyl-D-glucosamine	
14	D-glucosaminic acid	carboxylic and acetic acids
15	glucose-1-phosphate	carbohydrates
16	D-, L-α-glycerol phosphate	
17	D-galactonic acid-γ-lactone	carboxylic and acetic acids
18	D-galacturonic acid	
19	2-hydroxy benzoic acid	
20	4-hydroxy benzoic acid	
21	γ-hydroxybutyric acid	
22	itaconic acid	
23	α-ketobutyric acid	
24	D-malic acid	
25	L-arginine	amino acids
26	L-asparagine	
27	L-phenylalanine	
28	L-serine	
29	L-threonine	
30	glycyl-l-glutamic acid	
31	phenylethylamine	amines/amides
32	putrescine	

3.3.1.4 Root Exudate Analysis

This experiment occurs in the start-up phase of a CW, which therefore involves the growth and establishment of plant species. Plants have been known to exude chemicals from their roots which can alter the chemical and biological characteristics of the rhizosphere and therefore the microbial community contained within that region (Whipps and Lynch 1990). During the seedling stage, approximately 30-40% of carbon originating from photosynthesis products is spent on root exudates (Whipps and Lynch 1990). Therefore, reporting the contribution of root exudate utilization on the overall CSUPs of the microbial communities is important. Root exudates commonly identified from plants which are present on the BIOLOG EcoPlate™ have been noted in Table 3.2 (Campbell et al. 1997; Uren 2007; Badri and Vivanco 2009).

Carbon sources on the BIOLOG EcoPlate™ were identified as root exudates by cross referencing tables listing common plant root exudates in the literature, specifically from Campbell et al. (1997) Table 2a, Baris and Vivanco (2009) Table 1, and Uren (2008) Table 1.1. Additional common names for carbon sources on the BIOLOG EcoPlate™ were also screened in the same way (e.g. 2-hydroxy benzoic acid is also known as salicylic acid). If a specific isomer of the compound was list on the BIOLOG EcoPlate™ (e.g. L-arginine) but a reference list denoted the compound without an isomer (e.g. arginine) then it was ensured that the isomer listed on the BIOLOG EcoPlate™ was the common and naturally occurring isomer. After this initial screening was performed, if a carbon source listed on the BIOLOG EcoPlate™ was not identified as being a common root exudate a quick literature search was performed with the name of the carbon source (also using additional common names) and "root exudate" on the search engines Engineering Village, Web of Science, SciFinder and Google Scholar. If identified within the literature, the references were screened for applicability (plants in which the compound was identified as a root exudate were similar to wetland plants) and the root exudate was added to the list, if necessary.

The list of carbon sources from the BIOLOG EcoPlate™ which have been identified as root exudates can be found in Table 3.3. Carbon sources with grey shading indicate they have been identified as root exudates. The notes section of Table 1.1 details that D-galacturonic acid, N-acetyl-D-glucosamine, putrescine, α-D-lactose were identified in the literature search as being root exudates from specific plants (soy bean, rice or sunflower). It is unknown whether they are more common root exudates from widespread plant species so they will not be considered as root exudates in this analysis for that reason. The remaining compounds (10) which were selected as root exudates based on the literature survey include: 2-hydroxy benzoic acid, 4-hydroxy benzoic acid, D-malic acid, D-mannitol, D-xylose, L-arginine, L-asparagine, L-phenylalanine, L-serine, and L-threonine.

Table 3.3: Identification of root exudates from BIOLOG™ EcoPlate carbon sources.

Carbon source	Notes	Root exudate selection	Reference for root exudate selection
2-hydroxy benzoic acid	Identified as root exudate in the literature under common name salicylic acid	✓	Badri & Vivanco (2009) Table 1; Campbell (1997) Table 2a
4-hydroxy benzoic acid	Identified as root exudate in the literature under common name: p-hydroxybenzoic acid	✓	Uren (2007) Table 1.1; Campbell (1997) Table 2a
D-malic acid	Identified as root exudate in the literature. L-malic acid is naturally occurring isomer. Mixture of L- and D-malic acid produced synthetically.	✓	Badri & Vivanco (2009) Table 1; Campbell (1997) Table 2a
D-mannitol	Identified as root exudate in the literature. Naturally occurring isomer.	✓	
D-xylose		✓	
L-arginine	Identified as root exudates in the literature.	✓	Uren (2007) Table 1.1; Campbell (1997) Table 2a
L-asparagine		✓	
L-phenylalanine		✓	
L-serine		✓	
L-threonine		✓	
D-galacturonic acid	Root exudate from soy bean (Tawaraya et al. 2014)		
N-acetyl-D-glucosamine			
putrescine	Root exudate from sunflower (Bowsher et al. 2015) and rice (Suzuki et al. 2009)		
α-D-lactose	Root exudate from rice (Suzuki et al. 2009)		
D-, L-α-glycerol phosphate	No mention in literature search as root exudate.		
D-cellobiose			
D-galactonic acid-γ-lactone			
D-glucosaminic acid			
glucose-1-phosphate			
glycogen			
glycyl-l-glutamic acid	Glutamic acid identified as root exudate but not glycyl-l-glutamic acid.		
i-erythritol	No mention in literature search as root exudate.		
itaconic acid			
phenylethylamine			
pyruvic acid methyl ester			
Tween 40			
Tween 80			
α-cyclodextrin			
α-ketobutyric acid			
β-methyl-D-glucoside			
γ-hydroxybutyric acid			

Root exudates were analyzed by averaging the response from the wells identified to be root exudates, essentially creating an AWCD for root exudates alone. 2-hydroxy benzoic acid, 4-hydroxy benzoic acid, D-malic acid, D-mannitol, D-xylose, L-arginine, L-asparagine, L-phenylalanine, L-serine and L-threonine were identified as common root exudates in numerous plants in a literature survey (Table 3.3). Therefore 10 carbon sources on the BIOLOG™ EcoPlate were identified as root exudates. Therefore, the contribution of AWCD from identified root exudates is calculated as:

$$\frac{1}{10}\sum_{i=1}^{10}(A_{REi} - A_0) \quad (16)$$

where A_{REi} represents the absorbance reading of well containing an identified root exudate *i* and A_0 is the absorbance reading of blank well.

3.3.1.5 Principal Component Analysis

Principal component analysis (PCA) is used to ordinate data with a large amount of variables onto a two dimensional plane. PCA was performed using the covariance (n-1) matrix of the mean CSUP (average from three replicates) data (Weber and Legge 2010) to further elucidate trends in the microbial community development and stabilization. Datasets were subjected to logarithmic (ln(x+1)) data transformations based on assessment of normality, homoscedasticity and linear correlations following the recommendations of Weber et al. (2007). PCA analysis was completed using the covariance (n-1) matrix with XLSTAT 2017 (Addinsoft New York, NY).

3.3.2 Statistical Analysis

Statistical analyses were performed using SPSS (version 23, IBM Corporation, New York, USA) and Microsoft Excel (Microsoft Office 2010, New Mexico, USA). Data was tested for normality using the Shapiro-Wilk test and for variability with the Mauchly's Test of Sphericity and Levene's Test of Equality of Error Variances. Data was analyzed over time with a repeated measures analysis of variance (ANOVA) with a significance level of $p = 0.05$. Data was also analyzed for differences between the aerated and non-aerated system replicates each week with Student's t-test with a significance level of $p = 0.05$.

3.4 Results and Discussion

3.4.1 Plant Growth

All twelve systems were initially seeded on the day of wetland inoculation in week 1 with 100 mg of *Phalaris arundinacea* seeds. Initially, the plants were growing well in all systems but by week 4 the plant growth appeared to have stalled in terms of number of plant stems (Figure 3.6). By week 8 it was very evident that plant growth had stalled. This is likely due to a lack to adequate light to facilitate plant growth. The mesocosms were in a lab with ample natural light but this was not enough to sustain adequate plant development. Therefore, in week 9, all plant seedlings were removed from the mesocosms and the systems were reseeded with 500 mg of *Phalaris arundinacea*, from which point plant growth accelerated in weeks 10 to 12. Plants lights (with a 12-hour photo period) were also added at the time of reseeding to help stimulate growth. Additionally, the seeding ratio was increased for the second seeding due to the lack of establishment (number of stems) noted after the first round of seeding.

Plant growth was significantly better in non-aerated mesocosms compared to aerated mesocosms (Figure 3.6) (Student's t-test, $p<0.05$). *Phalaris arundinacea* is naturally found in wetlands which are typically more anaerobic environments (USDA 2002). Artificial aeration negatively impacted plant growth, causing a yellowing in the leaves, less overall stems and shorter height (Figure 3.7). This is consistent with a review of *Phragmites australis*, another common wetland plant, which found artificial aeration to cause chlorosis in the species (Weedon 2014). In addition to colour change, reduced stem height, reduced biomass density and increased susceptibility to disease and infestation were also noted in this review, which is consistent with observations from the present study.

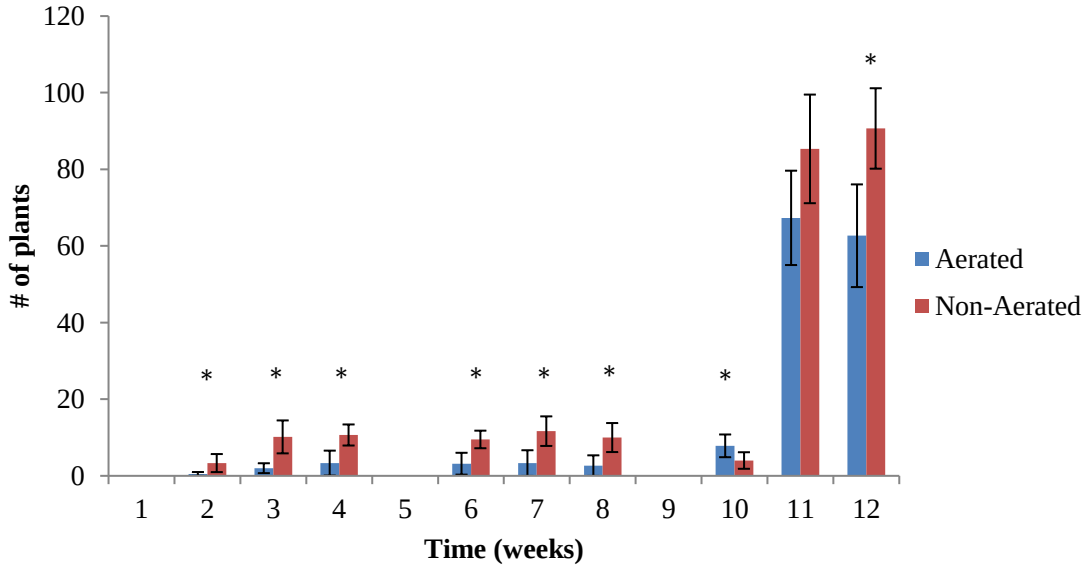

Figure 3.6: Plant count, recorded as number of stems per system, during the wetland start-up period. Poor plant growth is noted initially for the first 8 weeks. All stems were removed in week 9 and systems were replanted. Plant grow lights were also added to help with plant growth after week 9. Plant count was recorded once a week and data shown is the average from six replicates

of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

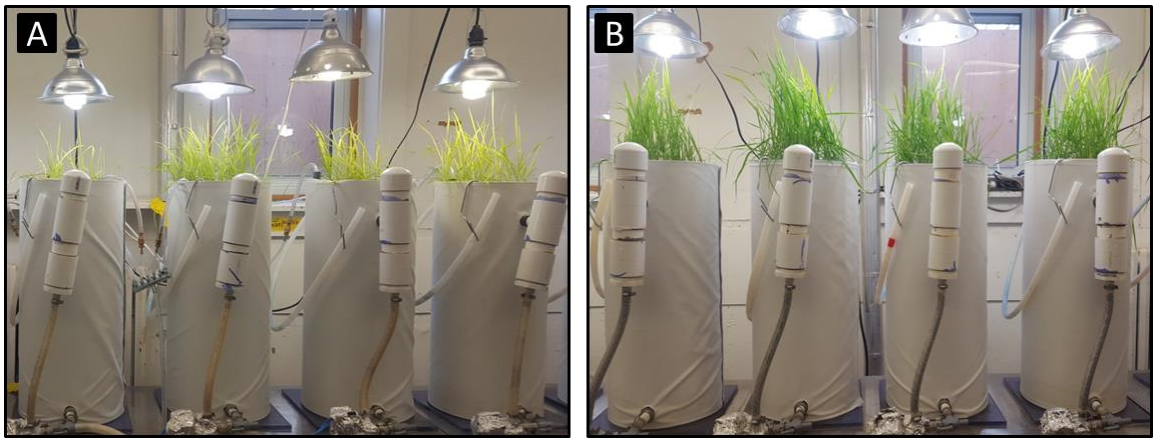

Figure 3.7: Sample image of plant growth in aerated (A) versus non-aerated (B) CW mesocosms. The addition of aeration is not favourable for the health and growth of *Phalaris arundinacea* causing the stems to be bleached relative to those grown in a non-aerated environment.

3.4.2 **Water Chemistry**

The temperature in aerated and non-aerated systems differed slightly over the course of the wetland start-up period with values ranging from 18-24 °C (Figure 3.8). The temperature trend observed in Figure 3.8 is likely as a reflection of changes in air temperature within the room the mesocosms were housed. The mesocosms were initially set-up and seeded with a microbial community on November 16th, 2015, therefore the decline in temperature from week 2 to week 9 fits with the onset of winter. CWs often display seasonal pollutant removal trends based on temperature trends (Werker et al. 2002; Ouellet-Plamondon et al. 2006). However, these are over much larger temperature differences of 15-20 °C therefore effects of temperature on pollutant removal and microbial community are not expected here.

The significant temperature resolution between aerated and non-aerated systems may be due a difference in metabolic heat generation between microbial communities (Student's t-test, $p<0.05$). There is the potential for an increased microbial biomass, and therefore activity, in the non-aerated systems as the water agitation and shear force from the aeration pump is not present. Alternatively, the increased mixing and the addition of cooler room temperature air via the artificial aeration in the aerated systems may cause this temperature differential.

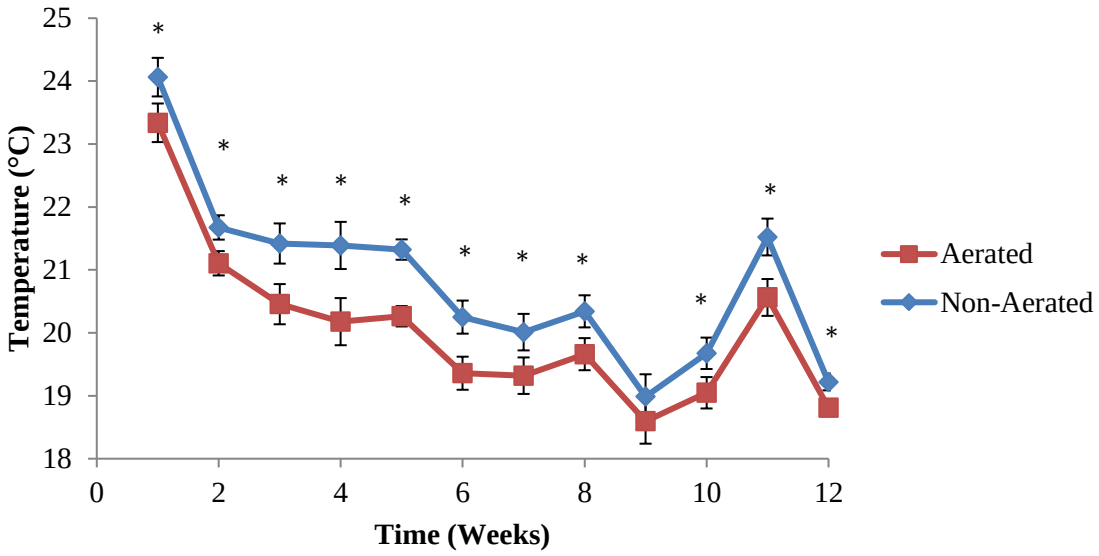

Figure 3.8: Weekly average water temperature (°C) during start-up period for six aerated and six non-aerated constructed wetland mesocosms. The onset of this experimental period was in late November, therefore the decreasing trend correlates with the onset of winter. Data points are made up of weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

The conductivity in both the aerated and non-aerated mesocosms significantly declined over the wetland development period (Figure 3.9) (repeated measures ANOVA, $p<0.05$). These changes may be caused by an uptake of ions into the wetland biofilm as development occurs throughout the system over time. A decline in conductivity has been observed in other full scale wetlands over a start-up period of two years (Kadlec and Wallace 2008). Additionally, other studies have reported conductivity values between 700-900 µS/cm for a full scale systems treating municipal wastewater (Leschisin et al. 1992; Hemming et al. 2001). Therefore, the conductivity values observed in these mesocosm CWs are related to values observed in full-scale constructed wetland systems.

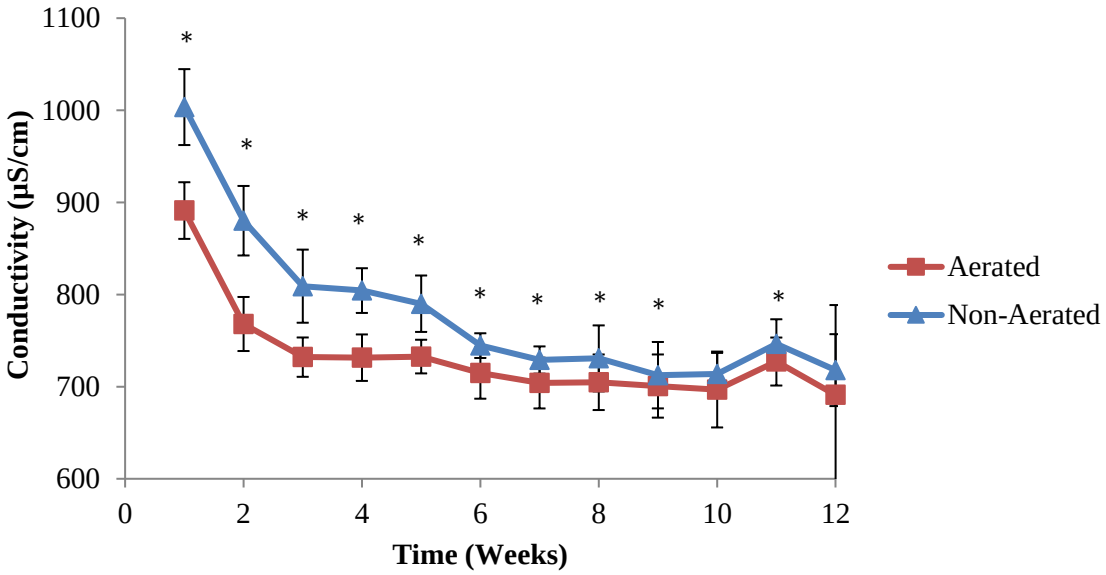

Figure 3.9: Weekly average conductivity (µS/cm) during start-up period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

The pH of the water in aerated and non-aerated systems was consistent throughout the start-up period (Figure 3.10) (repeated measures ANOVA, $p>0.05$). The pH stability within the systems is likely due to the use of limestone pea gravel as the subsurface substrate. The pH for both systems was between 7 and 8.25 for the duration of the start-up period. A possible explanation for the aerated mesocosm consistently having higher pH values than non-aerated systems is the *in-situ* formation of calcium bicarbonate ($Ca(HCO_3)_2$) (Student's t-test, $p<0.05$). Limestone is composed of calcium carbonate ($CaCO_3$), which can react with water that is saturated with carbon dioxide (CO_2) to form soluble calcium bicarbonate. Calcium bicarbonate only exists in aqueous solution containing the calcium (Ca^{2+}), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) ions, together with dissolved carbon dioxide (CO_2) (Benjamin 2002).

$$CaCO_{3\,(s)} + CO_{2\,(aq)} + H_2O \rightleftharpoons Ca(HCO_3)_{2\,(aq)} \qquad \text{(d)}$$

$$Ca(HCO_3)_{2\,(aq)} \rightleftharpoons {Ca^{2+}}_{(aq)} + {HCO_3}^-_{(aq)} + CO_{2\,(aq)} \qquad \text{(e)}$$

The addition of artificial aeration to the wetland systems is done to add dissolved oxygen to the water, but this likely adds dissolved carbon dioxide at the same time, allowing the above reactions to proceed. Bicarbonate is a well-known weak base and would contribute to the increased basicity of the aerated systems.

The weekly average pH of the circulating water in the non-aerated systems was similar to the average outlet pH from a worldwide survey of HSSF CWs (7.12 – 7.43) (Kadlec and Wallace 2008). The weekly average pH of the circulating water in aerated systems is comparable to the

outlet pH of an aerated HSSF wetland treating landfill leachate (seasonal fluctuations between 7.7 – 7.9) (Nivala et al. 2007). Another study also reported more alkaline pH values in aerated CWs than in non-aerated CWs treating domestic wastewater (Zhang et al. 2010).

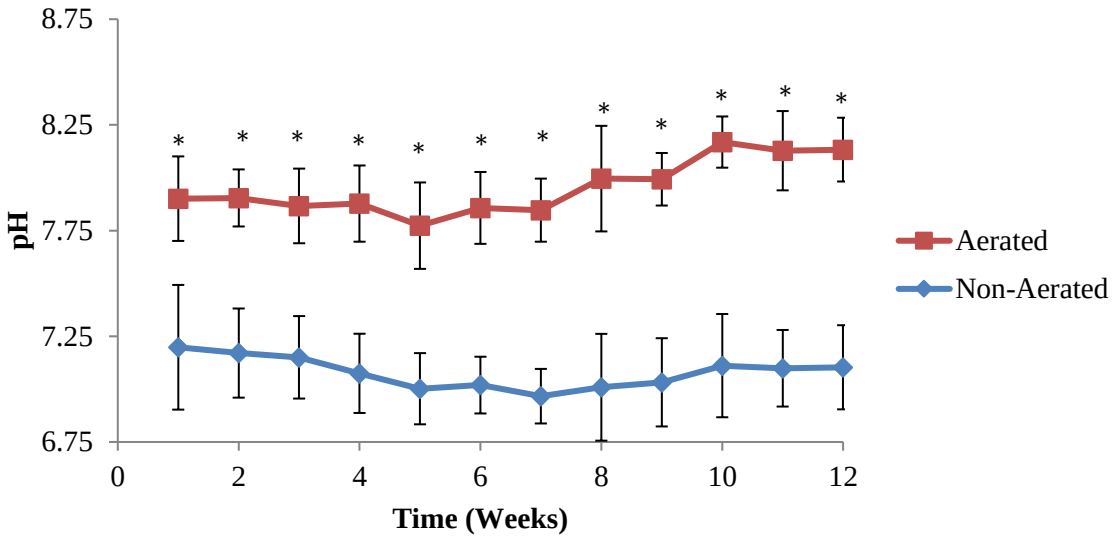

Figure 3.10: Weekly average pH during start-up period for six aerated and six non-aerated constructed wetland mesocosms. Data points from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

The aerated systems had significantly higher (6-8 mg/L) dissolved oxygen (DO) than non-aerated systems (0-0.1 mg/L) (Figure 3.11) (Student's t-test, $p<0.05$). This outcome was expected based on the addition of artificial aeration to the aerated systems. DO concentrations in both systems were stable over the development period (repeated measures ANOVA, $p>0.05$). Over the course of a weekly drainage/feeding cycle, DO remained consistent in aerated or non-aerated systems. DO in the artificially aerated CW mesocosms is higher than those reported in the literature. A laboratory scale horizontal flow CW reported DO concentrations between 3.0 to 3.5 mg/L in areas supplemented with aeration (Li et al. 2014), and a laboratory scale vertical flow CW with continuous aeration reported a DO concentration of 4.4 mg/L (Dong et al. 2012). A pilot scale HSSF CW described DO in a bed without aeration to be close to zero and one with aeration between 7 to 8 mg/L of DO (Uggetti et al. 2016). The higher DO observed in this study compared with those in the literature is likely due to the rapid diffusion of air bubbles provided by the air pump and aeration stone used. This may limit the anaerobic microenvironments available in the biofilm in the aerated systems which may have implications for the removal of total nitrogen.

Oxidative-reductive potential (ORP or redox) refers to the tendency of a chemical species to gain or lose electrons. This potential is measured on a scale where a low redox potential is associated with reducing conditions which favour anaerobic processes, and a high redox potential is characteristic of an oxidized environment and promotes aerobic processes (Table 1.2). The redox potential in aerated systems is positive with values between 50 to 100 mV while the redox potential in non-aerated systems is negative with values between -150 to -200 mV (Figure 3.12). The

difference in ORP values between the aerated and non-aerated mesocosms is statistically significant at every time point (Student's t-test, $p<0.05$). This is to be expected for systems with such drastically different dissolved oxygen profiles (Figure 3.11). Data was not collected for ORP between weeks 2 and 7 due to a malfunction with the probe used for data collection. ORP within the aerated and non-aerated mesocosms did not change significantly over the course of the start-up period (repeated measures ANOVA, $p<0.05$)

The redox potential in the aerated systems is in the range for nitrate reduction (Table 1.2), while the non-aerated systems supplied redox conditions suitable for denitrification (Cheng et al. 2012), based on values reported in the literature. Based on the differences in dissolved oxygen and redox potential between aerated and non-aerated systems it is expected that differences will be present for nutrient removal and microbial community characteristics between the systems. Nitrification is expected to proceed in the aerated systems, while denitrification is expected to take place in the non-aerated systems.

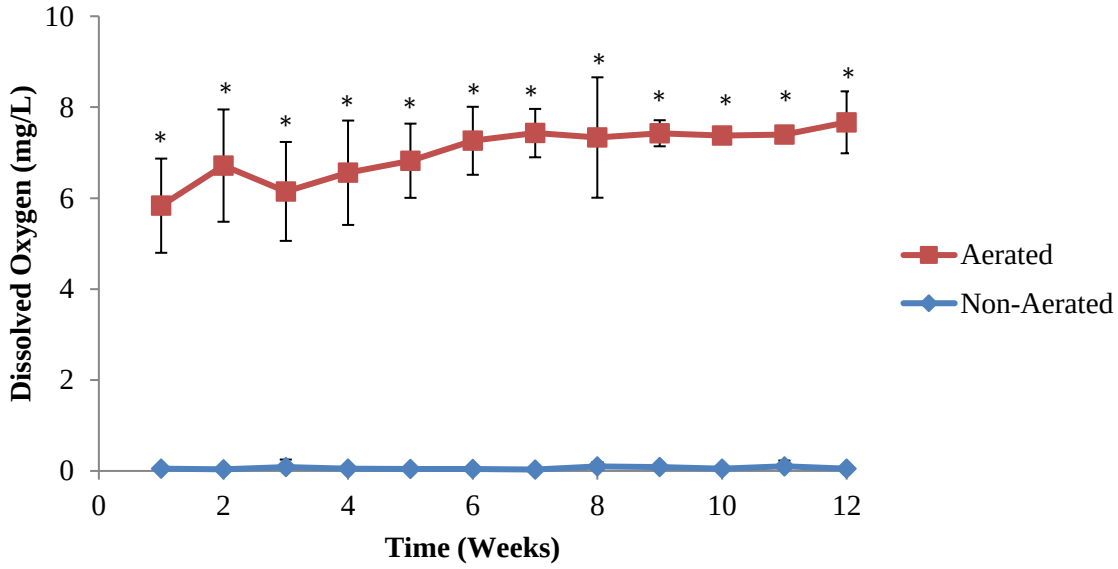

Figure 3.11: Weekly average dissolved oxygen (mg/L) during start-up period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

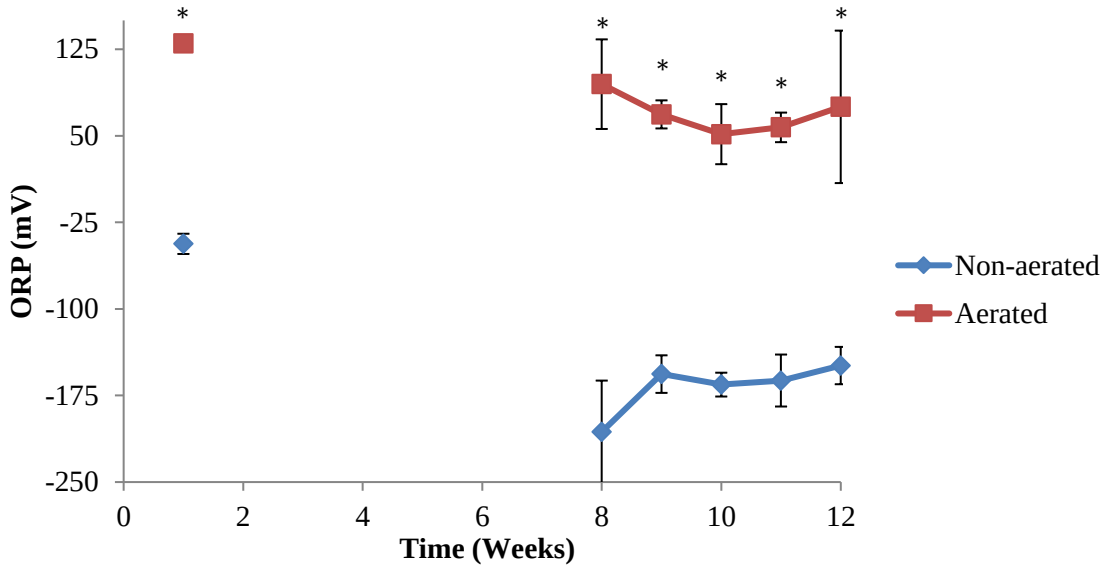

Figure 3.12: Weekly average oxidative-reductive potential (ORP) (mV) during start-up period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

Nitrogen is present in wastewater as organic nitrogen, ammonium, nitrite and nitrate (Halling-Sørensen and Jorgensen 1993). Microbial and biological processes dominate the nitrogen cycling within CWs (Garcia et al. 2010). Organic nitrogen can be mineralized into ammonium through the ammonification reaction; then ammonium is converted to nitrate via nitrification. Both reactions are aerobic processes. Further, denitrification converts nitrate to nitrogen gas via microbial processes under anaerobic conditions and is typically the rate limiting step in nitrogen removal in CWs (Faulwetter et al. 2009; Garcia et al. 2010). In these systems the weekly inlet ammonium was approximately 10 mg/L, while nitrate was approximately 60 mg/L (Table 3.4). During the development period of these CWs the aerated mesocosms show significantly lower ammonium levels (2-3 mg/L) than non-aerated mesocosms (6-8 mg/L) (Student's t-test, $p<0.05$) (Figure 3.13); while non-aerated systems have significantly lower nitrate levels (2-10 mg/L) than aerated systems (50-80 mg/L) (Figure 3.14) (Student's t-test, $p<0.05$). This is in line with the fundamental differences between the redox and dissolved oxygen conditions between the aerated and non-aerated systems. As these are microbially-mediated processes it is expected that there may be differences between the microbial consortium between the aerated and non-aerated mesocosms.

Despite the lack of oxygen recorded in the non-aerated mesocosms (<0.5 mg/L), there is some removal of ammonium provided from these systems based on an inlet ammonium concentration of 10 mg/L (Table 3.4). The oxygen for the nitrification process may come from root-mediated oxygen release or exchange with the atmosphere (less likely). The dissolved oxygen values which are reported for the system are just the values which can be captured in a reading and may not fully represent the situation *in-situ*. Dissolved oxygen may be utilized within the biofilm as soon as it becomes available. The non-aerated mesocosms are anaerobic (Figure 3.12), but not to an extreme. Therefore, the *in-situ* rate of oxygen utilization may equal the rate at which it

becomes available in the wetland mesocosms. Additionally, volatilization of ammonia (NH_3) from the systems is possible, especially as the pH increases. However, this is not thought to be a major removal pathway for ammonium within constructed wetlands as ammonium (NH_4^+) prevails in aqueous solutions.

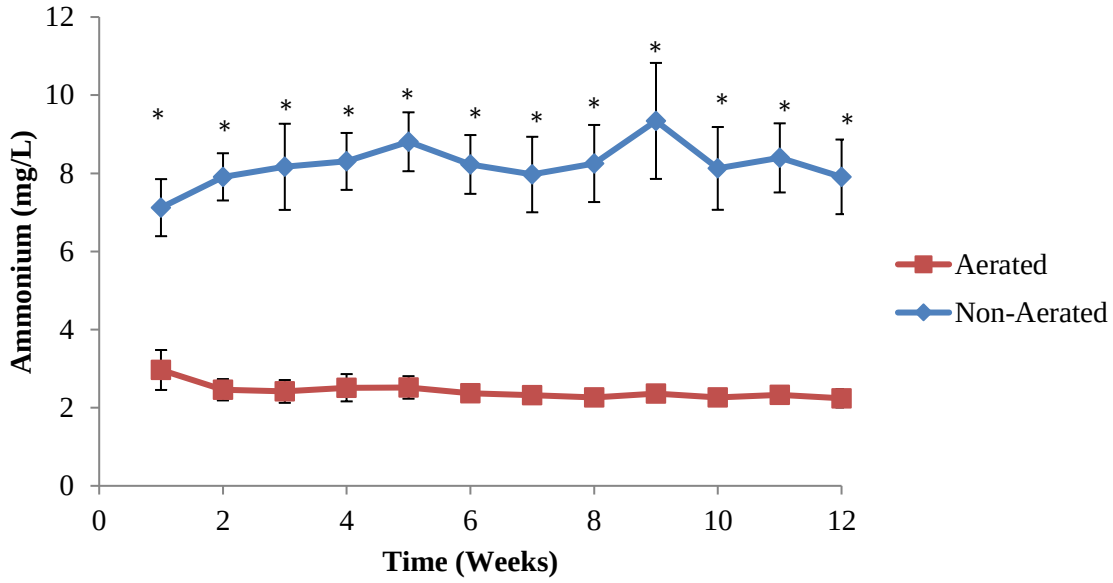

Figure 3.13: Weekly average ammonium (mg/L) during start-up period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (five days of sampling) from six replicates of aerated and non-aerated mesocosms. Data collected on the day of feeding was omitted as it was fluctuating due to nitrogen utilization by the microbial community. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

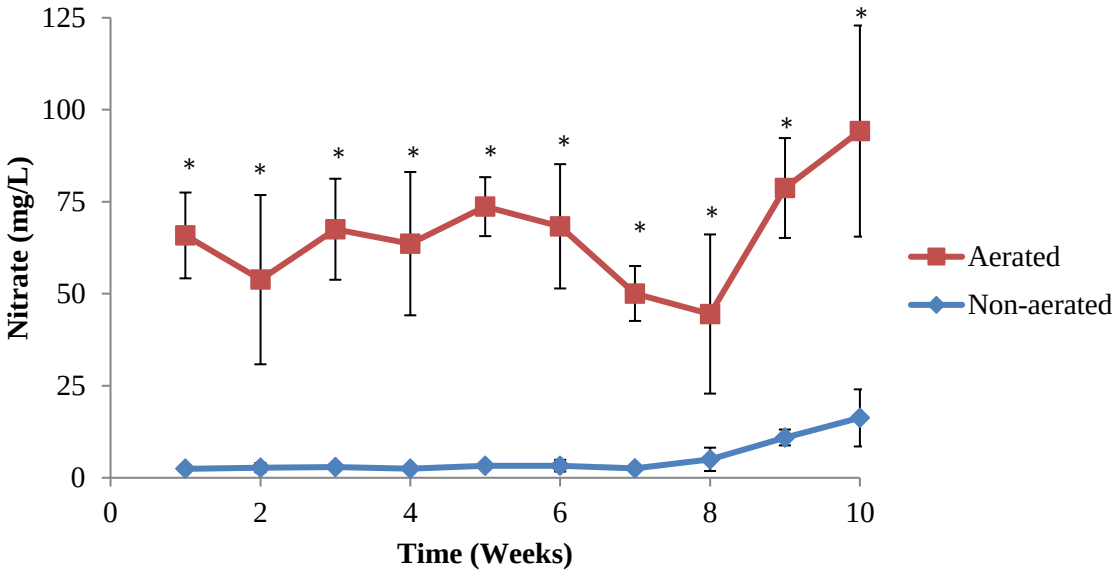

Figure 3.14: Weekly average nitrate (mg/L) during start-up period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (five days of sampling) from six replicates of aerated and non-aerated mesocosms. Data was not analyzed for weeks 11 and 12 as the nitrate probe was outside of its useable product life span. Data collected on the day of feeding was omitted as it was fluctuating due to nitrogen utilization by the microbial community. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

3.4.3 Wastewater Treatment

Water treatment was evaluated for these mesocosm wetlands based on total organic carbon (TOC) and total nitrogen removal (TN) kinetics. Ammonia removal is also a metric used to evaluate the efficacy of wastewater treatment in some jurisdictions. However, TN is more commonly regulated and expected to be so moving forward. Removals were calculated based on first order rate kinetics using the k-C* model (Kadlec and Wallace 2008). Table 3.4 provides average inlet concentrations of the bulk simulated wastewater and average outlet concentrations after 4 days.

Table 3.4: Average inlet loading concentration of the bulk simulated wastewater compared to average outlet concentrations for aerated and non-aerated mesocosms after 4 days. All data is presented in mg/L.

Pollutant	Average Inlet Concentration (mg/L)	Average Outlet Concentration AERATED (mg/L)	Average Outlet Concentration NON-AERATED (mg/L)
TOC	220.0 ± 63.5	13.1 ± 5.2	27.8 ± 14.9
TN	191.3 ± 14.3	153.1 ± 9.7	33.1 ± 5.1
Ammonia	10.7 ± 5.2	2.4 ± 0.2	7.9 ± 0.6
Nitrate	58.3 ± 8.3	51.4 ± 17.6	8.9 ± 3.2

Aerated and non-aerated mesocosms showed similar TOC removal kinetics at the beginning of the start-up period, but started to significantly diverge after 5 weeks (Figure 3.15) (Student's t-test, $p<0.05$). After 5 weeks, the aerated mesocosms ability to remove TOC greatly increases while the non-aerated mesocosms increased only slightly. The significant increase (repeated measures ANOVA, $p<0.05$) in TOC removal rate for both systems over the course of the start-up period can be attributed to the growth and development of the biofilm microbial community within the CW mesocosms. This microbial consortium is largely responsible for the breakdown of organic pollutants within CWs (Faulwetter et al. 2009). Organic pollutants are broken down by microorganisms via the processes of respiration and fermentation into simpler substances (H_2O, CO_2, NO_3, CH_4, alcohols). Respiration processes are dependent on the oxidation-reduction (redox) conditions in the wetland environment (Weber and Gagnon 2014). Aerobic respiration requires oxygen and is a much faster process than fermentation. Therefore, the aerated mesocosms have an advantage because of the higher dissolved oxygen content in the water. This is demonstrated by the greater reaction rate for the removal of TOC in aerated mesocosms. It should be noted that the rate of removal of TOC does appear to be stabilizing between weeks 10 to 12 which could indicate that the microbial community in the mesocosms of both aerated and non-aerated systems is stabilizing as well. This is in agreement with Rajabzadeh et al. (2014) who found the consumption rate of readily biodegradable organic matter to be 31% faster in the tenth week of study versus the second week of study. In subsequent weeks, the consumption rate remained unchanged. It should also be noted that the mass removal of TOC was greater than 90% in aerated systems for entire wetland start-up period (Table 3.4). In non-aerated systems, TOC mass removal was between 60 – 92%, increasing over the start-up period. A study which looked at the start-up performance of laboratory scale aerated and non-aerated vertical flow CWs treating decentralized domestic wastewater found aerated systems could handle shock loads of organic matter over shorter retention times compared to non-aerated systems (Zhu et al. 2013).

An estimation was performed to determine the proportion of TOC utilized by the wetland microbial community for processes of catabolism (energy/respiration) and anabolism (growth of microbial matter). Average porosity trends for both aerated and non-aerated mesocosms began to stabilize after eight weeks of the start-up period (Figure 3.17). An assumption is made that this change in volume is due to the growth of an active, living biofilm. Table 3.4 lists the average inlet concentration of TOC in the simulated wastewater as 220 mg/L. Average outlet concentrations, after 4 days, are listed as 13 mg/L and 28 mg/L for aerated and non-aerated systems, respectively.

Therefore, on average, 207 mg/L and 192 mg/L of TOC are removed each week from aerated and non-aerated systems, respectively. Over 8 weeks of start-up where porosity is decreasing this equates to 1.656 kg/L and 1.536 kg/L of TOC removed (mass of TOC removed per litre of system volume) in total from aerated and non-aerated systems, respectively. To more easily perform this calculation, an assumption is made that the average pore volume over 8 weeks of start-up is 9.5 and 9 L in aerated and non-aerated systems, respectively. In reality, the pore volume is decreasing slowly over this time period. Therefore the total mass of TOC removed in 8 weeks is 15.7 kg and 13.8 kg from aerated and non-aerated systems, respectively. Pore volume of the aerated and non-aerated systems changes by 0.947 L and 0.704 L, respectively over the first 8 weeks of the start-up period. The 0.947 L and 0.704 L of pore space change attributed to biofilm growth over the start-up period can be converted to a mass of biofilm using a density of biofilm of 0.08 kg/L (Rajabzadeh et al. 2015) as biofilm is largely made up of water. Therefore, anabolic transformation of TOC over 8 weeks in the wetland start-up period translated to 0.076 kg and 0.056 kg of (dry) biofilm in aerated and non-aerated systems, respectively.

Microbial biomass has a general formula of $C_5H_7O_2NP_{0.1}$ (Rittmann and McCarty 2001) and thus is approximately 55% carbon by mass. Converting to mass of carbon from dry biofilm mass gives 0.042 kg and 0.031 kg of carbon in the biofilm biomass created for aerated and non-aerated systems, respectively. This amount of carbon in the biofilm equates to 0.3 % and 0.2% of the TOC removed from the aerated and non-aerated systems, respectively, over the first 8 weeks of the wetland start up period. Therefore, the large majority of overall TOC removed went to respiration processes for the microbial community, rather than anabolic processes. This is positive for CW technology and design as subsurface media is prone to clogging, so it is advantageous for CWs to remove matter from the system without excessive biofilm growth preferring methods which remove pollutants by respiration processes.

The wastewater treatment performance for the aerated and non-aerated CWs was also evaluated in terms of total nitrogen (TN) removal. The non-aerated mesocosms consistently have a higher TN removal rate than the aerated mesocosms (Figure 3.16) (Student's t-test, $p<0.05$). The non-aerated mesocosms start to diverge after 5 weeks and increase more rapidly than the aerated mesocosms. The significant (repeated measures ANOVA, $p<0.05$) increase in TN removal for both system types over the course of the start-up period can again be attributed to the growth and development of the biofilm microbial community within the CW mesocosms. For the removal of TN, ammonia-nitrogen species must be converted to nitrate species (in an aerobic environment) and secondly, nitrate must be converted to nitrogen gas without the presence of oxygen (reactions (a) (b) and (c)). The non-aerated mesocosms appear to have an advantage over the aerated mesocosms for the removal of TN. Organic nitrogen and ammonia can be converted to nitrate in microenvironments containing dissolved oxygen in the wetland biofilm. The non-aerated systems then provide ideal conditions for the conversion of nitrate to nitrogen gas under anaerobic conditions, with residual organic carbon. In the aerated systems an increase in the conversion of organic nitrogen and ammonia-nitrogen to nitrate is possible because of the addition of artificial aeration (increased dissolved oxygen in water). However, nitrogen species may remain as nitrate if the environmental conditions for the conversion of nitrate to nitrogen gas (anaerobic conditions in the presence of organic carbon) are not present.

Alternatively, anaerobic ammonia oxidation (Anammox) may be possible in the non-aerated mesocosms. The Anammox process occurs in two steps: first, ammonia-oxidizing bacteria partially oxidize ammonia to nitrite; secondly, Anammox bacteria use nitrite to oxidize ammonia directly

to nitrogen gas (Wallace and Austin 2008). As the influent wastewater already contains a large proportion of nitrate (Table 3.4) and the non-aerated systems have low dissolved oxygen levels (Figure 3.11) this may be a perfect condition for this type of process to occur. The Anammox process requires only 20% of the oxygen demand required by the typical nitrification-denitrification nitrogen removal (Jetten et al. 2005). Additionally, the bacterial species involved in the Anammox process are slow growing in comparison to classical nitrogen cycling bacteria which perform nitrification and denitrification (Wallace and Austin 2008), which may explain why the large increase in total nitrogen removal rate was not reported until weeks 11 and 12 of the start-up period.

The dissolved oxygen values observed in the aerated mesocosms (Figure 3.11) are quite high in comparison to other studies, which typically report values between 1 to 5 mg/L of DO (Cottingham et al. 1999; Zhu et al. 2013; Boog et al. 2014; Zhai et al. 2016). Wu et al. (2014) discuss continuous application of aeration (24 hours a day), may cause a contradiction between the removal of organic and ammonium nitrogen, and TN because of the lack of anaerobic conditions for denitrification. This may explain why there is not much of an increase in removal rate over the start-up period for the aerated mesocosms. Reducing the frequency of aeration in the wetland to timed cycle or to be activated when the DO reaches a determined threshold can increase the removal of TN in aerated systems (Boog et al. 2014). Therefore, this method of aeration could be applied in CWs where high organic carbon and TN removal is required in the same stage of treatment.

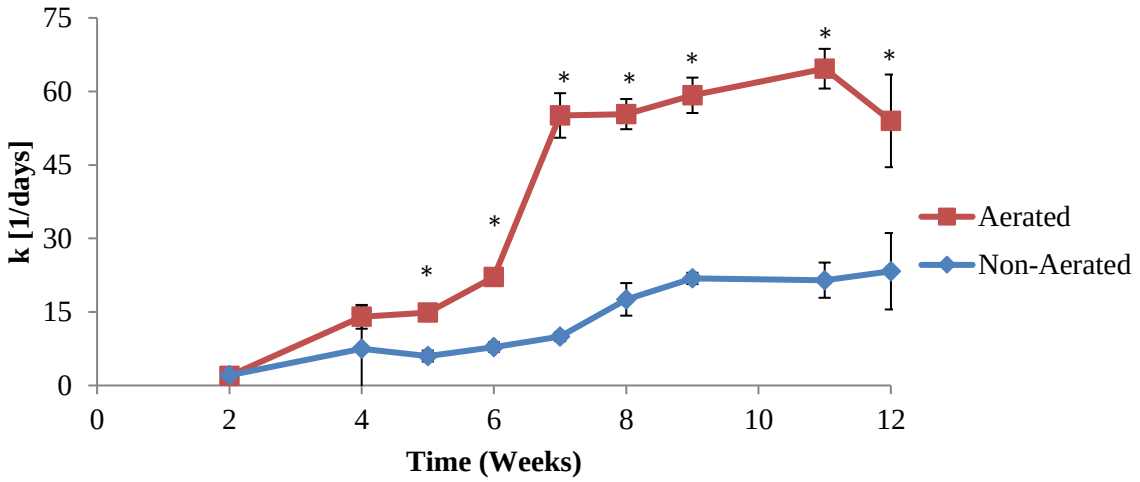

Figure 3.15: Total organic carbon (TOC) first order removal rate for aerated and non-aerated CW mesocosms based on the k-C* model. Samples for TOC removal rate were not collected in week 1. Other missing data points can be attributed to instrument malfunction during analysis. Data points represent six replicates of aerated and non-aerated mesocosms, respectively. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

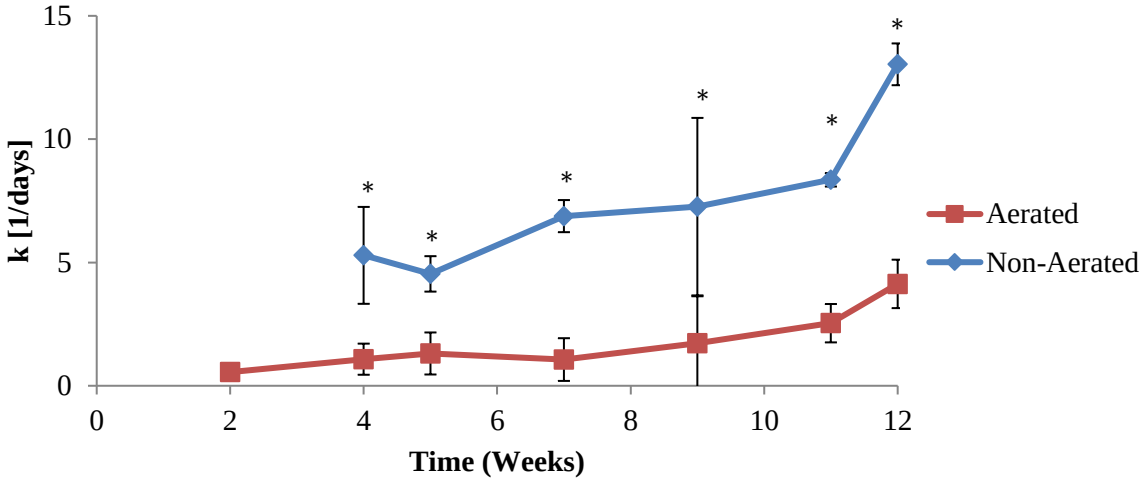

Figure 3.16: Total nitrogen (TN) removal first order removal rate for aerated and non-aerated CW mesocosms based on the k-C* model. Samples for TN removal rate were not collected in week 1. Other missing data points can be attributed to instrument malfunction during analysis. Data points represent six replicates of aerated and non-aerated mesocosms, respectively. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

3.4.4 Hydrological Parameters

The hydrological characteristics of the aerated and non-aerated mesocosms were similar over the course of the start-up period. The porosity of the aerated systems was between 0.275 – 0.325 and the non-aerated between 0.290 – 0.320 (Figure 3.17). The porosity decreased slightly for both system types (more so for aerated) over the development period. This change is statistically significant in reference to time (repeated measures ANOVA, $p<0.05$). This is attributed to the development of biofilm in the system, although the amount of the biofilm per system was not directly measured. This porosity change is consistent with changes reported in the literature for systems of similar size (Weber and Legge 2011). Consolidation of gravel may have also occurred over time within the mesocosms systems over the start-up period influencing the porosity of the systems. Care was taken to wash the gravel prior to addition into the systems and grain sizes were quite large as pea gravel was used in this study. Additionally, there were no obvious depressions noted in the gravel or "sinking" of the gravel media from the additional fill level.

A decrease in porosity within the wetland bed will increase the flow rate of the water through the system. This may decrease the contact time available between the wastewater and biofilm. Physical contact time between the wastewater and biofilm microbial community is essential for wastewater treatment. Additionally, a decrease in porosity in the wetland that is attributed to the development of biofilm has the potential to impact the hydraulic performance of the wetland due to clogging. After week 10, the porosity does appear to come to a steady state value for both aerated and non-aerated systems.

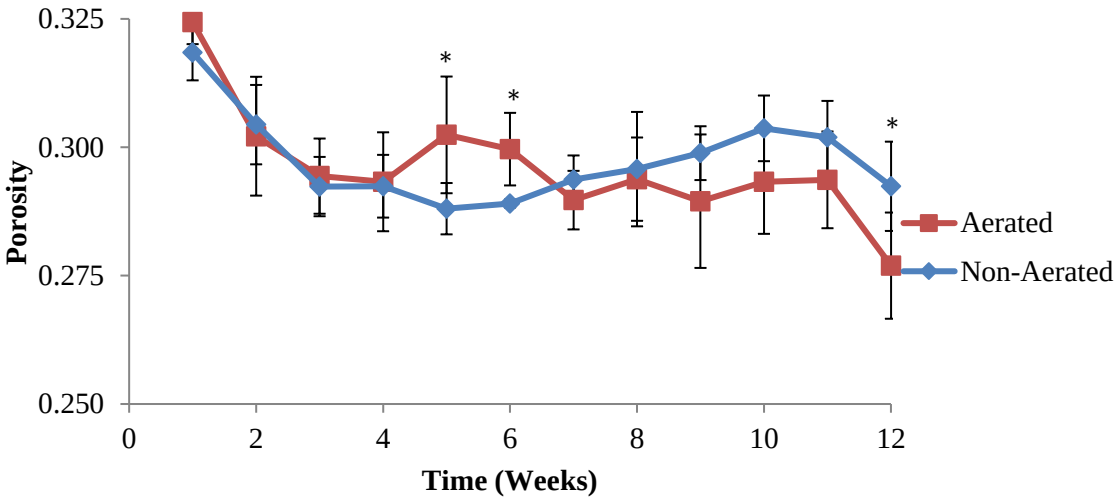

Figure 3.17: Average porosity (recorded weekly) per system during wetland start-up period. Averages are from six aerated (red) and six non-aerated (blue) constructed wetland mesocosms. Porosity was recorded once a week and data shown is the average from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

The evapotranspiration rates within the aerated and non-aerated systems were similar over the development period with a decreasing trend noted (repeated measures ANOVA, $p<0.05$). Fluctuations occurred week to week between aerated and non-aerated systems, and as well between system replicates as evidenced in the large standard deviations some weeks (Figure 3.18). The fluctuations are likely caused by temperature variations initially (Figure 3.8). A larger effect on evapotranspiration was expected from *Phalaris arundinacea* but due to poor growth in this study no noticeable affects were observed. Plants only begin to establish themselves properly from week 11 to 12 (Figure 3.6).

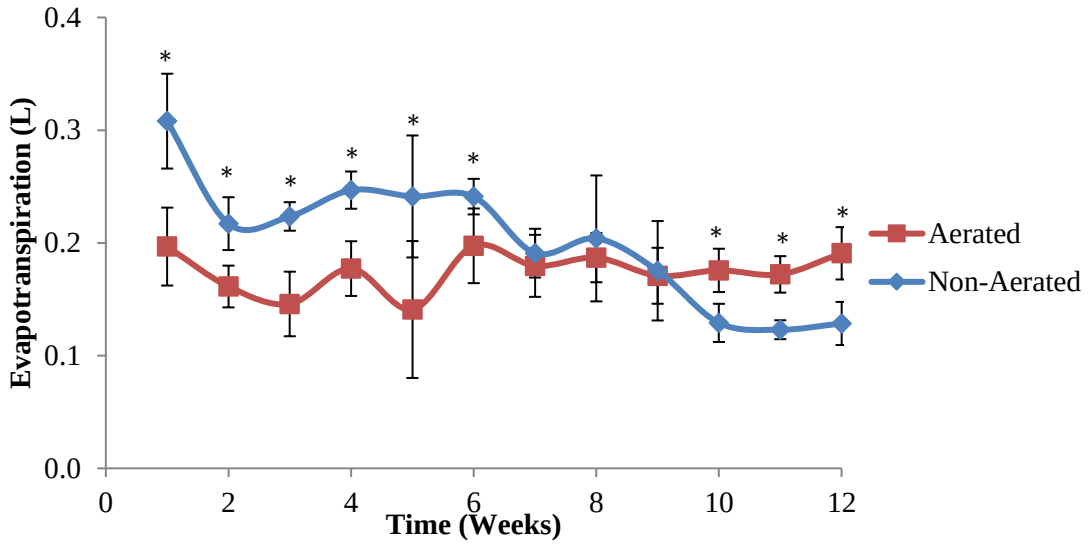

Figure 3.18: Average evapotranspiration (L) system during wetland start-up period. Data points depict weekly averages (four sampling days) from six aerated (red) and six non-aerated (blue) constructed wetland mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

The dispersion coefficient remained relatively stable over the course of the wetland development period (Figure 3.19). This is likely due to the water velocity provided by the pump. The dispersion coefficient was anticipated to increase over the wetland development period as observed in a previous study using a similar system design. Weber and Legge (2011) observed an increase in the dispersion coefficient as biofilm and plant roots established within the system resulting in increased hydrological mixing. The addition of biofilm and plant roots to the system provide increased disturbance points within the subsurface resulting in velocity variations as well as increased tortuosity and therefore greater dispersion.

The water velocity provided by the pump in this experiment may have been too high and could have caused biofilm to shear from the gravel medium. In this study water was recirculated at approximately 10 L/minute with a total system recirculation time of approximately 1 minute. This value was calculated in the lab and confirmed with tracer test data. This was set based on the pump rating, and the 10 L/min recirculation time was calculated from timing the pump moving 10 L of water in consecutive trials. The mesocosms from Weber and Legge (2011) recirculated at approximately 2.4 L/min which resulted in a total recirculation time of approximately 4 to 5 minutes. The latter is more relevant for flows expected in CWs.

The dispersion coefficient was not statistically different between the aerated and non-aerated mesocosms at any time point (Student's t-test, $p<0.05$). The large error bars associated with the dispersion coefficients from the aerated mesocosms are due to the increased mixing involved with the addition of air to the systems. The tracer tests from the aerated mesocosms had greater root mean squared error than the non-aerated mesocosms when applied to the 1D-advection-dispersion equation. This caused the data fit to be less consistent between system replicates, increasing the inter-system variability.

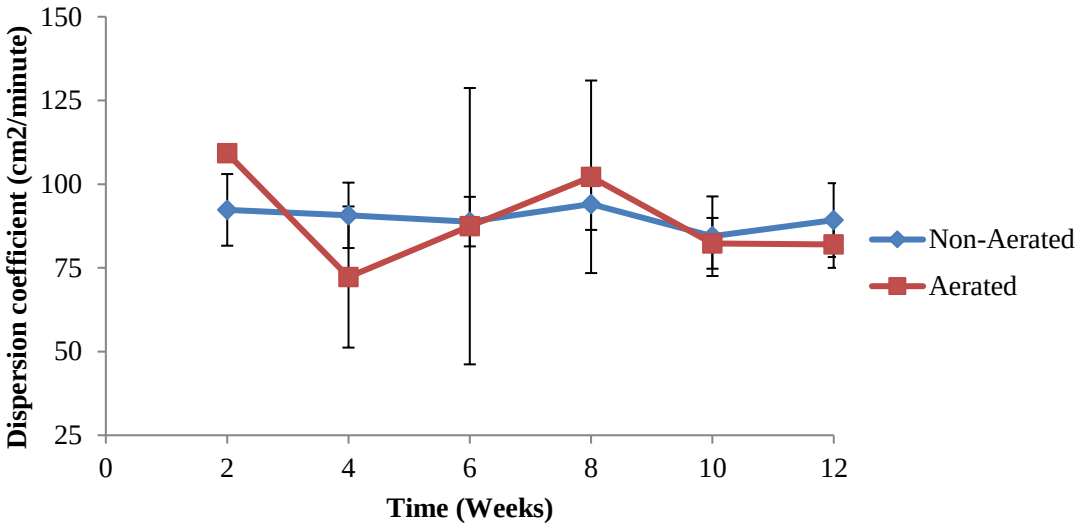

Figure 3.19: Average dispersion coefficient (cm2/minute) calculated from sodium bromide tracer test data using a 1D advection-dispersion equation. Data points depict averages of six aerated (red) and six non-aerated (blue) constructed wetland mesocosms from a single tracer event. Error bars represent one standard deviation.

3.4.5 Microbial Community Analysis

The wetland microbial communities were evaluated for activity using a fluorescein di-acetate hydrolysis (FDA) test and for function using community level physiological profiling (CLPP) data (metrics average well colour development and richness). *In-situ* microbial activity based on FDA hydrolysis increased significantly (repeated measures ANOVA, $p<0.05$) over the course of the wetland development period for microbial communities in both aerated and non-aerated systems (Figure 3.20). From weeks 3 to 7 microbial activities increased for both system types. This increase comes at the same time as the increase in TOC removal rate (Figure 3.15); therefore, this may indicate a maturity of the biofilm microbial community. Between weeks 7 to 12 the activity remained steady, which could indicate the stabilization of the microbial community within the aerated and non-aerated systems. This increase in microbial activity over the development period is consistent with other data in the literature (Weber and Legge 2011). Weber and Legge (2011) reported microbial activity from FDA hydrolysis between 10 – 20 (µg/L fluorescein)/minute for both planted and unplanted systems for the first 75 days of study. Translated to the general field of applied CWs, microbial activity can be expected to stabilize after 8 to 12 weeks.

The microbial activity based on FDA hydrolysis was not expected to be the same in the aerated and non-aerated systems (not significantly different at any time point, Student's t-test, $p<0.05$). It was expected to be higher in aerated systems because of the faster TOC removal rate observed in aerated systems as a result of aerobic respiration. The hydrolysis of FDA involves the cleavage of acetate groups from the bound fluorescein molecule by esterases, lipases, and proteases (Schnürer and Rosswall 1982). It could be that these acetate groups are very bioavailable to the microbial community, making the breakdown of FDA easy, and not indicative of major differences of a well-developed biofilm microbial community. Variability associated with FDA hydrolysis

could indicate the development of slightly different microbial populations between system replicates, which is inherent to biological systems, such as constructed wetlands.

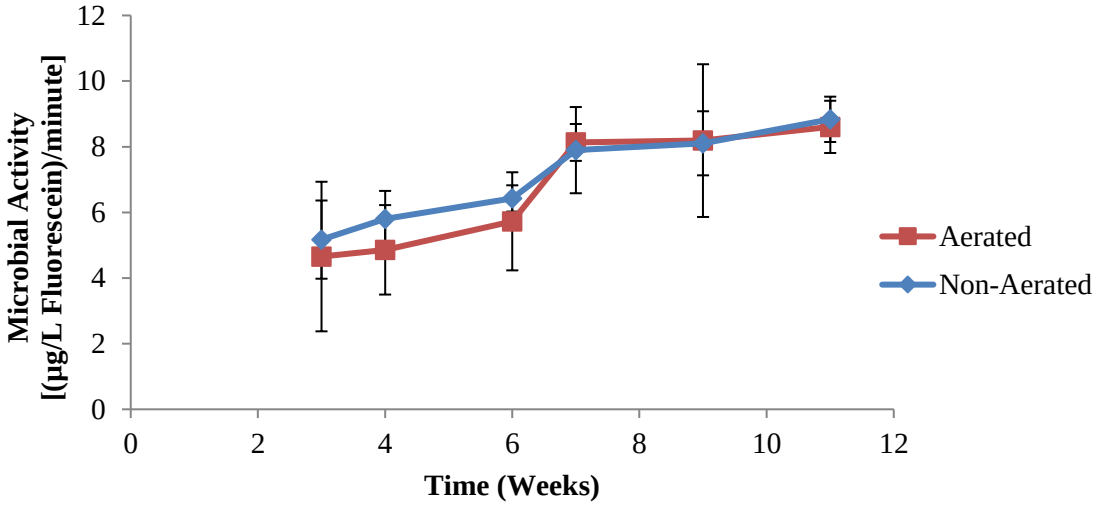

Figure 3.20: Microbial activity for aerated and non-aerated microbial communities during start-up. Data points depict averages of six aerated (red) and six non-aerated (blue) constructed wetland mesocosms from a single sampling event. Error bars represent one standard deviation.

In addition to evaluating the *in-situ* activity of the microbial communities in the mesocosm systems, BIOLOG EcoPlates™ were also used to characterize the mesocosm interstitial water microbial community function. As described in Section 3.3.1, standard ecological parameters can be calculated using the BIOLOG EcoPlate™ data. Figure 3.21 summarizes the CLPP parameters over the course of the wetland development period.

Average well colour development (AWCD) is summarized in Figure 3.21 (A). The AWCD in all mesocosms was similar in weeks 2 and 4 (Student's t-test, $p>0.05$), with a large increase in AWCD noted in week 4. This could be a result of a temperature flux in the room in which the plates are incubated as it is not temperature controlled. After week 4, AWCD of aerated and non-aerated microbial communities diverge and begin to level off to a pseudo steady-state value. A resolution between AWCDs from the aerated and non-aerated systems is noted after week 4 but it is not significant until week 8 (Student's t-test, $p<0.05$). This resolution could be due to differences in plant root zone development or biofilm development (Weber and Legge 2011). Over the start-up period a slightly larger decrease in porosity was noted in aerated systems versus non-aerated systems (Figure 3.17). This decrease in porosity can be attributed to the growth and development of a subsurface wetland biofilm (Weber and Legge 2011). In the case of the aerated systems, additional external forces exerting shear stress are present from the constant bubbling of air through the system. The biofilm which grows in the aerated systems may be adapting to this constant shear stress by producing more EPS to create a more favourable and stable environment for the microbial community more closely associated with the subsurface media (gravel) (Weber 2016). Additionally, this could mean that free floating microbes found in the interstitial water of the wetland do not represent the greater biofilm microbial community in this case as they may be bound more tightly to the subsurface media (gravel). This is supported by the fact that the AWCD of the aerated microbial communities is half that of the non-aerated microbial communities by week 12

of the start-up period (Figure 3.21, A). This would also explain why drastically different trends are observed between two microbial metrics presented in this study (activity from FDA hydrolysis versus function from CLPP). FDA hydrolysis is an *in-situ* characterization method, whereas CLPP is an *ex-situ* characterization method. The water sampled from the CWs for CLPP is not completely representative of the *in-situ* microbial dynamics but it gives a good estimation (Weber and Legge 2013). The difference in findings between microbial methods stresses the importance of characterizing the microbial communities within CWs by various metrics to gain a holistic insight to the function which they provide to wastewater treatment (Weber 2016). Greater insight into the trends observed over the wetland start-up period could be gained with additional microbial analysis including DNA sequencing. Bi-weekly samples were prepared and frozen for future microbial community analysis by Next Generation Sequencing using an Illumina MiSeq. The microbial community structure is expected to be diverse at the beginning of the start-up period due to seeding with activated sludge from a wastewater treatment plant. Over time a slight loss of diversity is expected, while further stabilization of the microbial community population is anticipated over time.

Figure 3.21 (B) depicts the substrate richness for the mesocosm interstitial water microbial communities over the start-up period. A similar trend is observed as for AWCD with all mesocosms showing incredibly similar richness in weeks 2 (Student's t-test, $p>0.05$), with a large increase in richness noted in week 4. After week 4, the richness of the aerated and non-aerated microbial communities diverge and begin to level off to a pseudo steady-state value. The significant difference (Student's t-test, $p<0.05$) in richness between the aerated and non-aerated microbial communities at later weeks is a result of the data analysis method where richness (# of wells with an absorbance over 0.25) is intrinsically tied to the activity of the microbial community (Weber and Legge 2010). By weeks 10 and 12, the AWCD (activity metric for 31 wells) of the aerated microbial communities is close to half that of the non-aerated microbial communities. The same trend is noted for the richness. Therefore, the microbial communities from the aerated systems are likely less active in the breakdown of the range of carbon source provided on the BIOLOG EcoPlate™ than in the non-aerated systems. It may be that the population of microorganisms in the aerated mesocosms developed over time to breakdown a smaller variety of carbon sources by processes of ecological succession or the microbes which degrade a larger variety of carbon sources may be those bound tightly to the subsurface media within the mesocosms and not those in the interstitial water. The main organic carbon source in the simulated wastewater is molasses; therefore, aerated microbial communities are expected to breakdown carbohydrates and polymers effectively.

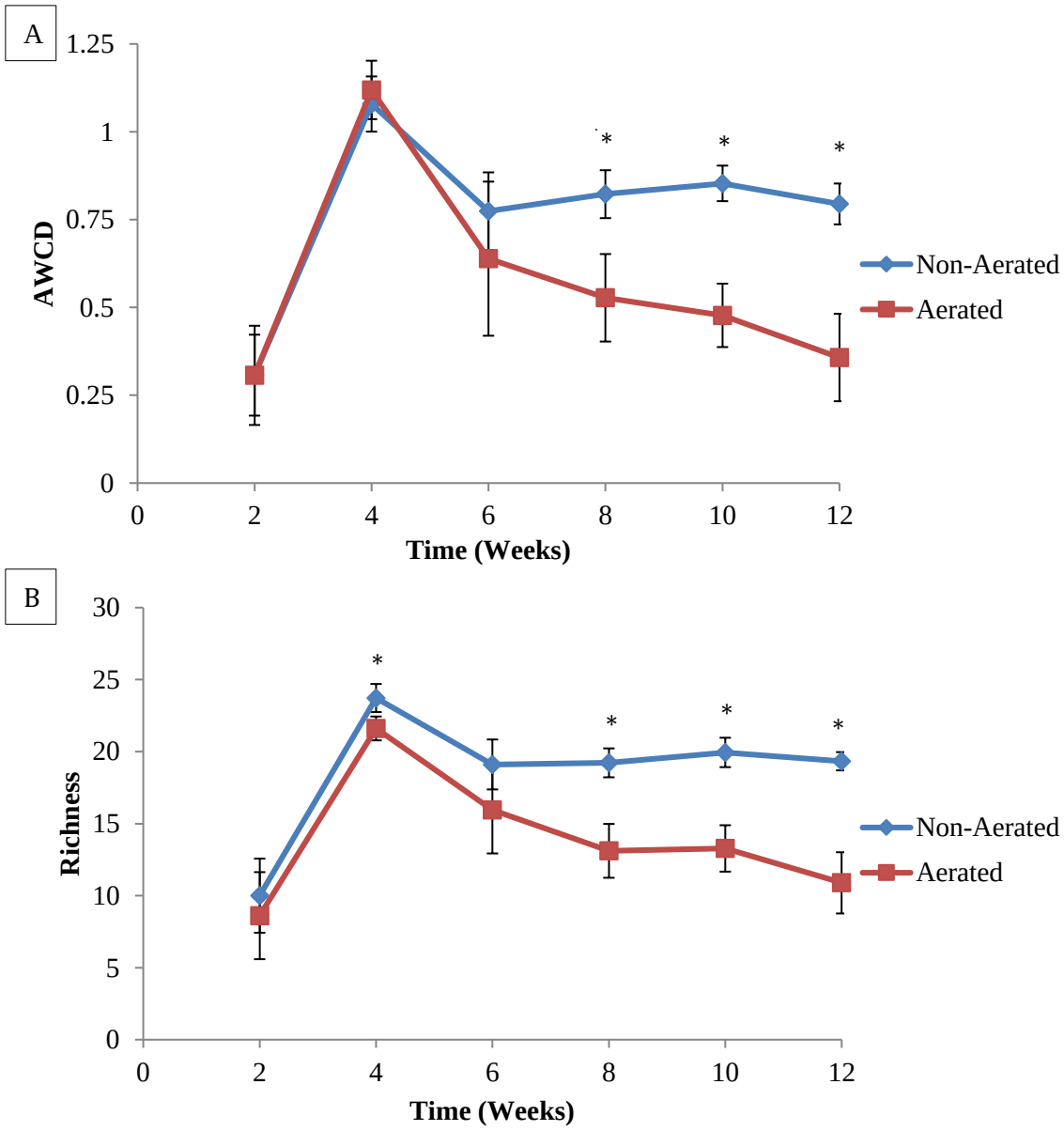

Figure 3.21: Summary of CLPP data for aerated and non-aerated mesocosms (A) average well colour development (B) richness over the wetland start-up period. Data displayed are averages from interstitial water microbial community samples taken from six replicates of aerated and non-aerated CW mesocosms. Each data points represents one sampling event. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

Characterization of the carbon source utilization patterns (CSUPs) of the interstitial water microbial communities was conducted every two weeks over the start-up period. This type of analysis provides information on the utilization of 31 different carbon sources for 12 mesocosm wetlands each time it is performed. Principal component analysis (PCA) is a powerful statistical tool which can show similarities of different objects, in this case carbon sources on BIOLOG

EcoPlates™, on a 2D plane. Information extracted from PCA ordinations can be extensive and is commonly used to classify CSUPs into groups based on their proximity on the PCA ordination (Weber and Legge 2011). For this study, CLPP was performed 6 times, and the same number of PCA ordinations could be used to represent analysis events. However, four plots were chosen to represent the major trends in CSUPs over the start-up period.

PCA was used to examine the CSUPs of the aerated and non-aerated systems. Figure 3.22 depicts PCA ordinations for four time points over the start-up period: week 2, week 6, week 8 and week 12, for aerated and non-aerated wetland mesocosms. For week 2, one distinct group is observed for CSUPs of the interstitial water microbial communities from non-aerated mesocosms, plotted on the right side of the graph. On the other hand, three distinct groups can be visualized for the aerated mesocosm CSUPs. The CSUPs were expected to be differentiated for aerated and non-aerated mesocosms as the environments (ORP, dissolved oxygen, pH) are distinct between the two systems and would likely favour the development of different microbial communities. The CSUPs for both aerated and non-aerated systems in week 6 are more aligned together. The CSUPs from non-aerated mesocosms are starting to group closer together, again on the right side of the ordination. The CSUPs from aerated mesocosms appear to be starting to form two or three groups. In week 8, the CSUPs for non-aerated mesocosms form a tight group on the left side of the ordination, while the aerated CSUPs form two groups which are not closely assembled on the right side of the ordination. In week 12, CSUPs for non-aerated mesocosms are tightly bound together in a group on the right side of the ordination. The CSUPs for aerated mesocosms have once again diverged and are scattered throughout the ordination with no discernable grouping between the six system replicates. There is one CSUP from an aerated mesocosm which is very close to the non-aerated CSUP grouping. It is very interesting to note that throughout the start-up period there was limited time where the CSUPs from the six replicates of aerated mesocosms could easily be grouped together. It may be that the addition of aeration causes such an ecological stress to the microbial community in aerated systems that it's constantly evolving and created a much more diverse microbial population over the six replicates than in the non-aerated replicates. Overall, the CSUPs for interstitial water microbial communities from the non-aerated systems converge over the course of the start-up period implying that replicates have similar abilities to utilize a variety of carbon sources, while CSUPs for interstitial water microbial communities from the aerated systems at times allow loose groupings but more often than not display a varied response. It appears the addition of artificial aeration to the wetland bed destabilizes the microbial population and drastically affects its ability to utilize carbon sources on a per system basis.

To the knowledge of the author, this is the first time CLPP data has been displayed over time for aerated and non-aerated CW mesocosms; however other studies have performed CLPP analysis in a CW setting. Weber and Legge (2011) performed a start-up study on planted and unplanted CW mesocosms seeded with activated sludge from a wastewater treatment plant or dairy farm wastewater. The systems used were a very similar design to the mesocosms used in this study. They found grouping to change over a start-up period of 232 days (a much longer time scale than this study). Initially, differences in CSUPs were accounted for based on the different seeding microbial communities, and over time with the growth of plants, CSUPs from planted mesocosms could be differentiated from unplanted mesocosms (Weber and Legge 2011). The groupings, as in this study, were quite fluid over the development period as the microbial populations stabilized. Another study of CSUPs from CW microbial communities was performed at the pilot scale to assess differences across the wetland flow path and wetland system type (Button et al. 2015). Differences in CSUPs were observed between the HSSF and VF wetlands, and as well over the

course of the flow path of HSSF wetlands. A small difference in CSUPs appears between aerated and non-aerated HSSF wetlands (Button et al. 2015), which support the findings of the current study.

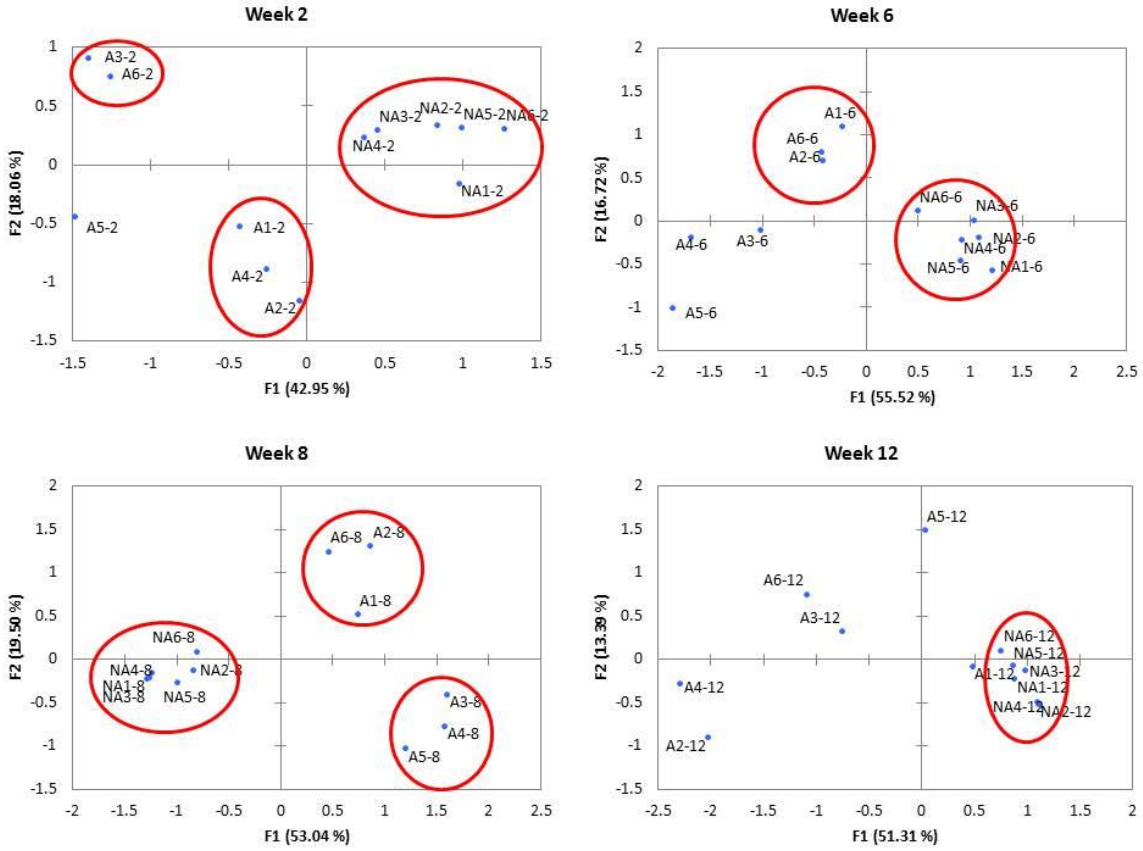

Figure 3.22: Principal component analysis ordinations computed for mean carbon source utilization patterns (all 31 carbon sources) collected with BIOLOG EcoPlates™ for aerated (A) and non-aerated (NA) constructed wetlands. Data is presented for 4 time points during the start-up period: week 2, week 6, week 8 and week 12. Output generated with XLSTAT 2017.

In Figure 3.23, AWCD is presented as stacked column containing the sum of the contributions from each carbon source guild (Section 3.1.4 Table 4) alongside the AWCD coming from the wells which have been identified as root exudates (Section 3.1.4 Table 4). When looking at the carbon source utilization (by guild) (Figure 3.23) it can be observed that the overall utilization pattern for both aerated and non-aerated systems changed with respect to time (repeated measures ANOVA, $p<0.05$). For aerated systems, initially, a large contribution to the AWCD was provided by carbohydrates, but over time this decreased and an increase in the utilization of carboxylic/acetic acids and amino acids was observed, proportionally. For non-aerated systems, an increase in the utilization of carbohydrates is apparent over the course of the wetland start-up period.

No statistically significant differences (Student's t-test, $p>0.05$) between the utilization of carbohydrates was observed between the aerated and non-aerated microbial communities. For polymers and carboxylic and acetic acids, a statistically significant difference (Student's t-test,

p<0.05) was only observed for weeks 2 and 10. Amino acids, amines and amides and root exudates had statistically significant differences (Student's t-test, p<0.05) between their microbial utilization in weeks 2, 4, 6 and 12. These differences may be due to changes in the microbial population in the constructed wetland mesocosms over time as the consortium develops and adapts to treat the simulated wastewater. Differences between the aerated and non-aerated microbial community carbon utilization are likely a function of the different chemical and physical environments observed within the two system types.

The contribution from root exudates makes up approximately 30% of the colour development on the plate for both aerated and non-aerated systems. This may be influenced by the stage of plant growth during the wetland development period. As noted previously, during the seedling stage, approximately 30-40% of carbon originating from photosynthesis products is spent on root exudates (Whipps and Lynch 1990). In this study, the plants in both aerated and non-aerated systems did not grow favourably due to low seeding density and lack of light. Therefore, effects from plants and root exudates on the microbial community were muted from what was expected and no significant changes in microbial root exudate utilization were observed over time (repeated measures ANOVA, p>0.05). Plants were in seedling stage for the first 4 weeks and again from week 10 to week 12.

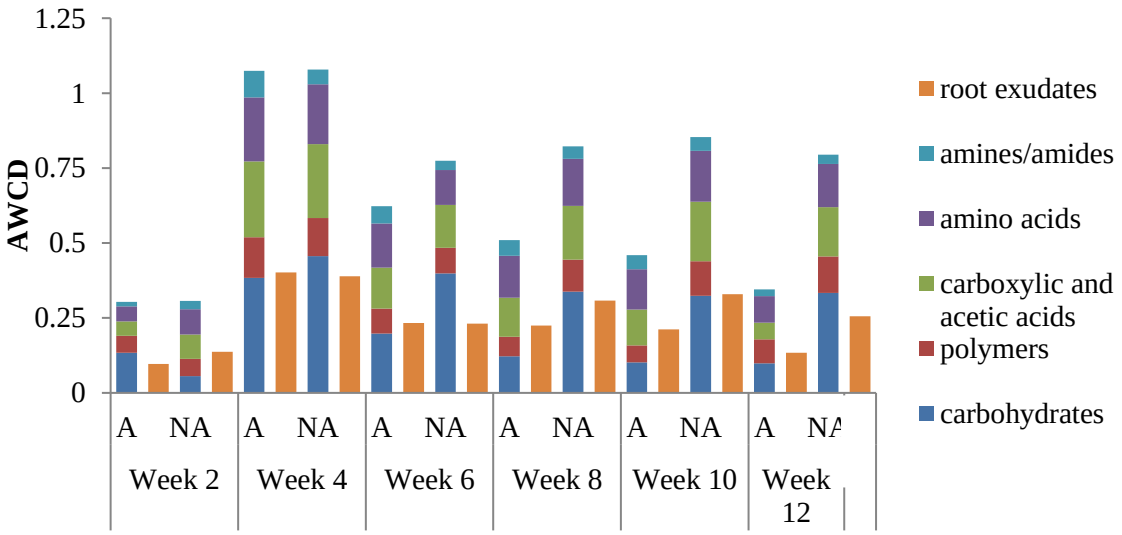

Figure 3.23: Summary of AWCD proportion by guild and AWCD of identified root exudates, for aerated (A) and non-aerated (NA) systems, over the start-up period. Data displayed are averages from interstitial water microbial community samples taken from six replicates of aerated and non-aerated constructed wetland mesocosms. Each data bar represents one sampling event.

3.5 Conclusions

The objective of this study was to investigate the effects of aeration on the start-up of recirculating saturated vertical flow CWs in terms of water chemistry, water treatment, hydrological and microbial parameters. This was performed for six replicates of aerated and non-aerated CW mesocosms. Overall, stabilization of water chemistry parameters occurred readily. In some cases, differentiation was evident between aerated and non-aerated mesocosms (ORP, pH,

nitrate, ammonia, dissolved oxygen). Plant health (*Phalaris arundinacea*) suffered in aerated systems, with a decrease in plant stem count observed. In addition, a distinct yellowing of the leaves was noted for plants grown in the aerated mesocosms. The two system types had similar hydrological properties (porosity, evapotranspiration, dispersivity). Porosity decreased for both system types over the start-up period which alluded to the formation of a subsurface biofilm microbial community over the same time.

The different physical conditions (pH, ORP, dissolved oxygen, constant air bubble movement) between aerated and non-aerated microbial communities likely fostered the development of different microbial communities in the different system regimes. This theory is backed up by the divergence of microbial function after four weeks between the non-aerated (higher) and aerated (lower). However, the microbial activity based on FDA hydrolysis had a similar trend for both system types. These results may indicate that the microbial communities are of the same general activity in aerated and non-aerated systems but have specialized in the breakdown of different pollutants. It will be important to follow up with the samples taken for DNA sequencing to identify the species of bacteria present in the wetlands. In future experiments, performing the CLPP analysis with the interstitial water and microbial communities from the non-aerated mesocosms under conditions closer to those found in the mesocosms (e.g. low oxygen) could provide interesting information regarding the function of those microbial communities.

No enhancement of start-up efficiency was noted for aerated systems, as was the hypothesis. The time required for the microbial community to stabilize in both systems appears to be the same, requiring approximately 60 to 75 days. This finding is similar to those published in other CW start-up papers for non-aerated CWs in the literature, between 75 to 100 days (Weber and Legge 2011; Ramond et al. 2012; Oopkaup et al. 2016). The addition of artificial aeration to the CW mesocosms created a more volatile microbial population which was frequently changing and not similar to system replicates. In non-aerated systems, this was not observed; the CSUPs between system replicates were more stable and converged together over the start-up period. TOC removal was enhanced by artificial aeration and rate of removal increased greatly as the microbial community had time to establish. TN removal was somewhat hindered in aerated mesocosms, as anaerobic conditions required to convert nitrate to nitrogen gas, and remove TN were not available. The results presented here can be applied to CW design and optimization when considering the addition of artificial aeration to a wetland system. They can hopefully be used to increase the use of artificial aeration in wetlands in order to decrease wetland size and increase usage of CWs for new complex wastewater applications. Additionally, these results may be applied in situations where the wetland has been perturbed from its steady-state value by an external environmental factor.

4 CHAPTER 4: EFFECTS OF PLANT ESTABLISHMENT ON MICROBIAL COMMUNITIES, WATER CHEMISTRY, AND HYDROLOGY IN AERATED AND NON-AERATED CONSTRUCTED WETLAND MESOCOSMS

4.1 Introduction

Constructed wetlands (CWs) are an environmentally friendly and cost effective alternative to more traditional wastewater treatment technologies. CWs provide wastewater treatment services for many pollutant types. Treatment mechanisms rely on naturally occurring wetland functions such as physical, chemical and biological processes.

Wetland vegetation plays an important role in the ecology of CWs and can have a positive influence on pollutant removal. Plants can uptake pollutants (nitrogen, phosphorus) through their roots, while root structures can contribute to the removal of suspended solids (Brix 1994). However, the most important contribution of plants in CWs is to provide conditions which favour the establishment of a diverse microbial community. Microbial communities in CWs perform wastewater treatment services invariably through metabolic processes which result in the general degradation of wastewater components (Weber 2016). The microbial community utilizes wastewater components, to survive, for either cellular mass and reproduction (anabolism) or energy (catabolism).

Plants can influence microbial communities in CWs by: providing surface (root structures) for microbial establishment and biofilm development and increasing dissolved oxygen near roots (within mm) which can accelerate aerobic respiration processes for pollution removal (Münch et al. 2007). In addition, plants are known to exude enzymes, chemicals and nutrients from their roots which can be beneficial for nearby microbial communities (Bais et al. 2006) and consequently increase water treatment performance. These factors combine to create a "rhizosphere effect" where the plant root system influences the surrounding environment, creating favorable conditions for the microbial ecosystem. The majority of studies comparing planted and unplanted systems show that the presence of plants has a positive effect on nitrogen and phosphorus removal in CWs (IWA 2000; Allen et al. 2002; Jing et al. 2002; Weber and Legge 2013). The presence of plants in CWs correlated with an increase in microbial community diversity (Zhang et al. 2010) and microbial activity (Gagnon et al. 2007; Weber and Legge 2013).

Macrophyte species selection is important during CW design as pollutant removal efficiencies differ between plant species (Brisson and Chazarenc 2009). A typical emergent vegetation species found in wetlands is reed canary grass (*Phalaris arundinacea*) (Vymazal 2013). Previous research found that microbial community activity and metabolic richness is influenced by plant species (*Phragmites australis* and *Phalaris arundinacea*) in HSSF CWs (Button et al. 2016). A study by Gagnon et al. (2007) reported higher microbial density and activity in wetland microcosms planted with *Phalaris arundinacea* than those planted with *Phragmites australis* and *Typha angustifolia* or the unplanted controls.

CWs are usually planted directly after construction and plants establish themselves over the same time frame as the microbial population, during a start-up phase. However, it is hard to distinguish the effects of natural changes during a start-up period to those coming from plant establishment. Plants can supply oxygen to the microorganisms in the rhizosphere, but it can be

limited for some concentrated wastewaters or water with stringent discharge regulations. Therefore, some CW designs use artificial aeration to enhance wastewater treatment performance, where added oxygen favours aerobic microbial communities which are responsible for organic matter and ammonia removal. The objective of this study is to investigate the effects of plant establishment (*Phalaris arundinacea*) on a set of well stabilized recirculating saturated vertical flow CWs. Six replicates of aerated and non-aerated CWs were allowed to develop naturally, unplanted for four months prior to planting with *Phalaris arundinacea.*

4.2 Materials and Methods

4.2.1 Experimental Design

Twelve CW mesocosms were seeded with activated sludge (500 mL/mesocosm) from a local wastewater treatment plant (Cataraqui Bay Waste Water Treatment Plant, Kingston, ON) on March 16th, 2016. The activated sludge was applied in layers during the gravel filling stage. Six systems were artificially aerated and six systems were not aerated. The unplanted mesocosms were fed with a simulated wastewater solution once a week and allowed a four-month development and stabilization period which was not characterized. The mesocosms were not planted during this development and stabilization period. Four months was chosen for the development period based on previous work, which indicated ecological stabilization of CW mesocosm systems, similar to the ones used in this study, after ninety days (Weber and Legge 2011).

On July 4th, 2016 a rigorous characterization was performed to holistically evaluate the twelve mesocosms over a two-week period. Metrics assessed are detailed in Table 4.1. On July 18th, 2016 all systems were seeded with red canary grass (*Phalaris arundinacea*) (Figure 4.1) at a seeding density of 500 mg/mesocosm.

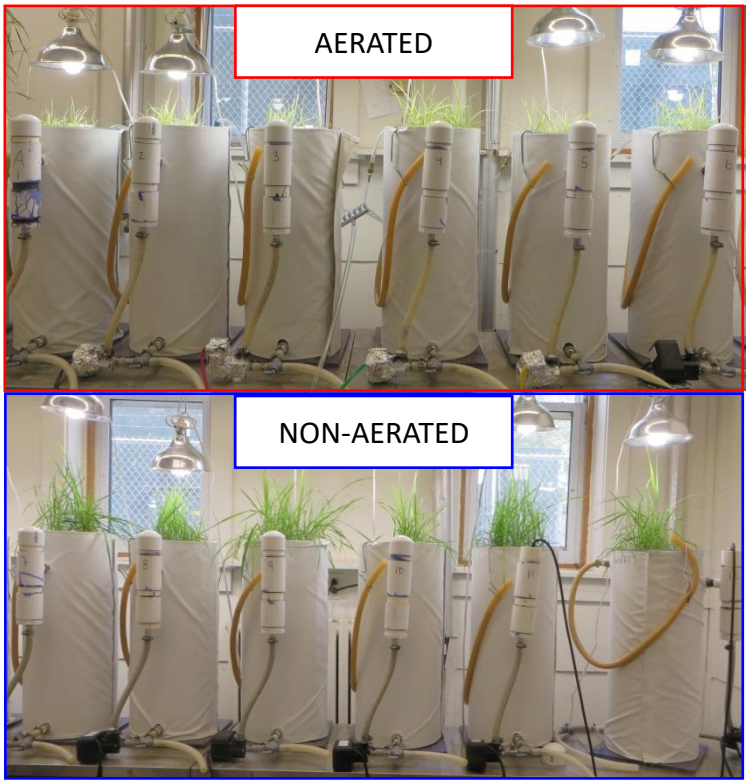

Figure 4.1: Mesocosm experimental design showing six aerated and six non-aerated CW mesocosms, planted with Phalaris arundinacea.

4.2.2 **Mesocosm Set-Up and Maintenance**

Please refer to Section 3.2.2 for mesocosm sizing, design and weekly maintenance regime.

The CW mesocosms were sampled frequently for fifteen weeks to monitor potential changes after seeding with red canary grass (*Phalaris arundinacea*). Two weeks of monitoring occurred prior to seeding to evaluate the starting point of the mesocosms and monitoring continued for thirteen weeks after seeding. Mesocosms were sampled frequently for twelve weeks to monitor their development. Chemical, hydrological and microbial parameters were monitored over this time and pollutant removal was characterized. The parameters monitored during the wetland development period and their frequencies are listed in Table 4.1. Water chemistry was typically analyzed daily and data was analyzed so that the entire week was compared for aerated and non-aerated replicates, unless otherwise specified. Water treatment was analyzed for TOC and TN removal rates on a weekly basis between aerated and non-aerated replicates. Sampling times are detailed in Section 4.2.3.1 which were used to calculate the rates of removals. Porosity was determined from a single weekly measurement and compared between aerated and non-aerated replicates. Evapotranspiration was measured 5 times a week and combined to determine a weekly average over all six replicates of the aerated and non-aerated systems. Microbial activity and microbial function were evaluated on a bi-weekly basis with a single measurement which was evaluated for six replicates of the aerated and non-aerated systems.

Table 4.1: Wetland characterization methods and sampling frequency for the 15-week plant addition and development study.

Parameter Measured	Method	Frequency
Water Chemistry (pH, ORP, NO_3^-, NH_4^+, Cl^-, DO, temperature)	YSI Professional Plus Field Probe	5x per week
Water Treatment (TOC/TN)	Analytik Jena TOC/TN analyzer	Weekly cycle (7x in a week)
Evapotranspiration	Water loss from mesocosm/day	5x per week
Plant Health	Growth & Colour	1x per week
Porosity	Drainable volume	1x per week
Microbial Community Activity	1. Community Level Physiological Profiling 2. FDA Hydrolysis	Bi-weekly

4.2.3 Water Quality

Please refer to Section 0 for water quality method information.

4.2.3.1 Total Organic Carbon and Total Nitrogen Removal

Total Organic Carbon (TOC) and Total Nitrogen (TN) concentrations in simulated wastewater/mesocosm interstitial water were monitored to assess the water treatment capacity of the wetland mesocosms. Water samples were collected from the simulated wastewater solution before introduction into mesocosms (time 0) and from the interstitial water at time points of 0.5, 1, 2, 3, 5, 24, and ~96 hours post mesocosm refill to assess TOC and TN degradation within the wetland mesocosms. Water samples were analyzed using a TOC/TN analyzer (Analytik Jena, TOC/TNb: multi N/C® Series, Germany).

TOC and TN removal from the wetland system was analyzed using a percent removal calculation:

$$\%\, removal = 100\, x\, \left(\frac{C_i - C_o}{C_i}\right) \tag{17}$$

where, C_i is the initial concentration and C_o is the outlet concentration. For this assessment C_o was taken as the 96-hour time point.

Table 4.2: Average inlet concentrations of the bulk simulated wastewater. Data is presented in mg/L.

Pollutant	Average Inlet Concentration (mg/L)
TOC	271.2 ± 66.9
TN	79.9 ± 11.3
Ammonia	6.73 ± 6.5
Nitrate	58.2 ± 7.5

4.2.4 Hydrological Measurements

Please refer to Section 3.2.4 for method information on wetland hydrological parameters.

4.2.5 Plant Growth

Photos of plants were taken weekly to qualitatively monitor changes in growth and colour. A count of the number of plant stems in each mesocosm was performed weekly. Plant height was also recorded weekly based on the maximum height of a stem in each mesocosm.

4.2.6 Microbial Community Analysis

Please refer to Section 3.2.6 for the methods performed to analyze the wetland microbial communities.

4.3 Data Analysis

4.3.1 Community Level Physiological Profiling

Please refer to Section 3.3.1 for a full description of the analysis performed on Community Level Physiological Profiling data.

A time point of 48 hours was chosen for the calculation of the average well colour development (AWCD) and richness in this study.

PCA was performed using the covariance (n-1) matrix of the mean CSUP (average from three replicates) data (Weber and Legge 2010) to further elucidate trends in the microbial community development and stabilization. Datasets were subjected to Taylor data transformations based on assessment of normality, homoscedasticity and linear correlations following the recommendations of Weber et al. (2007). PCA analysis was completed using a covariance (n-1) matrix with XLSTAT 2017 (Addinsoft New York, NY).

4.3.2 Statistical Analysis

Statistical analyses were performed using SPSS (version 23, IBM Corporation, New York, USA) and Microsoft Excel (Microsoft Office 2010, New Mexico, USA). Data was tested for normality using the Shapiro-Wilk test and for variability with the Mauchly's Test of Sphericity and Levene's Test of Equality of Error Variances. Data was analyzed over time with a repeated

measures analysis of variance (ANOVA) with a significance level of p = 0.05. Data was also analyzed for differences between the aerated and non-aerated system replicates each week with Student's t-test with a significance level of p = 0.05.

4.4 Results

Within the results presented in this chapter the terms "unplanted", "planted" and "plants established" are frequently used. Unplanted refers to the time before seeing with *Phalaris arundinacea*. "Planted" refers to any time after seeding with *Phalaris arundinacea.* "Plants established" refers to the time (week 7 and later) after which the number of plant stems in the mesocosms began to level off and the presence of plants in the mesocosms began to affect other wetland parameters (notably evapotranspiration).

4.4.1 Water Chemistry

Water chemistry parameters and temperature were measured daily (Monday to Friday), including water temperature, pH, conductivity, dissolved oxygen, ammonia, nitrate and redox potential. Results from these parameters are displayed in Figure 4.2 to Figure 4.8. The aerated and non-aerated systems display the same temperature trend and variations over the course of the plant establishment period (Figure 4.2). Temperatures ranged from 21 to 27 °C, the decrease was statistically significant with respect to time (repeated measures ANOVA, $p<0.05$). CWs often display seasonal pollutant removal trends based on temperature trends (Werker et al. 2002; Ouellet-Plamondon et al. 2006). However, these are over much larger temperature differences of 15 to 20 °C therefore effects of temperature on pollutant removal and microbial community are not expected.

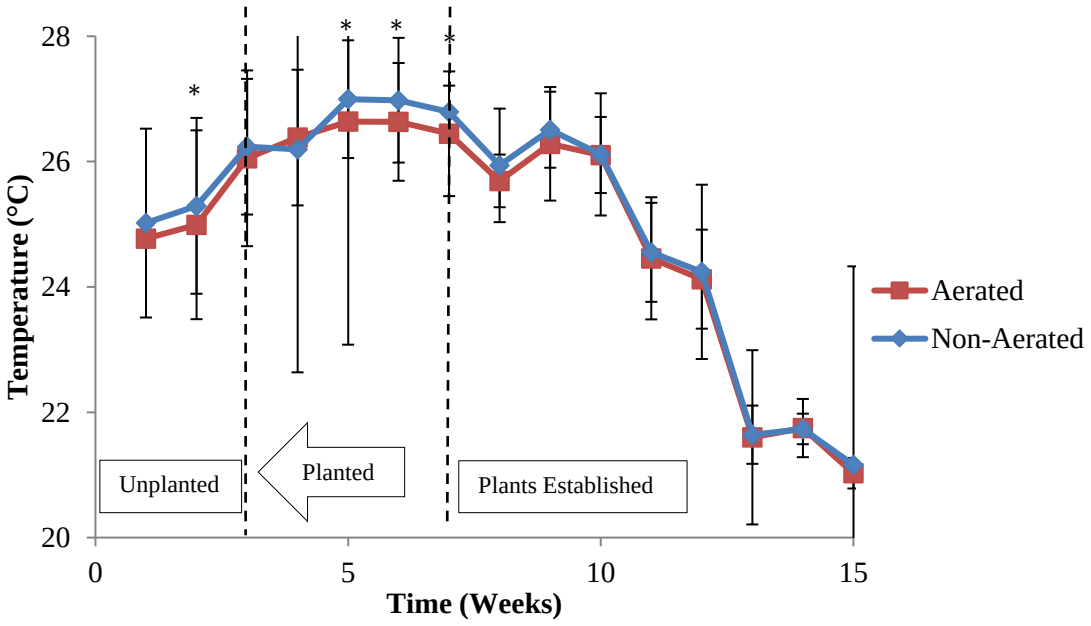

Figure 4.2: Weekly average water temperature (°C) during plant establishment period for six aerated and six non-aerated constructed wetland mesocosms. The onset of this experimental period was in late July, therefore the decreasing trend correlates with the onset of fall. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated

mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

The conductivity in both the aerated and non-aerated mesocosms was relatively stable over the plant establishment period (Figure 4.3), but a dependence of time was reported with the repeated measures ANOVA ($p<0.05$). This may be due to changes in ion content flowing in and out of the biological media (both plants and biofilm) over time.

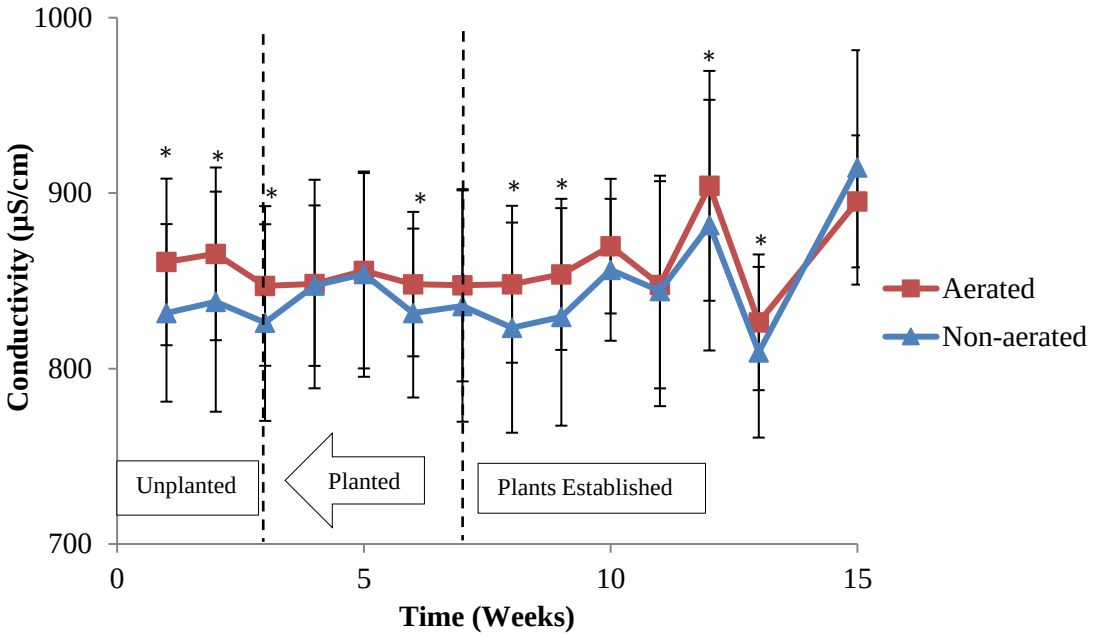

Figure 4.3: Weekly average conductivity (µS/cm) during plant establishment period for six aerated and six non-aerated constructed wetland mesocosms. The large increase in conductivity in 15 week was caused by an error in the addition of nutrients in the simulated wastewater in that week. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

The pH of the water in aerated and non-aerated systems was consistent throughout the plant establishment period (Figure 4.4). The addition of plants had no significant effect on the pH of the wetland water (repeated measures ANOVA, $p>0.05$). The pH for both systems was between 7 and 7.75 for the duration of the plant establishment period, with the aerated systems having significantly higher pH values than the non-aerates systems (Student's t-test, $p<0.05$). This was expected as the pH in the mesocosms is continuously regulated by the substrate material used, limestone pea gravel. The aerated systems had significantly higher (6-9 mg/L) dissolved oxygen than non-aerated systems (0-0.2 mg/L) (Figure 4.5) (Student's t-test, $p<0.05$). DO concentrations in non-aerated systems were very stable over the course of the plant establishment period, while DO started to increase slightly in aerated systems after 10 weeks (repeated measures ANOVA, $p<0.05$). The redox potential in aerated systems is positive with values between 25 to 100 mV

while the redox potential in non-aerated systems is negative with values between -150 to -200 mV (Figure 4.6). The difference in ORP values between the aerated and non-aerated mesocosms is statistically significant at every time point (Student's t-test, $p<0.05$). After the plants established in the systems, around week 7, the redox potential in aerated systems became more positive (repeated measures ANOVA, $p<0.05$), but the redox potential in the in non-aerated systems remains consistent (within error).

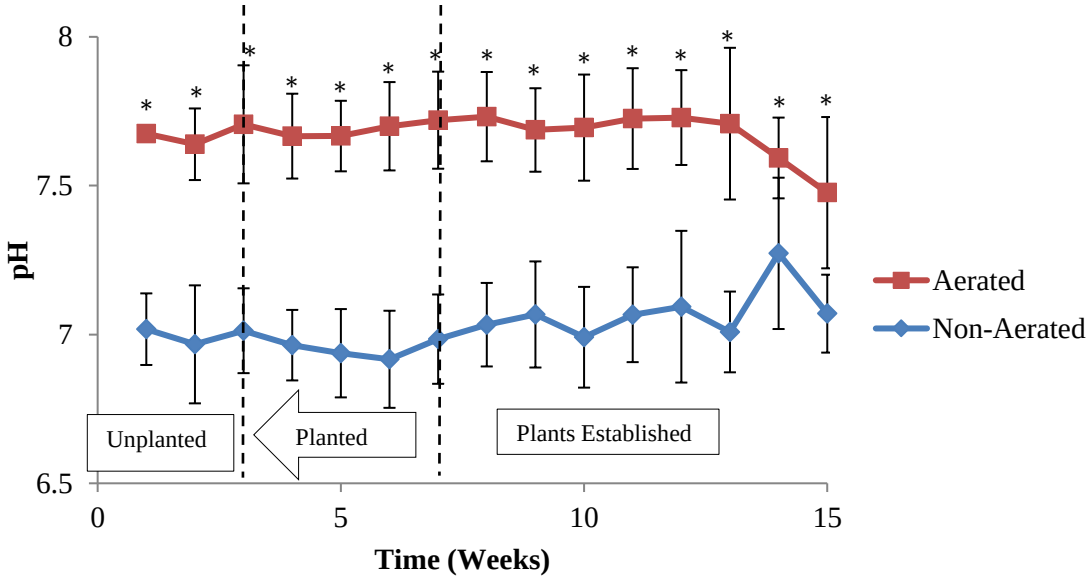

Figure 4.4: Weekly average pH during the plant establishment period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

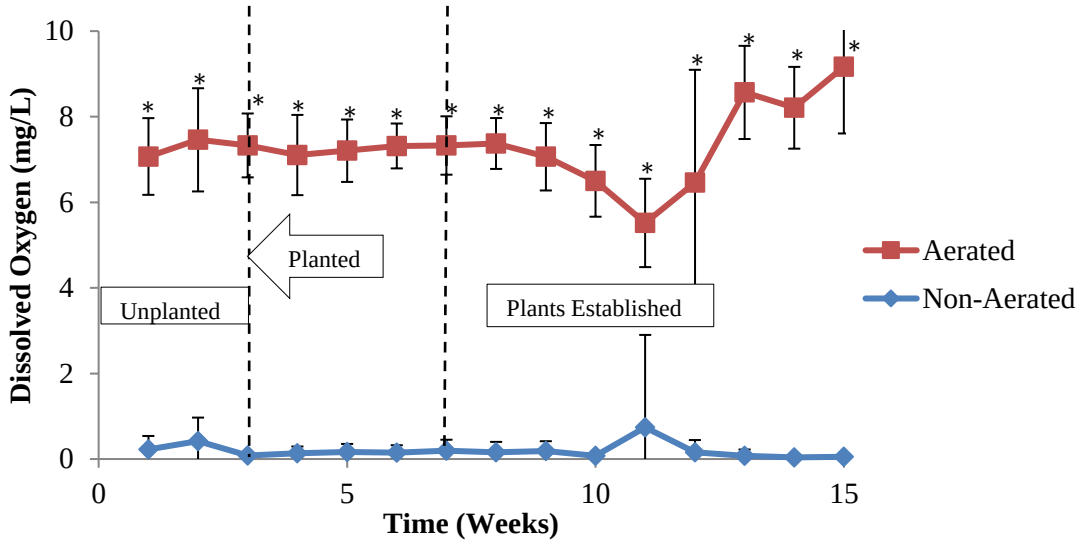

Figure 4.5: Weekly average dissolved oxygen (mg/L) during the plant establishment period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

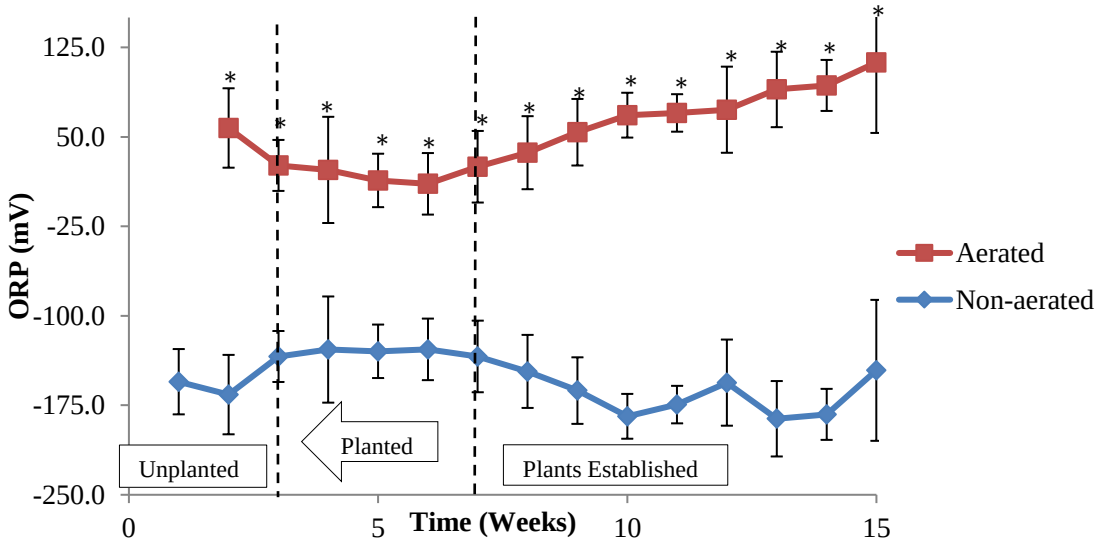

Figure 4.6: Weekly average oxidative-reductive potential (ORP) (mV) during the plant establishment period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (six days of sampling) from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

In this study, the aerated mesocosms show significantly lower ammonium levels (2-4 mg/L) than non-aerated mesocosms (6-8 mg/L) (Student's t-test, $p<0.05$) (Figure 4.7); while non-aerated systems have significantly lower nitrate levels (2-10 mg/L) than aerated systems (50-80 mg/L) (Student's t-test, $p<0.05$) (Figure 4.8). By week 7, the plants had reached a point in their establishment where they began to exert significant effects on the wetland system (repeated measures ANOVA, $p<0.05$, for both nitrate and ammonium). For nitrogen species, this resulted in lower ammonium levels in non-aerated systems and lower nitrate levels in aerated systems.

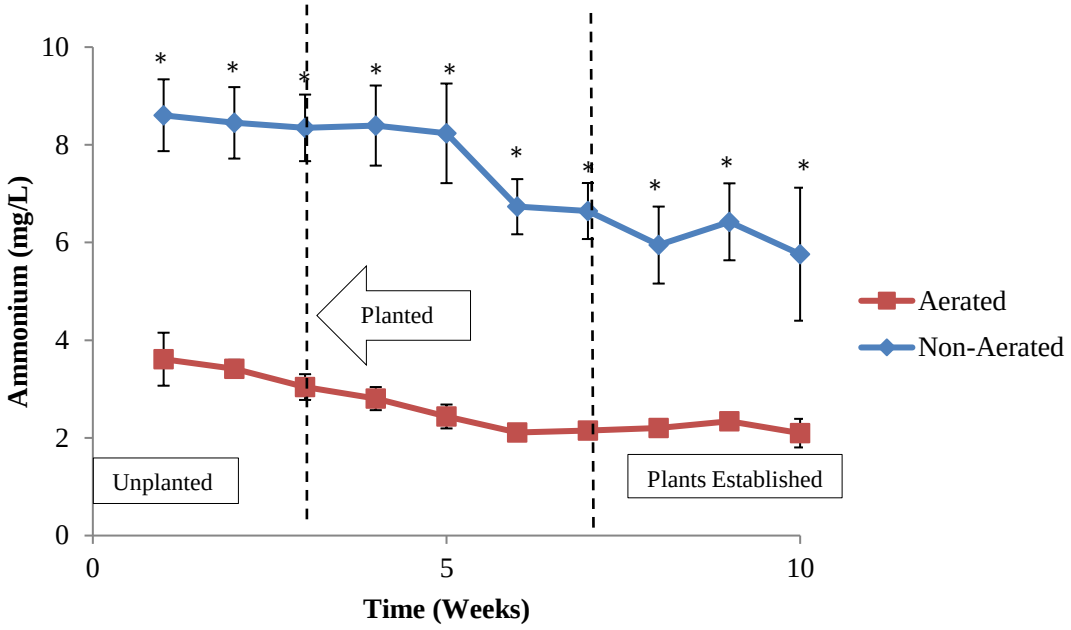

Figure 4.7: Weekly average ammonium (mg/L) during the plant establishment period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (five days of sampling) from six replicates of aerated and non-aerated mesocosms. Data collected on the day of feeding was omitted as it was fluctuating due to nitrogen utilization by the microbial community. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

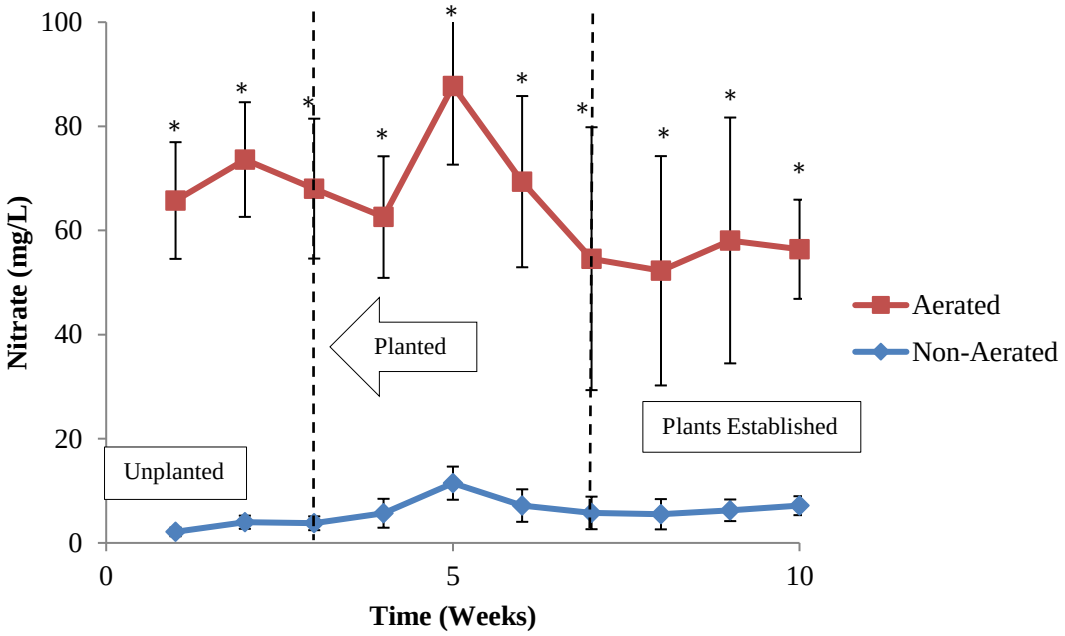

Figure 4.8: Weekly average nitrate (mg/L) during plant establishment period for six aerated and six non-aerated constructed wetland mesocosms. Data points are from weekly averages (five days of sampling) from six replicates of aerated and non-aerated mesocosms. Data collected on the day of feeding was omitted as it was fluctuating due to nitrogen utilization by the microbial community. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

4.4.2 Wastewater Treatment

Water treatment was evaluated for the mesocosm wetlands in terms of total organic carbon (TOC) and total nitrogen (TN) removal, based on percent mass removal calculations (equation 17). Aerated and non-aerated mesocosms showed similar TOC percent removal over the course of the plant establishment period, but the TOC removal in aerated mesocosms is consistently greater (Student's t-test, $p<0.05$) (Figure 4.9). The percent removal of TOC changes significantly with respect to time over the plant establishment period (repeated measures ANOVA, $p<0.05$). This significance is likely a result of the increase in TOC removal observed in the non-aerated systems before and after the addition of plants. The reverse trend is apparent for TN percent removal in aerated and non-aerated mesocosms. The non-aerated mesocosms have a much more consistent and significantly higher (Student's t-test, $p<0.05$) TN removal percentage than the aerated mesocosms (Figure 4.10). The removal of TN from non-aerated mesocosms is relatively stable before and after the addition of plants to the system but an increasing trend can be noted between week 9 and 15 (repeated measures ANOVA, $p<0.05$). An increase from 83% to 96% mass removal of TN occurs over the plant establishment period. TN removal rate in aerated systems was less stable, fluctuating between 0% and 45% removal of TN from the system. Variations in data associated with weeks 9, 11 and 14 are likely a result of discrepancies in stock solution preparation with lower than typical TOC values observed in these weeks. This may be a result of natural

fluctuations in the TOC content provided by molasses or issues with mixing the simulated wastewater. As background levels of TOC are reached every week in both aerated and non-aerated mesocosms, a drop in the initial concentration will result in a lower overall percent removal of TOC.

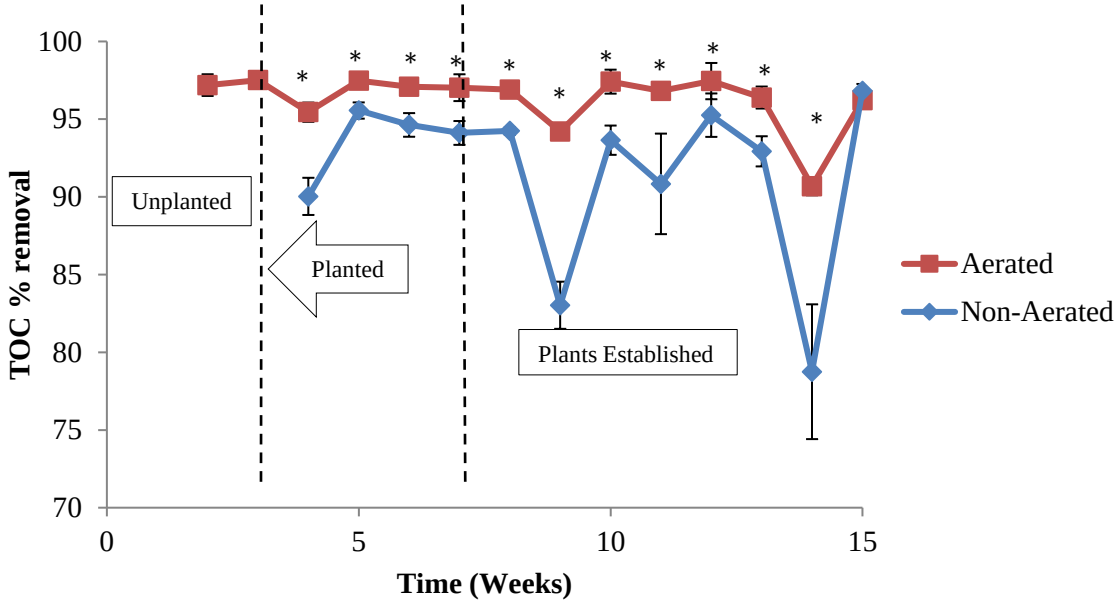

Figure 4.9: Total organic carbon (TOC) removal from aerated and non-aerated CW mesocosms based on percent mass removal over 4 days. Data points represent six replicates of aerated and non-aerated mesocosms, respectively. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

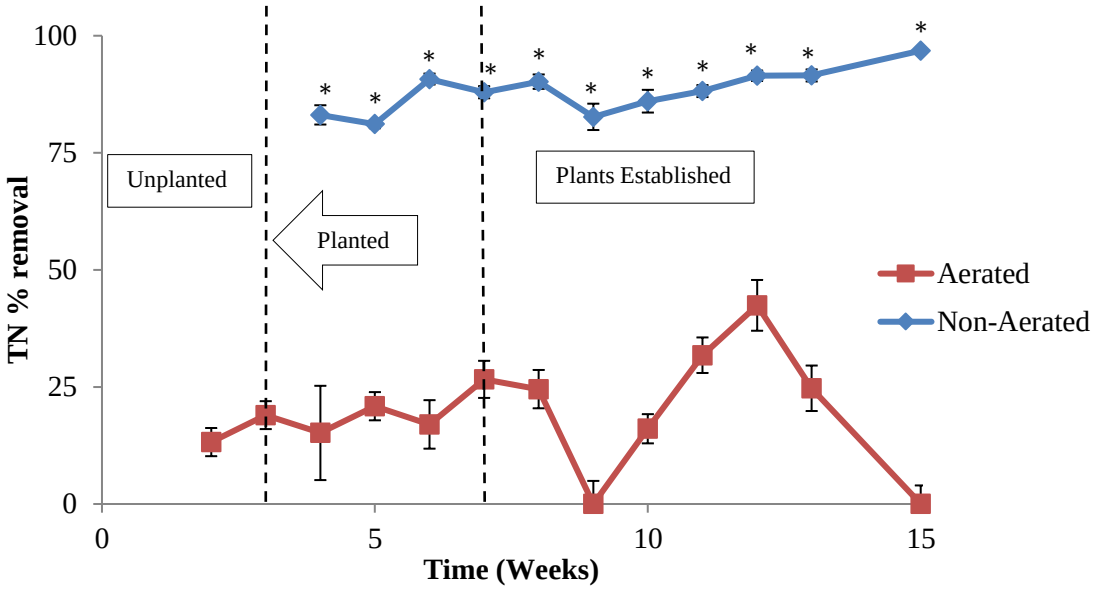

Figure 4.10: Total nitrogen (TN) removal from aerated and non-aerated CW mesocosms based on based on percent mass removal over 4 days. Data points represent six replicates of aerated and non-aerated mesocosms, respectively. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

4.4.3 Hydrological Parameters

The hydrological characteristics of the aerated and non-aerated mesocosms were similar over the course of the plant establishment period. The porosity of the aerated systems was between 0.280 – 0.300 and the non-aerated between 0.285 – 0.310 (Figure 4.11). The mean porosity increased slightly for both system types (more so for non-aerated) with the addition of plants to the system however the increase was not statistically significant from before planting to the end of the plant establishment period (repeated measures ANOVA, $p>0.05$). After the initial increase the porosity in both system types generally decreased with time, with the non-aerated mesocosms consistently displaying significantly larger porosity than the aerated mesocosms (Student's t-test, $p<0.05$). The evapotranspiration (ET) rates within the aerated and non-aerated systems were similar before seeding, after which the ET in the non-aerated mesocosms was significantly higher than the ET in the aerated mesocosms in most weeks (Student's t-test, $p<0.05$) (Figure 4.12). The increase in the mean ET after week 7 is accounted for by the accelerated plant growth in non-aerated systems compared to the aerated systems (Figure 4.13). The increase in ET over the plant establishment period is significant with time (repeated measures ANOVA, $p<0.05$). Large variability in evapotranspiration was reported in the literature for other planted CW mesocosms (Lv et al. 2016).

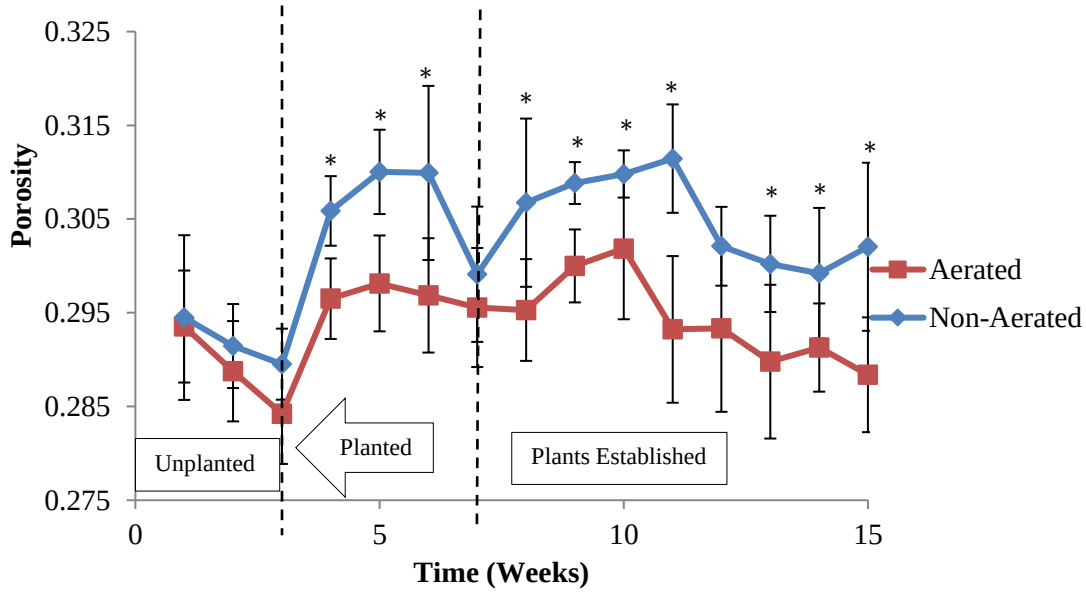

Figure 4.11: Average porosity (recorded weekly) per system during the plant establishment period. Averages are from six aerated (red) and six non-aerated (blue) constructed wetland mesocosms. Porosity was recorded once a week and data shown is the average from six replicates of aerated and non-aerated mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

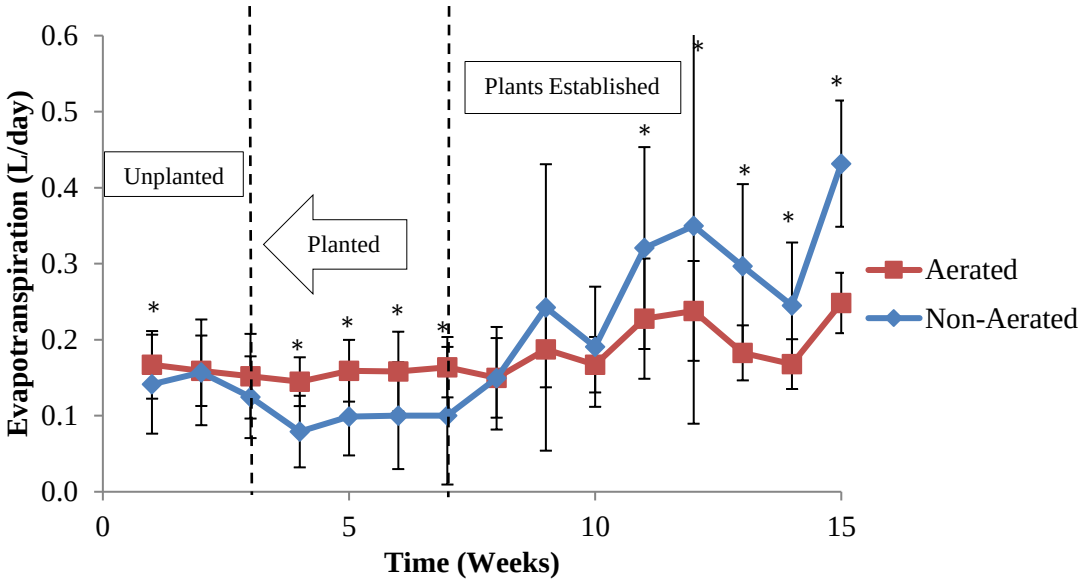

Figure 4.12: Evapotranspiration during plant establishment period. Weekly average values are depicted for aerated (red) and non-aerated (blue) wetland mesocosms. Data points depict weekly averages (four sampling days) from six aerated (red) and six non-aerated (blue) constructed wetland mesocosms. Error bars represent one standard deviation. Asterisks represent weeks where

there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

Figure 4.13: Plant growth in week 13 of the plant establishment period. Larger plants are evident in the non-aerated systems (right six, dark green) than in the aerated systems (left six, light green).

4.4.4 Plant Growth

Plants grew well in both system types as evidenced by the plant count and plant height trends in Figure 4.14 and Figure 4.15. For both plant count and plant height, the values in all weeks are statistically significant from week 4 when evidence of plant growth first appeared (repeated measures ANOVA, $p<0.05$). In both instances plant growth was significantly better in non-aerated mesocosms compared to the aerated mesocosms (Student's t-test, $p<0.05$). Statistically different values for plant growth metrics between the aerated and non-aerated mesocosms are depicted with an asterisk (*) in Figure 4.14 and Figure 4.15. Plants in the aerated mesocosms were not as healthy as those in the non-aerated mesocosms. A yellowing in the leaves, less overall stems and shorter height (Figure 4.16) was observed in aerated systems. An invasion of pests was noted throughout the plant establishment period in aerated systems, spreading to non-aerated systems.

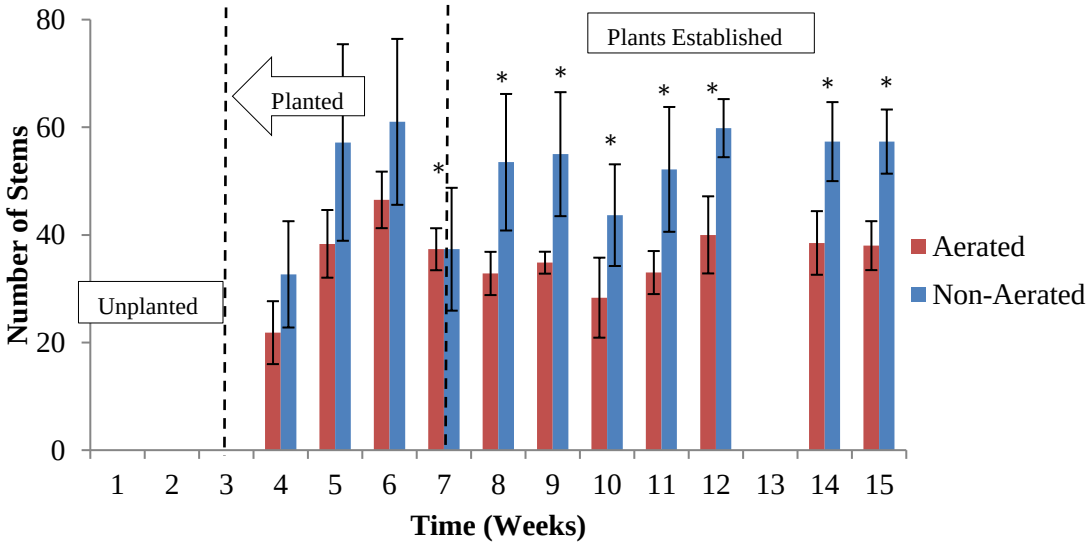

Figure 4.14: Plant count, recorded as number of stems per system, during the plant establishment period. Data was not collected in Week 13. Plant count was recorded once a week and data shown is the average from six replicates of aerated and non-aerated mesocosms. Error bars represent one

standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

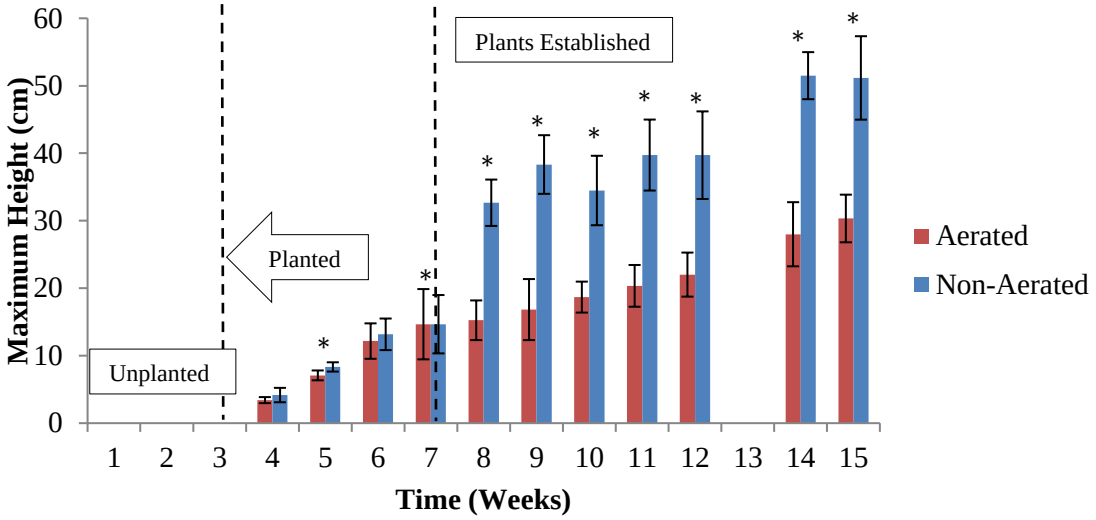

Figure 4.15: Maximum stem height during plant establishment period. Data was not collected in Week 13. Plant height was recorded once a week and data shown is the average from six replicates of aerated and non-aerated mesocosms.. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

Figure 4.16: Plant growth progression in aerated (left six, light green) versus non-aerated (right six, dark green) constructed wetland mesocosms. Pictures are included from week 7, 8, 10, 13 and 15.

4.4.5 **Microbial Community Analysis**

The mean *in-situ* microbial activity (based on FDA hydrolysis) increased after planting over the course of the wetland development period for microbial communities in both aerated and non-aerated systems (Figure 4.17). Throughout the planting experiment, microbial activity increased very slightly for both system types but stabilized after week 10. In all weeks apart from week 11, a statistically significant difference is observed between the microbial communities from the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$). Over time, before and after the addition of plants there is no statistically significant effect of the plants on the microbial activity (repeated measures ANOVA, $p>0.05$).

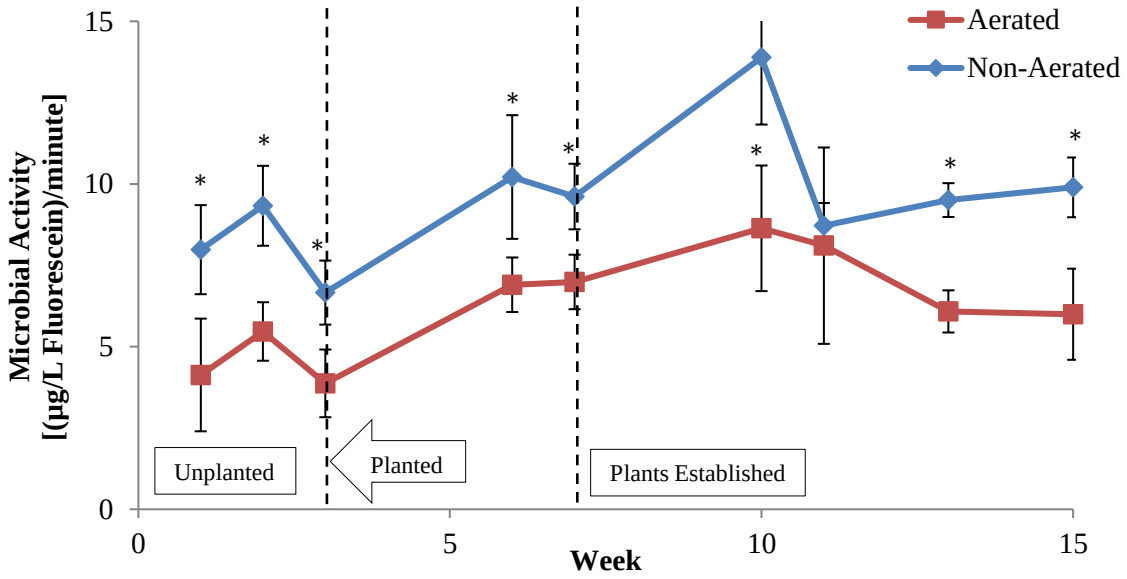

Figure 4.17: Microbial activity for aerated and non-aerated microbial communities over the course of the plant establishment period. Data points depict averages of six aerated (red) and six non-aerated (blue) constructed wetland mesocosms from a single sampling event. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

In addition to tracking the *in-situ* activity of the microbial communities in the mesocosm systems, an *ex-situ* method using BIOLOG EcoPlates™, was used to characterize the mesocosm interstitial water microbial community function. Figure 4.18 (A) and (B) summarize the AWCD and richness over the course of the plant establishment period. Figure 4.18 (A) summaries the AWCD for the microbial communities from aerated and non-aerated mesocosms during the plant establishment study. AWCD for aerated and non-aerated systems vary after the addition of plants to the systems in week 3 until week 7. After week 7, AWCD in the aerated and non-aerated started to converge resulting in very similar values by week 15 (not statistically different from weeks 7 to 15, Student's t-test, $p>0.05$). Figure 4.18 (B) depicts the substrate richness of the mesocosm interstitial water microbial communities over the plant establishment period. A similar trend is observed for richness as for AWCD with all mesocosms showing a slight variation between weeks 3 to 7, post plant addition. One difference between the richness and AWCD, is that for richness resolution can be observed between the systems types with non-aerated mesocosms displaying

significantly higher richness values than the aerated mesocosms at later weeks (6 to 13) (Student's t-test, $p<0.05$). Richness begins to stabilize and form a steady-state value between weeks 7 to 15 (repeated measures ANOVA, $p>0.05$). The variation in both richness and AWCD observed for both systems types (Figure 4.18, A and B) from weeks 3 to 7 coincides with the increase in porosity (Figure 4.11) over the same time period. Despite the immediate changes caused by the addition of plants to the microbial community catabolic function and richness, before and after the addition of plants there is no statistically significant effect of the plants on the microbial function metrics over time (repeated measures ANOVA, $p>0.05$).

Principal component analysis (PCA) was used to examine the carbon source utilization patterns (CSUPs) of the aerated and non-aerated systems over the plant establishment period. Figure 4.19 depicts PCA ordinations for four time points over the start-up period: week 2, week 9, week 11 and week 15, for aerated and non-aerated wetland mesocosms. Before planting, in week 2, one distinct group is observed for CSUPs of the interstitial water microbial communities from non-aerated mesocosms, plotted on the right side of the graph, and a more loosely defined group is plotted on the left side of the graph for the aerated mesocosms. The CSUPs were expected to be differentiated for aerated and non-aerated mesocosms as the environments (ORP, dissolved oxygen, pH) are distinct between the two systems and would likely favour the development of different microbial communities. In week 9, the CSUPs for both aerated and non-aerated mesocosms are dispersed together and no clear groupings emerge. At this point, the plants have grown enough to start to influence properties in the wetland mesocosms. In week 11, the CSUPs for non-aerated and aerated mesocosms are again mixed, and on both side of the ordination. Two tighter groupings of aerated and non-aerated CSUPs can be distinguished. Finally, in week 15, 8 of the 12 CSUPs for both systems types can realistically be placed in a group together in the middle of the ordination. Two CSUPs for aerated mesocosms and two CSUPs for non-aerated mesocosms remained outliners from this central grouping. Overall, the CSUPs for interstitial water microbial communities from the aerated and non-aerated systems converge into one grouping over the course of the plant establishment period implying that replicates have similar abilities to utilize a variety of carbon sources.

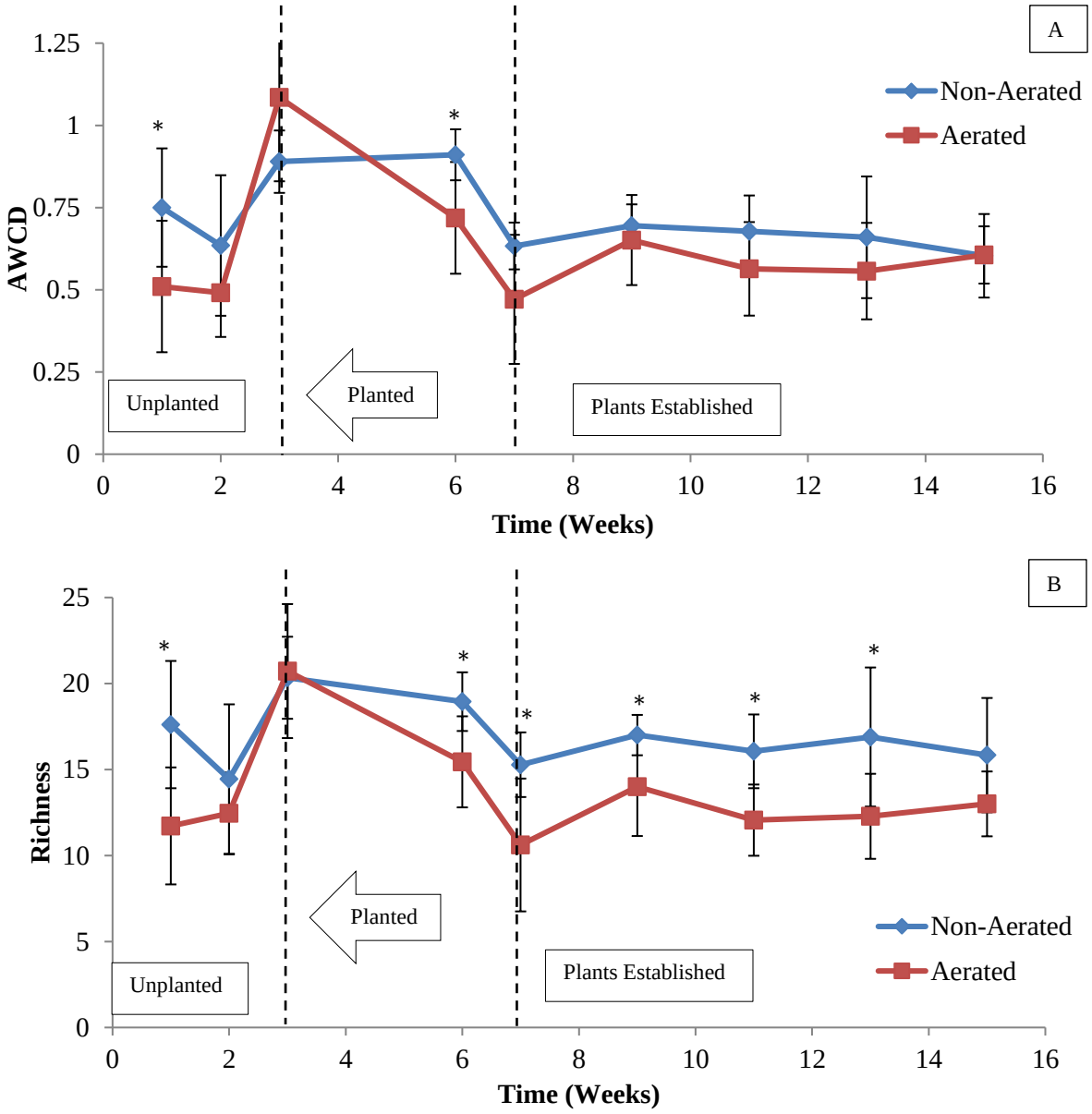

Figure 4.18: Summary of CLPP data for aerated and non-aerated mesocosms (A) average well colour development (B) richness over the plant establishment period. Data displayed are averages from interstitial water microbial community samples taken from six replicates of aerated and non-aerated constructed wetland mesocosms. Each data point represents one sampling event. Error bars represent one standard deviation. Asterisks represent weeks where there was a statistically significant difference between the aerated and non-aerated mesocosms (Student's t-test, $p<0.05$).

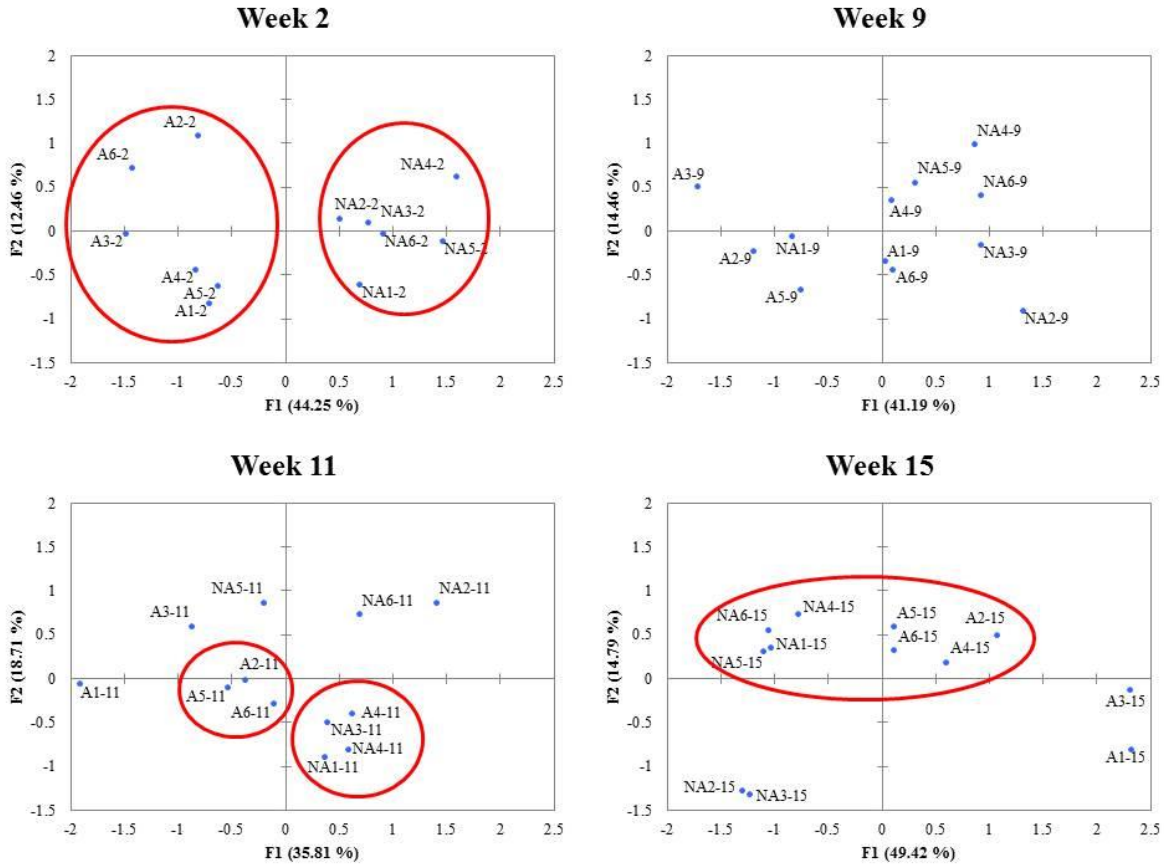

Figure 4.19: Principal Component Analysis ordinations computed for mean carbon source utilization patterns (all 31 carbon sources) collected with BIOLOG EcoPlates™ for aerated (A) and non-aerated (NA) constructed wetlands. Data is presented for 4 time points during the plant establishment period: week 2, week 9, week 11 and week 15. Output generated with XLSTAT 2017.

Figure 4.20 presents AWCD as stacked column containing the sum of the contributions from each carbon source guild (Section 2.3.1.3 Table 2.2) alongside the AWCD coming from the wells which have been identified as root exudates (Section 2.3.1.4 Table 2.3). Carbohydrates appear to be better utilized (as a proportion of AWCD) in non-aerated systems (Student's t-test, $p<0.05$). Over time, the non-aerated systems appear to lose their functionality for the breakdown of amines and amides. For aerated systems, prior to plant addition the ability to utilize carbohydrate was low and amino acids were high (repeated measures ANOVA, $p<0.05$). After the establishment of plants this trend reverses. The ability of the microbial communities to utilize root exudates does not change significantly over time (repeated measures ANOVA, $p<0.05$).

A statistically significant difference (Student's t-test, $p<0.05$) between the utilization of carbohydrates was observed between the aerated and non-aerated microbial communities in every week except week 3. Utilization of carboxylic and acetic acids was significantly different between aerated and non-aerated mesocosm replicates in weeks 3 and 6 (Student's t-test, $p<0.05$). For polymers, a statistically significant difference (Student's t-test, $p<0.05$) was only observed for

weeks 1 and 6. For root exudates the only week for which the utilization patterns were significantly different was week 3 (Student's t-test, $p<0.05$). For amino acids no weeks were significantly different (Student's t-test, $p>0.05$). Amines and amides displayed the greatest differences between aerated and non-aerated utilization with statistically significant differences (Student's t-test, $p<0.05$) observed in weeks 2, 3, 6 and 15. These differences may be due to changes in the microbial population in the constructed wetland mesocosms over time as the consortium develops and adapts to treat the simulated wastewater. Differences between the aerated and non-aerated microbial community carbon utilization are likely a function of the different chemical and physical environments observed within the two system types.

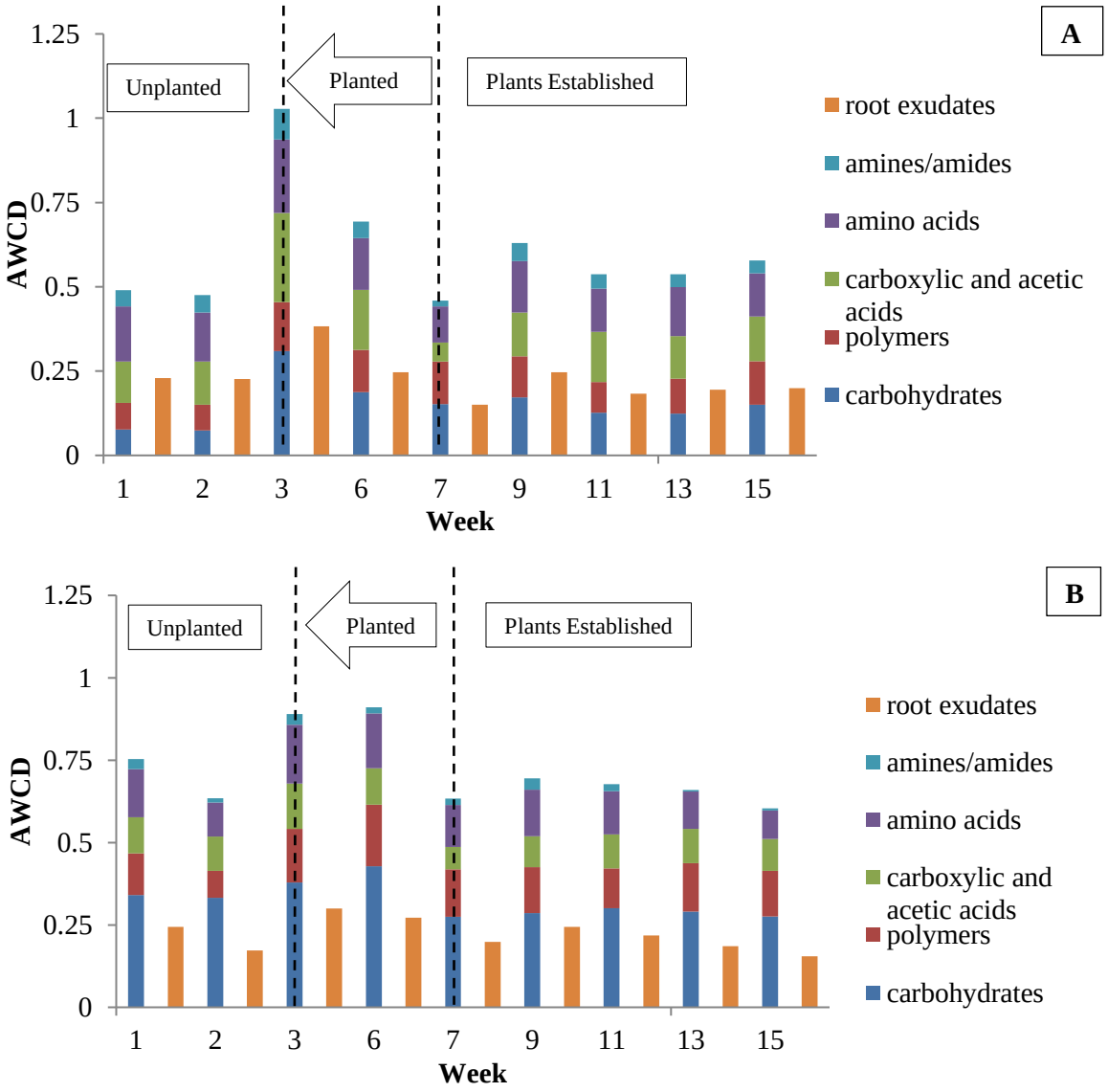

Figure 4.20: Summary of AWCD proportion by guild and AWCD of identified root exudates, for aerated (A) and non-aerated (B) systems, over the plant establishment period. Data displayed are averages from interstitial water microbial community samples taken from six replicates of aerated and non-aerated constructed wetland mesocosms. Each bar represents one sampling event. Error bars represent one standard deviation.

4.5 Discussion

4.5.1 Plant Growth

Plant growth was healthier in non-aerated mesocosms compared to aerated mesocosms. *Phalaris arundinacea* is typically found within anaerobic environments in the wild (USDA 2002). In this plant establishment study, as with the wetland development study, the artificial aeration appears to negatively impact plant growth, causing a yellowing in the leaves, less overall stems

and shorter height (Figure 4.16), refer to Section 3.4.1. Poor establishment of other wetland plants (*Phragmites australis* and *Tyhpa latifolia*) in aerated environments has been reported in the literature frequently (Nivala 2012; Butterworth et al. 2013; Weedon 2014; Butterworth et al. 2016). Reduced height, growth rate and leaf length were reported, in addition to yellowing of the leaves. Butterworth et al. (2016) also reported visual differences in root structures between *Phragmites australis* in aerated systems. Shallower roots and fewer fine root structures were noted in aerated systems. The authors attributed this to stress derived growth inhibition due to root disturbances from the aeration bubbles (Butterworth et al. 2016). An analysis of the root growth structures and root biomass between the aerated and non-aerated mesocosms should be performed for the twelve wetland mesocosms in this study when they are eventually disassembled. An invasion of pests was noted throughout the plant establishment period in aerated systems, spreading to the non-aerated systems, which is consistent with a review by Weedon (2014) of artificial aeration effects on *Phragmites australis,* another common wetland plant.

After seven weeks, the plants have established enough to affect the evapotranspiration rates from the mesocosms. The plant growth in the non-aerated systems is greater, and therefore exerts a larger effect on the evapotranspiration after week 7 (Figure 4.12). Fluctuations occurred week to week between aerated and non-aerated systems. Between weeks 7 to 15, plant count was approximately 50 stems in non-aerated systems, while only between 35 to 40 stems in aerated systems. Over the same time period, plants in non-aerated systems were 35 to 50 cm tall and only 15 to 30 cm tall in aerated systems.

The stem counts from the end of the plant establishment period (38 and 48 for aerated and non-aerated mesocosms, respectively) can be divided by the area of the CW in m^2 (0.05 m^2) in order to compare with literature values. This equates to 760 and 960 stems per m^2 for aerated and non-aerated mesocosms, respectively. A study analyzing growth pattern of *Phalaris arundinacea* reports average number of stems per m^2 between 300 and 600 for eight full scale (non-aerated) CWs in the Czech Republic (Březinová and Vymazal 2015). Therefore, stem density in this study is greater than for field scale CWs. The seeding ratio of 500 mg of seeds per mesocosm is admittedly high; however poor plant growth was noted in the previous study at a lower seeding ratio (100 mg/mesocosm). Additionally, *Phalaris arundinacea* is reported to grow 1 to 3 m tall in the wild (Vymazal 2011). In this study, plants in the non-aerated mesocosms reached 50 cm and in aerated systems 40 cm after two months. Rapid growth is apparent but the plants may need longer than two months to reach their full height/biomass. CWs planted with *Phalaris arundinacea* may not reach full wastewater treatment potential for more than two months after planting and start-up. The rapid growth of *Phalaris arundinacea* was also reported in laboratory microcosm wetlands (Gagnon et al. 2007), but also in field applications, reaching maximum biomass after only 1 to 2 years (Vymazal and Krőpfelová 2005).

4.5.2 Effects of Plant Establishment on the Microbial Community

Plants can impact the microbial community in CWs by providing an environment which is beneficial for microbial development. Plants provide a surface for microbial attachment and biofilm development in their root structures, dissolved oxygen near their roots (Münch et al. 2007), and plants are known to exude enzymes, chemicals and nutrients from their roots which can be beneficial for nearby microbial communities (Bais et al. 2006). These factors combine to create a "rhizosphere effect" where the plant root system influences the surrounding environment, creating favorable conditions for the microbial ecosystem. *Phalaris arundinacea* is a common wetland plant

which has previously been reported to increase microbial density and activity (Gagnon et al. 2007), as well as microbial catabolic function and richness (Button et al. 2016) in CWs.

Following seeding with *Phalaris arundinacea* in the wetland mesocosms, an initial increase in the microbial activity (*in-situ*, based on FDA hydrolysis) was observed. As the plants had time to grow and establish within the systems, the microbial activity varied, finding a stabilization point after week 10. A similar trend can be noted in both the aerated and non-aerated systems (Figure 4.17). The changes in microbial activity with the addition of plants is likely caused by the plants creating a more dynamic ecology in the wetland subsurface, causing subsequent ecological succession of the previous (unplanted) microbial community. Similar temporal undulating trends with respect to microbial community changes have been reported in the literature for planted and unplanted wetland mesocosms over a wetland start-up phase (Weber and Legge 2011). Microbial activity in non-aerated mesocosms is consistently greater than in aerated mesocosms. This may be as a result of a local dissolved oxygen effects in the rhizosphere influencing the microbial community in the non-aerated mesocosms to a greater extent than in aerated mesocosms, as the dissolved oxygen is already near saturation in aerated systems. The opposite was reported for planted, aerated and non-aerated pilot scale CWs where microbial activity was generally greater in the aerated pilot scale system (Chazarenc et al. 2009).

Mesocosm interstitial water microbial community catabolic function and richness was also calculated over the course of the plant establishment period. Again, an initial increase in the microbial catabolic function and richness was observed after seeding with *Phalaris arundinacea*, followed by a decrease and successive stabilization. Both richness and AWCD display the same overall trends with respect to plant establishment (Figure 4.18, A and B). After week 9, the initial impact of the addition of plants to the system may have stabilized and the microbial community function began to level off to a pseudo steady-state value. The trends in both richness and AWCD observed for both systems types are likely a result of ecological succession at the microbial scale as a result of the developing plant rhizosphere and associated ecological stresses. This idea is enforced by porosity data, for which a slight increase is noted for both system variations (more so for non-aerated) with the addition of plants to the system. This initial increase is possibly due to an initial die-off of bacterial species which could not adapt favourably to the addition of plant seeds to the wetland. After this initial increase in porosity for both system types, porosity generally decreased with time. This can be attributed to the reestablishment of bacteria and the growth and development of plant roots throughout the wetland sub-surface.

The changes in microbial community abundance and composition could arise due to the root exudation of compounds which are not favourable or do not support the growth of the current rhizospheric microbial community. A variety of wetland plants (*Scirpus lacustris, Mentha Aquatica, Phragmites australis*) have been reported to exude antimicrobial compounds from their roots (Seidel 1976; Vincent et al. 1994). Tannic and gallic acids, among other compounds, may be responsible for the antimicrobial activity of root exudates from wetland plants (Gopal and Goel 1993). However, the effect observed is not solely based on an antimicrobial effect it is more a microbial succession. Changing AWCD and richness over an adaptation period, has been noted in the literature after seeding mesocosm systems with an activated sludge microbial community and allowing the systems to naturally develop thereafter (Weber and Legge 2011). The ecological succession at the microbial scale hypothesis can be confirmed later with additional microbial analysis including DNA sequencing. Bi-weekly samples were prepared and frozen for future microbial community analysis by Next Generation Sequencing using an Illumina MiSeq. A recent

study reported effects on bacterial community compositions being affected by the presence of plants and plant litter (Chen et al. 2015). An initial change in microbial community structural composition is expected with the onset of plant development. Additionally, an increase in facultative bacteria in the non-aerated systems is expected over the plant establishment period.

The microbial communities from the non-aerated mesocosms display greater function and richness than those from the aerated mesocosms, however not as pronounced as the differences in activity. These differences could be due to differences in plant root zone development or biofilm development (Weber and Legge 2011). The differences may also be because of a less robust biofilm microbial community forming in the aerated systems because of the constant agitation and shear force from the air movement through the wetland bed media. This could also explain why the *in-situ* microbial activity values are lower for aerated mesocosms.

Principal component analysis (PCA) performed on the microbial carbon source utilization patterns (CSUPs) of the aerated and non-aerated systems over the plant establishment period (Figure 4.19) also support the idea that plants provide a regulating and stabilizing environment for the microbial community. In terms of CSUPs over the plant establishment period this amounts to separated groups of aerated and non-aerated microbial CSUPs converging to a single grouping of CSUPs by week 15. This implies that mesocosm replicates have similar abilities to utilize a variety of carbon sources. The non-aerated microbial communities may be developing functionality close to that of the aerated microbial communities due to the localized dissolved oxygen effects in the rhizosphere biofilm region.

Figure 4.20 presents AWCD as stacked column containing the sum of the contributions from each carbon source guild (Section 2.3.1.3 Table 2.2) alongside the AWCD coming from the wells which have been identified as root exudates (Section 2.3.1.4 Table 2.3). Carbohydrates appear to be better utilized (as a proportion of AWCD) in non-aerated systems. Over time, the non-aerated systems appear to lose their functionality for the breakdown of amines and amides. For aerated systems, prior to plant addition the ability to utilize carbohydrates was low and amino acids were high. After the establishment of plants in the system, this trend reverses. Identification of carbon source groups which wetlands containing *Phalaris arundinacea* and their associated microbial communities are partial to breaking down is important to identify future applications for CW technology (Button et al. 2016). A study looked at CWs in series, planted with *Phalaris arundinacea.* They found microbial communities from CWs planted with *Phalaris arundinacea* in wetland position 1 were functionally diverse, and able to break down many carbon sources from all guilds, while microbially communities in wetland position 2 lost some ability to utilize amino acids and carboxylic and acetic acids. Therefore, environmental conditions of the system, whether aerated or non-aerated, high nutrients or low nutrients, plants or no plants, truly impact the microbial communities and their ability to breakdown various carbon source types. The differences in the carbon source utilization between the aerated and non-aerated mesocosms in this study are likely as result of different microbial consortiums which developed based on the environmental conditions within the mesocosms. The varying environmental conditions (pH, ORP, DO) as evidenced from the water quality data set gathered in this study will influence energy sources (nutrients, carbon, etc.) available to the microbial consortium which will therefore shape the microbial population which is found in the different mesocosm system designs.

The contribution from root exudates makes up approximately 30% of the colour development on the plate for both aerated and non-aerated systems over the plant establishment period. As noted previously, during the seedling stage, approximately 30-40% of carbon

originating from photosynthesis products is spent on root exudates (Whipps and Lynch 1990). The mesocosms in this study are operating under a continuously recirculating flow regime, which could allow root exudates to be well mixed within the wetland subsurface rather than residing solely within the plant rhizosphere (Weber and Legge 2013). This supports the notion that root exudates may be responsible for the differences in microbial community function before and after plant establishment. In this study, seedlings germinated at week 3, where a small (5-10%) increase in AWCD from root exudates can be noted (Figure 4.20). Root exudates may also drive changes in microbial community structure within the wetland which influences the catabolic capability, as noted in the changes of carbon source utilization over time.

4.5.3 Effects of Plants on Water Chemistry and Wastewater Treatment

The addition of aeration to the wetland bed creates a very different physiochemical environment than that of the non-aerated mesocosms. This is evidenced by the differences in water chemistry parameters between the two system types. The pH, dissolved oxygen, ORP, ammonium and nitrate trends are very clearly defined by the level of aeration in the wetland mesocosm. This in turn affects the water treatment mechanisms available in the mesocosms. The addition and establishment of plants to the wetland mesocosms adds another layer to the wastewater treatment mechanics.

The pH of the water in aerated and non-aerated systems was consistent throughout the plant establishment period (Figure 4.4). The addition of plants had no significant effect on the pH of the wetland water. The pH stability within the systems is likely due to the use of limestone pea gravel as the subsurface substrate. The pH for both systems was between 7 and 7.75 for the duration of the plant establishment period. A possible explanation for the aerated mesocosm consistently having higher pH values than non-aerated systems is the *in-situ* formation of calcium bicarbonate ($Ca(HCO_3)_2$), as outlined in Section 3.4.2.

The aerated systems had higher (6-9 mg/L) dissolved oxygen than non-aerated systems (0-0.2 mg/L) (Figure 4.5), which is consistent with the wetland development study (Section 3.4.2). DO concentrations in non-aerated systems were very stable over the course of the plant establishment period, while DO started to increase slightly in aerated systems after 10 weeks. The increase in DO in aerated systems later in the plant establishment period can be partially explained by the drop in temperature observed in the mesocosms due to environmental conditions (Figure 4.2). Temperatures dropped ranged from 27 to 21 °C with the onset of fall over the experimental period. This temperature range corresponds to a 1 mg/L change in dissolved oxygen saturation. Additional dissolved oxygen is likely a result of root-mediated oxygen release adding oxygen to the rhizosphere and biofilm in the mesocosms (Brix 1994). An increase in dissolved oxygen is not observed in the non-aerated mesocosms over the same time period as the wetland is completely anaerobic and any added dissolved oxygen to the rhizosphere and biofilm would be subsequently utilized in microbial processes. The redox potential in aerated systems is positive with values between 25 to 100 mV while the redox potential in non-aerated systems is negative with values between -150 to -200 mV (Figure 4.6). After the plants established in the systems, after around 7 weeks the redox potential in aerated systems becomes more positive, but the redox potential in the non-aerated systems remains consistent (within error). Therefore, the plants in the systems appear to be causing the redox potential to diverge. This may be caused by the increase in dissolved oxygen recorded in the aerated systems over the same time frame (Figure 3.11, weeks 10 – 15). Based on the differences in dissolved oxygen and redox potential between aerated and non-aerated

systems we expect to see differences in nutrient removal and microbial community characteristics between system types.

In this study, the aerated mesocosms show lower ammonium levels (2-3 mg/L) than non-aerated mesocosms (6-8 mg/L) (Figure 4.7); while non-aerated systems have lower nitrate levels (2-10 mg/L) than aerated systems (50-80 mg/L) (Figure 4.8). This is completely in line with the fundamental differences between the redox and dissolved oxygen conditions between the aerated and non-aerated systems. As these are microbially mediated processes it is expected that there may be differences between the microbial consortium between the aerated and non-aerated mesocosms (Section 0). By week 7, the plants had reached a point in their establishment where they began to exert effects on the wetland system. For nitrogen species, this resulted in lower ammonium levels in non-aerated systems and lower nitrate levels in non-aerated systems. The establishment of plants in the wetlands increases oxygen flux to the subsurface via the plants roots (Brix 1994). For ammonium transformations in non-aerated systems, the plants roots would supply the oxygen required to transform ammonium to nitrate (reaction (a)). The decrease in nitrate levels in aerated systems can be explained by uptake of nitrogen by plants as well as the requirement of nitrogen for plant growth.

Changes in ammonium and nitrate concentrations are also reflected in the TN mass removal from the mesocosm wetlands, used to evaluate wastewater treatment performance in this study. The non-aerated mesocosms have a much more consistent TN removal rate than the aerated mesocosms (Figure 4.10). The removal of TN from non-aerated mesocosms is relatively stable before and after the addition of plants to the system but a slight increasing trend can be noted between week 9 and 15. TN removal rate in aerated systems was less stable, fluctuating between 0% and 45% removal of TN from the system. The simulated wastewater in this experiment contains more nitrate than ammonium (Table 4.2) therefore the non-aerated mesocosms have a fundamental advantage in the removal of TN from the system as the last step in the nitrogen cycle is denitrification of nitrate to nitrogen gas (reaction c) which requires an anaerobic environment and organic carbon (Faulwetter et al. 2009). Additional organic carbon may be added to the system in the form of root exudates from the plant rhizosphere (Bais et al. 2006), which can facilitate the transformation of nitrate to nitrogen gas. The increase mass removal of TN between weeks 9 and 15 in the non-aerated systems may also be related to the flux of oxygen provided by the introduction of plant roots to the system. *Phalaris arundinacea* has previously been reported to exude high concentrations of organic carbon from its roots, facilitating denitrification in low carbon environments (Zhu and Sikora 1995). A decrease in ammonium was noted in non-aerated systems (Figure 4.7). Ammonia could be converted to nitrate within the wetland rhizosphere due to root-mediated oxygen release by the plants (reaction (a) and (b)). In the non-aerated systems, both the aerobic and anaerobic processes can occur within the wetland simultaneously because oxygen is only present close to the plant root structures (Münch et al. 2005), while the overall wetland has very low oxygen. The fluctuating TN mass removals experienced in aerated systems may be a result of increased oxygen saturation in the mesocosms (Figure 4.5) over the plant establishment period. This may equate to the removal of anaerobic microenvironments in the biofilm by the release of oxygen to the rhizosphere from the plant roots.

The high dissolved oxygen levels in the aerated mesocosm in this study (6-8 mg/L) may hinder the availability of anaerobic denitrification processes. This may be a result of the continuous application of aeration used within the aerated mesocosms in this study. DO values in the aerated mesocosms (Figure 4.5) were high in comparison to other studies, which typically report values

between 1 to 5 mg/L of DO (Cottingham et al. 1999; Zhu et al. 2013; Boog et al. 2014; Zhai et al. 2016). The continuous application of aeration (24 hours a day), may cause a contradiction between the removal of organic and ammonium nitrogen and TN because of the lack of anaerobic conditions for denitrification (Wu et al. 2015). This may explain the fluctuating removal values for TN observed in the aerated mesocosms after the addition and establishment of plants (Figure 4.10) and the further addition of dissolved oxygen provided by plant root mediated oxygen release. Reducing the frequency of aeration in the wetland to timed cycles or to be activated when the DO reaches a determined threshold can increase the removal of TN in aerated systems (Boog et al. 2014).

Plants may also uptake nitrogen at certain times in their growth cycle. The amount of nitrogen uptake depends on the species of the plant. Uptake by *Phalaris arundinacea* is often lower than other wetland species (Kadlec and Wallace 2008; Maltais-Landry et al. 2009). Plant uptake of nitrogen is reported to drastically different degrees in the literature contributing from 1% (Kadlec and Wallace 2008), 7% (Maltais-Landry et al. 2009) and 21 % (Borin and Salvato 2012) of the removal of the total nitrogen load. Uptake by *Phalaris arundinacea* has been reported on the order of 163 g N/m^2 (Salvato et al. 2012) and 27.7 g N/m^2 (Maltais-Landry et al. 2009) per growing season. Computed for the mesocosms used in this study (0.05 m^2) it would be roughly 1-8 g N per growing season, which is a very small amount of the incoming TN each week (Table 4.2).

Total organic carbon (TOC) removal was also assessed as a measure of the wastewater treatment performance of the wetland mesocosms. Aerated and non-aerated mesocosms showed similar TOC mass percent removal over the course of the plant establishment period (Figure 4.9). TOC removal was dominated more by the nature of the system (aerated versus non-aerated) than by the addition of plants to the system. The removal of TOC in wetlands can be attributed to the biofilm microbial community within the CW mesocosms. The microbial consortium within CWs use the constituents of the simulated wastewater for survival (catabolism) (Weber and Gagnon 2014; Weber 2016). Organic pollutants are broken down by microorganisms via the processes of respiration and fermentation. The aerated mesocosms display a higher mass removal of TOC in relation to the non-aerated systems because aerobic respiration requires oxygen and is a much faster process than anaerobic fermentation. Therefore, the aerated mesocosms have an advantage because of the higher dissolved oxygen content in the water. Nonetheless, the non-aerated CW had slightly lower removal of TOC (1-5% less than the aerated CW), which may in part be explained by the readily biodegradable source of carbon within the artificial wastewater. The percent mass removal of TOC is stable throughout the plant establishment period with no significant effects observed by the addition of plants for both system types. The dips in performance in weeks 9 and 14 for the aerated and non-aerated systems can be attributed to a lower than average inlet concentration in those weeks. As the outlet concentration of TOC was relatively stable week to week a lower inlet concentration gives a false sense of a decrease in overall performance.

4.6 Conclusions

The objective of this study was to investigate the effects of the addition of plants (*Phalaris arundinacea*) on a set of stabilized recirculating saturated vertical flow CWs in terms of water chemistry, water treatment, hydrological and microbial parameters. *Phalaris arundinacea* germinated within the first week after seeding and quickly established within the CW mesocosms. Artificial aeration was not favourable towards the growth of *Phalaris arundinacea*, causing a yellowing in the leaves, less overall stems and shorter height compared to plants in non-aerated mesocosms. The establishment of plants (*Phalaris arundinacea*) in the aerated and non-aerated CW mesocosms exerted a stabilizing effect on the microbial community, starting to bridge the

microbial catabolic functionality between the two system types. A 15% increase in the mass removal of TN and a 6% increase in the mass removal of TOC were observed in non-aerated systems over the plant establishment period. In aerated systems, TOC removal was high (>95%) independent of plant addition and TN removal fluctuated more after plant establishment.

This method of holistically evaluating changes to metrics encompassing overall wetland ecosystem dynamics elucidated the subtle effects of the addition of plants to developed wetland mesocosms. Complex interactions between plants, water chemistry (ORP and DO) and microbial communities lead to changes in pollutant removal (TN and TOC) in the aerated and non-aerated systems. The effect of plants on the mesocosms was more defined in non-aerated systems than in aerated systems. The ability of plants to mediate oxygen release to the wetland subsurface via their roots is likely the cause of this difference. This study validates the importance of rigorous characterization of wetland health and function. Studying wetlands from several angles informs research and industry on subtle interactions between microbial communities, plants and wastewater components. Understanding solely the effects of the addition of plants to CWs and the timescale at which plants start to effect CWs is novel.

5 CHAPTER 5: EFFECTS OF SILVER NANOPARTICLES ON CONSTRUCTED WETLAND MICROBIAL COMMUNITIES

5.1 Introduction

Silver nanoparticles (Ag NPs) are increasingly incorporated into a wide variety of consumer products based on the innate antimicrobial properties of silver (Vance et al. 2015). Therefore, their introduction into the environmental is likely and can occur at any stage in their lifecycle, such as during manufacturing, product usage and final disposal (Fabrega et al. 2011). Studies have shown that silver can leach quite readily from Ag NP containing consumer products, in both particulate and ionic forms (Benn and Westerhoff 2008). The released silver is expected to reach wastewater treatment plants and potentially be discharged to surface waters, including natural wetlands, in unknown magnitudes (Blaser et al. 2008). Current models predict environmental concentrations of Ag NPs in the ng/L range, which may be sufficient to pose a risk to aquatic ecosystems and biota (Fabrega et al. 2011; Gottschalk and Nowack 2011).

The study of Ag NP release from consumer products is relatively well documented in the literature. Benn *et al.* (2010) reported releases of 1 – 46 µg Ag/g product for a range of consumer products after washing for 1 hour with tap water. Two silver fibres commonly incorporated into clothing and textiles, X-STATIC™ and AgKilBact™, were reported to release approximately 314 µg Ag/g fabric and 377 µg Ag/g fabric, respectively (Geranio et al. 2009). A recent calculation estimated laundry wastewater (from clothing containing X-STATIC™ or AgKilBact™ silver treated fabrics) could realistically contain 1.5 mg Ag/L (Button et al. 2016). The authors noted that the 1.5 mg Ag/L laundry wastewater may be diluted by the other household greywater to around 0.1 mg Ag/L over the course of a day (Button et al. 2016). Wash water from consumer products containing Ag NPs is likely to reach both wastewater treatment facilities and natural aquatic ecosystems. Recent estimates show concentrations of Ag NPs in water entering wastewater treatment facilities between 0.06 and 1.5 µg/L (Li et al. 2013).

Toxicity of Ag NPs has been attributed to several factors including to the release of Ag+ ions from the nanoparticle shell (Navarro et al. 2008; Miao et al. 2009) particle-size specific interactions (Choi and Hu 2008; Fabrega et al. 2009) or due to the ability to generate reactive oxygen species (ROS) (Foldbjerg et al. 2009; Massarsky et al. 2014). Ag^+ ions interact strongly with thiol groups, which can inactivate important enzymes, including those involved with the electron-transport chain, which in turn affects cellular oxidation, RNA translation and DNA replication (Morones et al. 2005; Kim et al. 2007). Several studies concluded that Ag NPs alone have minimal toxicity and serve mostly as a source of Ag^+ ions (Navarro et al. 2008; Miao et al. 2009). In contrast, Fabrega *et al.* (2009) concluded that the effect of released Ag^+ ions is not significant, therefore the dominating factor of toxicity is bacterial contact with the nanoparticles themselves. In additional studies, smaller nanoparticles were found to be most toxic, often explained by easier uptake (Choi and Hu 2008) and larger surface area per mass of silver (Johnston et al. 2010), which can facilitate faster dissolution and release of silver ions. Research has also supported the generation of ROS by Ag NPs which can induce oxidative damage at the cell membrane (Massarsky et al. 2014).

The toxicity and reactivity of Ag NPs in environmental compartments, such as water, also depends on the aqueous chemistry of silver. Ag NPs will additionally undergo environmental transformations including changes in aggregation state, changes in oxidation state, precipitation,

and sorption to natural organic matter and inorganic species (Levard et al. 2012) which will affect their toxicity and reactivity. Metallic silver is thermodynamically unstable under most environmental conditions and will oxidize or react with natural organic matter and inorganic ligands, such as sulphide and chloride (Liu and Hurt 2010; Fabrega et al. 2011; Levard et al. 2011; Xiu et al. 2011). In freshwater, silver is likely to complex with sulphur to form solid Ag_2S (Fabrega et al. 2011; Levard et al. 2012). The formation of solid AgCl is also likely based the availability of chloride in natural waters (Fabrega et al. 2011; Levard et al. 2012). Ag NPs show increased reactivity and therefore environmental transformations are expected to be faster than for bulk silver (Levard et al. 2012). These environmental transformations of silver will affect the surface chemistry of Ag NPs and therefore their transport, reactivity and toxicity in the environment.

In recent years, substantial research has been conducted regarding the fate of Ag NPs in environmental compartments and the toxicological effects on microbial communities and other small organisms (Marambio-Jones and Hoek 2010; Reidy et al. 2013). The majority of this research was conducted with pristine, manufactured nanoparticles in laboratory media (distilled water, growth substrates, etc.) on single species. More recently, toxicity studies have been performed on groups of microorganisms and bacteria, such as those occurring in biofilms. For a laboratory grown biofilm containing exclusively *Pseudomonas putida*, a decrease in the biofilm volume was observed when exposed to uncoated Ag NPs (65 nm) at 0.02 – 2 mg/L (Fabrega et al. 2009). Another study looked at interactions of Ag NPs with *E. coli* cells in planktonic and biofilm cultures. The minimum bactericidal concentrations of nanosilver, defined as the lowest concentration that kills at least 99.9% of a population, were 38 and 10 mg/L Ag for particles sized 15 to 21 nm, respectively. Planktonic and biofilm bacteria were more strongly affected by silver ions than Ag NPs. Ag NPs aggregated in the presence of planktonic and biofilms cells, causing an increase of the average silver particle size. The authors suggested that the biofilm resistance to Ag NPs could be partially a result of nanoparticle aggregation (Choi et al. 2010). While the study of pristine NPs was a necessary first step to understand the toxicity associated with Ag NPs, there has been a call for research using environmentally relevant concentrations and conditions as well as more relevant Ag NPs, including those weathered/released from consumer products or those aged by environmental processes (Selck et al. 2016). Furthermore, Ag NPs released from consumer products are likely to enter biologically-mediated wastewater treatment facilities (activated sludge plants, constructed wetlands, etc.) and thus the study of the fate, removal and possible negative effects of Ag NPs on these treatment methods are crucial to ensure safe water management.

Constructed wetlands (CWs) are an alternative to classical wastewater treatment plants, which engineer natural wetland processes to provide enhanced water treatment services. As a wastewater technology, CWs are at the forefront of Ag NP exposure and thus are an ideal system to study NP removal and their impact on the wetland ecosystem. Furthermore, pollution removal within CWs relies heavily on microbial processes (Weber 2016), so there may be some concern for the treatment efficiency of those systems with the presence of Ag NP in the wastewater. For Ag NP toxicity on natural and CWs, limited information is available in the literature. In natural wetlands, interstitial water microbial communities displayed similar ecotoxicity for ionic silver and CMC-coated Ag NPs, with complete inhibition of microbial activity at 1 mg/L. The uncoated and PVP-coated Ag NPs displayed a lower toxicity with partial or complete inhibition not occurring until 10 mg/L of Ag (Schneider 2015). In CWs, low doses of Ag (0.1 mg/L Ag NPs) added *in-situ* did not appear to exert any significant toxicity effects in the short term (1 month) whether dissolved or in nanoparticle form. This may suggest some natural ability to adapt to the stress from the addition of silver. However, when dosing interstitial water microbial communities from the same

CWs (*ex-situ* toxicity testing), silver concentrations higher than 0.5 mg/L significantly reduced microbial community catabolic activity for citrate-coated, biogenic NPs and ionic silver (Button et al. 2016). In both studies, only pristine, manufactured or readily synthesized Ag NPs were used to evaluate toxicity to the microbial communities.

Community Level Physiological Profiling (CLPP) is a convenient method for characterizing the overall function of a microbial population (Garland and Mills 1991). Recently, an *ex-situ* ecotoxicity method was developed using CLPP to assess the effects of a toxicant on the overall catabolic capabilities and function of a wetland microbial community (Weber et al. 2014; Button et al. 2016). The method uses BIOLOG EcoPlates™, with multiple sole carbon sources, to accurately and rapidly to determine differences in microbial community function, carbon utilization intensity and overall catabolic capability (Weber and Legge 2010). A carbon source on the BIOLOG EcoPlate™ may be utilized by a single species or a group of species (Weber et al. 2014). The advantage of using this method to evaluate ecotoxicity is that it represents the effect to the entire microbial community as opposed to the effect to a single species, which is more typical of a natural ecosystem, e.g. biofilm. Additionally, the method is rapid and a range of concentrations and Ag NP types can be analyzed easily without specialized experience or equipment. Furthermore, the information collected regarding the ability of microbial communities to utilize carbon sources based on Ag NP loading is invaluable in CW technology. This would allow researchers and industry professionals an insight into how active CW systems and the associated wastewater treatment performance may be affected by incoming Ag NP loads.

Subsurface CW technologies generally have low oxygen and favour anaerobic microbial processes to remove pollutants such as denitrification and sulfate reduction (Faulwetter et al. 2009). To improve wastewater treatment capacity, CWs may use certain intensification designs, such as artificial aeration (Kadlec and Wallace 2008). Artificial aeration permits microbial processes such as ammonification, nitrification and aerobic organic matter degradation. The presence of oxygen alters the environmental conditions within the wetland and will therefore affect the fate and toxicity of Ag NPs. CWs with or without artificial aeration may be affected to different extents by Ag NPs. Microbial communities in CWs play a significant role in the overall function and pollutant removal abilities of the wastewater treatment technology (Weber and Gagnon 2014). Understanding the effect of Ag NPs to these microbial communities is essential for safe operation of CWs in a world of growing nanoparticle use and therefore, environmental exposure (Button et al. 2016). The objective of this study was to evaluate the effect of various types of Ag NPs (pristine and leached) in addition to ionic silver on the microbial communities associated with interstitial water from CWs. Pristine Ag NPs with a variety of coating types, in addition to two more environmentally relevant particles (wash water from an Ag-containing sock and a sulphidized Ag NP) were applied to microbial communities sampled from aerated and non-aerated CW mesocosms. This study will utilize the *ex-situ* method derived by Weber et al. (2014) to assess the effects of Ag NPs on the overall catabolic capabilities and function of mesocosm wetland microbial communities. Dose-response curves (0 – 10 mg/L) were created based on the ability of microbial communities to utilize carbon sources on commercially available BIOLOG EcoPlates™ when exposed to varying amounts of Ag NPs.

5.2 Materials and Methods

5.2.1 Experimental Design

Twelve CW mesocosms were sampled (interstitial water) for this experiment. Six of which were artificially aerated and six were not aerated, all mesocosms were planted with *Phalaris arundinacea*. The system design and operation of these mesocosms was described in Chapter 3. Interstitial water samples from both aerated and non-aerated CW mesocosms were evaluated as the different environmental regimes may influence the fate and toxicity of Ag NPs. A range of Ag NP types were exposed to interstitial water microbial community samples including polyvinylpyrrolidone (PVP) coated, citrate stabilized, uncoated and carboxymethyl cellulous (CMC) coated Ag NPs. Additionally, two weathered particles (from consumer athletic socks and an artificially sulphidized Ag NP) were also evaluated. Ionic silver in the form of silver nitrate ($AgNO_3$) was used as a positive control. A soap solution, at the concentration used for washing the athletic socks (Section 5.2.4), was also included as a control. The pristine particles were selected as they are commonly cited in the literature and therefore allow comparison to other studies. Weathered particles were additionally selected as they represent more environmentally relevant scenarios (laundry wash water of silver containing fabrics and sulphidized Ag NPs which have been reported in wastewater streams and natural wetlands).

A dose response curve was created for each type of nanoparticle using a wide range of concentrations between 0 mg/L and 10 mg/L where possible. This concentration range was chosen based on *ex-situ* dose response curves performed by Button et al. (2016) which showed a missing window of data between 0 mg/L and 1 mg/L. Six concentrations were used for each nanoparticle: 0 mg/L, 0.1 mg/L, 0.25 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L. Due to availability limitations the 5 mg/L and 10 mg/L concentrations had to be omitted for certain nanoparticle types (Table 5.1).

Table 5.1: Concentrations evaluated for ex-situ silver nanoparticle (and ionic silver) exposures on constructed wetland interstitial water microbial communities. An "X" depicts a concentration which was used.

Silver Nanoparticle Type	**Concentration (mg/L)**					
	0.1	**0.25**	**0.5**	**1**	**5**	**10**
Ag+	X	X	X	X	X	X
PVP	X	X	X	X	X	X
Citrate	X	X	X	X		
CMC	X	X	X	X	X	X
Uncoated	X	X	X	X	X	X
Sock Wash	X	X	X	X	X	
Sulphidized	X	X	X	X	X	

5.2.2 Nanoparticle Types

A range of pristine silver nanoparticles were use in this study including polyvinylpyrrolidone (PVP) coated (99.95 % Ag, 20 to 30 nm, SkySpring Nanomaterials Inc.), citrate stabilized (20 nm,

Sigma-Aldrich), uncoated (99.95 % Ag, 20 to 30 nm, SkySpring Nanomaterials Inc.) and carboxymethyl cellulous (CMC) coated silver nanoparticles (3.7 g/L in 1 % CMC solution, 20 to 30 nm, provided by The University of Western Ontario, London, Canada. Synthesis involved the combination of CMC and $AgNO_3$ in the presence of sodium borohydride ($NABH_4$) as described in Molnar et al. (2014)). Artificially weathered silver nanoparticles were also were use in this study. Artificially sulphidized silver nanoparticles (see Section 5.2.3) and the wash water from artificially washing socks containing a silver nano fibre (Men's T.H.E. Sock with X-STATIC® technology, lululemon, Canada) (see Section 5.2.4). Ionic silver in the form of silver nitrate, $AgNO_3$ (1000 µg/mL $AgNO_3$ in 4 % HNO_3, SCP Science, PlasmaCal ICP-AES & ICP-MS Standard) was used as a positive control as it is known to be a potent form of silver.

A variety of nanoparticle types were selected for analysis as physical properties (such as coating and charge) and environmental conditions (use of pristine or aged/weathered nanoparticles) have previously been reported to influence the toxicity associated with silver nanoparticles. The nanoparticles selected for analysis here are by no means exhaustive but were selected based on their ability to be compared to other studies (PVP, citrate and uncoated Ag NPs), influence of synthesis method (high residual ionic content in CMC Ag NPs) and applicability to relevant environmental scenarios associated with constructed wetlands (artificially sulphidized Ag NPs and sock wash water from silver nano-containing commercially available socks in Canada).

5.2.3 Silver Nanoparticle Sulphidation

The method for the artificial sulphidation of silver nanoparticles was adapted from Levard et al. (2011) and Reinsch et al. (2012). Manufactured PVP-coated silver nanoparticles (99.95 % Ag, 20 to 30 nm, SkySpring Nanomaterials Inc.) were reacted with Na_2S to artificially sulphidize the Ag NPs. 9 mg of Ag NPs was dispersed in 49.93 mL of deionized water (dH_2O), while 246 mg of $Na_2S{\cdot}9H_2O$ was dissolved in 25.0175 mL of dH_2O. Both solutions were added to a 250 mL Erlenmeyer flask. The initial water level was recorded on the flask. The flask was sealed with parafilm and then wrapped with aluminum foil to reduce exposure to light. An air pump with a flow rate of 0.5 L/min was inserted with a pipette tip at the end of the tubing to supply oxygen for the reaction. The reaction proceeded on a bench top at room temperature for one week. Each day, the solution volume was adjusted to that of the initial reaction volume to accommodate evaporation effects.

After one week, the resulting solution was pipetted into two 50 mL centrifuge tubes. To remove excess ionic silver and Na_2S the nanoparticles were rinsed. The tubes were centrifuged at 3800 g for one hour, after which the supernatant was removed. The nanoparticle solution was then re-suspended in dH_2O. To re-suspend the nanoparticles this solution was sonicated (Fischer Scientific Model 505 Sonic Dismembrator) for 30 seconds. This process was repeated for a total of three washings (Levard et al. 2011; Reinsch et al. 2012).

5.2.4 Sock Washing Procedure

Standardized washing procedures, adapted from ISO 105-C06, have been developed to study the release of nanoparticles from clothes and textiles (Geranio et al. 2009; Lorenz et al. 2012; Windler et al. 2012; Mitrano et al. 2014). A pair of socks (Men's T.H.E. Sock with X-STATIC® technology, lululemon, Canada) was washed in 300 mL of tap water with a liquid detergent (Tide, Original Liquid Detergent, P&G, Canada) concentration of 4 g/L for 45 minutes at 40°C while shaking at 150 rpm. 8 polypropylene balls were included in the wash to simulate fiction. Following

washing, each sock was wrung out three times by rolling the sock and squeezing out as much excess water as possible. The wash water was transferred from the washing container into sampling bottles. The socks were rinsed using the same wash bottle with 300 mL of tap water and 8 polypropylene balls for 5 minutes at 40°C and 150 rpm. The rinse solution was transferred into another sampling bottle and a second rinse was performed under the same conditions. The negative control for the sock washing procedure consisted of 300 mL of tap water and liquid detergent at 4 g/L. The solutions from the sock washing procedure and the liquid detergent were freeze-dried to reduce sample volume. Prior to dosing, the freeze-dried wash water was reconstituted to obtain final concentrations presented in Table 5.1. A laundry detergent control solution was treated the same as the sock wash water containing silver, therefore it was freeze-dried, reconstituted in a lower water volume and serially diluted into wetland water as well.

5.2.5 Water Sampling

Wetland interstitial water samples (100 mL) were collected from the sampling port from each mesocosm the day after the wetland renewal/feeding was performed. Composite samples were created between the six mesocosm replicates from both the aerated and non-aerated mesocosms, respectively, creating a 600 mL total solution volume for each water type. Samples were agitated lightly to ensure complete mixing of the water/microbial community. These water stock solutions were dosed with silver in order to create dose response curves for each nanoparticle type. A single sample was created at each nanoparticle concentration in each water type, aerated and non-aerated. Water samples containing a mixed microbial community were taken directly before use and were not exposed to light prior to dosing with Ag NPs.

5.2.6 Stock Solution Preparation

For each nanoparticle type, stock solutions were prepared the day prior to the *ex-situ* experiment. The stock solutions were prepared in deionized water (dH_2O) to reduce impact on the microbial matrix. Nanoparticle stock solutions were prepared in 50 mL centrifuge tubes and wrapped in aluminum foil to reduce expose to light. The stock solutions were sonicated for 30 minutes in a bath sonicator (Tabletop Ultrasonic Cleaner, FS140H, Fisher Scientific, Waltman, MA, USA) after addition of nanoparticles and prior to any serial dilution. Serial dilutions were performed on the main stock solutions (for each nanoparticle type and ionic silver) to make solutions which were ten times more concentrated than the final solutions to be created with water from the wetland mesocosms. This was done, where possible, to ensure the same volumes of nanoparticle solution and wetland water were combined in each instance. Samples were stored in the fridge (dark, 4-8 °C) prior to use.

Final solutions containing silver nanoparticles (or Ag^+) and a mixed microbial community in wetland water were not sonicated to limit disturbance to the microbial community. Samples were lightly agitated by turning the tubes end over end prior to use.

5.2.7 Community Level Physiological Profiling

Community Level Physiological Profiling (CLPP) is a method used to characterize heterotrophic microbial function based on carbon source utilization patterns (CSUPs) (Weber and Legge 2010). The method uses BIOLOG EcoPlates™ to accurately and rapidly determine differences in microbial community function, carbon utilization intensity and overall catabolic capability (Weber and Legge 2010). The BIOLOG EcoPlate™ (Biolog Inc., Hayward CA., USA)

is 96-well plate which contains 31 carbon sources and a blank, in triplicate. Along with the carbon source, each well contains a redox dye indicator, tetrazolium violet. When a mixed microbial community sample is inoculated into the well and starts to utilize the carbon source, the production of NADH via cell respiration reduces the tetrazolium dye to formazan. The development of formazan induces a change in colour from clear to purple which can be monitored over time to evaluate the microbial community function. This colour development is evaluated photometrically with a spectrophotometer (Eon microplate reader, BioTek Instruments, Inc., Winooski, Vermont, United States) at 590 nm.

Interstitial water from the aerated and non-aerated CWs was dosed with Ag NPs in varying concentrations to create dose-dependent series. Samples were lightly agitated to ensure proper mixing. Samples were then inoculated onto the BIOLOG EcoPlates™ using aseptic techniques inside a clean hood. After gentle agitation of the sample and with the use of sterile pipette tips, 100 µL of the interstitial sample was inoculated into each well on the BIOLOG EcoPlate™. Each wetland water sample received its own BIOLOG EcoPlate™ and was incubated in the dark at room temperature. Microplates were read photometrically at 4-hour time intervals for 96 hours using a Eon microplate reader equipped with a Biostack 3 microplate stacker and Gen5 All-in-One Microplate Reader Software (version 2.05.5) (all from BioTek Instruments, Inc., Winooski, Vermont, United States). The microplates were read individually at 590 nm following a 3 second shake at medium setting to ensure each well was well mixed.

5.2.8 Nanoparticle Characterization

Characterization of nanoparticles in this study was performed on representative stock solutions and nanoparticle spiked composite water samples from aerated and non-aerated wetland mesocosms. Ag NP solutions were analyzed for total Ag concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) on a quadrupole inductively coupled plasma-mass spectrometry instrument (Elan DRC II, Perkin Elmer, Inc., Waltham, MA, USA). Instrument specifications for ICP-MS: Nebulizer Gas Flow (1.08 L/min), lens voltage (7), and ICP RF power (1300). Prior to analysis, 250 µL of the sample to be analyzed was digested with 250 µL of concentrated nitric acid (TraceMetal Grade, Fisher Scientific, Waltham, MA, USA). Samples were allowed 1 hour for digestion at room temperature. The solutions were then diluted to 2% nitric acid in a deionized water matrix.

The percentage of ionic Ag in silver nanoparticle stock solutions (defined here as <10 kDa molecular weight cut-off (MWCO)) was determined using centrifugal ultrafiltration devices (Amicon Utlra-4, EMD Millipore Corporation, Billerica, MA, USA; MWCO of 10 kDa) (Van Koetsem et al. 2017). For this, 2 mL of each solution was pipetted into an ultrafiltration device and subjected to centrifugation at 4000 rpm for 30 minutes (Sorvall Legend RT Centrifuge, Thermo Electron Corporation, Waltman, MA, USA). Afterwards, the filtrate was digested with concentrated nitric acid and diluted by 40 times in deionized water. Samples were then analyzed for total Ag content as above. External calibration standards were used for ICP-MS analyses. Blank samples and triplicates were included every 10 samples in each batch.

5.3 Data Analysis

5.3.1 Community Level Physiological Profiling

Please refer to Section 3.3.1 for a full description of the analysis performed on Community Level Physiological Profiling data.

A time point of 40 hours was chosen for the calculation of the average well colour development (AWCD) and richness in this experiment.

The statistical significance of differences in AWCD and richness data was assessed using a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey HSD ($p<0.05$) for differences between each treatment, and a one-way analysis of variance (ANOVA) followed by a 2-sided Dunnett's test ($p<0.05$) comparing each dose concentration against the control. Statistical analyses were performed using XLSTAT 2017 (Addinsoft New York, NY) and SPSS (version 23, IBM Corporation, New York, USA).

5.4 Results and Discussion

It is important to evaluate the potential effects of Ag NPs to CW microbial communities. CWs rely on inherent microbial processes to provide wastewater treatment services. The interstitial water of mesocosm CWs was sampled for this study as it is a quick and easy method to gather information related to the microbial community, by proxy. The interstitial water microbial community in these mesocosms will comprise of free-floating bacteria as well as elements of the biofilm which have sheared off into solution. A variety of Ag NP types were evaluated in this study, as size, coating and ionic content have all been factors proposed to drive Ag NP toxicity. Silver has a complex aqueous chemistry, reacts readily with many constituents of wastewater (chloride, sulphide, organic matter) and is also influenced by oxidation (Levard et al. 2012). Therefore, evaluation of silver toxicity to microbial communities from typical non-aerated CWs is important, but also those from artificially aerated CWs. Aeration is commonly added to CWs to increase wastewater treatment for a variety of factors and will influence the fate and toxicity of Ag NPs.

5.4.1 Silver Analysis and Nanoparticle Characterization

Silver (nanoparticle and ionic) solutions used to dose the wetland microbial communities were analyzed to determine total and dissolved silver content of the samples. Dissolved versus particulate content in the stock solutions (deionized water) of each silver treatment are displayed in Figure 5.1. The ionic silver (as $AgNO_3$) stock solution had a small fraction of particulates (< 10%), which can be attributed to instrument error. CMC-coated Ag NPs are reported as 50% dissolved ionic silver, which is likely an artifact of the synthesis process. Citrate-stabilized, PVP-coated, uncoated, and sulphidized nanoparticles as well as the sock wash solution contained negligible dissolved silver. Appendix C (Table C.10) lists the raw total silver data from dosed wetland interstitial water samples used to create dose response curves in this study.

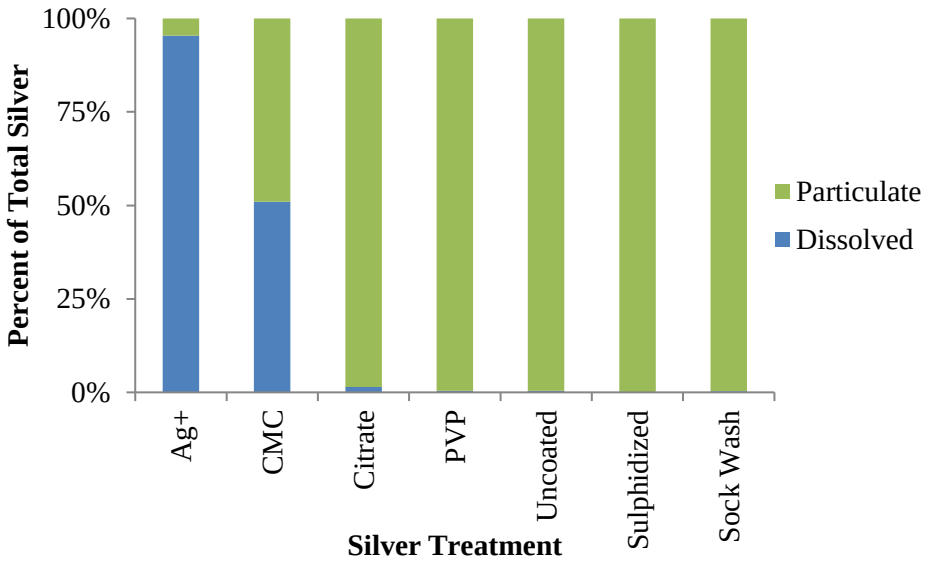

Figure 5.1: Particulate versus dissolved (ionic) fraction of silver contained in the stock solutions of each silver treatment mixed in deionized water. Dissolved defined here as <10 kDa molecular weight cut-off. Each bar represents the value from a single sample; replicates were not performed for this analysis.

5.4.2 Effects of Nanoparticle Type on Constructed Wetland Microbial Communities

The ecotoxicological effects of various types of Ag NPs were evaluated on wetland interstitial water microbial communities from aerated and non-aerated CW mesocosms. It is important to evaluate both aerated and non-aerated CW microbial communities as the presence of oxygen alters the environmental conditions within the wetland and will therefore affect the fate and toxicity of Ag NPs. Additionally, due to the different environmental conditions, different wastewater treatment mechanisms are available between aerated and non-aerated mesocosms which likely fosters different microbial communities. Overall, CWs with or without artificial aeration may be affected to different extents by Ag NPs. A range of concentrations (0 to 10 mg/L) were examined in this study to quantify the potential effects of Ag NPs on CW microbial communities.

The dose response curves for the overall catabolic function (AWCD) and the number of carbon sources utilized (richness) for microbial communities from non-aerated mesocosms are depicted in Figure 5.2 (A and B) (Tukey-HSD groupings of silver treatment in Table C.4 and Table C.5). Both the AWCD and richness decreased with increasing silver concentration for the CMC-coated Ag NPs, the silver-containing sock wash water ("sock wash") and ionic silver, Ag^+. Complete catabolic inhibition of the non-aerated microbial communities was observed for Ag^+ and CMC-coated Ag NPs at 10 mg/L of Ag. The sock wash water showed significant (ANOVA, $p<0.05$) inhibition to the microbial community catabolic function at 0.5 mg/L and greater. Little to no effect was observed from citrate stabilized, uncoated, PVP-coated and artificially sulphidized Ag NPs.

The large effect observed by CMC-coated particles is likely an artifact of the synthesis process and residual ionic silver that remains in solution. Evaluation of the ionic content of CMC-coated particles revealed they may be up to 50% Ag^+ (Figure 5.1). Schneider (2015) used the same particles for their ecotoxicity screening of Canadian soils and wetlands and found similar effects between Ag^+ and CMC-coated Ag NPs. They reported total inhibition of natural wetland microbial communities between 0.1 to 5 mg/L for Ag^+ and 0.5 to 1 mg/L for CMC-coated Ag NPs. In this study, complete inhibition of the microbial community catabolic function was not observed until 5 mg Ag/L for both Ag^+ and CMC-coated Ag NP treatments. This may imply an increased resistance to silver in the CW microbial community versus those from a natural wetland.

The mild positive effect (greater than 1, therefore greater than the control) for citrate-stabilized Ag NPs can be attributed to the microbial communities' ability to utilize the citrate coating. Weber et al. (2014) explains that citrate is a key component of the Kreb's cycle which is readily metabolized by many microorganisms. Therefore, the added citrate coating on the nanoparticles provides an additional source of carbon for the microbial community in these wells. An increase in catabolic activity was observed when natural wetland microbial communities were exposed to citrate-stabilized gold nanoparticles (Weber et al. 2014).

The washing procedure performed on the athletic socks used liquid detergent (Tide Liquid Original), which represents the most environmentally realistic scenario for release of silver from Ag NP containing textiles. Figure 5.3 depicts the toxicity of the sock wash water and the laundry detergent control in terms of laundry detergent concentration. There is some toxicity from the laundry detergent itself but the sock wash water containing silver exerts more toxicity at concentrations above 3 g/L of laundry detergent. At lower concentrations, the sock wash water displays less toxicity than the laundry detergent. It may be that the laundry detergent provides carbon sources for the microbial community to utilize but also substances that are harmful to the microbial community. Based on the ingredient list from P&G (Table C.1) multiple ingredients may be sources of carbon for the wetland microbial community including the polymers and enzymes listed. Conversely, borax and sodium hydroxide are toxic to microbially communities. At lower concentrations of silver and laundry detergent the positive effects from the laundry detergent are noted, but at higher concentrations the toxicity from silver and constituents of the laundry detergent become apparent.

Little to no effect was observed on the microbial communities from non-aerated CWs when exposed to uncoated, PVP-coated and artificially sulphidized Ag NPs. This can be attributed to a combination of three things. Firstly, the coating on the nanoparticle itself hindering bioavailability of silver and decreasing toxicity (Silva et al. 2014). Coatings may inhibit direct contact of the nanoparticle with cellular components, reducing the particle-size specific interactions which cause Ag NPs to be toxic. Secondly, inorganic ligands such as sulphide (Levard et al. 2011) or chloride (Fabrega et al. 2011) may react with silver and again hinder bioavailability and block toxicity mechanisms (Reinsch et al. 2012). Finally, the anaerobic conditions found in the non-aerated wetlands (low dissolved oxygen, low redox see Section 3.4.2) prevent metallic silver, Ag(0), oxidation and subsequent Ag^+ release (Xiu et al. 2012). This could explain the lack of toxicity observed from uncoated Ag NPs. A large portion of the literature agrees with the theory that Ag NPs are a large reservoir for Ag^+ and toxicity to microbial communities is actually due to the release of Ag^+ from the nanoparticle (Miao et al. 2009).

The overall trends observed in this study align with those of Schneider (2015). They found PVP-coated Ag NPs to be the least toxic, followed by uncoated and CMC-coated Ag NPs. Ag^+ was

reported as acutely toxic. Button et al. (2016) found citrate Ag NPs to be more toxic than PVP-coated Ag NPs to biofilm microbial communities from CW microcosms, with a significant catabolic inhibition of the microbial community was observed at 5 mg Ag/L for citrate Ag NPs. They also found limited toxicity from PVP-coated Ag NPs with only slight catabolic inhibition (20%) at the highest concentration tested, 5 mg Ag/L. Ionic silver trends were consistent with those of Schneider (2015) with complete catabolic inhibition observed at 1 mg/L. In this study, the toxicity generally followed the amount of ionic silver in the treatment (Table 5.1), with ionic silver and CMC-coated Ag NPs being the most toxic, while the sock wash water affected the microbial community in terms of silver content but also due to the toxicity of the added laundry detergent.

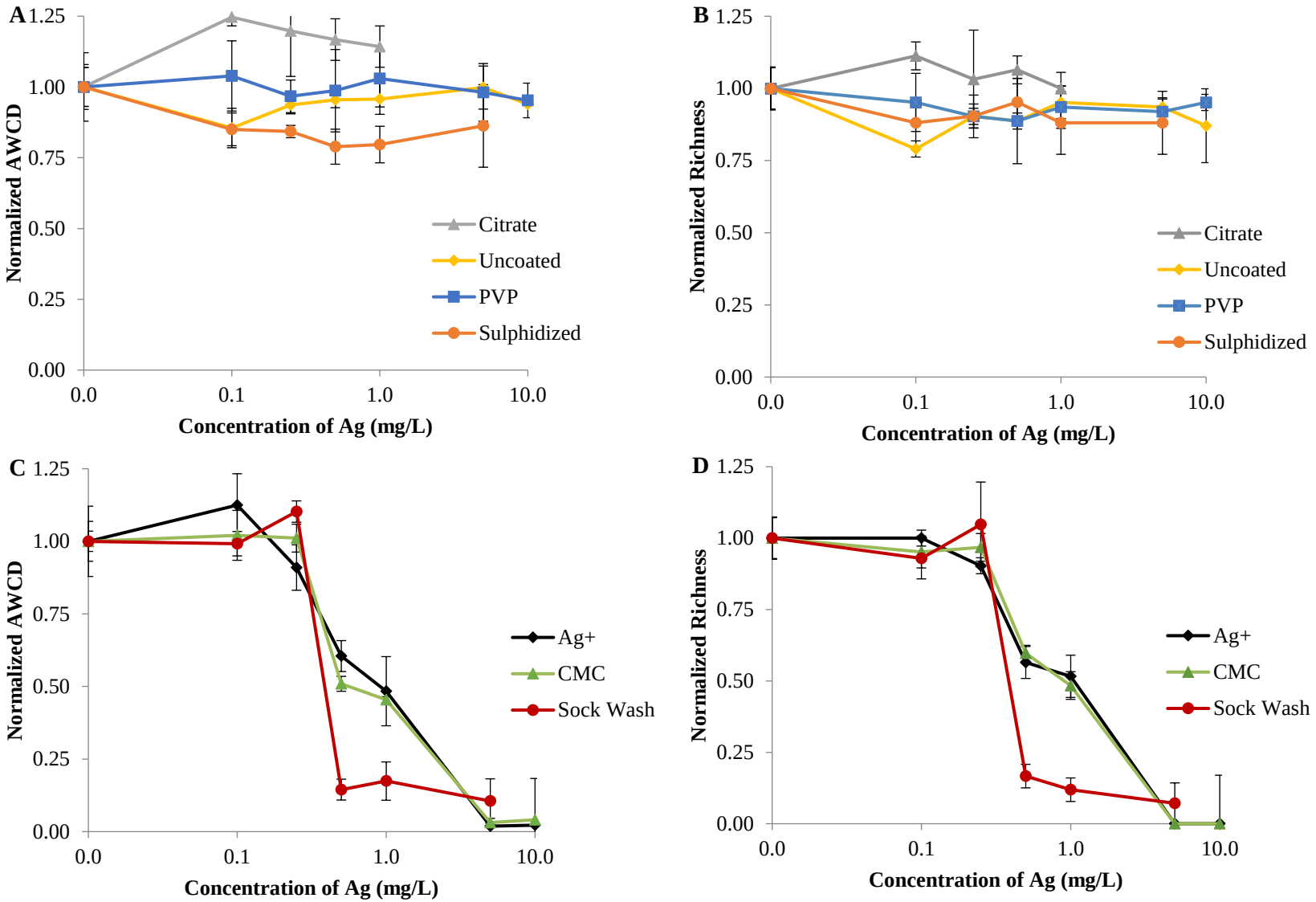

Figure 5.2: Dose-response curves for microbial communities from non-aerated constructed wetlands. (A, B) Average well colour development (AWCD) and (B, D) richness, calculated as number of carbon sources utilized, for interstitial water microbial communities from non-aerated constructed wetlands over increasing silver dose. Data points are the average of triplicate measurements (on the same plate) and error bars indicate standard deviation of the mean. X-axis is represented in the log scale.

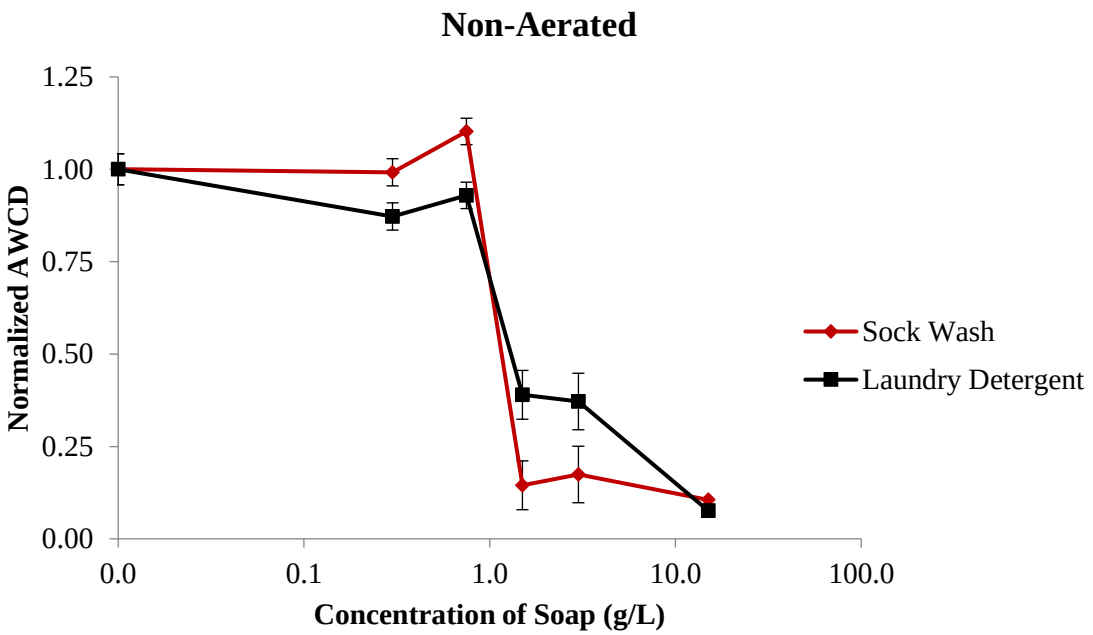

Figure 5.3: Dose-response curves for average well colour development (AWCD) from interstitial water microbial communities from non-aerated constructed wetlands over increasing laundry detergent. Data points are the average of triplicate measurements (on the same plate) and error bars indicate standard deviation of the mean. X-axis is represented in the log scale.

5.4.3 Effects of Artificial Aeration on Silver Toxicity to Constructed Wetland Microbial Communities

Artificial aeration is employed in CW technology in oxygen-limited sub-surface flow wetlands to favor microbial catabolic processes which are limited by the availability of oxygen, namely nitrification, but it may also increase the speed of ammonification and organic matter degradation (Butterworth et al. 2013). The addition of aeration to the wetland bed cause changes to the oxidative-reductive (redox) potential, and allows aerobic wastewater treatment processes to take place (Section 0 and Section 3.4.2). While this changes pollutant removal processes within the CW, it also creates a different chemical and biological environment for Ag NP transformations, in comparison to the non-aerated CW.

Dose response curves for the overall catabolic function (AWCD) and the number of carbon sources utilized (richness) for microbial communities from aerated mesocosms are displayed in Figue 5.4, A and B (Tukey-HSD groupings of silver treatment in Table C.6 and Table C.7). In general, the toxicity observed in the aerated microbial communities is considerably greater than that for non-aerated microbial communities for both AWCD and richness.

A non-monotonic trend can be observed for the silver-containing sock wash water for both the AWCD and richness for aerated microbial communities over increasing silver concentration (Figure 5.4). This trend is not observed for the laundry detergent control solutions (Figure 5.5). The term non-monotonic dose-response describes a curve whose slope changes direction within the range of tested doses (Lagarde et al. 2015). Non-monotonic dose-response trends are often reported in the literature for hormones and endocrine disruptors (Lagarde et al. 2015), but have also been reported for silver nanomaterials (Bicho et al. 2016). Opposing effects (agonist vs. antagonist) across a range of tested doses are often proposed to explain this phenomenon. For instance, a molecular target which is differentially activated by the same substance at different concentration levels. A compound may interact with one receptor at low concentrations and another at higher concentrations, which provides opposing effects for the test organism, creating a non-monotonic dose-response curve (Lagarde et al. 2015). In the present study, this may be the result of laundry detergent constituents (Table C.1) and silver providing both positive and negative inputs for the microbial community. Additionally, it could be a function of microbial population changes at varying concentrations of silver and laundry detergent. A competitive community, in which certain slow growing bacteria are supressed by other bacteria, may change as species are inhibited allowing carbon utilization by other species. At low concentrations, the laundry detergent is non-toxic and can serve as a carbon source for the microbial community (Figure 5.5). At higher silver/laundry detergent concentrations, the sock wash solution and the laundry detergent solution display the same trend. Therefore, the overall toxicity effect observed is likely a result of the detergent rather than the silver in the sock wash solution.

For all silver treatments except sulphidized Ag NPs and silver-containing sock wash water, AWCD and richness decreased with increasing silver concentrations. Complete inhibition of microbial community catabolic activity was observed at 5 mg Ag/L for PVP-coated and uncoated Ag NPs, and 0.1 mg/L for Ag^+ and CMC-coated Ag NPs, which was the lowest concentration, tested initially. Additional analysis was performed to analyze the effect of Ag^+ and CMC-coated Ag NPs at lower concentrations (0.005 to 0.05 mg/L), results of which are depicted in Figure 5.4. At 0.005 mg Ag/L of ionic silver, Ag^+, the microbial community is still significantly inhibited,

while for CMC-coated Ag NPs close to complete inhibition is observed even at this very low concentration.

No negative effects were observed from artificially sulphidized Ag NPs to the AWCD and richness of aerated microbial communities. This supports results of the toxicity testing on microbial communities from non-aerated wetlands exhibiting limited toxicity (Figure 5.2), likely due to *in-situ* sulphidation to varying degrees. Reinsch et al. (2012) reported reduced bacterial growth inhibition with increasing sulphidation of PVP-coated Ag NPs. This may be a result of decreased dissolution of Ag^+ from the sulphidized Ag NPs (Levard et al. 2011) or a decrease in contact between microbes and metallic silver, due to coating with sulphur and subsequent loss of surface area (Reinsch et al. 2012).

The aqueous chemistry of silver and Ag NPs is complex. Metallic silver is thermodynamically unstable under most environmental conditions and will oxidize or react with natural organic matter and inorganic ligands (Liu and Hurt 2010; Xiu et al. 2011). Many silver complexes involve Ag in an oxidation state of +1. Therefore, the Ag^0 core in Ag NPs requires oxidation prior to complexation with inorganic and organic compounds (Levard et al. 2012). With the added oxygen from the artificial aeration in the CW, the oxidation of Ag^0 to Ag^+ will occur even more readily. Ag^+ ions interact strongly with thiol groups, which can inactivate important enzymes, including those involved with the electron-transport chain, which in turn affects cellular oxidation, RNA translation and DNA replication (Morones et al. 2005; Kim et al. 2007; Gordon et al. 2010; Massarsky et al. 2014). Navarro et al. (2008) concluded that Ag NPs alone have minimal toxicity and serve mostly as a source of Ag^+ ions. Another study came to a similar conclusion, that the dissolution of silver ions from the nanoparticle dictate their toxicity (Miao et al. 2009). The dissolution of Ag^+ ions from Ag NPs requires an aerobic, oxidizing environment. Elemental Ag NPs are considered by some to be a reservoir of Ag^+ especially when in oxidized aqueous environments where the following reaction can proceed (Tolaymat et al. 2010):

$$O_{2\,(aq)} + 4H_3O^+ + 4Ag_{(s)} \rightarrow 4Ag^+_{(aq)} + 6H_2O \qquad \text{(f)}$$

For future experimentation, an evaluation of pH changes over the experimental period is recommended which may inform whether reaction (f) is taking place.

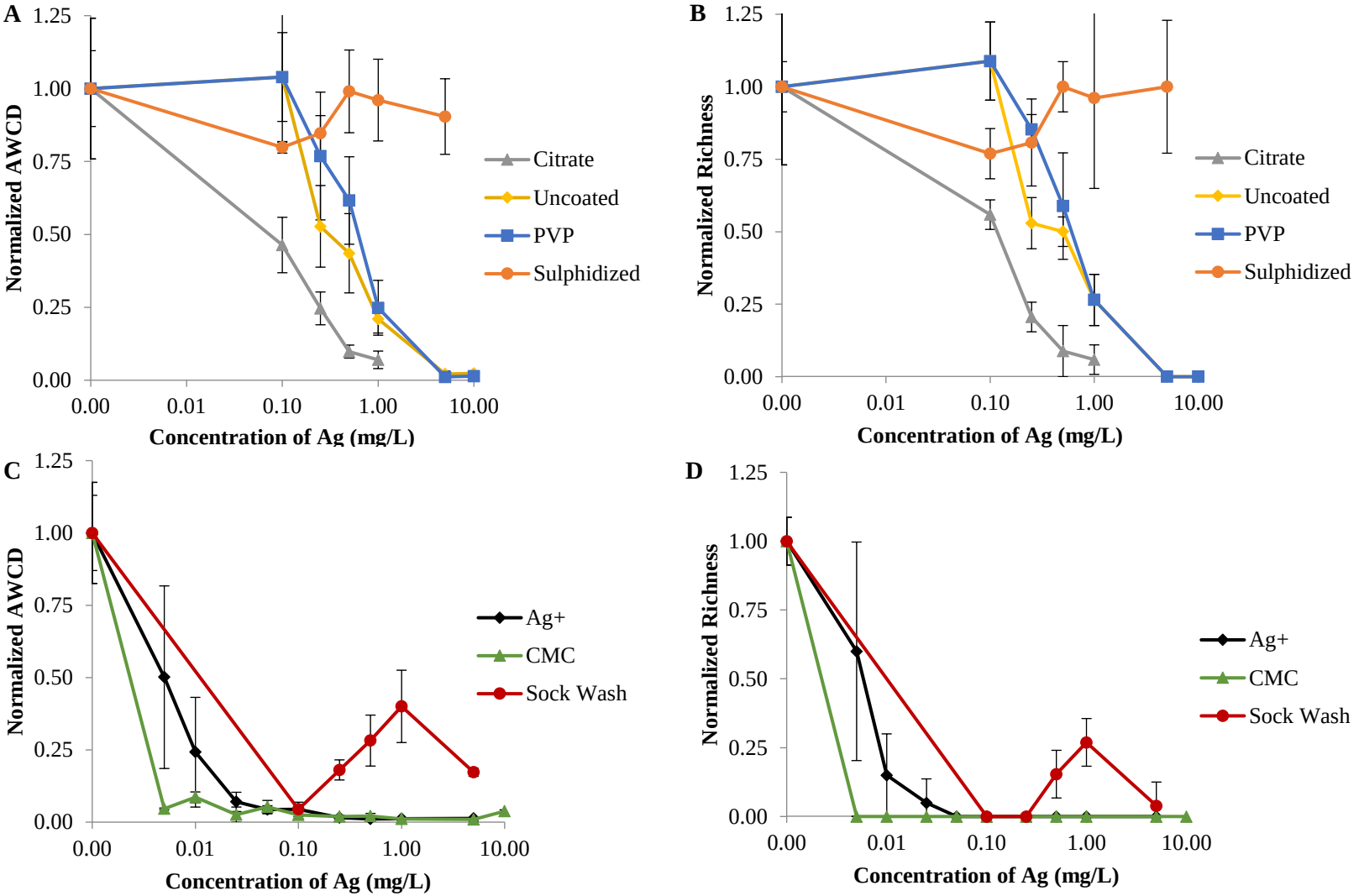

Figure 5.4: Dose-response curves for microbial communities from aerated constructed wetlands. (A, C) Average well colour development (AWCD) and (B, D) richness, calculated as number of carbon sources utilized, for interstitial water microbial communities from aerated constructed wetlands over increasing silver dose. Data points are the average of triplicate measurements (on the same plate) and error bars indicate standard deviation of the mean. X-axis is represented in the log scale.

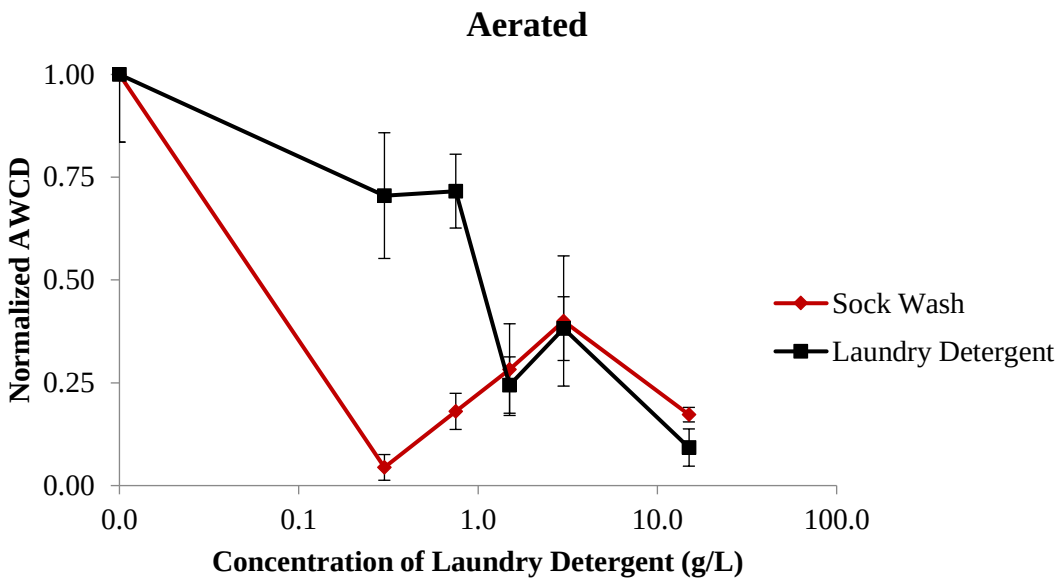

Figure 5.5: Dose-response curves for average well colour development from aerated interstitial water microbial communities from constructed wetlands over increasing laundry detergent. Data points are the average of triplicate measurements (on the same plate) and error bars indicate standard deviation of the mean. X-axis is represented in the log scale.

5.4.4 Potential Effects of Silver Nanoparticles on Wastewater Treatment

This subsection will relate the toxicity observed by Ag NPs on microbial communities from aerated and non-aerated CW mesocosms to potential effects of Ag NPs to wastewater treatment. First, a minimum community level catabolic effect concentration (MCLCEC) is used to define the minimum tested concentration of silver which causes a statistically significant reduction in the catabolic capabilities of the microbial community studied. This measure is appropriate for assessing the risk associated with Ag NPs on CW microbial communities as any reduction in catabolic activity/function will have implication for the viability of wastewater treatment. At the MCLCEC, the catabolic activity/function of the microbial community is significantly different from the control, which when thought of in terms of wastewater treatment could result in a significant decrease in wastewater treatment performance. Secondly, an EC_{50} was calculated for each nanoparticle type as this is a common way to compare toxicity in risk assessment. Finally, silver toxicity to the microbial communities will be evaluated based on carbon source guild utilization (according to chemical structure as carbohydrates, polymers, carboxylic acids, amino acids, and amines/amides) and related to wastewater treatment performance.

A summary of the minimum community level catabolic effect concentration (MCLCEC) for each nanoparticle type, as defined by Weber et al. (2014), is listed in Table C.8 and Table C.9. Ag NPs have been estimated to reach household CWs at approximately 0.1 Ag mg/L (Button et al. 2016). Based on the MCLCEC's presented in Table C.8 and Table C.9 microbial communities from CWs which are artificially aerated could be at risk for catabolic inhibition at concentrations which are relevant to the environment. Ionic silver, CMC-coated Ag NPs and citrate-stabilized Ag NPs have MCLCEC's at or below 0.1 mg/L in aerated environments, for either catabolic function (AWCD) or catabolic richness. Therefore, the ability to degrade a variety of contaminants at an appreciable level could be at risk. The most alarming result is the MCLCEC's for the Ag NP-containing sock wash water. Microbial communities from aerated mesocosms are inhibited in terms of both AWCD and richness at 0.1 mg/L. This is the most environmentally relevant scenario and concentration tested. Household wastewater containing silver nano-wash water discharged directly to an aerated CW could impact the microbial community within the system and thus pollution removal provided by the CW.

A MCLCEC was calculated for a positive response (citrate Ag NPs, 0.1 mg/L) for the AWCD associated with non-aerated microbial communities. After which, no significant affect (positive or negative) was observed for citrate Ag NPs. As previously discussed, this may be due to the utilization of the citrate coating used to stabilize the nanoparticle in solution as a carbon source by the microbial community.

No MCLCEC could be determined for sulphidized particles for either AWCD or richness for both aerated and non-aerated microbial communities, as there was no significant catabolic affect to the microbial communities at any concentration tested, even 10 mg/L. For non-aerated microbial communities no MCLCEC was recorded for uncoated (AWCD), PVP-coated (both AWCD and richness) or citrate-stabilized (richness), again even at the highest concentrations tested, 10 mg/L (1 mg/L for citrate). Therefore, there was no significant level of toxicity from the Ag NPs to the microbial communities at the concentrations tested. It is proposed that this is a result of the *in-situ* sulphidation of the nanoparticles within the experimental media (non-aerated wetland water) which hinders the contact of silver with bacteria and the dissolution of Ag^{+} from the nanoparticle shell.

Another more common way to look at the toxicity of Ag NPs from an ecotoxicity perspective is with an effective concentration (EC_{50}) (Table 5.2). An EC_{50}, represents the concentration at which the population observes a 50 percent effect from the toxicant. For this experiment, this was calculated for both AWCD and richness (Figure 5.6, Figure C.1). In most cases the EC_{50} for the richness and the AWCD are in agreement. As noted above for the MCLCECs, ionic silver, CMC-coated Ag NPs and citrate-stabilized Ag NPs have EC_{50}s at or below 0.1 mg/L in aerated environments, for both catabolic function (AWCD) and catabolic richness. Therefore, the ability to degrade a variety of contaminants at an adequate level could be very much at risk. Again, the Ag NP-containing sock wash water had an EC_{50} of much less than 0.1 mg/L for microbial communities from aerated mesocosms. Therefore, the microbial communities will be inhibited by over 50 percent in terms of both AWCD and richness at 0.1 mg/L. EC_{50}s which could be calculated for the non-aerated microbial communities where much higher between 0.4 and 1 mg/L. However, Button et al. (2016) calculated undiluted wastewater from washing silver containing fibres could contain up to 1.5 mg/L of silver. If this undiluted wastewater were to end up in a CW it may significantly affect the ability of the microbial community to treat wastewater.

No EC_{50}s could be calculated for sulphidized particles for either AWCD or richness for both aerated and non-aerated microbial communities as the highest concentrations tested in this study did not provide a 50 percent effect to the microbial community. For non-aerated microbial communities, no EC_{50}s could be calculated for citrate, uncoated and PVP AgNPs for both AWCD and richness.

Table 5.2: Summary of EC50s for aerated and non-aerated microbial communities treated with various types of silver and silver nanoparticles. EC_{50}s were calculated by linear interpolation for both AWCD and richness. N/A = EC_{50} could not be calculated for that treatment as concentrations applied in this study were not high enough to create a 50% effect. EC_{50}s were calculated using raw data (Appendix C, Table C.10).

Silver Treatment	**EC_{50} (AWCD)**		**EC_{50} (Richness)**	
	Aerated	**Non-Aerated**	**Aerated**	**Non-Aerated**
Ag+	0.005	0.930	0.006	1.183
CMC	0.003	0.500	0.002	0.500
Sock Wash	0.052	0.407	0.050	0.405
Citrate	0.134	N/A	0.125	N/A
Uncoated	0.325	N/A	0.501	N/A
PVP	0.658	N/A	0.637	N/A
Sulphidized	N/A	N/A	N/A	N/A

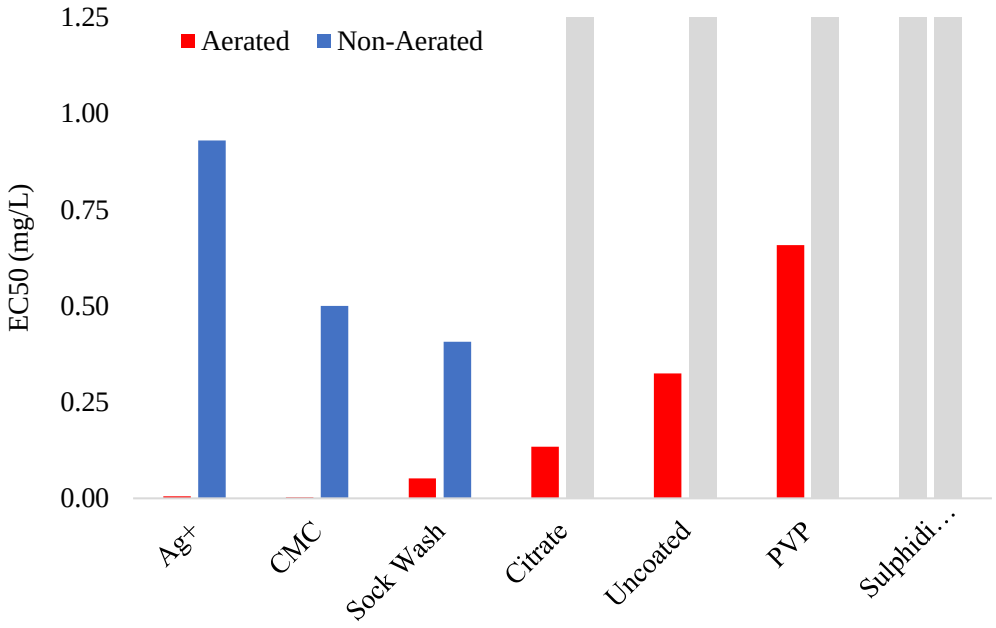

Figure 5.6: Summary of EC50s for aerated (red) and non-aerated (blue) microbial communities treated with various types of silver and silver nanoparticles. EC_{50}s were calculated by linear interpolation for AWCD. Grey bars = EC_{50} could not be calculated for concentrations within the scope of this study as silver dosing was not high enough to create a 50% effect. EC_{50}s were calculated using raw data (Appendix C, Table C.10). Data is compiled from triplicated measurements which are built into the BIOLOG EcoPlates™.

Community Level Physiological Profiling is a powerful tool for CW research. As previously mentioned, it involves the use of BIOLOG EcoPlates™, which contain 31 carbon sources. In addition to the AWCD and richness metrics presented previously as dose-response curves, carbon-source specific dose-response curves can also be visualized, for example (Figure 5.7). Figure 5.7 provides information about the microbial community's ability to utilize each individual carbon source. The dose-response for each carbon source is unique with respect to the effects of increasing silver concentration and the type of silver dosed. The information provided in this type of analysis is extensive. The carbon sources on the BIOLOG EcoPlate™ can be simplified by their carbon source guild as described in Section 3.3.1.3 and plotted graphically (example plotted in Figure 5.8) or in a table format (Table 5.3) with respect to the dose of silver. This analysis allows for rapid investigations to understand whether one general type of catabolic function is being hindered or eliminated with respect to Ag NP toxicity. This provides information for wastewater treatment performance by identifying which types of pollutants (chemical structure) will be affected by Ag NPs preferentially.

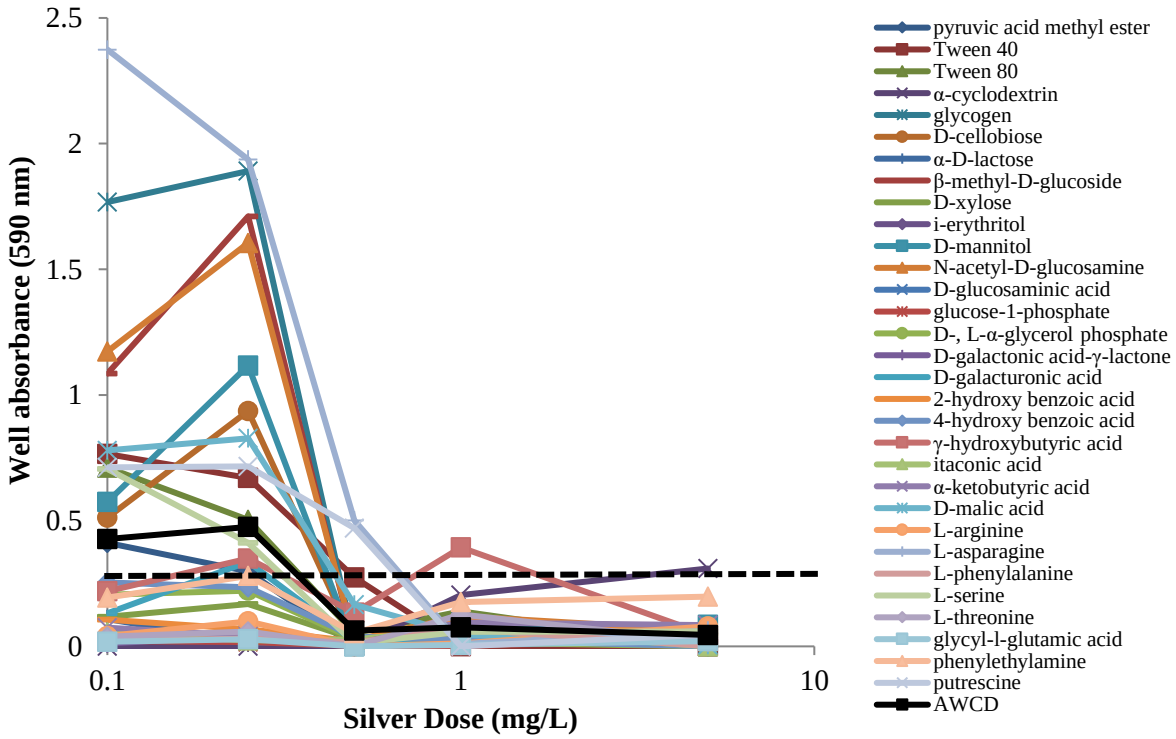

Figure 5.7: Well absorbance (colour development measured at 590 nm) for each individual carbon source (31 in total) after 40 hours plotted against increasing silver dose (mg/L). This is a representative graph from the sock wash water and the microbial community from an aerated mesocosm wetland. The data plotted in black is the overall AWCD for all 31 carbon sources. The dotted black line represents the richness cut-off of 0.25. X-axis is in the log scale.

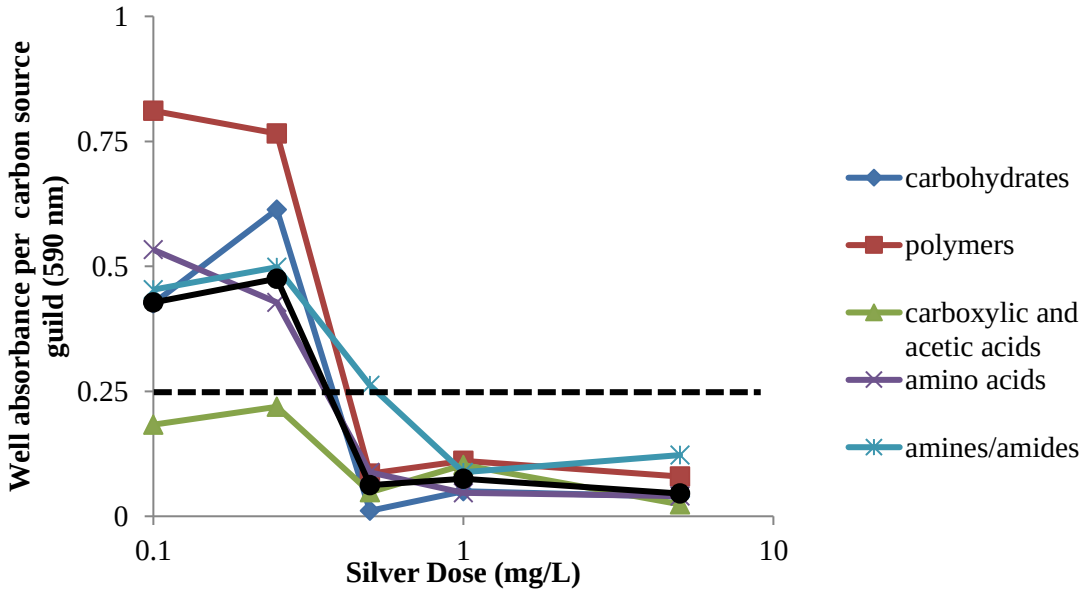

Figure 5.8: Well absorbance calculated per carbon source guild (colour development measured at 590 nm). This is a representative graph from the sock wash water and the microbial community from an aerated mesocosm wetland. The data plotted in black is the overall AWCD for all 31 carbon sources. The dotted black line represents the richness cut-off of 0.25. X-axis is in the log scale.

The information from the carbon source guild analysis is summarized in Table 5.3, where carbon sources were considered utilized if absorbance of the well colour development was over 0.25. This was given a check mark (✓) in the case that the carbon source was not utilized it was given an X. The utilization of carbon source guilds changed drastically with respect to concentration of silver, type of silver and whether the microbial community was from an aerated or non-aerated CW mesocosm. PVP-coated Ag NPs were not toxic to microbial communities from non-aerated mesocosms and all carbon source guilds were utilized at all concentrations. For aerated mesocosms, PVP-coated Ag NPs reduced catabolic function to carboxylic and acetic acids first, followed by carbohydrates, polymers, amino acids and finally amines and amides. Uncoated Ag NPs followed the same carbon source utilization pattern (CSUP) as PVP-coated Ag NPs both for microbial communities from aerated and non-aerated mesocosms. Citrate-stabilized Ag NPs did not affect the utilization of any carbon source guild for microbial communities from non-aerated mesocosms. For aerated microbial communities, amino acids were the only carbon source utilized and this was only at 0.1 and 0.25 mg/L Ag. Ionic silver and CMC-coated Ag NPs produced the same carbon source utilization trends for microbial communities from both aerated and non-aerated mesocosms. Inhibition of the utilization of carbohydrates was first at 0.5 mg/L Ag, followed by all other guilds at 5 mg/L Ag for non-aerated mesocosms. No carbon sources utilization for any guild was recorded for aerated mesocosms. In non-aerated mesocosms, artificially sulphidized Ag NPs inhibited microbial utilization of carboxylic and acetic acids at all concentrations tested (0.1 – 5 mg/L Ag) but no other guilds were affected. In aerated mesocosms, polymers and amines/amides were utilized at all concentrations but all other guilds were completely inhibited at all concentrations. The sock wash water inhibited the utilization of all carbon source guilds at all concentrations in aerated mesocosms. In non-aerated mesocosms, utilization of carboxylic and

acetic acids were inhibited at all concentrations tested (0.1 – 5 mg/L Ag), carbohydrates, polymers and amino acids were inhibited completely by 0.5 mg/L Ag and amines/amides by 1 mg/L Ag.

Trends in carbon source utilization vary with respect to concentration of silver, type of silver and whether the microbial community was from an aerated or non-aerated CW mesocosm. In general, it appears that the utilization of carbohydrates and carboxylic and acetic acids by microbial communities from non-aerated systems are preferentially inhibited, while amines/amides are the most readily utilized with increasing silver concentration. Utilization of carbon sources by microbial communities from aerated CWs are more acutely affected by Ag NPs, with carboxylic and acetic acids the guild group most affected while polymers, amino acids, and amines/amides the lesser affected guild groups. The silver nanoparticles may preferentially affect fast growing bacterial species such as those which are proficient in the breakdown of easily degradable organics. This may be why the several guild metabolic functionalities are reduced first while the ability to break down amines/amides, which contain nitrogen, remains functional at higher concentrations. Bacterial species which cycle nitrogen are known to be slower growing. Reduction in the ability to degrade carboxylic and acetic acids, carbohydrates and polymers may affect a CWs ability to remove both simple and complex carbon inputs from wastewater.

Table 5.3: Utilization of carbon source guilds on the BIOLOG EcoPlate™ with respect to silver dose. A carbon source was considered utilized when a blank-corrected absorbance value was above 0.25. ✓ = carbon source utilized. X = carbon source not utilized. Grey box = concentration not used for that specific silver treatment. Silver treatment as list down the left side of the table.

		NON-AERATED						**AERATED**					
		Concentration of Ag (mg/L)						**Concentration of Ag (mg/L)**					
		0.1	**0.25**	**0.5**	**1**	**5**	**10**	**0.1**	**0.25**	**0.5**	**1**	**5**	**10**
	GUILD												
PVP	carbohydrates	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X
	polymers	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X
	carboxylic and acetic acids	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
	amino acids	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X
	amines/amides	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
CMC	carbohydrates	✓	✓	X	X	X	X	X	X	X	X	X	X
	polymers	✓	✓	✓	✓	X	X	X	X	X	X	X	X
	carboxylic and acetic acids	✓	✓	✓	✓	X	X	X	X	X	X	X	X
	amino acids	✓	✓	✓	✓	X	X	X	X	X	X	X	X
	amines/amides	✓	✓	✓	✓	X	X	X	X	X	X	X	X
UNCOATED	carbohydrates	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X
	polymers	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X
	carboxylic and acetic acids	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X
	amino acids	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
	amines/amides	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	X
Ag+	carbohydrates	✓	✓	X	X	X	X	X	X	X	X	X	X
	polymers	✓	✓	✓	✓	X	X	X	X	X	X	X	X
	carboxylic and acetic acids	✓	✓	✓	✓	X	X	X	X	X	X	X	X
	amino acids	✓	✓	✓	✓	X	X	X	X	X	X	X	X

SULPHIDIZED	amines/amides	✓	✓	✓	✓	X	X		X	X	X	X	X	X
	carbohydrates	✓	✓	✓	✓	✓			X	X	✓	X	X	
	polymers	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
	carboxylic and acetic acids	X	X	X	X	X			X	X	X	X	X	
	amino acids	✓	✓	✓	✓	✓			X	X	X	X	X	
	amines/amides	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
SOCK WASH	carbohydrates	✓	✓	X	X	X			X	X	X	X	X	
	polymers	✓	✓	X	X	X			X	X	X	X	X	
	carboxylic and acetic acids	X	X	X	X	X			X	X	X	X	X	
	amino acids	✓	✓	X	X	X			X	X	X	X	X	
	amines/amides	✓	✓	✓	X	X			X	X	X	X	X	
CITRATE	carbohydrates	✓	✓	✓	✓				X	X	X	X		
	polymers	✓	✓	✓	✓				X	X	X	X		
	carboxylic and acetic acids	✓	✓	✓	✓				X	X	X	X		
	amino acids	✓	✓	✓	✓				✓	✓	X	X		
	amines/amides	✓	✓	✓	✓				X	X	X	X		

5.5 Conclusions

The ecotoxicological effects of various types of Ag NPs were evaluated in reference to wetland interstitial water microbial communities from CW mesocosms in artificially aerated and non-aerated environments. A range of concentrations (0 to 10 mg/L) were examined in this study to quantify the potential effects of Ag NPs on CW microbial communities.

The aerated and non-aerated environments from which water and interstitial water microbial communities were sampled for this experiment had very different physiochemical properties which influenced the toxicity of the different types of Ag NPs studied. In general, the ecotoxicological effects of Ag NPs and ionic silver were more acute in the aerated environment. This can be explained by the fundamental aqueous chemistry of silver. The increased dissolved oxygen in water sampled from aerated mesocosms allows the Ag^0 core of the Ag NPs to become oxidized to Ag^+, and exert toxicity mechanisms related to those of ionic silver. The overall toxicity trend of silver treatments tested on interstitial water microbial communities from aerated CWs from least toxic to most toxic silver treatment: sulphidized Ag NPs, uncoated = PVP-coated Ag NPs, citrate-stabilized Ag NPs, sock wash water, Ag^+ and CMC-coated Ag NPs. The wash water from Ag NP containing socks catabolically inhibited the microbial community at a concentration which is environmentally relevant for wastewater release of silver (0.1 mg/L).

Little to no effect was observed on the microbial communities from non-aerated CWs when exposed to uncoated, PVP-coated and artificially sulphidized Ag NPs. This can be attributed to a combination of three things. Firstly, the coating on the nanoparticle itself hindering bioavailability of silver and decreasing toxicity. Secondly, inorganic ligands such as sulphide and chloride can react with silver and hinder bioavailability, blocking toxicity mechanisms. Finally, the anaerobic conditions found in the non-aerated wetlands (low dissolved oxygen, low redox) prevent metallic silver, Ag(0), oxidation and subsequent Ag^+ release reducing the toxicity observed for many nanoparticles tested.

This *ex-situ* method of analysis, utilizing CLPP, was sensitive enough to differentiate the effects of a range of silver concentrations on microbial communities and their overall functional catabolic activity as well as distinct carbon source utilization patterns. Ag NPs preferentially reduced the utilization of carbohydrates and carboxylic and acetic acids which should be a note of caution for CW engineers designing systems receiving wastewater containing municipal waste or Ag NP industrial waste.

Moving forward, the use of weathered Ag NPs is recommended for ecotoxicity studies with microbial communities from CWs. Using the most relevant nanoparticles to the release situation and environmental conditions will provide the most pertinent information to the field. Ag NPs are hard to study as any transformations which take place will significantly affect their toxicity, therefore weather Ag NPs should be studied over pristine particles were applicable.

6 CHAPTER 6: PRINCIPAL OUTCOMES AND RECOMMENDATIONS

6.1 Research Objectives

The overall objective of this thesis was to observe and quantify constructed wetland mesocosm dynamics during development periods, and the anti-microbial effects of silver nanoparticles.

A. Characterize the development (start-up) period of aerated and non-aerated constructed wetland mesocosms planted with *Phalaris arundinacea*.

B. Characterize the plant initialization and establishment of *Phalaris arundinacea* in unplanted, well-developed aerated and non-aerated constructed wetland mesocosms.

C. Quantify the effects of various types of silver nanoparticles (pristine and weathered) on interstitial microbial communities from constructed wetlands.

Figure 6.1 outlines a timeline for project work and experiment work associated with this Master's thesis from Winter 2015 to Fall 2016.

Figure 6.1: Research Timeline

6.2 Objective A: Characterize the development period of aerated and non-aerated constructed wetland mesocosms planted with *Phalaris arundinacea*.

Twelve constructed wetland mesocosms were built and inoculated with activated sludge from a local wastewater treatment plant. Six mesocosms were artificially aerated and the other six remained non-aerated. All mesocosms were seeded with *Phalaris arundinacea* on the day of inoculation. The twelve mesocosms naturally developed for 12 weeks while water chemistry, system hydrology, pollutant removal and microbial community metrics were characterized.

Overall, stabilization of water chemistry parameters occurred readily. Differentiation was evident between aerated and non-aerated mesocosms from the outset for a number of water parameters (ORP, pH, nitrate, ammonia, dissolved oxygen). Start-up efficiency was not enhanced for aerated systems. The time required for the microbial community to stabilize, in terms of catabolic function and overall activity, in both aerated and non-aerated mesocosm was equal,

requiring approximately 60 to 75 days. The addition of artificial aeration enhanced the rate of removal of total organic carbon (TOC) but hindered the removal of total nitrogen (TN) in comparison to the non-aerated environment. The addition of artificial aeration did not affect wetland hydrology (porosity, evapotranspiration, dispersivity) over the wetland start-up period. Porosity generally decreased in all mesocosms over the start-up period which can be attributed to the development of biofilm on the wetland bed media, which coincides with an increase in microbial activity and catabolic function. The addition of artificial aeration to the CW mesocosms created a more volatile microbial population which often did not display as similar trends in carbon source utilization between system replicates. In non-aerated systems, this was not observed; the CSUPs between system replicates were more stable and converged together over the start-up period.

6.3 Objective B: Characterize the plant initialization and establishment of *Phalaris arundinacea* in unplanted, well-developed aerated and non-aerated constructed wetland mesocosms.

Twelve recirculating saturated vertical flow constructed wetlands were allowed to develop naturally, unplanted for four months prior to planting with *Phalaris arundinacea.* Six replicates were artificially aerated and six remained non-aerated. CWs are usually planted directly after construction and plants establish themselves over the same time frame as the microbial population, during a start-up phase. Therefore, it is difficult to distinguish the effects of natural changes during a start-up period to those coming from plant establishment. The experimental design allowed for an holistic evaluation of changes to metrics encompassing overall wetland dynamics (water chemistry, water treatment, hydrological and microbial parameters) as a result of the addition of vegetation (from seed).

Phalaris arundinacea growth was not as healthy in the aerated mesocosms. Artificial aeration caused a yellowing in the leaves, less overall stems and shorter height in comparison to plants in non-aerated mesocosms. The establishment of plants (*Phalaris arundinacea*) in the aerated and non-aerated CW mesocosms exerted a stabilizing effect on the microbial community, which effectively bridged the microbial catabolic functionality between the two system types (aerated and non-aerated). An increase in both the mass removal of TN and TOC was observed in non-aerated systems over the plant establishment period. In aerated systems, TOC removal was high (>95%) independent of plant addition, TN removal fluctuated more after plant establishment. The effect of plants on the mesocosms was more defined in non-aerated systems than in aerated systems. The ability of plants to mediate oxygen release to the wetland subsurface via their roots is likely the cause of this difference.

6.4 Objective C: Quantify the effects of various types of silver nanoparticles (pristine and weathered) on interstitial microbial communities from constructed wetlands.

An *ex-situ* ecotoxicity assessment was performed to gain a better understanding of the potential effects of various types of silver nanoparticles on constructed wetland interstitial microbial communities. Microbial communities from constructed wetland mesocosms in artificially aerated and non-aerated environments were exposed to silver nanoparticles over a range of concentrations (0 to 10 mg/L). Four pristine silver nanoparticles were exposed to interstitial water microbial community samples including polyvinylpyrrolidone (PVP) coated, citrate

stabilized, uncoated and carboxymethyl cellulous (CMC) coated silver nanoparticles. Additionally, two weathered particles (from silver containing consumer athletic socks and an artificially sulphidized Ag NP) were also evaluated. Ionic silver in the form of silver nitrate, $AgNO_3$, was used as a positive control.

In general, the ecotoxicological effects of silver nanoparticles and ionic silver were much more acute in the aerated environments. This is likely a result of fundamental differences in water chemistry between wetland types and the nature of aqueous chemistry of silver. CMC-coated Ag NPs were identified as 50% ionic silver and were the most toxic nanoparticle to interstitial water microbial communities from aerated constructed wetland mesocosms. The silver nanoparticle-containing sock wash water was the next most toxic, followed by citrate-stabilized Ag NPs, uncoated and PVP-coated Ag NPs. Artificially sulphidized Ag NPs were not toxic to the microbial community. The wash water from silver nanoparticle containing socks catabolically inhibited the microbial community at a concentration which is environmentally relevant for wastewater release of silver (0.1 mg/L).

No effect was observed on the microbial communities from non-aerated constructed wetlands when exposed to uncoated, PVP-coated and artificially sulphidized silver nanoparticles at concentrations up to 10 mg/L. This can be attributed to a combination of three things: coating effects decreasing bioavailability and toxicity of silver, silver binding with inorganic ligands (sulphide and chloride) in the anaerobic environment and the anaerobic conditions found in the non-aerated wetlands (low dissolved oxygen, low redox) preventing silver, Ag(0), oxidation and subsequent Ag^+ release. However, Ag^+, CMC-coated Ag NPs and the sock wash water still completely inhibited the non-aerated microbial community catabolic activity within the concentration range tested (by 5 mg/L for all silver treatments).

Upon examination of the carbon source utilization patterns of the wetland microbial communities, silver nanoparticles preferentially reduced the utilization of carbohydrates and carboxylic and acetic acids. This finding should be a note of caution for constructed wetland engineers designing systems receiving wastewater containing municipal waste or silver nanoparticle industrial waste.

6.5 Scientific Contribution of Thesis

The goal of this thesis was to gain a rigorous, fundamental understanding of the differences between aerated and non-aerated constructed wetlands and apply this understanding in the assessment of a new emerging contaminant, silver nanoparticles, on constructed wetland microbial communities. The largest scientific contribution of this thesis is the data gained from the start-up monitoring of the aerated and non-aerated constructed wetland mesocosms. Prior to this thesis no direct comparison between aerated and non-aerated constructed wetlands (of any size) had gone into this amount of detail to compare the technologies. This will further the use of aeration, which is a developing technology, to more widespread application in constructed wetlands. Also, the information gained regarding pollutant removal in aerated constructed wetlands may allow constructed wetlands to be used to treat more complex wastewaters and take on new wastewater applications. Insight was gained into the operation of aerated constructed wetlands, in that they can be planned the same as non-aerated systems in terms of start-up length.

New information was gained in regards to the effects of plants on constructed wetlands. According to previous literature, the benefits and effects of plants on constructed wetland treatment

performance has not been assessed in detail. This thesis revealed that the microbial community in constructed wetlands is much more important to the total system function of constructed wetlands than plants.

Additionally, insight was gained as to the effects of silver nanoparticles on the microbial communities from constructed wetlands. Constructed wetlands, which are currently in operation and actively treating wastewater, may be at risk from this new emerging contaminant as effects were observed on interstitial water microbial communities in this thesis. Further research should investigate the effects of silver nanoparticles on constructed wetlands and their microbial communities based on the results from the interstitial water microbial screening performed in this thesis. Moreover, the data from this thesis may be extended to inform policy makers on the potential effects of silver nanoparticles to natural wetland microbial communities. Silver nanoparticles are not currently regulated in Canada in terms of wastewater discharge and the results from Chapter 5 of this thesis may help in the decision making regarding their regulation.

6.6 Conclusions and Recommendations for Further Research

The three studies performed in this thesis may also be thought of as perturbation studies on the wetland steady-state. For the start-up study, the initialization of the steady state was monitored for a variety of wetland characteristics. The wetland chemical and physical characteristics influenced the microbial community which was the driving force in the development of the wetland ecosystem over time. Tracking the addition of plants to well-developed constructed wetland systems allows resolution from changes to the microbial community. Typically, the effect of plants on constructed wetlands are evaluated at the same time as the wetland initialization, therefore the microbial community is changing naturally and trying to develop a steady state. By adding plants to a well-developed unplanted constructed wetland the plants themselves cause the perturbation to the wetland system and their effects can be further explored. The final study in this thesis looked at the effects of silver nanoparticles on wetland microbial communities and the potential effects on wastewater treatment. This again can be thought of as an antimicrobial perturbation on the wetland steady state.

This thesis also focused on understanding fundamental differences between aerated and non-aerated constructed wetlands, as well as the effects of adding plants to stabilized constructed wetlands. These differences were applied in understanding the risk of an emerging contaminant, silver nanoparticles, and the potential negative effects it may cause on microbial community health and subsequent pollutant removal in constructed wetlands. The aerated and non-aerated wetland environments more likely than not foster entirely different microbial communities which will respond to the stresses of a toxicant, such as silver nanoparticles, differently as evidenced in this thesis. The physiochemical parameters of the constructed wetland will also play into transformations of and toxicity mechanisms available to silver nanoparticles. The effect silver nanoparticles have on the microbial community in a constructed wetland as a whole will affect the wastewater treatment of that wetland, and potentially the safety of the surface water to which it discharges. It is therefore critically important to understand the toxicity mechanisms of silver nanoparticles in constructed wetlands.

Based on the knowledge gained in this thesis, recommendations for future work include:

1) Observe the impact of variation in aeration regimes (constant, variable) on development of microbial communities and wastewater treatment performance in constructed wetlands.

2) Study constructed wetlands from a variety of perspectives (physical, chemical, microbial, vegetation, water treatment potential) using multiple lines of evidence for each perspective is recommended to elucidate subtle interactions between microbial communities, plants and wastewater components.

3) Perform bacterial species sequencing on water samples from the aerated and non-aerated constructed wetlands to evaluate which species are present for pollutant removal purposes, but also to evaluate the presence of sulphur reducing bacteria which could sulphidize Ag.

4) Continue to assess the effects of silver nanoparticles on constructed wetlands and their microbial communities. Quantify the effects and risk from silver nanoparticles to wastewater treatment processes in constructed wetlands through *in-situ* silver nanoparticle exposures. Examine the fate (location and chemical nature) of silver nanoparticles in constructed wetlands to help quantify risk and validate/invalidate the need for further research. Identify whether wetland intensification designs, such as the use of artificial aeration, impact the fate of silver nanoparticles in constructed wetlands. Examine the concentrations of Ag NPs within the microbial biofilms. Quantify the effects of silver nanoparticles on the genetic diversity of bacteria in constructed wetlands.

5) Examine the uptake of silver nanoparticles by a variety of wetland plants. Evaluate the effects of silver nanoparticles on the health of wetland plants in environmentally relevant concentrations.

6) Use non-pristine silver nanoparticles in future toxicity testing which are in an environmentally relevant form to the test species/population.

7) Develop a 96-well plate (similar to that of the BIOLOG EcoPlate™) which has more appropriate carbon sources for the characterization of microbial communities used for biological wastewater treatment. It would be useful for rapid characterization of the health of microbial communities in various wastewater treatment applications and reveal new applications for the use of constructed wetlands for the treatment of complex wastewater.

REFERENCES

Adam, G. and H. Duncan (2001). "Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils." Soil Biology and Biochemistry **33**(7): 943-951.

Allen, W. C., P. B. Hook, et al. (2002). "Temperature and wetland plant species effects on wastewater treatment and root zone oxidation." Journal of Environmental Quality **31**(3): 1010-1016.

Armstrong, J., W. Armstrong, et al. (1992). "Phragmites australis: Venturi-and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation." New Phytologist: 197-207.

Asharani, P., Y. L. Wu, et al. (2008). "Toxicity of silver nanoparticles in zebrafish models." Nanotechnology **19**(25): 255102.

Auffan, M., J. Rose, et al. (2009). "Chemical stability of metallic nanoparticles: A parameter controlling their potential cellular toxicity in vitro." Environmental Pollution **157**(4): 1127-1133.

Auvinen, H., I. Havran, et al. (2017). "Removal of pharmaceuticals by a pilot aerated sub-surface flow constructed wetland treating municipal and hospital wastewater." Ecological Engineering **100**: 157-164.

Ávila, C., J. M. Bayona, et al. (2015). "Emerging organic contaminant removal in a full-scale hybrid constructed wetland system for wastewater treatment and reuse." Ecological Engineering **80**: 108-116.

Ávila, C., V. Matamoros, et al. (2014). "Attenuation of emerging organic contaminants in a hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater." Science of the Total Environment **470**: 1272-1280.

Badri, D. V. and J. M. Vivanco (2009). "Regulation and function of root exudates." Plant, Cell & Environment **32**(6): 666-681.

Bais, H. P., T. L. Weir, et al. (2006). "The role of root exudates in rhizosphere interactions with plants and other organisms." Annu. Rev. Plant Biol. **57**: 233-266.

Bar-Ilan, O., K. M. Louis, et al. (2012). "Titanium dioxide nanoparticles produce phototoxicity in the developing zebrafish." Nanotoxicology **6**(6): 670-679.

Battin, T. J. (1997). "Assessment of fluorescein diacetate hydrolysis as a measure of total esterase activity in natural stream sediment biofilms." Science of the Total Environment **198**(1): 51-60.

Benjamin, M. M. (2002). Water Chemistry, Waveland Press, Incorporated.

Benn, T., B. Cavanagh, et al. (2010). "The release of nanosilver from consumer products used in the home." Journal of Environmental Quality **39**(6): 1875-1882.

Benn, T. M. and P. Westerhoff (2008). "Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics." Environmental Science & Technology **42**(11): 4133-4139.

Berglund, B., G. A. Khan, et al. (2014). "Efficient removal of antibiotics in surface-flow constructed wetlands, with no observed impact on antibiotic resistance genes." Science of the Total Environment **476**: 29-37.

Bicho, R. C., T. Ribeiro, et al. (2016). "Effects of Ag nanomaterials (NM300K) and Ag salt (AgNO3) can be discriminated in a full life cycle long term test with Enchytraeus crypticus." Journal of Hazardous Materials **318**: 608-614.

Blaser, S. A., M. Scheringer, et al. (2008). "Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles." Science of the Total Environment **390**(2-3): 396-409.

Boog, J., J. Nivala, et al. (2014). "Hydraulic characterization and optimization of total nitrogen removal in an aerated vertical subsurface flow treatment wetland." Bioresource Technology **162**: 166-174.

Borin, M. and M. Salvato (2012). "Effects of five macrophytes on nitrogen remediation and mass balance in wetland mesocosms." Ecological Engineering **46**: 34-42.

Boutin, C. (1987). "Domestic wastewater treatment in tanks planted with rooted macrophytes: case study; description of the system; design criteria; and efficiency." Water Science & Technology **19**(10): 29-40.

Brar, S. K., M. Verma, et al. (2010). "Engineered nanoparticles in wastewater and wastewater sludge – Evidence and impacts." Waste Management **30**(3): 504-520.

Březinová, T. and J. Vymazal (2015). "Seasonal growth pattern of Phalaris arundinacea in constructed wetlands with horizontal subsurface flow." Ecological Engineering **80**: 62-68.

Brisson, J. and F. Chazarenc (2009). "Maximizing pollutant removal in constructed wetlands: should we pay more attention to macrophyte species selection?" Science of the Total Environment **407**(13): 3923-3930.

Brix, H. (1994). "Functions of macrophytes in constructed wetlands." Water Science and Technology **29**(4): 71-78.

Brix, H. (1994). "Use of constructed wetlands in water pollution control: historical development, present status, and future perspectives." Water Science and Technology **30**(8): 209-224.

Brix, H. (1997). "Do macrophytes play a role in constructed treatment wetlands?" Water Science and Technology **35**(5): 11-17.

Brix, H. and C. A. Arias (2005). "Danish guidelines for small-scale constructed wetland systems for onsite treatment of domestic sewage." Water Science & Technology **51**(9): 1-9.

Brunetti, G., E. Donner, et al. (2015). "Fate of zinc and silver engineered nanoparticles in sewerage networks." Water Research **77**(0): 72-84.

Butterworth, E., G. Dotro, et al. (2013). "Effect of artificial aeration on tertiary nitrification in a full-scale subsurface horizontal flow constructed wetland." Ecological Engineering **54**: 236-244.

Butterworth, E., A. Richards, et al. (2016). "Impact of aeration on macrophyte establishment in sub-surface constructed wetlands used for tertiary treatment of sewage." Ecological Engineering **91**: 65-73.

Button, M., H. Auvinen, et al. (2016). "Susceptibility of constructed wetland microbial communities to silver nanoparticles: A microcosm study." Ecological Engineering **97**: 476-485.

Button, M., J. Nivala, et al. (2015). "Microbial community metabolic function in subsurface flow constructed wetlands of different designs." Ecological Engineering **80**: 162-171.

Button, M., M. Rodriguez, et al. (2016). "Use of two spatially separated plant species alters microbial community function in horizontal subsurface flow constructed wetlands." Ecological Engineering **92**: 18-27.

Button, M., K. Weber, et al. (2016). "Community-level physiological profiling of microbial communities in constructed wetlands: effects of sample preparation." Applied Biochemistry and Biotechnology **178**(5): 960-973.

Campbell, C. D., S. J. Grayston, et al. (1997). "Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities." Journal of Microbiological Methods **30**(1): 33-41.

Canada, E. (2011). 2011 Municipal Water Use Report: Municipal Water Use 2009 Statistics, Environment Canada, Government of Canada.

Carlson, C., S. M. Hussain, et al. (2008). "Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species." The journal of physical chemistry B **112**(43): 13608-13619.

Chazarenc, F., V. Gagnon, et al. (2009). "Effect of plant and artificial aeration on solids accumulation and biological activities in constructed wetlands." Ecological Engineering **35**(6): 1005-1010.

Chen, Y., Y. Wen, et al. (2015). "Effects of plant biomass on bacterial community structure in constructed wetlands used for tertiary wastewater treatment." Ecological Engineering **84**: 38-45.

Cheng, H.-B., M. Kumar, et al. (2012). "Interpretation of redox potential variation during biological denitrification using linear non-equilibrium thermodynamic model." International Biodeterioration & Biodegradation **67**: 28-39.

Cho, K.-H., J.-E. Park, et al. (2005). "The study of antimicrobial activity and preservative effects of nanosilver ingredient." Electrochimica Acta **51**(5): 956-960.

Choi, O., T. E. Clevenger, et al. (2009). "Role of sulfide and ligand strength in controlling nanosilver toxicity." Water Research **43**(7): 1879-1886.

Choi, O. and Z. Hu (2008). "Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria." Environmental Science & Technology **42**(12): 4583-4588.

Choi, O., C.-P. Yu, et al. (2010). "Interactions of nanosilver with Escherichia coli cells in planktonic and biofilm cultures." Water Research **44**(20): 6095-6103.

Cleveland, D., S. E. Long, et al. (2012). "Pilot estuarine mesocosm study on the environmental fate of silver nanomaterials leached from consumer products." Science of the Total Environment **421**: 267-272.

Colman, B. P., C. L. Arnaout, et al. (2013). "Low concentrations of silver nanoparticles in biosolids cause adverse ecosystem responses under realistic field scenario." Plos One **8**(2): e57189.

Colman, B. P., M. Auffan, et al. (2009). "Impacts of nanosilver on microbial activity in wetlands and streams." Geochimica et Cosmochimica Acta **73**(13S): A238.

Colman, B. P., B. Espinasse, et al. (2014). "Emerging Contaminant or an Old Toxin in Disguise? Silver Nanoparticle Impacts on Ecosystems." Environ. Sci. Technol. **48**(9): 5229-5236.

Cooper, P. (2005). "The performance of vertical flow constructed wetland systems with special reference to the significance of oxygen transfer and hydraulic loading rates." Water Science & Technology **51**(9): 81-90.

Cosway, K. (2014). Fate and Effect of Triclosan and Sulfamethoxazole Within Meso-Scale Vertical Flow Constructed Wetlands, Royal Military College of Canada.

Cottingham, P., T. Davies, et al. (1999). "Aeration to promote nitrification in constructed wetlands." Environmental Technology **20**(1): 69-75.

Crites, R. W., G. D. Dombeck, et al. (1997). "Removal of metals and ammonia in constructed wetlands." Water Environ. Res. **69**(2): 132-135.

Dale, A. L., E. A. Casman, et al. (2015). "Modeling nanomaterial environmental fate in aquatic systems." Environmental Science & Technology **49**(5): 2587-2593.

Dale, A. L., G. V. Lowry, et al. (2013). "Modeling Nanosilver Transformations in Freshwater Sediments." Environ. Sci. Technol. **47**(22): 12920-12928.

Das, P., C. J. Williams, et al. (2012). "Changes in bacterial community structure after exposure to silver nanoparticles in natural waters." Environmental Science and Technology **46**(16): 9120-9128.

Das, P., M. A. Xenopoulos, et al. (2012). "Effects of silver nanoparticles on bacterial activity in natural waters." Environmental Toxicology and Chemistry **31**(1): 122-130.

Dobias, J., A. Costanza, et al. (2011). "Toxicity of silver nanoparticles to environmental microbial consortia." Mineralogical Magazine **75**(3): 768.

Dong, H., Z. Qiang, et al. (2012). "Effect of artificial aeration on the performance of vertical-flow constructed wetland treating heavily polluted river water." Journal of Environmental Sciences **24**(4): 596-601.

Drizo, A., C. Frost, et al. (1999). "Physico-chemical screening of phosphate-removing substrates for use in constructed wetland systems." Water Research **33**(17): 3595-3602.

Durán, N., P. D. Marcato, et al. (2007). "Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment." Journal of biomedical nanotechnology **3**(2): 203-208.

El Badawy, A. M., R. G. Silva, et al. (2010). "Surface charge-dependent toxicity of silver nanoparticles." Environmental Science & Technology **45**(1): 283-287.

EPA (1988). Constructed Wetlands and Aquatic Plant Systems for Municipal Wastewater Treatment. Design Manual, Office of Research and Development. United States Environmental Protection Agency.

EPA (2004). Primer for Municipal Wastewater Treatment Systems, United States Environmental Protection Agency, Office of Water, Office of Wastewater Management.

Fabrega, J., S. N. Luoma, et al. (2011). "Silver nanoparticles: Behaviour and effects in the aquatic environment." Environment International **37**(2): 517-531.

Fabrega, J., J. C. Renshaw, et al. (2009). "Interactions of Silver Nanoparticles with Pseudomonas putida Biofilms." Environmental Science & Technology **43**(23): 9004-9009.

Farkas, J., H. Peter, et al. (2011). "Characterization of the effluent from a nanosilver producing washing machine." Environment International **37**(6): 1057-1062.

Farkas, J., H. Peter, et al. (2011). "Characterization of the effluent from a nanosilver producing washing machine." Environment International **37**(6): 1057-1062.

Faulwetter, J. L., V. Gagnon, et al. (2009). "Microbial processes influencing performance of treatment wetlands: a review." Ecological Engineering **35**(6): 987-1004.

Fetter, C., W. Sloey, et al. (1976). "Potential Replacement of Septic Tank Drain Fields by Artificial Marsh Wastewater Treatment Systemsa." Groundwater **14**(6): 396-402.

Foldbjerg, R., P. Olesen, et al. (2009). "PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes." Toxicology letters **190**(2): 156-162.

Frimmel, F. and M. Delay (2010). Introducing the "Nano-world". Nanoparticles in the Water Cycle. F. H. Frimmel and R. Niessner, Springer Berlin Heidelberg**:** 1-11.

Gagnon, V., F. Chazarenc, et al. (2007). "Influence of macrophyte species on microbial density and activity in constructed wetlands." Water Science and Technology **56**(3): 249-254.

Gao, J., S. Youn, et al. (2009). "Dispersion and Toxicity of Selected Manufactured Nanomaterials in Natural River Water Samples: Effects of Water Chemical Composition." Environmental Science & Technology **43**(9): 3322-3328.

Garcia, A., L. Delgado, et al. (2012). "Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment." Journal of Hazardous Materials **199-200**: 64-72.

García, A., L. Delgado, et al. (2012). "Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment." Journal of Hazardous Materials **199–200**: 64-72.

Garcia, J., D. P. Rousseau, et al. (2010). "Contaminant removal processes in subsurface-flow constructed wetlands: a review." Critical Reviews in Environmental Science and Technology **40**(7): 561-661.

Garland, J. L. and A. L. Mills (1991). "Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization." Applied and Environmental Microbiology **57**(8): 2351-2359.

Geranio, L., M. Heuberger, et al. (2009). "The behavior of silver nanotextiles during washing." Environmental Science and Technology **43**(21): 8113-8118.

Gopal, B. and U. Goel (1993). "Competition and allelopathy in aquatic plant communities." The Botanical Review **59**(3): 155-210.

Gordon, O., T. V. Slenters, et al. (2010). "Silver coordination polymers for prevention of implant infection: thiol interaction, impact on respiratory chain enzymes, and hydroxyl radical induction." Antimicrobial agents and chemotherapy **54**(10): 4208-4218.

Gottschalk, F. and B. Nowack (2011). "The release of engineered nanomaterials to the environment." Journal of Environmental Monitoring **13**(5): 1145-1155.

Gottschalk, F., T. Sonderer, et al. (2009). "Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions." Environmental Science & Technology **43**(24): 9216-9222.

Gregersen, P. and H. Brix (2001). "Zero-discharge of nutrients and water in a willow dominated constructed wetland." Water Science & Technology **44**(11): 407-412.

Halling-Sørensen, B. and S. Jorgensen (1993). The removal of nitrogen compounds from wastewater, Elsevier.

He, W., Y. Liu, et al. (2014). "Electron spin resonance spectroscopy for the study of nanomaterial-mediated generation of reactive oxygen species." Journal of Food and Drug Analysis **22**(1): 49-63.

Helt, C. D., K. P. Weber, et al. (2012). "Antibiotic resistance profiles of representative wetland bacteria and faecal indicators following ciprofloxacin exposure in lab-scale constructed mesocosms." Ecological Engineering **39**: 113-122.

Hemming, J. M., W. T. Waller, et al. (2001). "Assessment of the estrogenicity and toxicity of a domestic wastewater effluent flowing through a constructed wetland system using biomarkers in male fathead minnows (Pimephales promelas rafinesque, 1820)." Environmental Toxicology and Chemistry **20**(10): 2268-2275.

Hendren, C. O., X. Mesnard, et al. (2011). "Estimating Production Data for Five Engineered Nanomaterials As a Basis for Exposure Assessment." Environmental Science & Technology **45**(7): 2562-2569.

Imfeld, G., M. Braeckevelt, et al. (2009). "Monitoring and assessing processes of organic chemicals removal in constructed wetlands." Chemosphere **74**(3): 349-362.

Impellitteri, C. A., S. Harmon, et al. (2013). "Transformation of silver nanoparticles in fresh, aged, and incinerated biosolids." Water Research **47**(12): 3878-3886.

IWA (2000). "Constructed Wetland for Pollution Control: Processes, Performance, Design and Operation." International Water Association (IWA)(Scientific and Technical Report No. 8, IWA Publishing, London, UK.).

Jagtap, U. B. and V. A. Bapat (2013). "Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. seed extract and its antibacterial activity." Industrial crops and products **46**: 132-137.

Jenssen, P., T. Maehlum, et al. (1993). "Potential use of constructed wetlands for wastewater treatment in northern environments." Water Science & Technology **28**(10): 149-157.

Jetten, M., I. Cirpus, et al. (2005). 1994–2004: 10 years of research on the anaerobic oxidation of ammonium, Portland Press Limited.

Jiang, H.-S., M. Li, et al. (2012). "Physiological analysis of silver nanoparticles and AgNO3 toxicity to Spirodela polyrhiza." Environmental Toxicology and Chemistry **31**(8): 1880-1886.

Jiang, H.-S., X.-N. Qiu, et al. (2014). "Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant Spirodela polyrhiza." Environmental Toxicology and Chemistry **33**(6): 1398-1405.

Jiang, H. S., M. Li, et al. (2012). "Physiological analysis of silver nanoparticles and AgNO3 toxicity to Spirodela polyrhiza." Environmental Toxicology and Chemistry **31**(8): 1880-1886.

Jiang, W., B. Y. Kim, et al. (2008). "Nanoparticle-mediated cellular response is size-dependent." Nature nanotechnology **3**(3): 145-150.

Jing, S.-R., Y.-F. Lin, et al. (2002). "Microcosm wetlands for wastewater treatment with different hydraulic loading rates and macrophytes." Journal of Environmental Quality **31**(2): 690-696.

Johnson, A. C., M. D. Jürgens, et al. (2014). "Particulate and colloidal silver in sewage effluent and sludge discharged from British wastewater treatment plants." Chemosphere **112**(0): 49-55.

Johnston, B. D., T. M. Scown, et al. (2010). "Bioavailability of Nanoscale Metal Oxides TiO2, CeO2, and ZnO to Fish." Environ. Sci. Technol. **44**(3): 1144-1151.

Jörg, E. D. and S. S. Laurence (2001). Concerns about Pharmaceuticals in Water Reuse, Groundwater Recharge, and Animal Waste. Pharmaceuticals and Care Products in the Environment, American Chemical Society. **791:** 206-228.

Kadlec, R. H. and R. L. Knight (1996). Treatment wetlands, Lewis Publishers, Boca Raton, FL.

Kadlec, R. H. and S. Wallace (2008). Treatment wetlands, CRC press.

Kaegi, R., B. Sinnet, et al. (2010). "Release of silver nanoparticles from outdoor facades." Environmental Pollution **158**(9): 2900-2905.

Kaegi, R., A. Voegelin, et al. (2013). "Fate and transformation of silver nanoparticles in urban wastewater systems." Water Research **47**(12): 3866-3877.

Kaegi, R., A. Voegelin, et al. (2011). "Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant." Environmental Science & Technology **45**(9): 3902-3908.

Kawata, K., M. Osawa, et al. (2009). "In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells." Environmental Science & Technology **43**(15): 6046-6051.

Keller, A., S. McFerran, et al. (2013). "Global life cycle releases of engineered nanomaterials." Journal of Nanoparticle Research **15**(6): 1-17.

Keller, A. A. and A. Lazareva (2014). "Predicted releases of engineered nanomaterials: from global to regional to local." Environmental Science & Technology Letters **1**(1): 65-70.

Kennedy, G. and T. Mayer (2002). "Natural and constructed wetlands in Canada: An overview." Water Quality Research Journal of Canada **37**(2): 295-325.

Kim, B., C.-S. Park, et al. (2010). "Discovery and Characterization of Silver Sulfide Nanoparticles in Final Sewage Sludge Products." Environmental Science & Technology **44**(19): 7509-7514.

Kim, J. S., E. Kuk, et al. (2007). "Antimicrobial effects of silver nanoparticles." Nanomedicine: Nanotechnology, Biology and Medicine **3**(1): 95-101.

Kivaisi, A. K. (2001). "The potential for constructed wetlands for wastewater treatment and reuse in developing countries: a review." Ecological Engineering **16**(4): 545-560.

Knowles, P., G. Dotro, et al. (2011). "Clogging in subsurface-flow treatment wetlands: occurrence and contributing factors." Ecological Engineering **37**(2): 99-112.

Krishnaraj, C., E. G. Jagan, et al. (2012). "Effect of biologically synthesized silver nanoparticles on Bacopa monnieri (Linn.) Wettst. plant growth metabolism." Process Biochemistry **47**(4): 651-658.

Kulthong, K., S. Srisung, et al. (2010). "Research Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat."

Laban, G., L. F. Nies, et al. (2010). "The effects of silver nanoparticles on fathead minnow (Pimephales promelas) embryos." Ecotoxicology **19**(1): 185-195.

Lagarde, F., C. Beausoleil, et al. (2015). "Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment." Environmental Health **14**: 13.

Leschisin, D. A., G. L. Williams, et al. (1992). "Factors affecting waterfowl use of constructed wetlands in northwestern Minnesota." Wetlands **12**(3): 178-183.

Levard, C., E. M. Hotze, et al. (2012). "Environmental transformations of silver nanoparticles: Impact on stability and toxicity." Environmental Science and Technology **46**(13): 6900-6914.

Levard, C., B. C. Reinsch, et al. (2011). "Sulfidation Processes of PVP-Coated Silver Nanoparticles in Aqueous Solution: Impact on Dissolution Rate." Environ. Sci. Technol. **45**(12): 5260-5266.

Levard, C., B. C. Reinsch, et al. (2011). "Sulfidation processes of PVP-coated silver nanoparticles in aqueous solution: impact on dissolution rate." Environmental Science & Technology **45**(12): 5260-5266.

Li, F., L. Lu, et al. (2014). "Enhanced nitrogen removal in constructed wetlands: Effects of dissolved oxygen and step-feeding." Bioresource Technology **169**: 395-402.

Li, L., G. Hartmann, et al. (2013). "Quantification of Nanoscale Silver Particles Removal and Release from Municipal Wastewater Treatment Plants in Germany." Environmental Science & Technology **47**(13): 7317-7323.

Liu, J. and R. H. Hurt (2010). "Ion release kinetics and particle persistence in aqueous nano-silver colloids." Environmental Science & Technology **44**(6): 2169-2175.

Lombi, E., E. Donner, et al. (2013). "Transformation of four silver/silver chloride nanoparticles during anaerobic treatment of wastewater and post-processing of sewage sludge." Environmental Pollution **176**: 193-197.

Lorenz, C., L. Windler, et al. (2012). "Characterization of silver release from commercially available functional (nano) textiles." Chemosphere **89**(7): 817-824.

Lowry, G. V. and E. A. Casman (2009). Nanomaterial transport, transformation, and fate in the environment. NATO Advanced Research Workshop on Nanomaterials: Environmental Risks and Benefits, 27-30 April 2008, Dordrecht, Netherlands, Springer.

Lowry, G. V., B. P. Espinasse, et al. (2012). "Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland." Environmental Science and Technology **46**(13): 7027-7036.

Lowry, G. V., E. M. Hotze, et al. (2010). "Environmental occurrences, behavior, fate, and ecological effects of nanomaterials: An introduction to the special series." Journal of Environmental Quality **39**(6): 1867-1874.

Lv, T., Y. Zhang, et al. (2016). "Microbial community metabolic function in constructed wetland mesocosms treating the pesticides imazalil and tebuconazole." Ecological Engineering.

Lv, T., Y. Zhang, et al. (2016). "Removal of the pesticides imazalil and tebuconazole in saturated constructed wetland mesocosms." Water Research **91**: 126-136.

Ma, R., C. Levard, et al. (2014). "Fate of zinc oxide and silver nanoparticles in a pilot wastewater treatment plant and in processed biosolids." Environmental Science and Technology **48**(1): 104-112.

Maltais-Landry, G., R. Maranger, et al. (2009). "Nitrogen transformations and retention in planted and artificially aerated constructed wetlands." Water Research **43**(2): 535-545.

Marambio-Jones, C. and E. M. Hoek (2010). "A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment." Journal of Nanoparticle Research **12**(5): 1531-1551.

Massarsky, A., L. Strek, et al. (2014). "Acute embryonic exposure to nanosilver or silver ion does not disrupt the stress response in zebrafish (Danio rerio) larvae and adults." Sci. Total Environ. **478**: 133-140.

Matamoros, V. and J. M. Bayona (2006). "Elimination of Pharmaceuticals and Personal Care Products in Subsurface Flow Constructed Wetlands." Environmental Science & Technology **40**(18): 5811-5816.

Maynard, A. D. (2006). "A research strategy for addressing risk." Nanotechnology, Woodrow Wilson International Center for Scholars.

Metcalf and Eddy (2003). Wastewater Engineering, Treatment and Reuse. Toronto, Canada, McGraw-Hill.

Miao, A.-J., K. A. Schwehr, et al. (2009). "The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances." Environmental Pollution **157**(11): 3034-3041.

Mirzajani, F., H. Askari, et al. (2013). "Effect of silver nanoparticles on Oryza sativa L. and its rhizosphere bacteria." Ecotoxicology and Environmental Safety **88**(0): 48-54.

Mitrano, D. M., J. F. Ranville, et al. (2014). "Tracking dissolution of silver nanoparticles at environmentally relevant concentrations in laboratory, natural, and processed waters using single particle ICP-MS (spICP-MS)." Environ. Sci.: Nano **1**: 248-259.

Molnar, I. L., C. S. Willson, et al. (2014). "Method for Obtaining Silver Nanoparticle Concentrations within a Porous Medium via Synchrotron X-ray Computed Microtomography." Environmental Science & Technology **48**(2): 1114-1122.

Monjeau, C. (1901). Purifying water, Google Patents.

Morones, J. R., J. L. Elechiguerra, et al. (2005). "The bactericidal effect of silver nanoparticles." Nanotechnology **16**(10): 2346.

Münch, C., P. Kuschk, et al. (2005). "Root stimulated nitrogen removal: only a local effect or important for water treatment?" Water Science and Technology **51**(9): 185-192.

Münch, C., T. Neu, et al. (2007). "The root surface as the definitive detail for microbial transformation processes in constructed wetlands – a biofilm characteristic." Water Science and Technology **56**(3): 271-276.

Murphy, C., A. R. Rajabzadeh, et al. (2016). "Nitrification cessation and recovery in an aerated saturated vertical subsurface flow treatment wetland: Field studies and microscale biofilm modeling." Bioresource Technology **209**: 125-132.

Murty, B. S., P. Shankar, et al. (2013). Textbook of nanoscience and nanotechnology, Springer Science & Business Media.

Navarro, E., F. Piccapietra, et al. (2008). "Toxicity of Silver Nanoparticles to Chlamydomonas reinhardtii." Environ. Sci. Technol. **42**(23): 8959-8964.

Nivala, J., M. B. Hoos, et al. (2007). "Treatment of landfill leachate using an aerated, horizontal subsurface-flow constructed wetland." Science of the Total Environment **380**(1–3): 19-27.

Nivala, J. and D. P. Rousseau (2009). "Reversing clogging in subsurface-flow constructed wetlands by hydrogen peroxide treatment: two case studies." Water Science and Technology **59**(10): 2037-2046.

Nivala, J. A. (2012). Effect of design on treatment performance, plant, nutrition and clogging in subsurface flow treatment wetlands Ph.D., Det Naturvidenskabelige Fakultet, Aarhus Universitet.

Nowack, B., J. F. Ranville, et al. (2012). "Potential scenarios for nanomaterial release and subsequent alteration in the environment." Environmental Toxicology and Chemistry **31**(1): 50-59.

Oopkaup, K., M. Truu, et al. (2016). "Dynamics of Bacterial Community Abundance and Structure in Horizontal Subsurface Flow Wetland Mesocosms Treating Municipal Wastewater." Water **8**(10): 457.

Ouellet-Plamondon, C., F. Chazarenc, et al. (2006). "Artificial aeration to increase pollutant removal efficiency of constructed wetlands in cold climate." Ecological Engineering **27**(3): 258-264.

Park, B. J. and K. Kidd (2005). "Effects of the synthetic estrogen ethinylestradiol on early life stages of mink frogs and green frogs in the wild and in situ." Environmental Toxicology and Chemistry **24**(8): 2027-2036.

Piao, M. J., K. A. Kang, et al. (2011). "Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis." Toxicology letters **201**(1): 92-100.

Piccinno, F., F. Gottschalk, et al. (2012). "Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world." Journal of Nanoparticle Research **14**(9): 1-11.

Preston-Mafham, J., L. Boddy, et al. (2002). "Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles–a critique." FEMS Microbiology Ecology **42**(1): 1-14.

Quadros, M. E., R. Pierson, et al. (2013). "Release of Silver from Nanotechnology-Based Consumer Products for Children." Environmental Science & Technology **47**(15): 8894-8901.

Radniecki, T. S., D. P. Stankus, et al. (2011). "Influence of liberated silver from silver nanoparticles on nitrification inhibition of Nitrosomonas europaea." Chemosphere **85**(1): 43-49.

Raffi, M., F. Hussain, et al. (2008). "Antibacterial characterization of silver nanoparticles against E. coli ATCC-15224." Journal of Materials Science and Technology **24**(2): 192-196.

Ragusa, S., D. McNevin, et al. (2004). "Indicators of biofilm development and activity in constructed wetlands microcosms." Water Research **38**(12): 2865-2873.

Rajabzadeh, A. R., R. L. Legge, et al. (2015). "Multiphysics modelling of flow dynamics, biofilm development and wastewater treatment in a subsurface vertical flow constructed wetland mesocosm." Ecological Engineering **74**: 107-116.

Ramond, J.-B., P. J. Welz, et al. (2012). "Microbial community structure stability, a key parameter in monitoring the development of constructed wetland mesocosms during start-up." Research in Microbiology **163**(1): 28-35.

Ratte, H. T. (1999). "Bioaccumulation and toxicity of silver compounds: a review." Environmental Toxicology and Chemistry **18**(1): 89-108.

Reddy, K. and E. D'angelo (1997). "Biogeochemical indicators to evaluate pollutant removal efficiency in constructed wetlands." Water science and technology **35**(5): 1-10.

Reidy, B., A. Haase, et al. (2013). "Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications." Materials **6**(6): 2295-2350.

Reinsch, B. C., C. Levard, et al. (2012). "Sulfidation of silver nanoparticles decreases Escherichia coli growth inhibition." Environmental Science and Technology **46**(13): 6992-7000.

Reyes-Contreras, C., M. Hijosa-Valsero, et al. (2012). "Temporal evolution in PPCP removal from urban wastewater by constructed wetlands of different configuration: a medium-term study." Chemosphere **88**(2): 161-167.

Richards, R. (1980). "Antimicrobial action of silver nitrate." Microbios **31**(124): 83-91.

Richardson, S. D. and T. A. Ternes (2011). "Water analysis: emerging contaminants and current issues." Analytical chemistry **83**(12): 4614-4648.

Rittmann, B. E. and P. L. McCarty (2001). "Environmental biotechnology: principles and applications." New York: McGrawHill **400**.

Sakadevan, K. and H. Bavor (1998). "Phosphate adsorption characteristics of soils, slags and zeolite to be used as substrates in constructed wetland systems." Water Research **32**(2): 393-399.

Salvato, M., M. Borin, et al. (2012). "Wetland plants, micro-organisms and enzymatic activities interrelations in treating N polluted water." Ecological Engineering **47**: 36-43.

Samsó, R. and J. Garcia (2013). "BIO_PORE, a mathematical model to simulate biofilm growth and water quality improvement in porous media: Application and calibration for constructed wetlands." Ecological Engineering **54**: 116-127.

Schneider, M. (2015). Investigation of the effects of emerging contaminants on microbial communities within different ecosystems, Beuth Hochschule für Technik Berlin.

Schnürer, J. and T. Rosswall (1982). "Fluorescein Diacetate Hydrolysis as a Measure of Total Microbial Activity in Soil and Litter." Applied and Environmental Microbiology **43**(6): 1256-1261.

Seidel, K. (1976). "Macrophytes and water purification." Biological Control of Water Pollution. J. Tourbier & Rw Pierson, Jr., eds.

Selck, H., R. D. Handy, et al. (2016). "Nanomaterials in the aquatic environment: A European Union–United States perspective on the status of ecotoxicity testing, research priorities, and challenges ahead." Environmental Toxicology and Chemistry **35**(5): 1055-1067.

Sepúlveda, V. V. (2014). Fate of silver nanoparticles in treatment wetlands, Ghent University.

Shafer, M. M., J. T. Overdier, et al. (1998). "Removal, partitioning, and fate of silver and other metals in wastewater treatment plants and effluent-receiving streams." Environmental Toxicology and Chemistry **17**(4): 630-641.

Sharif, F., P. Westerhoff, et al. (2013). "Sorption of trace organics and engineered nanomaterials onto wetland plant material." Environ. Sci.: Processes Impacts **15**(1): 267-274.

Sheng, Z. and Y. Liu (2011). "The effects of silver nanoparticles on wastewater biofilms." Mineralogical Magazine **75**(3): 1854.

Silva, T., L. R. Pokhrel, et al. (2014). "Particle size, surface charge and concentration dependent ecotoxicity of three organo-coated silver nanoparticles: comparison between general linear model-predicted and observed toxicity." Science of the Total Environment **468**: 968-976.

Sintubin, L., B. De Gusseme, et al. (2011). "The antibacterial activity of biogenic silver and its mode of action." Applied microbiology and biotechnology **91**(1): 153-162.

Sklarz, M. Y., A. Gross, et al. (2009). "A recirculating vertical flow constructed wetland for the treatment of domestic wastewater." Desalination **246**(1–3): 617-624.

Sondi, I. and B. Salopek-Sondi (2004). "Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria." Journal of Colloid and Interface Science **275**(1): 177-182.

Song, K., S.-H. Lee, et al. (2011). "Denitrification rates and community structure of denitrifying bacteria in newly constructed wetland." European Journal of Soil Biology **47**(1): 24-29.

Stein, O., P. Hook, et al. (2003). "Does batch operation enhance oxidation in subsurface constructed wetlands?" Water Science & Technology **48**(5): 149-156.

Stensberg, M. C., Q. Wei, et al. (2011). "Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging." Nanomedicine **6**(5): 879-898.

Stottmeister, U., A. Wießner, et al. (2003). "Effects of plants and microorganisms in constructed wetlands for wastewater treatment." Biotechnology Advances **22**(1): 93-117.

Tolaymat, T. M., A. M. El Badawy, et al. (2010). "An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers." Science of the Total Environment **408**(5): 999-1006.

Truu, M., J. Juhanson, et al. (2009). "Microbial biomass, activity and community composition in constructed wetlands." Science of the Total Environment **407**(13): 3958-3971.

Uggetti, E., T. Hughes-Riley, et al. (2016). "Intermittent aeration to improve wastewater treatment efficiency in pilot-scale constructed wetland." Science of the Total Environment **559**: 212-217.

Uren, N. C. (2007). "Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants." The rhizosphere. Biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York: 1-21.

USDA (2002). Plant Fact Sheet: Reed Canary Grass, United States Department of Agriculture.

Van Koetsem, F., S. Verstraete, et al. (2017). "Use of filtration techniques to study environmental fate of engineered metallic nanoparticles: Factors affecting filter performance." Journal of Hazardous Materials **322, Part A**: 105-117.

Vance, M. E., T. Kuiken, et al. (2015). "Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory." Beilstein journal of nanotechnology **6**(1): 1769-1780.

Vert, M., K.-H. Hellwich, et al. (2012). "Terminology for biorelated polymers and applications (IUPAC Recommendations 2012)." Pure and Applied Chemistry **84**(2): 377-410.

Vertelov, G., Y. A. Krutyakov, et al. (2008). "A versatile synthesis of highly bactericidal Myramistin® stabilized silver nanoparticles." Nanotechnology **19**(35): 355707.

Vincent, G., S. Dallaire, et al. (1994). "Antimicrobial properties of roots exudate of three macrophytes." Mentha aquatica: 290-296.

Vohla, C., E. Poldvere, et al. (2005). "Alternative filter media for phosphorous removal in a horizontal subsurface flow constructed wetland." Journal of Environmental Science and Health **40**(6-7): 1251-1264.

von Goetz, N., C. Lorenz, et al. (2013). "Migration of Ag- and TiO2-(Nano)particles from Textiles into Artificial Sweat under Physical Stress: Experiments and Exposure Modeling." Environmental Science & Technology **47**(17): 9979-9987.

Vymazal, J. (2007). "Removal of nutrients in various types of constructed wetlands." Science of the Total Environment **380**(1): 48-65.

Vymazal, J. (2010). "Constructed wetlands for wastewater treatment." Water **2**(3): 530-549.

Vymazal, J. (2010). "Constructed wetlands for wastewater treatment: five decades of experience†." Environmental science & technology **45**(1): 61-69.

Vymazal, J. (2011). "Plants used in constructed wetlands with horizontal subsurface flow: a review." Hydrobiologia **674**(1): 133-156.

Vymazal, J. (2013). "Emergent plants used in free water surface constructed wetlands: a review." Ecological Engineering **61**: 582-592.

Vymazal, J. and T. Březinová (2015). "The use of constructed wetlands for removal of pesticides from agricultural runoff and drainage: A review." Environment international **75**: 11-20.

Vymazal, J., T. D. Březinová, et al. (2017). "Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic–the first year of monitoring." Ecological Engineering **98**: 354-364.

Vymazal, J. and L. Krőpfelová (2005). "Growth of Phragmites australis and Phalaris arundinacea in constructed wetlands for wastewater treatment in the Czech Republic." Ecological Engineering **25**(5): 606-621.

Wallace, S. and D. Austin (2008). "Emerging models for nitrogen removal in treatment wetlands." Journal of environmental health **71**(4): 10.

Wallace, S. D. (2001). System for removing pollutants from water, Google Patents.

Weber, K. P. (2016). "Microbial Community Assessment in Wetlands for Water Pollution Control: Past, Present, and Future Outlook." Water **8**(11): 503.

Weber, K. P. and V. Gagnon (2014). "Microbiology in treatment wetlands." Sust Sanit Pract **18**: 25-30.

Weber, K. P., M. Gehder, et al. (2008). "Assessment of changes in the microbial community of constructed wetland mesocosms in response to acid mine drainage exposure." Water Research **42**(1): 180-188.

Weber, K. P., J. A. Grove, et al. (2007). "Data transformations in the analysis of community-level substrate utilization data from microplates." Journal of Microbiological Methods **69**(3): 461-469.

Weber, K. P. and R. L. Legge (2010). Community-Level Physiological Profiling. Bioremediation: Methods and Protocols. P. S. Cummings. Totowa, NJ, Humana Press**:** 263-281.

Weber, K. P. and R. L. Legge (2011). "Dynamics in the bacterial community-level physiological profiles and hydrological characteristics of constructed wetland mesocosms during start-up." Ecological Engineering **37**(5): 666-677.

Weber, K. P. and R. L. Legge (2013). "Comparison of the catabolic activity and catabolic profiles of rhizospheric, gravel-associated and interstitial microbial communities in treatment wetlands." Water Science and Technology **67**(4): 886-893.

Weber, K. P., M. R. Mitzel, et al. (2011). "Effect of ciprofloxacin on microbiological development in wetland mesocosms." Water Research **45**(10): 3185-3196.

Weber, K. P., E. J. Petersen, et al. (2014). "Effect of gold nanoparticles and ciprofloxacin on microbial catabolism: a community-based approach." Environ. Toxicol. Chem. **33**(1): 44-51.

Weedon, C. (2014). "Yellow Phragmites: Significance, cause, and remedies." Sustainable Sanit. Pract. **18**: 37-42.

Werker, A., J. Dougherty, et al. (2002). "Treatment variability for wetland wastewater treatment design in cold climates." Ecological Engineering **19**(1): 1-11.

Westerhoff, P. K., M. A. Kiser, et al. (2013). "Nanomaterial removal and transformation during biological wastewater treatment." Environmental Engineering Science **30**(3): 109-117.

Wetzel, R. G. (2001). Limnology: lake and river ecosystems, Gulf Professional Publishing.

Whipps, J. and J. Lynch (1990). "Carbon economy." The rhizosphere.: 59-97.

Windler, L., C. Lorenz, et al. (2012). "Release of titanium dioxide from textiles during washing." Environmental Science & Technology **46**(15): 8181-8188.

Wu, J.-P., X.-J. Luo, et al. (2008). "Bioaccumulation of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in wild aquatic species from an electronic waste (e-waste) recycling site in South China." Environment International **34**(8): 1109-1113.

Wu, S., P. Kuschk, et al. (2014). "Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review." Water Research **57**: 40-55.

Wu, S., S. Wallace, et al. (2015). "Treatment of industrial effluents in constructed wetlands: Challenges, operational strategies and overall performance." Environmental Pollution **201**: 107-120.

Xiu, Z.-M., J. Ma, et al. (2011). "Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions." Environmental Science & Technology **45**(20): 9003-9008.

Xiu, Z.-m., Q.-b. Zhang, et al. (2012). "Negligible particle-specific antibacterial activity of silver nanoparticles." Nano letters **12**(8): 4271-4275.

Yang, Y., J. Quensen, et al. (2014). "Pyrosequencing reveals higher impact of silver nanoparticles than Ag+ on the microbial community structure of activated sludge." Water Research **48**(1): 317-325.

Yang, Y., Y. Wang, et al. (2014). "Metal and nanoparticle occurrence in biosolid-amended soils." Science of the Total Environment **485-486**(1): 441-449.

YeonáLee, H., H. KunáPark, et al. (2007). "A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications." Chemical Communications(28): 2959-2961.

Yin, L., B. P. Colman, et al. (2012). "Effects of Silver Nanoparticle Exposure on Germination and Early Growth of Eleven Wetland Plants." Plos One **7**(10).

Zak, J. C., M. R. Willig, et al. (1994). "Functional diversity of microbial communities: a quantitative approach." Soil Biology and Biochemistry **26**(9): 1101-1108.

Zhai, J., J. Xiao, et al. (2016). "Seasonal Variation of Nutrient Removal in a Full-Scale Artificial Aerated Hybrid Constructed Wetland." Water **8**(12): 551.

Zhang, C.-B., J. Wang, et al. (2010). "Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland." Ecological Engineering **36**(1): 62-68.

Zhang, L.-y., L. Zhang, et al. (2010). "Effect of limited artificial aeration on constructed wetland treatment of domestic wastewater." Desalination **250**(3): 915-920.

Zhang, W., Y. Li, et al. (2013). "Photogeneration of reactive oxygen species on uncoated silver, gold, nickel, and silicon nanoparticles and their antibacterial effects." Langmuir **29**(15): 4647-4651.

Zhao, C.-M. and W.-X. Wang (2011). "Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to Daphnia magna." Environ. Toxicol. Chem. **30**(4): 885-892.

Zhu, L., J. Takala, et al. (2013). "Comparison of vertical-flow constructed wetlands with and without supplementary aeration treating decentralized domestic wastewater." Environmental Technology **34**(1): 53-60.

Zhu, T. and F. J. Sikora (1995). "Ammonium and nitrate removal in vegetated and unvegetated gravel bed microcosm wetlands." Water Science and Technology **32**(3): 219-228.

A. APPENDIX A

Supplementary Information for Chapter 3: WETLAND START-UP MONITORING OF AERATED AND NON-AERATED MESOCOSM CONSTRUCTED WETLANDS

Figure A.1: Sample output from Aquasim v.1.0.0.1 (Eawag Institute, Switzerland, 1995) used for NaBr tracer test modeling.

Statistical Tables for Chapter 3: **Table A.1** to **Table A.87**

Student's t-test p-values, Shapiro-Wilk's Test for Normality, Mauchly's Test of Sphericity, Levene's Test of Equality Error Variances, Repeated Measures ANOVA Pairwise Comparison.

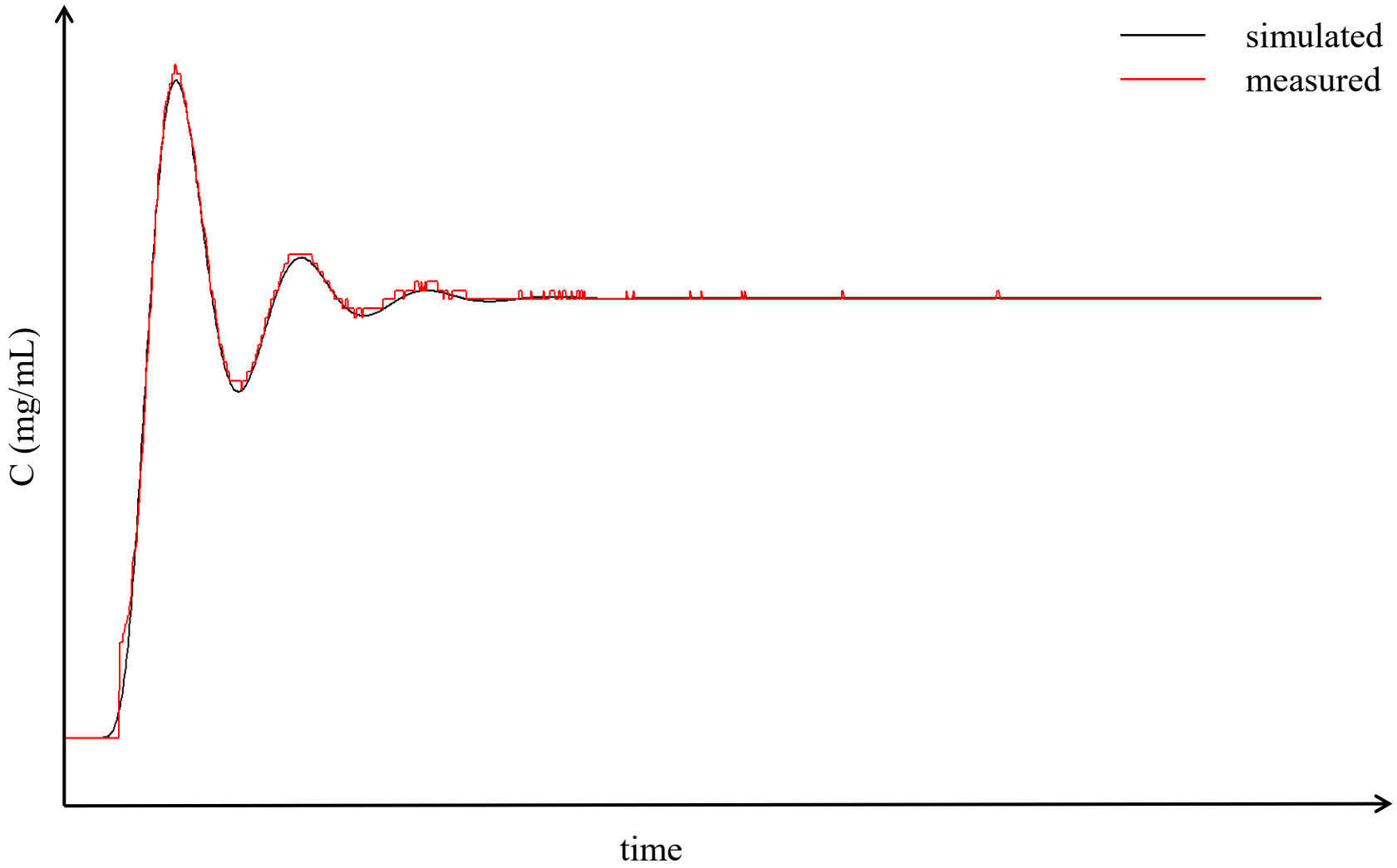

Figure A.1: Sample output from Aquasim v.1.0.0.1 (Eawag Institute, Switzerland, 1995) used for NaBr tracer test modeling. The black line represents the modeled curve while the red line represents the raw data.

Statistical Tables for Chapter 3: WETLAND START-UP MONITORING OF AERATED AND NON-AERATED MESOCOSM CONSTRUCTED WETLANDS

Plant Growth

Table A.1: Student's t-test p-values for plant stem data between aerated and non-aerated mesocosm system replicates. Significant weeks are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Plant Stems
2	0.042833702*
3	0.006830849*
4	0.003359803*
5	
6	0.003383325*
7	0.00463393*
8	0.006374389*
9	
10	0.043953853*
11	0.089836029
12	0.004678921*

Plant Stems

Table A.2: Shapiro-Wilk's Test of Normality for plant stem data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.683	6	.004
	NA	.741	6	.016
Week 3	A	.982	6	.960
	NA	.911	6	.445
Week 4	A	.855	6	.174
	NA	.816	6	.081
Week 6	A	.926	6	.548
	NA	.879	6	.264
Week 7	A	.847	6	.149
	NA	.986	6	.976
Week 8	A	.851	6	.162
	NA	.835	6	.118
Week 10	A	.949	6	.729
	NA	.947	6	.719
Week 11	A	.889	6	.314
	NA	.771	6	.032
Week 12	A	.934	6	.608

	NA	.950	6	.741

Table A.3: Mauchly's Test of Sphericity for plant stem data of aerated and non-aerated systems, α = 0.05.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	145.616	35	.000

Table A.4: Levene's Test of Equality of Error Variances for plant stem data of aerated and non-aerated systems, α = 0.05.

	F	df1	df2	Sig.
Week 2	151.250	1	10	.000
Week 3	7.926	1	10	.018
Week 4	.000	1	10	1.000
Week 6	.398	1	10	.542
Week 7	.438	1	10	.523
Week 8	3.378	1	10	.096
Week 10	.208	1	10	.658
Week 11	.715	1	10	.418
Week 12	.849	1	10	.379

Table A.5: Pairwise Comparisons from repeated measures ANOVA for plant stem data of aerated and non-aerated systems, α = 0.05. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 3	-4.167*	.828	.019	-7.790	-.543
	Week 4	-5.083*	.941	.011	-9.198	-.969
	Week 6	-4.417*	.941	.030	-8.531	-.302
	Week 7	-5.583*	1.265	.047	-11.119	-.047
	Week 8	-4.417	1.126	.103	-9.343	.510
	Week 10	-4.000	1.113	.176	-8.869	.869
	Week 11	-76.417*	5.915	.000	-102.294	-50.539
	Week 12	-74.750*	3.724	.000	-91.041	-58.459
Week 3	Week 2	4.167*	.828	.019	.543	7.790
	Week 4	-.917	.790	1.000	-4.371	2.538
	Week 6	-.250	.847	1.000	-3.957	3.457
	Week 7	-1.417	.898	1.000	-5.346	2.513

	Week 8	-.250	.857	1.000	-4.000	3.500
	Week 10	.167	1.334	1.000	-5.671	6.004
	Week 11	-72.250*	5.396	.000	-95.856	-48.644
	Week 12	-70.583*	3.634	.000	-86.482	-54.685
Week 4	Week 2	5.083*	.941	.011	.969	9.198
	Week 3	.917	.790	1.000	-2.538	4.371
	Week 6	.667	.530	1.000	-1.650	2.984
	Week 7	-.500	.500	1.000	-2.687	1.687
	Week 8	.667	.553	1.000	-1.752	3.085
	Week 10	1.083	1.530	1.000	-5.609	7.776
	Week 11	-71.333*	5.697	.000	-96.258	-46.409
	Week 12	-69.667*	4.024	.000	-87.271	-52.062
Week 6	Week 2	4.417*	.941	.030	.302	8.531
	Week 3	.250	.847	1.000	-3.457	3.957
	Week 4	-.667	.530	1.000	-2.984	1.650
	Week 7	-1.167	.728	1.000	-4.353	2.020
	Week 8	.000	.832	1.000	-3.638	3.638
	Week 10	.417	1.261	1.000	-5.100	5.933
	Week 11	-72.000*	5.411	.000	-95.672	-48.328
	Week 12	-70.333*	3.830	.000	-87.089	-53.578
Week 7	Week 2	5.583*	1.265	.047	.047	11.119
	Week 3	1.417	.898	1.000	-2.513	5.346
	Week 4	.500	.500	1.000	-1.687	2.687
	Week 6	1.167	.728	1.000	-2.020	4.353
	Week 8	1.167	.373	.385	-.464	2.797
	Week 10	1.583	1.615	1.000	-5.480	8.647
	Week 11	-70.833*	5.372	.000	-94.335	-47.331
	Week 12	-69.167*	4.024	.000	-86.771	-51.562
Week 8	Week 2	4.417	1.126	.103	-.510	9.343
	Week 3	.250	.857	1.000	-3.500	4.000
	Week 4	-.667	.553	1.000	-3.085	1.752
	Week 6	.000	.832	1.000	-3.638	3.638
	Week 7	-1.167	.373	.385	-2.797	.464
	Week 10	.417	1.524	1.000	-6.252	7.085
	Week 11	-72.000*	5.543	.000	-96.248	-47.752
	Week 12	-70.333*	4.092	.000	-88.235	-52.432
Week 10	Week 2	4.000	1.113	.176	-.869	8.869
	Week 3	-.167	1.334	1.000	-6.004	5.671
	Week 4	-1.083	1.530	1.000	-7.776	5.609
	Week 6	-.417	1.261	1.000	-5.933	5.100
	Week 7	-1.583	1.615	1.000	-8.647	5.480
	Week 8	-.417	1.524	1.000	-7.085	6.252
	Week 11	-72.417*	5.438	.000	-96.207	-48.626
	Week 12	-70.750*	3.784	.000	-87.303	-54.197

Week 11	Week 2	76.417*	5.915	.000	50.539	102.294
	Week 3	72.250*	5.396	.000	48.644	95.856
	Week 4	71.333*	5.697	.000	46.409	96.258
	Week 6	72.000*	5.411	.000	48.328	95.672
	Week 7	70.833*	5.372	.000	47.331	94.335
	Week 8	72.000*	5.543	.000	47.752	96.248
	Week 10	72.417*	5.438	.000	48.626	96.207
	Week 12	1.667	5.009	1.000	-20.246	23.579
Week 12	Week 2	74.750*	3.724	.000	58.459	91.041
	Week 3	70.583*	3.634	.000	54.685	86.482
	Week 4	69.667*	4.024	.000	52.062	87.271
	Week 6	70.333*	3.830	.000	53.578	87.089
	Week 7	69.167*	4.024	.000	51.562	86.771
	Week 8	70.333*	4.092	.000	52.432	88.235
	Week 10	70.750*	3.784	.000	54.197	87.303
	Week 11	-1.667	5.009	1.000	-23.579	20.246

Water Chemistry

Table A.6: Student's t-test p-values for water chemistry data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Temperature	pH	Conductivity	Dissolved Oxygen	Ammonium	Nitrate	ORP
1	0.005677779*	2.12902E-05*	6.07337E-05*	1.69975E-05*	7.55888E-09*	9.48113E-07*	7.5946E-11*
2	0.000806863*	1.58967E-06*	6.22963E-07*	1.62297E-05*	3.42434E-08*	9.94907E-07*	
3	0.001766739*	2.74359E-09*	0.000129802*	2.47146E-05*	2.22008E-05*	5.76924E-07*	
4	0.000120371*	4.59572E-10*	1.96845E-06*	1.99171E-05*	1.97249E-06*	2.43613E-07*	
5	6.99139E-07*	3.32091E-06*	1.11353E-05*	3.16702E-06*	9.8826E-08*	2.453E-07*	
6	0.000211203*	1.41002E-12*	2.89264E-05*	1.42492E-06*	2.03363E-06*	1.41746E-09	
7	0.017179214*	1.25162E-10*	0.000139183*	1.96735E-09*	4.70295E-06*	3.96293E-09*	
8	0.000669302*	1.25162E-10*	0.033932406*	1.29141E-06*	5.31856E-06*	2.98866E-09*	5.30059E-11*
9	0.145072902	1.84014E-11*	0.075889517	1.57946E-10*	6.53634E-06*	2.05564E-08*	7.66419E-13*
10	0.00251702*	2.3422E-08*	0.006404459*	3.4037E-12*	4.64503E-06*	2.22871E-07*	1.43957E-13*
11	0.000148901*	9.23275E-11*	0.000230152*	1.51168E-13*	8.26882E-06*	7.89632E-09*	1.33324E-10*
12	0.024365037*	2.4608E-09*	0.092703439	1.01114E-07*	6.26644E-10*	1.17032E-06*	2.61326E-07*

Temperature

Table A.7: Shapiro-Wilk's Test of Normality for temperature data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.894	6	.339
	NA	.796	6	.054
Week 2	A	.812	6	.075
	NA	.909	6	.433

Week 3	A	.925	6	.539
	NA	.958	6	.804
Week 4	A	.922	6	.523
	NA	.918	6	.492
Week 5	A	.982	6	.961
	NA	.920	6	.505
Week 6	A	.874	6	.242
	NA	.864	6	.204
Week 7	A	.845	6	.143
	NA	.875	6	.246
Week 8	A	.933	6	.600
	NA	.934	6	.610
Week 9	A	.842	6	.134
	NA	.952	6	.759
Week 10	A	.851	6	.162
	NA	.929	6	.570
Week 11	A	.767	6	.029
	NA	.875	6	.246
Week 12	A	.918	6	.492
	NA	.908	6	.421

Table A.8: Mauchly's Test of Sphericity for temperature data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	167.001	54	.000

Table A.9: Levene's Test of Equality of Error Variances for temperature data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.605	1	10	.455
Week 2	.017	1	10	.897
Week 3	1.178	1	10	.303
Week 4	.497	1	10	.497
Week 5	.125	1	10	.731
Week 6	.036	1	10	.854
Week 7	.280	1	10	.608
Week 8	.145	1	10	.711
Week 9	.141	1	10	.716
Week 10	.020	1	10	.890

Week 11	10.208	1	10	.010
Week 12	3.709	1	10	.083

Table A.10: Pairwise Comparisons from repeated measures ANOVA for temperature data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	2.300*	.093	.000	1.868	2.732
	Week 3	2.750*	.160	.000	2.007	3.493
	Week 4	2.883*	.157	.000	2.155	3.611
	Week 5	2.900*	.106	.000	2.407	3.393
	Week 6	3.875*	.085	.000	3.478	4.272
	Week 7	4.025*	.076	.000	3.671	4.379
	Week 8	3.683*	.082	.000	3.301	4.066
	Week 9	4.883*	.067	.000	4.573	5.193
	Week 10	4.325*	.066	.000	4.020	4.630
	Week 11	4.667*	.150	.000	3.969	5.364
Week 2	Week 1	-2.300*	.093	.000	-2.732	-1.868
	Week 3	.450	.129	.318	-.149	1.049
	Week 4	.583*	.097	.007	.132	1.035
	Week 5	.600*	.054	.000	.349	.851
	Week 6	1.575*	.061	.000	1.293	1.857
	Week 7	1.725*	.086	.000	1.326	2.124
	Week 8	1.383*	.073	.000	1.043	1.724
	Week 9	2.583*	.101	.000	2.115	3.051
	Week 10	2.025*	.062	.000	1.737	2.313
	Week 11	2.367*	.104	.000	1.885	2.848
Week 3	Week 1	-2.750*	.160	.000	-3.493	-2.007
	Week 2	-.450	.129	.318	-1.049	.149
	Week 4	.133	.104	1.000	-.351	.618
	Week 5	.150	.117	1.000	-.394	.694
	Week 6	1.125*	.135	.000	.495	1.755
	Week 7	1.275*	.172	.001	.476	2.074
	Week 8	.933*	.142	.004	.271	1.595
	Week 9	2.133*	.184	.000	1.277	2.989
	Week 10	1.575*	.144	.000	.904	2.246
	Week 11	1.917*	.078	.000	1.552	2.281
Week 4	Week 1	-2.883*	.157	.000	-3.611	-2.155
	Week 2	-.583*	.097	.007	-1.035	-.132
	Week 3	-.133	.104	1.000	-.618	.351
	Week 5	.017	.069	1.000	-.302	.335

	Week 6	.992*	.094	.000	.553	1.430
	Week 7	1.142*	.143	.001	.478	1.806
	Week 8	.800*	.121	.003	.235	1.365
	Week 9	2.000*	.157	.000	1.270	2.730
	Week 10	1.442*	.111	.000	.927	1.956
	Week 11	1.783*	.082	.000	1.401	2.166
Week 5	Week 1	-2.900*	.106	.000	-3.393	-2.407
	Week 2	-.600*	.054	.000	-.851	-.349
	Week 3	-.150	.117	1.000	-.694	.394
	Week 4	-.017	.069	1.000	-.335	.302
	Week 6	.975*	.043	.000	.775	1.175
	Week 7	1.125*	.093	.000	.692	1.558
	Week 8	.783*	.057	.000	.517	1.050
	Week 9	1.983*	.101	.000	1.513	2.453
	Week 10	1.425*	.056	.000	1.163	1.687
	Week 11	1.767*	.078	.000	1.402	2.131
Week 6	Week 1	-3.875*	.085	.000	-4.272	-3.478
	Week 2	-1.575*	.061	.000	-1.857	-1.293
	Week 3	-1.125*	.135	.000	-1.755	-.495
	Week 4	-.992*	.094	.000	-1.430	-.553
	Week 5	-.975*	.043	.000	-1.175	-.775
	Week 7	.150	.054	1.000	-.101	.401
	Week 8	-.192	.047	.127	-.412	.028
	Week 9	1.008*	.065	.000	.706	1.311
	Week 10	.450*	.022	.000	.346	.554
	Week 11	.792*	.105	.001	.303	1.281
Week 7	Week 1	-4.025*	.076	.000	-4.379	-3.671
	Week 2	-1.725*	.086	.000	-2.124	-1.326
	Week 3	-1.275*	.172	.001	-2.074	-.476
	Week 4	-1.142*	.143	.001	-1.806	-.478
	Week 5	-1.125*	.093	.000	-1.558	-.692
	Week 6	-.150	.054	1.000	-.401	.101
	Week 8	-.342	.074	.050	-.683	6.941E-5
	Week 9	.858*	.033	.000	.706	1.010
	Week 10	.300*	.044	.002	.096	.504
	Week 11	.642	.146	.073	-.036	1.319
Week 8	Week 1	-3.683*	.082	.000	-4.066	-3.301
	Week 2	-1.383*	.073	.000	-1.724	-1.043
	Week 3	-.933*	.142	.004	-1.595	-.271
	Week 4	-.800*	.121	.003	-1.365	-.235
	Week 5	-.783*	.057	.000	-1.050	-.517
	Week 6	.192	.047	.127	-.028	.412
	Week 7	.342	.074	.050	-6.941E-5	.683
	Week 9	1.200*	.071	.000	.869	1.531

	Week 10	.642*	.047	.000	.422	.862
	Week 11	.983*	.108	.000	.481	1.486
Week 9	Week 1	-4.883*	.067	.000	-5.193	-4.573
	Week 2	-2.583*	.101	.000	-3.051	-2.115
	Week 3	-2.133*	.184	.000	-2.989	-1.277
	Week 4	-2.000*	.157	.000	-2.730	-1.270
	Week 5	-1.983*	.101	.000	-2.453	-1.513
	Week 6	-1.008*	.065	.000	-1.311	-.706
	Week 7	-.858*	.033	.000	-1.010	-.706
	Week 8	-1.200*	.071	.000	-1.531	-.869
	Week 10	-.558*	.049	.000	-.786	-.330
	Week 11	-.217	.156	1.000	-.944	.511
Week 10	Week 1	-4.325*	.066	.000	-4.630	-4.020
	Week 2	-2.025*	.062	.000	-2.313	-1.737
	Week 3	-1.575*	.144	.000	-2.246	-.904
	Week 4	-1.442*	.111	.000	-1.956	-.927
	Week 5	-1.425*	.056	.000	-1.687	-1.163
	Week 6	-.450*	.022	.000	-.554	-.346
	Week 7	-.300*	.044	.002	-.504	-.096
	Week 8	-.642*	.047	.000	-.862	-.422
	Week 9	.558*	.049	.000	.330	.786
	Week 11	.342	.119	.900	-.210	.893
Week 11	Week 1	-4.667*	.150	.000	-5.364	-3.969
	Week 2	-2.367*	.104	.000	-2.848	-1.885
	Week 3	-1.917*	.078	.000	-2.281	-1.552
	Week 4	-1.783*	.082	.000	-2.166	-1.401
	Week 5	-1.767*	.078	.000	-2.131	-1.402
	Week 6	-.792*	.105	.001	-1.281	-.303
	Week 7	-.642	.146	.073	-1.319	.036
	Week 8	-.983*	.108	.000	-1.486	-.481
	Week 9	.217	.156	1.000	-.511	.944
	Week 10	-.342	.119	.900	-.893	.210

pH

Table A.11: Shapiro-Wilk's Test of Normality for pH data of aerated and non-aerated systems, α = 0.05.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.612	6	.001
	NA	.912	6	.452
Week 2	A	.870	6	.225
	NA	.955	6	.781
Week 3	A	.957	6	.800

	NA	.824	6	.096
Week 4	A	.961	6	.830
	NA	.901	6	.378
Week 5	A	.772	6	.033
	NA	.902	6	.384
Week 6	A	.816	6	.081
	NA	.871	6	.229
Week 7	A	.892	6	.331
	NA	.890	6	.318
Week 8	A	.892	6	.331
	NA	.890	6	.318
Week 9	A	.902	6	.387
	NA	.910	6	.435
Week 10	A	.892	6	.331
	NA	.945	6	.702
Week 11	A	.962	6	.838
	NA	.869	6	.222
Week 12	A	.940	6	.660
	NA	.749	6	.019

Table A.12: Mauchly's Test of Sphericity for pH data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	116.662	54	.000

Table A.13: Levene's Test of Equality of Error Variances for pH data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	3.945	1	10	.075
Week 2	10.991	1	10	.008
Week 3	1.189	1	10	.301
Week 4	1.777	1	10	.212
Week 5	7.713	1	10	.020
Week 6	1.596	1	10	.235
Week 7	.417	1	10	.533
Week 8	.417	1	10	.533
Week 9	.960	1	10	.350
Week 10	9.301	1	10	.012
Week 11	.021	1	10	.886

Week 12	1.820	1	10	.207

Table A.14: Pairwise Comparisons from repeated measures ANOVA for pH data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.013	.021	1.000	-.086	.111
	Week 3	.041	.027	1.000	-.083	.164
	Week 4	.072	.022	.460	-.030	.175
	Week 5	.162*	.012	.000	.106	.219
	Week 6	.110*	.021	.020	.013	.207
	Week 7	.018	.025	1.000	-.099	.136
	Week 8	.018	.025	1.000	-.099	.136
	Week 9	.037	.028	1.000	-.095	.168
	Week 10	-.091	.032	.910	-.238	.056
	Week 11	-.068	.025	1.000	-.183	.047
Week 2	Week 1	-.013	.021	1.000	-.111	.086
	Week 3	.028	.013	1.000	-.030	.087
	Week 4	.060	.015	.154	-.011	.131
	Week 5	.150*	.019	.001	.060	.240
	Week 6	.097*	.015	.005	.026	.169
	Week 7	.006	.015	1.000	-.065	.077
	Week 8	.006	.015	1.000	-.065	.077
	Week 9	.024	.017	1.000	-.053	.101
	Week 10	-.103	.023	.069	-.211	.005
	Week 11	-.081	.018	.076	-.167	.005
Week 3	Week 1	-.041	.027	1.000	-.164	.083
	Week 2	-.028	.013	1.000	-.087	.030
	Week 4	.032	.013	1.000	-.028	.092
	Week 5	.122*	.023	.020	.015	.229
	Week 6	.069*	.013	.015	.010	.128
	Week 7	-.022	.017	1.000	-.101	.056
	Week 8	-.022	.017	1.000	-.101	.056
	Week 9	-.004	.015	1.000	-.074	.066
	Week 10	-.132*	.022	.007	-.232	-.031
	Week 11	-.109*	.015	.002	-.181	-.037
Week 4	Week 1	-.072	.022	.460	-.175	.030
	Week 2	-.060	.015	.154	-.131	.011
	Week 3	-.032	.013	1.000	-.092	.028
	Week 5	.090	.020	.060	-.002	.182

	Week 6	.037*	.005	.001	.015	.060
	Week 7	-.054	.013	.087	-.113	.004
	Week 8	-.054	.013	.087	-.113	.004
	Week 9	-.036	.011	.418	-.086	.014
	Week 10	-.163*	.016	.000	-.237	-.090
	Week 11	-.141*	.009	.000	-.181	-.100
Week 5	Week 1	-.162*	.012	.000	-.219	-.106
	Week 2	-.150*	.019	.001	-.240	-.060
	Week 3	-.122*	.023	.020	-.229	-.015
	Week 4	-.090	.020	.060	-.182	.002
	Week 6	-.052	.019	1.000	-.143	.038
	Week 7	-.144*	.023	.005	-.252	-.036
	Week 8	-.144*	.023	.005	-.252	-.036
	Week 9	-.126*	.026	.037	-.246	-.005
	Week 10	-.253*	.030	.000	-.395	-.112
	Week 11	-.231*	.024	.000	-.341	-.121
Week 6	Week 1	-.110*	.021	.020	-.207	-.013
	Week 2	-.097*	.015	.005	-.169	-.026
	Week 3	-.069*	.013	.015	-.128	-.010
	Week 4	-.037*	.005	.001	-.060	-.015
	Week 5	.052	.019	1.000	-.038	.143
	Week 7	-.092*	.015	.006	-.162	-.022
	Week 8	-.092*	.015	.006	-.162	-.022
	Week 9	-.073*	.013	.010	-.132	-.014
	Week 10	-.201*	.016	.000	-.274	-.128
	Week 11	-.178*	.008	.000	-.215	-.141
Week 7	Week 1	-.018	.025	1.000	-.136	.099
	Week 2	-.006	.015	1.000	-.077	.065
	Week 3	.022	.017	1.000	-.056	.101
	Week 4	.054	.013	.087	-.004	.113
	Week 5	.144*	.023	.005	.036	.252
	Week 6	.092*	.015	.006	.022	.162
	Week 8	.000	.000	.	.000	.000
	Week 9	.018	.008	1.000	-.018	.055
	Week 10	-.109	.024	.065	-.223	.004
	Week 11	-.087*	.017	.028	-.167	-.007
Week 8	Week 1	-.018	.025	1.000	-.136	.099
	Week 2	-.006	.015	1.000	-.077	.065
	Week 3	.022	.017	1.000	-.056	.101
	Week 4	.054	.013	.087	-.004	.113
	Week 5	.144*	.023	.005	.036	.252
	Week 6	.092*	.015	.006	.022	.162
	Week 7	.000	.000	.	.000	.000
	Week 9	.018	.008	1.000	-.018	.055

	Week 10	-.109	.024	.065	-.223	.004
	Week 11	-.087*	.017	.028	-.167	-.007
Week 9	Week 1	-.037	.028	1.000	-.168	.095
	Week 2	-.024	.017	1.000	-.101	.053
	Week 3	.004	.015	1.000	-.066	.074
	Week 4	.036	.011	.418	-.014	.086
	Week 5	.126*	.026	.037	.005	.246
	Week 6	.073*	.013	.010	.014	.132
	Week 7	-.018	.008	1.000	-.055	.018
	Week 8	-.018	.008	1.000	-.055	.018
	Week 10	-.128*	.020	.005	-.222	-.033
	Week 11	-.105*	.015	.002	-.173	-.037
Week 10	Week 1	.091	.032	.910	-.056	.238
	Week 2	.103	.023	.069	-.005	.211
	Week 3	.132*	.022	.007	.031	.232
	Week 4	.163*	.016	.000	.090	.237
	Week 5	.253*	.030	.000	.112	.395
	Week 6	.201*	.016	.000	.128	.274
	Week 7	.109	.024	.065	-.004	.223
	Week 8	.109	.024	.065	-.004	.223
	Week 9	.128*	.020	.005	.033	.222
	Week 11	.023	.016	1.000	-.053	.098
Week 11	Week 1	.068	.025	1.000	-.047	.183
	Week 2	.081	.018	.076	-.005	.167
	Week 3	.109*	.015	.002	.037	.181
	Week 4	.141*	.009	.000	.100	.181
	Week 5	.231*	.024	.000	.121	.341
	Week 6	.178*	.008	.000	.141	.215
	Week 7	.087*	.017	.028	.007	.167
	Week 8	.087*	.017	.028	.007	.167
	Week 9	.105*	.015	.002	.037	.173
	Week 10	-.023	.016	1.000	-.098	.053

Conductivity

Table A.15: Shapiro-Wilk's Test of Normality for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.909	6	.431
	NA	.976	6	.928
Week 2	A	.969	6	.883
	NA	.935	6	.617
Week 3	A	.931	6	.585

	NA	.838	6	.126
Week 4	A	.902	6	.389
	NA	.932	6	.598
Week 5	A	.767	6	.029
	NA	.928	6	.568
Week 6	A	.984	6	.968
	NA	.926	6	.553
Week 7	A	.817	6	.083
	NA	.947	6	.714
Week 8	A	.914	6	.461
	NA	.867	6	.214
Week 9	A	.862	6	.196
	NA	.741	6	.016
Week 10	A	.932	6	.592
	NA	.950	6	.743
Week 11	A	.852	6	.164
	NA	.987	6	.980
Week 12	A	.801	6	.061
	NA	.911	6	.441

Table A.16: Mauchly's Test of Sphericity for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	111.989	54	.000

Table A.17: Levene's Test of Equality of Error Variances for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	1.312	1	10	.279
Week 2	.969	1	10	.348
Week 3	1.726	1	10	.218
Week 4	.148	1	10	.709
Week 5	.010	1	10	.921
Week 6	1.091	1	10	.321
Week 7	.109	1	10	.748
Week 8	2.025	1	10	.185
Week 9	.267	1	10	.617
Week 10	5.904	1	10	.035
Week 11	.016	1	10	.901

Week 12	.016	1	10	.901

Table A.18: Pairwise Comparisons from repeated measures ANOVA for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	123.167*	5.388	.000	98.118	148.216
	Week 3	176.583*	7.192	.000	143.146	210.020
	Week 4	179.250*	5.824	.000	152.173	206.327
	Week 5	185.917*	4.286	.000	165.988	205.845
	Week 6	217.583*	6.489	.000	187.414	247.752
	Week 7	230.583*	5.309	.000	205.901	255.266
	Week 8	229.500*	6.225	.000	200.560	258.440
	Week 9	240.583*	5.153	.000	216.627	264.540
	Week 10	241.917*	5.560	.000	216.065	267.768
	Week 11	210.333*	6.104	.000	181.952	238.714
Week 2	Week 1	-123.167*	5.388	.000	-148.216	-98.118
	Week 3	53.417*	3.581	.000	36.768	70.066
	Week 4	56.083*	4.184	.000	36.630	75.536
	Week 5	62.750*	3.579	.000	46.108	79.392
	Week 6	94.417*	4.828	.000	71.971	116.862
	Week 7	107.417*	3.607	.000	90.649	124.184
	Week 8	106.333*	5.508	.000	80.726	131.941
	Week 9	117.417*	3.859	.000	99.476	135.357
	Week 10	118.750*	3.178	.000	103.973	133.527
	Week 11	87.167*	4.543	.000	66.047	108.287
Week 3	Week 1	-176.583*	7.192	.000	-210.020	-143.146
	Week 2	-53.417*	3.581	.000	-70.066	-36.768
	Week 4	2.667	3.604	1.000	-14.089	19.423
	Week 5	9.333	4.561	1.000	-11.872	30.539
	Week 6	41.000*	4.760	.000	18.869	63.131
	Week 7	54.000*	4.980	.000	30.848	77.152
	Week 8	52.917*	5.345	.000	28.067	77.766
	Week 9	64.000*	5.410	.000	38.846	89.154
	Week 10	65.333*	4.870	.000	42.690	87.976
	Week 11	33.750*	5.096	.003	10.058	57.442
Week 4	Week 1	-179.250*	5.824	.000	-206.327	-152.173
	Week 2	-56.083*	4.184	.000	-75.536	-36.630
	Week 3	-2.667	3.604	1.000	-19.423	14.089
	Week 5	6.667	2.527	1.000	-5.082	18.416
	Week 6	38.333*	2.969	.000	24.531	52.136

	Week 7	51.333*	3.519	.000	34.971	67.696
	Week 8	50.250*	4.149	.000	30.961	69.539
	Week 9	61.333*	4.049	.000	42.509	80.158
	Week 10	62.667*	4.007	.000	44.039	81.294
	Week 11	31.083*	3.443	.000	15.074	47.092
Week 5	Week 1	-185.917*	4.286	.000	-205.845	-165.988
	Week 2	-62.750*	3.579	.000	-79.392	-46.108
	Week 3	-9.333	4.561	1.000	-30.539	11.872
	Week 4	-6.667	2.527	1.000	-18.416	5.082
	Week 6	31.667*	3.480	.000	15.487	47.847
	Week 7	44.667*	2.704	.000	32.096	57.238
	Week 8	43.583*	3.560	.000	27.032	60.135
	Week 9	54.667*	2.398	.000	43.515	65.818
	Week 10	56.000*	2.948	.000	42.295	69.705
	Week 11	24.417*	3.648	.003	7.457	41.376
Week 6	Week 1	-217.583*	6.489	.000	-247.752	-187.414
	Week 2	-94.417*	4.828	.000	-116.862	-71.971
	Week 3	-41.000*	4.760	.000	-63.131	-18.869
	Week 4	-38.333*	2.969	.000	-52.136	-24.531
	Week 5	-31.667*	3.480	.000	-47.847	-15.487
	Week 7	13.000*	2.241	.010	2.581	23.419
	Week 8	11.917	4.535	1.000	-9.169	33.002
	Week 9	23.000*	3.393	.003	7.224	38.776
	Week 10	24.333*	3.146	.001	9.709	38.958
	Week 11	-7.250	1.654	.075	-14.938	.438
Week 7	Week 1	-230.583*	5.309	.000	-255.266	-205.901
	Week 2	-107.417*	3.607	.000	-124.184	-90.649
	Week 3	-54.000*	4.980	.000	-77.152	-30.848
	Week 4	-51.333*	3.519	.000	-67.696	-34.971
	Week 5	-44.667*	2.704	.000	-57.238	-32.096
	Week 6	-13.000*	2.241	.010	-23.419	-2.581
	Week 8	-1.083	4.696	1.000	-22.918	20.752
	Week 9	10.000*	2.031	.033	.557	19.443
	Week 10	11.333*	1.380	.001	4.915	17.751
	Week 11	-20.250*	1.995	.000	-29.524	-10.976
Week 8	Week 1	-229.500*	6.225	.000	-258.440	-200.560
	Week 2	-106.333*	5.508	.000	-131.941	-80.726
	Week 3	-52.917*	5.345	.000	-77.766	-28.067
	Week 4	-50.250*	4.149	.000	-69.539	-30.961
	Week 5	-43.583*	3.560	.000	-60.135	-27.032
	Week 6	-11.917	4.535	1.000	-33.002	9.169
	Week 7	1.083	4.696	1.000	-20.752	22.918
	Week 9	11.083	3.409	.479	-4.768	26.934
	Week 10	12.417	4.488	1.000	-8.448	33.282

	Week 11	-19.167	4.589	.104	-40.503	2.170
Week 9	Week 1	-240.583*	5.153	.000	-264.540	-216.627
	Week 2	-117.417*	3.859	.000	-135.357	-99.476
	Week 3	-64.000*	5.410	.000	-89.154	-38.846
	Week 4	-61.333*	4.049	.000	-80.158	-42.509
	Week 5	-54.667*	2.398	.000	-65.818	-43.515
	Week 6	-23.000*	3.393	.003	-38.776	-7.224
	Week 7	-10.000*	2.031	.033	-19.443	-.557
	Week 8	-11.083	3.409	.479	-26.934	4.768
	Week 10	1.333	1.662	1.000	-6.396	9.063
	Week 11	-30.250*	3.016	.000	-44.272	-16.228
Week 10	Week 1	-241.917*	5.560	.000	-267.768	-216.065
	Week 2	-118.750*	3.178	.000	-133.527	-103.973
	Week 3	-65.333*	4.870	.000	-87.976	-42.690
	Week 4	-62.667*	4.007	.000	-81.294	-44.039
	Week 5	-56.000*	2.948	.000	-69.705	-42.295
	Week 6	-24.333*	3.146	.001	-38.958	-9.709
	Week 7	-11.333*	1.380	.001	-17.751	-4.915
	Week 8	-12.417	4.488	1.000	-33.282	8.448
	Week 9	-1.333	1.662	1.000	-9.063	6.396
	Week 11	-31.583*	2.537	.000	-43.377	-19.790
Week 11	Week 1	-210.333*	6.104	.000	-238.714	-181.952
	Week 2	-87.167*	4.543	.000	-108.287	-66.047
	Week 3	-33.750*	5.096	.003	-57.442	-10.058
	Week 4	-31.083*	3.443	.000	-47.092	-15.074
	Week 5	-24.417*	3.648	.003	-41.376	-7.457
	Week 6	7.250	1.654	.075	-.438	14.938
	Week 7	20.250*	1.995	.000	10.976	29.524
	Week 8	19.167	4.589	.104	-2.170	40.503
	Week 9	30.250*	3.016	.000	16.228	44.272
	Week 10	31.583*	2.537	.000	19.790	43.377
Week 12	Week 1	-119.667*	8.482	.000	-159.102	-80.231
	Week 2	-66.250*	8.416	.001	-105.380	-27.120
	Week 3	-63.583*	6.123	.000	-92.050	-35.116
	Week 4	-56.917*	7.370	.001	-91.182	-22.651
	Week 5	-25.250	6.054	.105	-53.397	2.897
	Week 6	-12.250	6.823	1.000	-43.971	19.471
	Week 7	-13.333	8.375	1.000	-52.272	25.605
	Week 8	-2.250	7.964	1.000	-39.274	34.774
	Week 9	-.917	7.611	1.000	-36.301	34.468
	Week 10	-32.500*	5.653	.010	-58.782	-6.218

Dissolved Oxygen

Table A.19: Shapiro-Wilk's Test of Normality for dissolved oxygen data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.960	6	.818
	NA	.955	6	.783
Week 2	A	.936	6	.625
	NA	.827	6	.101
Week 3	A	.880	6	.268
	NA	.603	6	.000
Week 4	A	.842	6	.136
	NA	.907	6	.415
Week 5	A	.638	6	.001
	NA	.827	6	.101
Week 6	A	.613	6	.001
	NA	.915	6	.473
Week 7	A	.890	6	.317
	NA	.866	6	.212
Week 8	A	.632	6	.001
	NA	.869	6	.221
Week 9	A	.904	6	.397
	NA	.938	6	.642
Week 10	A	.973	6	.913
	NA	.772	6	.033
Week 11	A	.929	6	.576
	NA	.957	6	.796
Week 12	A	.949	6	.730
	NA	.905	6	.405

Table A.20: Levene's Test of Equality of Error Variances for dissolved oxygen data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	15.250	1	10	.003
Week 2	18.020	1	10	.002
Week 3	14.838	1	10	.003
Week 4	40.501	1	10	.000
Week 5	5.822	1	10	.037
Week 6	5.839	1	10	.036
Week 7	11.135	1	10	.008
Week 8	5.031	1	10	.049
Week 9	4.554	1	10	.059

Week 10	9.670	1	10	.011
Week 11	6.667	1	10	.027
Week 12	7.510	1	10	.021

Repeated measures ANOVA performed to assess the effects of time on the dissolved oxygen levels in the aerated and non-aerated mesocosms and no significance was observed for the effect of time.

Ammonium

Table A.21: Shapiro-Wilk's Test of Normality for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.713	6	.008
	NA	.985	6	.974
Week 2	A	.819	6	.087
	NA	.872	6	.235
Week 3	A	.597	6	.000
	NA	.952	6	.759
Week 4	A	.720	6	.010
	NA	.884	6	.287
Week 5	A	.664	6	.003
	NA	.919	6	.499
Week 6	A	.870	6	.225
	NA	.900	6	.373
Week 7	A	.913	6	.460
	NA	.957	6	.795
Week 8	A	.874	6	.242
	NA	.959	6	.811
Week 9	A	.952	6	.757
	NA	.946	6	.710
Week 10	A	.850	6	.157
	NA	.971	6	.899
Week 11	A	.946	6	.707
	NA	.959	6	.815
Week 12	A	.937	6	.638
	NA	.916	6	.479

Table A.22: Mauchly's Test of Sphericity for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W		df	Sig.

		Approx. Chi-Square		
Time	.000	174.317	54	.000

Table A.23: Levene's Test of Equality of Error Variances for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.229	1	10	.643
Week 2	2.559	1	10	.141
Week 3	2.805	1	10	.125
Week 4	3.613	1	10	.087
Week 5	2.706	1	10	.131
Week 6	4.533	1	10	.059
Week 7	18.039	1	10	.002
Week 8	7.810	1	10	.019
Week 9	11.697	1	10	.007
Week 10	16.801	1	10	.002
Week 11	18.474	1	10	.002
Week 12	2.453	1	10	.148

Table A.24: Pairwise Comparisons from repeated measures ANOVA for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	-.140	.086	1.000	-.542	.262
	Week 3	-.247	.178	1.000	-1.072	.579
	Week 4	-.363	.119	.667	-.916	.189
	Week 5	-.618*	.102	.007	-1.094	-.143
	Week 6	-.251	.124	1.000	-.828	.326
	Week 7	-.101	.154	1.000	-.818	.617
	Week 8	-.213	.132	1.000	-.829	.403
	Week 9	-.806	.184	.075	-1.661	.049
	Week 10	-.147	.152	1.000	-.855	.561
	Week 11	-.316	.177	1.000	-1.140	.509
Week 2	Week 1	.140	.086	1.000	-.262	.542
	Week 3	-.107	.171	1.000	-.901	.687
	Week 4	-.223	.087	1.000	-.628	.182
	Week 5	-.478*	.054	.000	-.731	-.226
	Week 6	-.111	.066	1.000	-.416	.194
	Week 7	.039	.085	1.000	-.354	.433

	Week 8	-.073	.109	1.000	-.579	.432
	Week 9	-.666*	.106	.005	-1.157	-.175
	Week 10	-.007	.085	1.000	-.400	.387
	Week 11	-.176	.109	1.000	-.683	.332
Week 3	Week 1	.247	.178	1.000	-.579	1.072
	Week 2	.107	.171	1.000	-.687	.901
	Week 4	-.117	.093	1.000	-.549	.316
	Week 5	-.372	.141	1.000	-1.029	.286
	Week 6	-.004	.198	1.000	-.926	.918
	Week 7	.146	.235	1.000	-.947	1.238
	Week 8	.033	.102	1.000	-.439	.506
	Week 9	-.559	.210	1.000	-1.534	.416
	Week 10	.100	.220	1.000	-.923	1.123
	Week 11	-.069	.228	1.000	-1.128	.990
Week 4	Week 1	.363	.119	.667	-.189	.916
	Week 2	.223	.087	1.000	-.182	.628
	Week 3	.117	.093	1.000	-.316	.549
	Week 5	-.255	.062	.117	-.544	.034
	Week 6	.112	.113	1.000	-.411	.636
	Week 7	.263	.146	1.000	-.419	.944
	Week 8	.150	.068	1.000	-.164	.464
	Week 9	-.442	.135	.462	-1.071	.186
	Week 10	.217	.139	1.000	-.430	.863
	Week 11	.047	.152	1.000	-.658	.753
Week 5	Week 1	.618*	.102	.007	.143	1.094
	Week 2	.478*	.054	.000	.226	.731
	Week 3	.372	.141	1.000	-.286	1.029
	Week 4	.255	.062	.117	-.034	.544
	Week 6	.367	.095	.173	-.075	.810
	Week 7	.518	.113	.055	-.007	1.042
	Week 8	.405	.092	.074	-.023	.833
	Week 9	-.188	.115	1.000	-.723	.348
	Week 10	.472	.124	.190	-.104	1.048
	Week 11	.302	.145	1.000	-.372	.977
Week 6	Week 1	.251	.124	1.000	-.326	.828
	Week 2	.111	.066	1.000	-.194	.416
	Week 3	.004	.198	1.000	-.918	.926
	Week 4	-.112	.113	1.000	-.636	.411
	Week 5	-.367	.095	.173	-.810	.075
	Week 7	.150	.065	1.000	-.153	.453
	Week 8	.037	.121	1.000	-.527	.602
	Week 9	-.555*	.089	.005	-.968	-.142
	Week 10	.104	.060	1.000	-.175	.383
	Week 11	-.065	.091	1.000	-.486	.356

Week 7	Week 1	.101	.154	1.000	-.617	.818
	Week 2	-.039	.085	1.000	-.433	.354
	Week 3	-.146	.235	1.000	-1.238	.947
	Week 4	-.263	.146	1.000	-.944	.419
	Week 5	-.518	.113	.055	-1.042	.007
	Week 6	-.150	.065	1.000	-.453	.153
	Week 8	-.113	.160	1.000	-.858	.633
	Week 9	-.705*	.089	.001	-1.119	-.291
	Week 10	-.046	.072	1.000	-.378	.287
	Week 11	-.215	.087	1.000	-.621	.191
Week 8	Week 1	.213	.132	1.000	-.403	.829
	Week 2	.073	.109	1.000	-.432	.579
	Week 3	-.033	.102	1.000	-.506	.439
	Week 4	-.150	.068	1.000	-.464	.164
	Week 5	-.405	.092	.074	-.833	.023
	Week 6	-.037	.121	1.000	-.602	.527
	Week 7	.113	.160	1.000	-.633	.858
	Week 9	-.592	.134	.072	-1.216	.031
	Week 10	.067	.144	1.000	-.601	.734
	Week 11	-.103	.160	1.000	-.846	.641
Week 9	Week 1	.806	.184	.075	-.049	1.661
	Week 2	.666*	.106	.005	.175	1.157
	Week 3	.559	.210	1.000	-.416	1.534
	Week 4	.442	.135	.462	-.186	1.071
	Week 5	.188	.115	1.000	-.348	.723
	Week 6	.555*	.089	.005	.142	.968
	Week 7	.705*	.089	.001	.291	1.119
	Week 8	.592	.134	.072	-.031	1.216
	Week 10	.659*	.080	.001	.287	1.032
	Week 11	.490*	.100	.034	.026	.954
Week 10	Week 1	.147	.152	1.000	-.561	.855
	Week 2	.007	.085	1.000	-.387	.400
	Week 3	-.100	.220	1.000	-1.123	.923
	Week 4	-.217	.139	1.000	-.863	.430
	Week 5	-.472	.124	.190	-1.048	.104
	Week 6	-.104	.060	1.000	-.383	.175
	Week 7	.046	.072	1.000	-.287	.378
	Week 8	-.067	.144	1.000	-.734	.601
	Week 9	-.659*	.080	.001	-1.032	-.287
	Week 11	-.169	.042	.143	-.367	.028
Week 11	Week 1	.316	.177	1.000	-.509	1.140
	Week 2	.176	.109	1.000	-.332	.683
	Week 3	.069	.228	1.000	-.990	1.128
	Week 4	-.047	.152	1.000	-.753	.658

	Week 5	-.302	.145	1.000	-.977	.372
	Week 6	.065	.091	1.000	-.356	.486
	Week 7	.215	.087	1.000	-.191	.621
	Week 8	.103	.160	1.000	-.641	.846
	Week 9	-.490*	.100	.034	-.954	-.026
	Week 10	.169	.042	.143	-.028	.367
Week 12	Week 1	-.108	.107	1.000	-.605	.388
	Week 2	-.215	.230	1.000	-1.282	.852
	Week 3	-.332	.154	1.000	-1.047	.383
	Week 4	-.587	.134	.076	-1.209	.036
	Week 5	-.219	.084	1.000	-.611	.172
	Week 6	-.069	.105	1.000	-.556	.418
	Week 7	-.182	.155	1.000	-.903	.539
	Week 8	-.774*	.163	.043	-1.532	-.017
	Week 9	-.115	.120	1.000	-.671	.441
	Week 10	-.284	.140	1.000	-.933	.365

Nitrate

Table A.25: Shapiro-Wilk's Test of Normality for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.976	6	.929
	NA	.940	6	.659
Week 2	A	.944	6	.690
	NA	.984	6	.969
Week 3	A	.855	6	.174
	NA	.933	6	.603
Week 4	A	.914	6	.460
	NA	.753	6	.021
Week 5	A	.810	6	.072
	NA	.829	6	.105
Week 6	A	.871	6	.230
	NA	.837	6	.122
Week 7	A	.965	6	.856
	NA	.738	6	.015
Week 8	A	.900	6	.372
	NA	.696	6	.006
Week 9	A	.941	6	.670
	NA	.923	6	.524
Week 10	A	.836	6	.120
	NA	.956	6	.792

Table A.26: Mauchly's Test of Sphericity for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	181.324	44	.000

Table A.27: Levene's Test of Equality of Error Variances for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	10.928	1	10	.008
Week 2	18.016	1	10	.002
Week 3	20.881	1	10	.001
Week 4	31.833	1	10	.000
Week 5	13.684	1	10	.004
Week 6	7.671	1	10	.020
Week 7	6.347	1	10	.030
Week 8	6.586	1	10	.028
Week 9	3.924	1	10	.076
Week 10	2.258	1	10	.164

Table A.28: Pairwise Comparisons from repeated measures ANOVA for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	5.844	1.486	.127	-.872	12.561
	Week 3	-1.098	1.605	1.000	-8.348	6.153
	Week 4	1.075	1.486	1.000	-5.638	7.788
	Week 5	-4.325	1.543	.841	-11.295	2.645
	Week 6	-1.665	.980	1.000	-6.095	2.765
	Week 7	7.835*	1.127	.002	2.745	12.925
	Week 8	9.387*	1.146	.000	4.207	14.568
	Week 9	-10.706*	1.286	.000	-16.519	-4.893
	Week 10	-19.576*	1.932	.000	-28.305	-10.846
Week 2	Week 1	-5.844	1.486	.127	-12.561	.872
	Week 3	-6.942*	.692	.000	-10.070	-3.813
	Week 4	-4.769*	.323	.000	-6.231	-3.308
	Week 5	-10.169*	.610	.000	-12.926	-7.412

	Week 6	-7.509*	.855	.000	-11.371	-3.647
	Week 7	1.991	.847	1.000	-1.836	5.818
	Week 8	3.543	.818	.067	-.152	7.239
	Week 9	-16.550*	1.440	.000	-23.054	-10.046
	Week 10	-25.420*	1.873	.000	-33.884	-16.956
Week 3	Week 1	1.098	1.605	1.000	-6.153	8.348
	Week 2	6.942*	.692	.000	3.813	10.070
	Week 4	2.173*	.421	.019	.272	4.073
	Week 5	-3.227*	.317	.000	-4.659	-1.796
	Week 6	-.567	.849	1.000	-4.404	3.269
	Week 7	8.932*	.926	.000	4.750	13.115
	Week 8	10.485*	.775	.000	6.982	13.988
	Week 9	-9.608*	1.556	.005	-16.640	-2.577
	Week 10	-18.478*	1.878	.000	-26.962	-9.995
Week 4	Week 1	-1.075	1.486	1.000	-7.788	5.638
	Week 2	4.769*	.323	.000	3.308	6.231
	Week 3	-2.173*	.421	.019	-4.073	-.272
	Week 5	-5.400*	.423	.000	-7.311	-3.489
	Week 6	-2.740	.710	.142	-5.947	.467
	Week 7	6.760*	.710	.000	3.552	9.968
	Week 8	8.312*	.637	.000	5.435	11.190
	Week 9	-11.781*	1.343	.000	-17.849	-5.713
	Week 10	-20.651*	1.781	.000	-28.699	-12.602
Week 5	Week 1	4.325	1.543	.841	-2.645	11.295
	Week 2	10.169*	.610	.000	7.412	12.926
	Week 3	3.227*	.317	.000	1.796	4.659
	Week 4	5.400*	.423	.000	3.489	7.311
	Week 6	2.660	.795	.333	-.930	6.250
	Week 7	12.160*	.894	.000	8.123	16.197
	Week 8	13.712*	.730	.000	10.416	17.009
	Week 9	-6.381	1.504	.077	-13.177	.415
	Week 10	-15.251*	1.840	.000	-23.563	-6.939
Week 6	Week 1	1.665	.980	1.000	-2.765	6.095
	Week 2	7.509*	.855	.000	3.647	11.371
	Week 3	.567	.849	1.000	-3.269	4.404
	Week 4	2.740	.710	.142	-.467	5.947
	Week 5	-2.660	.795	.333	-6.250	.930
	Week 7	9.500*	.255	.000	8.349	10.651
	Week 8	11.052*	.204	.000	10.129	11.976
	Week 9	-9.041*	.799	.000	-12.650	-5.432
	Week 10	-17.911*	1.550	.000	-24.916	-10.906
Week 7	Week 1	-7.835*	1.127	.002	-12.925	-2.745
	Week 2	-1.991	.847	1.000	-5.818	1.836
	Week 3	-8.932*	.926	.000	-13.115	-4.750

	Week 4	-6.760*	.710	.000	-9.968	-3.552
	Week 5	-12.160*	.894	.000	-16.197	-8.123
	Week 6	-9.500*	.255	.000	-10.651	-8.349
	Week 8	1.553*	.248	.004	.434	2.671
	Week 9	-18.541*	.658	.000	-21.512	-15.569
	Week 10	-27.411*	1.505	.000	-34.209	-20.612
Week 8	Week 1	-9.387*	1.146	.000	-14.568	-4.207
	Week 2	-3.543	.818	.067	-7.239	.152
	Week 3	-10.485*	.775	.000	-13.988	-6.982
	Week 4	-8.312*	.637	.000	-11.190	-5.435
	Week 5	-13.712*	.730	.000	-17.009	-10.416
	Week 6	-11.052*	.204	.000	-11.976	-10.129
	Week 7	-1.553*	.248	.004	-2.671	-.434
	Week 9	-20.093*	.819	.000	-23.794	-16.392
	Week 10	-28.963*	1.494	.000	-35.712	-22.215
Week 9	Week 1	10.706*	1.286	.000	4.893	16.519
	Week 2	16.550*	1.440	.000	10.046	23.054
	Week 3	9.608*	1.556	.005	2.577	16.640
	Week 4	11.781*	1.343	.000	5.713	17.849
	Week 5	6.381	1.504	.077	-.415	13.177
	Week 6	9.041*	.799	.000	5.432	12.650
	Week 7	18.541*	.658	.000	15.569	21.512
	Week 8	20.093*	.819	.000	16.392	23.794
	Week 10	-8.870*	1.549	.009	-15.869	-1.871
Week 10	Week 1	19.576*	1.932	.000	10.846	28.305
	Week 2	25.420*	1.873	.000	16.956	33.884
	Week 3	18.478*	1.878	.000	9.995	26.962
	Week 4	20.651*	1.781	.000	12.602	28.699
	Week 5	15.251*	1.840	.000	6.939	23.563
	Week 6	17.911*	1.550	.000	10.906	24.916
	Week 7	27.411*	1.505	.000	20.612	34.209
	Week 8	28.963*	1.494	.000	22.215	35.712
	Week 9	8.870*	1.549	.009	1.871	15.869

ORP

Table A.29: Shapiro-Wilk's Test of Normality for ORP data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.845	6	.142
	NA	.941	6	.671
Week 8	A	.948	6	.724
	NA	.933	6	.603

Week 9	A	.970	6	.890
	NA	.887	6	.302
Week 10	A	.935	6	.620
	NA	.983	6	.964
Week 11	A	.827	6	.101
	NA	.832	6	.111
Week 12	A	.946	6	.710
	NA	.907	6	.418

Table A.30: Mauchly's Test of Sphericity for ORP data of aerated and non-aerated systems, α = 0.05.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.004	44.993	14	.000

Table A.31: Levene's Test of Equality of Error Variances for ORP data of aerated and non-aerated systems, α = 0.05.

	F	df1	df2	Sig.
Week 1	2.464	1	10	.148
Week 8	4.627	1	10	.057
Week 9	2.663	1	10	.134
Week 10	3.968	1	10	.074
Week 11	81.486	1	10	.000
Week 12	19.591	1	10	.001

Table A.32: Pairwise Comparisons from repeated measures ANOVA for ORP data of aerated and non-aerated systems, α = 0.05. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 8	99.126*	1.862	.000	92.001	106.251
	Week 9	83.186*	2.223	.000	74.679	91.693
	Week 10	97.027*	1.912	.000	89.708	104.345
	Week 11	98.127*	1.836	.000	91.101	105.153
	Week 12	80.208*	3.779	.000	65.744	94.672
Week 8	Week 1	-99.126*	1.862	.000	-106.251	-92.001
	Week 9	-15.940*	1.129	.000	-20.262	-11.618
	Week 10	-2.099	.926	.702	-5.643	1.445
	Week 11	-.999	.842	1.000	-4.221	2.223

	Week 12	-18.918*	3.265	.003	-31.414	-6.421
Week 9	Week 1	-83.186*	2.223	.000	-91.693	-74.679
	Week8	15.940*	1.129	.000	11.618	20.262
	Week 10	13.841*	.868	.000	10.519	17.162
	Week 11	14.941*	.758	.000	12.039	17.843
	Week 12	-2.978	3.544	1.000	-16.541	10.586
Week 10	Week 1	-97.027*	1.912	.000	-104.345	-89.708
	Week 8	2.099	.926	.702	-1.445	5.643
	Week 9	-13.841*	.868	.000	-17.162	-10.519
	Week 11	1.100	.548	1.000	-.996	3.196
	Week 12	-16.818*	3.309	.007	-29.483	-4.154
Week 11	Week 1	-98.127*	1.836	.000	-105.153	-91.101
	Week 8	.999	.842	1.000	-2.223	4.221
	Week 9	-14.941*	.758	.000	-17.843	-12.039
	Week 10	-1.100	.548	1.000	-3.196	.996
	Week 12	-17.918*	3.228	.004	-30.274	-5.562
Week 12	Week 1	-80.208*	3.779	.000	-94.672	-65.744
	Week 8	18.918*	3.265	.003	6.421	31.414
	Week 9	2.978	3.544	1.000	-10.586	16.541
	Week 10	16.818*	3.309	.007	4.154	29.483
	Week 11	17.918*	3.228	.004	5.562	30.274

Wastewater Treatment

Table A.33: Student's t-test p-values for wastewater treatment data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Total Organic Carbon Removal Rate	Total Nitrogen Removal Rate
4	0.131982776	0.002435942*
5	1.19852E-07*	3.84846E-05*
6	1.49973E-10*	
7	1.09176E-06*	2.59572E-07*
8	1.89705E-09*	
9	3.26721E-07*	0.011174532*
10		
11	3.33686E-09*	1.73695E-06*
12	0.00012842*	1.50844E-08*

Total Organic Carbon Removal Rate

Table A.34: Shapiro-Wilk's Test of Normality for total organic carbon removal rate of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week4	A	.803	6	.062
	NA	.856	6	.176
Week5	A	.902	6	.387
	NA	.945	6	.698
Week6	A	.976	6	.931
	NA	.965	6	.855
Week7	A	.833	6	.115
	NA	.849	6	.154
Week8	A	.855	6	.174
	NA	.918	6	.491
Week9	A	.922	6	.517
	NA	.885	6	.291
Week11	A	.889	6	.312
	NA	.926	6	.549
Week12	A	.734	6	.014
	NA	.837	6	.124

Table A.35: Mauchly's Test of Sphericity for total organic carbon removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
time	.003	44.210	27	.033

Table A.36: Levene's Test of Equality of Error Variances for total organic carbon removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 4	5.494	1	10	.041
Week 5	.008	1	10	.930
Week 6	.353	1	10	.566
Week 7	13.393	1	10	.004
Week 8	.024	1	10	.881
Week 9	6.853	1	10	.026
Week 11	.397	1	10	.543
Week 12	.003	1	10	.958

Table A.37: Pairwise Comparisons from repeated measures ANOVA for total organic carbon removal rate data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 4	Week 5	.320	1.916	1.000	-7.758	8.398
	Week 6	-4.224	1.768	1.000	-11.676	3.228
	Week 7	-21.783*	2.003	.000	-30.226	-13.339
	Week 8	-25.720*	2.309	.000	-35.453	-15.987
	Week 9	-29.772*	1.985	.000	-38.140	-21.403
	Week 11	-32.308*	1.701	.000	-39.478	-25.139
	Week 12	-27.902*	3.424	.000	-42.335	-13.468
Week 5	Week 4	-.320	1.916	1.000	-8.398	7.758
	Week 6	-4.544*	.491	.000	-6.614	-2.474
	Week 7	-22.103*	.976	.000	-26.215	-17.990
	Week 8	-26.040*	.973	.000	-30.141	-21.939
	Week 9	-30.092*	.926	.000	-33.993	-26.190
	Week 11	-32.628*	1.156	.000	-37.500	-27.757
	Week 12	-28.222*	2.612	.000	-39.232	-17.211
Week 6	Week 4	4.224	1.768	1.000	-3.228	11.676
	Week 5	4.544*	.491	.000	2.474	6.614

	Week 7	-17.558*	1.081	.000	-22.113	-13.003
	Week 8	-21.496*	.998	.000	-25.701	-17.290
	Week 9	-25.548*	.806	.000	-28.945	-22.150
	Week 11	-28.084*	1.190	.000	-33.100	-23.069
	Week 12	-23.678*	2.492	.000	-34.181	-13.174
Week 7	Week 4	21.783*	2.003	.000	13.339	30.226
	Week 5	22.103*	.976	.000	17.990	26.215
	Week 6	17.558*	1.081	.000	13.003	22.113
	Week 8	-3.937	1.335	.408	-9.565	1.690
	Week 9	-7.989*	1.346	.004	-13.663	-2.316
	Week 11	-10.526*	1.197	.000	-15.571	-5.481
	Week 12	-6.119	2.681	1.000	-17.418	5.179
Week 8	Week 4	25.720*	2.309	.000	15.987	35.453
	Week 5	26.040*	.973	.000	21.939	30.141
	Week 6	21.496*	.998	.000	17.290	25.701
	Week 7	3.937	1.335	.408	-1.690	9.565
	Week 9	-4.052	1.241	.238	-9.283	1.180
	Week 11	-6.588	1.573	.052	-13.219	.043
	Week 12	-2.182	2.645	1.000	-13.328	8.965
Week 9	Week 4	29.772*	1.985	.000	21.403	38.140
	Week 5	30.092*	.926	.000	26.190	33.993
	Week 6	25.548*	.806	.000	22.150	28.945
	Week 7	7.989*	1.346	.004	2.316	13.663
	Week 8	4.052	1.241	.238	-1.180	9.283
	Week 11	-2.537	1.462	1.000	-8.700	3.626
	Week 12	1.870	2.338	1.000	-7.983	11.723
Week 11	Week 4	32.308*	1.701	.000	25.139	39.478
	Week 5	32.628*	1.156	.000	27.757	37.500
	Week 6	28.084*	1.190	.000	23.069	33.100
	Week 7	10.526*	1.197	.000	5.481	15.571
	Week 8	6.588	1.573	.052	-.043	13.219
	Week 9	2.537	1.462	1.000	-3.626	8.700
	Week 12	4.407	3.258	1.000	-9.327	18.140
Week 12	Week 4	27.902*	3.424	.000	13.468	42.335
	Week 5	28.222*	2.612	.000	17.211	39.232
	Week 6	23.678*	2.492	.000	13.174	34.181
	Week 7	6.119	2.681	1.000	-5.179	17.418
	Week 8	2.182	2.645	1.000	-8.965	13.328
	Week 9	-1.870	2.338	1.000	-11.723	7.983
	Week 11	-4.407	3.258	1.000	-18.140	9.327

Total Nitrogen Removal Rate

Table A.38: Shapiro-Wilk's Test of Normality for total nitrogen removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 4	A	.904	6	.399
	NA	.881	6	.273
Week 5	A	.851	6	.161
	NA	.941	6	.671
Week 7	A	.826	6	.100
	NA	.946	6	.709
Week 9	A	.770	6	.031
	NA	.741	6	.016
Week11	A	.911	6	.443
	NA	.940	6	.658
Week12	A	.933	6	.601
	NA	.882	6	.279

Table A.39: Mauchly's Test of Sphericity for total nitrogen removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
time	.026	29.518	14	.011

Table A.40: Levene's Test of Equality of Error Variances for total nitrogen removal data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 4	5.262	1	10	.045
Week 5	.444	1	10	.520
Week 7	.187	1	10	.675
Week 9	.957	1	10	.351
Week 11	4.237	1	10	.067
Week 12	.007	1	10	.933

Table A.41: Pairwise Comparisons from repeated measures ANOVA for total nitrogen removal data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 4	Week 5	.258	.524	1.000	-1.746	2.263
	Week 7	-.791	.519	1.000	-2.777	1.195
	Week 9	-1.315	1.066	1.000	-5.393	2.763
	Week 11	-2.263*	.466	.010	-4.046	-.481
	Week 12	-5.402*	.420	.000	-7.008	-3.797
Week 5	Week 4	-.258	.524	1.000	-2.263	1.746
	Week 7	-1.049	.381	.306	-2.509	.410
	Week 9	-1.573	.882	1.000	-4.947	1.801
	Week 11	-2.522*	.260	.000	-3.516	-1.528
	Week 12	-5.661*	.323	.000	-6.899	-4.423
Week 7	Week 4	.791	.519	1.000	-1.195	2.777
	Week 5	1.049	.381	.306	-.410	2.509
	Week 9	-.524	.868	1.000	-3.848	2.800
	Week 11	-1.473*	.333	.019	-2.745	-.200
	Week 12	-4.612*	.303	.000	-5.772	-3.451
Week 9	Week 4	1.315	1.066	1.000	-2.763	5.393
	Week 5	1.573	.882	1.000	-1.801	4.947
	Week 7	.524	.868	1.000	-2.800	3.848
	Week 11	-.948	.861	1.000	-4.245	2.348
	Week 12	-4.087*	.881	.014	-7.458	-.717
Week 11	Week 4	2.263*	.466	.010	.481	4.046
	Week 5	2.522*	.260	.000	1.528	3.516
	Week 7	1.473*	.333	.019	.200	2.745
	Week 9	.948	.861	1.000	-2.348	4.245
	Week 12	-3.139*	.349	.000	-4.473	-1.805
Week 12	Week 4	5.402*	.420	.000	3.797	7.008
	Week 5	5.661*	.323	.000	4.423	6.899
	Week 7	4.612*	.303	.000	3.451	5.772
	Week 9	4.087*	.881	.014	.717	7.458
	Week 11	3.139*	.349	.000	1.805	4.473

Hydrological Parameters

Table A.42: Student's t-test p-values for porosity and evapotranspiration data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Porosity	Evapotranspiration
1	0.064767042	0.000595011*
2	0.701202166	0.0011686*
3	0.605797248	0.000538859*
4	0.856892963	0.000277058*
5	0.025723601*	0.01307568*
6	0.014184919*	0.022005704*
7	0.210898743	0.449659078
8	0.736494151	0.509827245
9	0.148786616	0.834599947
10	0.064480902	0.001278636*
11	0.11682772	0.000225662*
12	0.019253795*	0.000542094*

Porosity

Table A.43: Shapiro-Wilk's Test of Normality for porosity data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.961	6	.831
	NA	.808	6	.069
Week 2	A	.939	6	.647
	NA	.986	6	.977
Week 3	A	.962	6	.837
	NA	.887	6	.301
Week 4	A	.927	6	.555
	NA	.951	6	.751
Week 5	A	.884	6	.288
	NA	.953	6	.766
Week 6	A	.922	6	.518
	NA	.963	6	.844
Week 7	A	.940	6	.663
	NA	.945	6	.701
Week 8	A	.951	6	.752
	NA	.635	6	.001
Week 9	A	.931	6	.590
	NA	.991	6	.990

Week 10	A	.773	6	.033
	NA	.887	6	.304
Week 11	A	.907	6	.420
	NA	.900	6	.374
Week 12	A	.950	6	.737
	NA	.992	6	.994

Table A.44: Mauchly's Test of Sphericity for porosity data of aerated and non-aerated systems, α = 0.05.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
time	.001	47.702	54	.934

Table A.45: Levene's Test of Equality of Error Variances for porosity data of aerated and non-aerated systems, α = 0.05.

	F	df1	df2	Sig.
Week 1	.202	1	10	.663
Week 2	.518	1	10	.488
Week 3	.315	1	10	.587
Week 4	2.636	1	10	.136
Week 5	4.777	1	10	.054
Week 6	5.518	1	10	.041
Week 7	.000	1	10	.984
Week 8	.144	1	10	.712
Week 9	4.545	1	10	.059
Week 10	.540	1	10	.479
Week 11	.304	1	10	.593
Week 12	.117	1	10	.740

Table A.46: Pairwise Comparisons from repeated measures ANOVA for porosity data of aerated and non-aerated systems, α = 0.05. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.018*	.003	.008	.004	.032
	Week 3	.028*	.002	.000	.017	.039
	Week 4	.029*	.003	.000	.014	.043

	Week 5	.026*	.003	.000	.014	.039
	Week 6	.027*	.002	.000	.018	.036
	Week 7	.030*	.002	.000	.019	.041
	Week 8	.027*	.003	.000	.013	.041
	Week 9	.027*	.003	.000	.016	.039
	Week 10	.023*	.003	.003	.007	.039
	Week 11	.037*	.003	.000	.021	.053
Week 2	Week 1	-.018*	.003	.008	-.032	-.004
	Week 3	.010	.004	1.000	-.008	.028
	Week 4	.010	.003	.453	-.004	.025
	Week 5	.008	.004	1.000	-.010	.026
	Week 6	.009	.003	1.000	-.007	.025
	Week 7	.012	.003	.244	-.003	.026
	Week 8	.009	.004	1.000	-.011	.028
	Week 9	.009	.004	1.000	-.009	.027
	Week 10	.005	.004	1.000	-.012	.022
	Week 11	.019*	.003	.006	.005	.033
Week 3	Week 1	-.028*	.002	.000	-.039	-.017
	Week 2	-.010	.004	1.000	-.028	.008
	Week 4	.001	.003	1.000	-.015	.016
	Week 5	-.002	.003	1.000	-.015	.011
	Week 6	-.001	.002	1.000	-.010	.008
	Week 7	.002	.002	1.000	-.009	.013
	Week 8	-.001	.004	1.000	-.021	.018
	Week 9	-.001	.004	1.000	-.018	.017
	Week 10	-.005	.003	1.000	-.020	.010
	Week 11	.009	.004	1.000	-.010	.028
Week 4	Week 1	-.029*	.003	.000	-.043	-.014
	Week 2	-.010	.003	.453	-.025	.004
	Week 3	-.001	.003	1.000	-.016	.015
	Week 5	-.002	.003	1.000	-.017	.012
	Week 6	-.001	.004	1.000	-.018	.015
	Week 7	.001	.003	1.000	-.011	.014
	Week 8	-.002	.003	1.000	-.015	.011
	Week 9	-.001	.004	1.000	-.022	.019
	Week 10	-.006	.003	1.000	-.017	.006
	Week 11	.008	.004	1.000	-.009	.025
Week 5	Week 1	-.026*	.003	.000	-.039	-.014
	Week 2	-.008	.004	1.000	-.026	.010
	Week 3	.002	.003	1.000	-.011	.015
	Week 4	.002	.003	1.000	-.012	.017
	Week 6	.001	.003	1.000	-.013	.015
	Week 7	.003	.003	1.000	-.010	.017
	Week 8	.000	.004	1.000	-.018	.018

	Week 9	.001	.004	1.000	-.018	.021
	Week 10	-.003	.003	1.000	-.015	.008
	Week 11	.011	.005	1.000	-.011	.032
Week 6	Week 1	-.027*	.002	.000	-.036	-.018
	Week 2	-.009	.003	1.000	-.025	.007
	Week 3	.001	.002	1.000	-.008	.010
	Week 4	.001	.004	1.000	-.015	.018
	Week 5	-.001	.003	1.000	-.015	.013
	Week 7	.003	.002	1.000	-.006	.011
	Week 8	.000	.004	1.000	-.018	.017
	Week 9	.000	.003	1.000	-.012	.012
	Week 10	-.004	.003	1.000	-.019	.011
	Week 11	.010	.003	.403	-.004	.023
Week 7	Week 1	-.030*	.002	.000	-.041	-.019
	Week 2	-.012	.003	.244	-.026	.003
	Week 3	-.002	.002	1.000	-.013	.009
	Week 4	-.001	.003	1.000	-.014	.011
	Week 5	-.003	.003	1.000	-.017	.010
	Week 6	-.003	.002	1.000	-.011	.006
	Week 8	-.003	.003	1.000	-.017	.011
	Week 9	-.002	.003	1.000	-.015	.010
	Week 10	-.007	.003	1.000	-.022	.008
	Week 11	.007	.003	1.000	-.008	.022
Week 8	Week 1	-.027*	.003	.000	-.041	-.013
	Week 2	-.009	.004	1.000	-.028	.011
	Week 3	.001	.004	1.000	-.018	.021
	Week 4	.002	.003	1.000	-.011	.015
	Week 5	.000	.004	1.000	-.018	.018
	Week 6	.000	.004	1.000	-.017	.018
	Week 7	.003	.003	1.000	-.011	.017
	Week 9	.001	.004	1.000	-.018	.019
	Week 10	-.004	.004	1.000	-.021	.014
	Week 11	.010	.004	1.000	-.008	.028
Week 9	Week 1	-.027*	.003	.000	-.039	-.016
	Week 2	-.009	.004	1.000	-.027	.009
	Week 3	.001	.004	1.000	-.017	.018
	Week 4	.001	.004	1.000	-.019	.022
	Week 5	-.001	.004	1.000	-.021	.018
	Week 6	.000	.003	1.000	-.012	.012
	Week 7	.002	.003	1.000	-.010	.015
	Week 8	-.001	.004	1.000	-.019	.018
	Week 10	-.004	.005	1.000	-.028	.019
	Week 11	.010	.004	1.000	-.007	.026
Week 10	Week 1	-.023*	.003	.003	-.039	-.007

	Week 2	-.005	.004	1.000	-.022	.012
	Week 3	.005	.003	1.000	-.010	.020
	Week 4	.006	.003	1.000	-.006	.017
	Week 5	.003	.003	1.000	-.008	.015
	Week 6	.004	.003	1.000	-.011	.019
	Week 7	.007	.003	1.000	-.008	.022
	Week 8	.004	.004	1.000	-.014	.021
	Week 9	.004	.005	1.000	-.019	.028
	Week 11	.014	.004	.226	-.004	.031
Week 11	Week 1	-.037*	.003	.000	-.053	-.021
	Week 2	-.019*	.003	.006	-.033	-.005
	Week 3	-.009	.004	1.000	-.028	.010
	Week 4	-.008	.004	1.000	-.025	.009
	Week 5	-.011	.005	1.000	-.032	.011
	Week 6	-.010	.003	.403	-.023	.004
	Week 7	-.007	.003	1.000	-.022	.008
	Week 8	-.010	.004	1.000	-.028	.008
	Week 9	-.010	.004	1.000	-.026	.007
	Week 10	-.014	.004	.226	-.031	.004

Evapotranspiration

Table A.47: Shapiro-Wilk's Test of Normality for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.827	6	.102
	NA	.972	6	.905
Week 2	A	.949	6	.729
	NA	.906	6	.412
Week 3	A	.923	6	.529
	NA	.947	6	.715
Week 4	A	.964	6	.848
	NA	.948	6	.723
Week 5	A	.940	6	.658
	NA	.808	6	.069
Week 6	A	.950	6	.741
	NA	.979	6	.948
Week 7	A	.782	6	.040
	NA	.938	6	.640
Week 8	A	.826	6	.099
	NA	.947	6	.720
Week 9	A	.901	6	.377
	NA	.877	6	.255

Week 10	A	.952	6	.758
	NA	.924	6	.537
Week 11	A	.976	6	.932
	NA	.886	6	.300
Week 12	A	.885	6	.291
	NA	.927	6	.555

Table A.48: Mauchly's Test of Sphericity for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	84.694	54	.020

Table A.49: Levene's Test of Equality of Error Variances for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.638	1	10	.443
Week 2	.110	1	10	.747
Week 3	3.567	1	10	.088
Week 4	1.432	1	10	.259
Week 5	.184	1	10	.677
Week 6	4.710	1	10	.055
Week 7	.008	1	10	.928
Week 8	2.505	1	10	.145
Week 9	.425	1	10	.529
Week 10	.042	1	10	.842
Week 11	3.574	1	10	.088
Week 12	.059	1	10	.813

Table A.50: Pairwise Comparisons from repeated measures ANOVA for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.063*	.013	.041	.002	.125
	Week 3	.068*	.010	.003	.021	.114
	Week 4	.040	.011	.213	-.010	.091

	Week 5	.061	.021	.868	-.037	.160
	Week 6	.033	.014	1.000	-.032	.099
	Week 7	.067*	.014	.046	.001	.133
	Week 8	.057	.017	.356	-.020	.134
	Week 9	.079*	.014	.014	.012	.146
	Week 10	.100*	.012	.000	.046	.154
	Week 11	.105*	.011	.000	.052	.158
Week 2	Week 1	-.063*	.013	.041	-.125	-.002
	Week 3	.005	.007	1.000	-.026	.035
	Week 4	-.023*	.004	.017	-.042	-.003
	Week 5	-.002	.017	1.000	-.079	.075
	Week 6	-.030	.009	.385	-.071	.011
	Week 7	.004	.006	1.000	-.025	.032
	Week 8	-.006	.011	1.000	-.058	.046
	Week 9	.016	.012	1.000	-.039	.071
	Week 10	.037*	.007	.026	.003	.071
	Week 11	.042*	.008	.017	.006	.078
Week 3	Week 1	-.068*	.010	.003	-.114	-.021
	Week 2	-.005	.007	1.000	-.035	.026
	Week 4	-.027*	.005	.025	-.052	-.002
	Week 5	-.006	.017	1.000	-.087	.074
	Week 6	-.035	.008	.057	-.070	.001
	Week 7	-.001	.006	1.000	-.027	.026
	Week 8	-.011	.011	1.000	-.061	.040
	Week 9	.012	.011	1.000	-.041	.064
	Week 10	.032*	.007	.046	.000	.064
	Week 11	.037*	.007	.012	.006	.068
Week 4	Week 1	-.040	.011	.213	-.091	.010
	Week 2	.023*	.004	.017	.003	.042
	Week 3	.027*	.005	.025	.002	.052
	Week 5	.021	.019	1.000	-.066	.108
	Week 6	-.007	.008	1.000	-.043	.029
	Week 7	.027	.008	.298	-.008	.062
	Week 8	.017	.012	1.000	-.038	.071
	Week 9	.039	.012	.587	-.019	.097
	Week 10	.060*	.007	.000	.026	.093
	Week 11	.064*	.007	.000	.031	.098
Week 5	Week 1	-.061	.021	.868	-.160	.037
	Week 2	.002	.017	1.000	-.075	.079
	Week 3	.006	.017	1.000	-.074	.087
	Week 4	-.021	.019	1.000	-.108	.066
	Week 6	-.028	.018	1.000	-.113	.056
	Week 7	.006	.016	1.000	-.069	.080
	Week 8	-.004	.015	1.000	-.076	.068

	Week 9	.018	.017	1.000	-.061	.097
	Week 10	.039	.017	1.000	-.042	.120
	Week 11	.044	.017	1.000	-.037	.124
Week 6	Week 1	-.033	.014	1.000	-.099	.032
	Week 2	.030	.009	.385	-.011	.071
	Week 3	.035	.008	.057	-.001	.070
	Week 4	.007	.008	1.000	-.029	.043
	Week 5	.028	.018	1.000	-.056	.113
	Week 7	.034	.008	.066	-.001	.069
	Week 8	.024	.012	1.000	-.032	.079
	Week 9	.046	.011	.117	-.006	.098
	Week 10	.067*	.009	.001	.027	.107
	Week 11	.072*	.007	.000	.041	.102
Week 7	Week 1	-.067*	.014	.046	-.133	-.001
	Week 2	-.004	.006	1.000	-.032	.025
	Week 3	.001	.006	1.000	-.026	.027
	Week 4	-.027	.008	.298	-.062	.008
	Week 5	-.006	.016	1.000	-.080	.069
	Week 6	-.034	.008	.066	-.069	.001
	Week 8	-.010	.012	1.000	-.067	.047
	Week 9	.012	.010	1.000	-.036	.060
	Week 10	.033	.007	.061	-.001	.067
	Week 11	.038*	.007	.022	.004	.072
Week 8	Week 1	-.057	.017	.356	-.134	.020
	Week 2	.006	.011	1.000	-.046	.058
	Week 3	.011	.011	1.000	-.040	.061
	Week 4	-.017	.012	1.000	-.071	.038
	Week 5	.004	.015	1.000	-.068	.076
	Week 6	-.024	.012	1.000	-.079	.032
	Week 7	.010	.012	1.000	-.047	.067
	Week 9	.022	.014	1.000	-.041	.086
	Week 10	.043	.011	.165	-.008	.095
	Week 11	.048	.012	.116	-.006	.102
Week 9	Week 1	-.079*	.014	.014	-.146	-.012
	Week 2	-.016	.012	1.000	-.071	.039
	Week 3	-.012	.011	1.000	-.064	.041
	Week 4	-.039	.012	.587	-.097	.019
	Week 5	-.018	.017	1.000	-.097	.061
	Week 6	-.046	.011	.117	-.098	.006
	Week 7	-.012	.010	1.000	-.060	.036
	Week 8	-.022	.014	1.000	-.086	.041
	Week 10	.021	.008	1.000	-.016	.057
	Week 11	.026	.008	.703	-.014	.065
Week 10	Week 1	-.100*	.012	.000	-.154	-.046

	Week 2	-.037*	.007	.026	-.071	-.003
	Week 3	-.032*	.007	.046	-.064	.000
	Week 4	-.060*	.007	.000	-.093	-.026
	Week 5	-.039	.017	1.000	-.120	.042
	Week 6	-.067*	.009	.001	-.107	-.027
	Week 7	-.033	.007	.061	-.067	.001
	Week 8	-.043	.011	.165	-.095	.008
	Week 9	-.021	.008	1.000	-.057	.016
	Week 11	.005	.003	1.000	-.010	.020
Week 11	Week 1	-.105*	.011	.000	-.158	-.052
	Week 2	-.042*	.008	.017	-.078	-.006
	Week 3	-.037*	.007	.012	-.068	-.006
	Week 4	-.064*	.007	.000	-.098	-.031
	Week 5	-.044	.017	1.000	-.124	.037
	Week 6	-.072*	.007	.000	-.102	-.041
	Week 7	-.038*	.007	.022	-.072	-.004
	Week 8	-.048	.012	.116	-.102	.006
	Week 9	-.026	.008	.703	-.065	.014
	Week 10	-.005	.003	1.000	-.020	.010
Week 12	Week 1	-.093*	.011	.000	-.145	-.041
	Week 2	-.030	.010	.737	-.075	.016
	Week 3	-.025	.009	1.000	-.067	.017
	Week 4	-.052*	.009	.013	-.096	-.009
	Week 5	-.031	.018	1.000	-.116	.053
	Week 6	-.060*	.010	.007	-.105	-.014
	Week 7	-.026	.010	1.000	-.070	.019
	Week 8	-.036	.014	1.000	-.102	.030
	Week 9	-.013	.009	1.000	-.056	.029
	Week 10	.007	.004	1.000	-.013	.027

Microbial Community Analysis

Table A.51: Student's t-test p-values for microbial community data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

			Carbon Source Guilds						
Week	AWCD	Richness	Carbohydrates	Polymers	Carboxylic and Acetic Acids	Amino Acids	Amines and Amides	Root Exudates	FDA
2	0.98169783 5	0.41190752	0.09861862	0.01997145*	0.00629964 8*	0.00012481 6*	2.49373E-06*	0.00085752 6*	
3									0.66644059 6
4	0.41024081 7	0.00248396 4*	0.99914984 9	0.62711325 9	0.90182295 1	0.03525536 *	6.35794E-05*	0.03711929 7*	0.22276120 7
6	0.21115220 9	0.05631381	0.27363561 3	0.75510194 2	0.79517661 4	0.04421133 *	0.00393054 4*	0.00058769 6*	0.35450264 4
7									0.72438510 3
8	0.00097035 9*	0.00011607 7*	0.19671161 7	0.32622518 5	0.19601789 9	0.41563785 7	0.04593921 1*	0.00094375 9*	
9									0.943943
10	2.22544E-05*	1.90021E-05*	0.17603414 4	0.00014619 7*	0.00638908 1*	0.12978129	0.78648288	0.25855894 7	
11									0.63600003 4
12	8.8534E-05*	9.69512E-05*	0.42070967 2	0.63687272 2	0.97018254 2	0.04143423 5*	0.00427969 1*	4.31339E-05*	

Average Well Colour Development

Table A.52: Shapiro-Wilk's Test of Normality for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.924	6	.536
	NA	.893	6	.337
Week 4	A	.940	6	.663
	NA	.947	6	.714
Week 6	A	.953	6	.761
	NA	.954	6	.775
Week 8	A	.879	6	.264
	NA	.988	6	.983
Week 10	A	.731	6	.013
	NA	.926	6	.548
Week 12	A	.821	6	.090
	NA	.770	6	.031

Table A.53: Mauchly's Test of Sphericity for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.112	17.766	14	.236

Table A.54: Levene's Test of Equality of Error Variances for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 2	.561	1	10	.471
Week 4	.027	1	10	.873
Week 6	2.461	1	10	.148
Week 8	3.219	1	10	.103
Week 10	.760	1	10	.404
Week 12	.969	1	10	.348

Table A.55: Pairwise Comparisons from repeated measures ANOVA for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 4	-.792*	.037	.000	-.935	-.648
	Week 6	-.398*	.041	.000	-.556	-.241
	Week 8	-.367*	.030	.000	-.483	-.251
	Week 10	-.358*	.041	.000	-.514	-.201
	Week 12	-.270*	.034	.000	-.399	-.141
Week 4	Week 2	.792*	.037	.000	.648	.935
	Week 6	.393*	.049	.000	.206	.581
	Week 8	.425*	.038	.000	.280	.570
	Week 10	.434*	.027	.000	.332	.536
	Week 12	.522*	.034	.000	.391	.653
Week 6	Week 2	.398*	.041	.000	.241	.556
	Week 4	-.393*	.049	.000	-.581	-.206
	Week 8	.032	.037	1.000	-.111	.174
	Week 10	.040	.037	1.000	-.101	.182
	Week 12	.128	.043	.201	-.036	.293
Week 8	Week 2	.367*	.030	.000	.251	.483
	Week 4	-.425*	.038	.000	-.570	-.280
	Week 6	-.032	.037	1.000	-.174	.111
	Week 10	.009	.029	1.000	-.101	.119
	Week 12	.097	.027	.079	-.008	.201
Week 10	Week 2	.358*	.041	.000	.201	.514
	Week 4	-.434*	.027	.000	-.536	-.332
	Week 6	-.040	.037	1.000	-.182	.101
	Week 8	-.009	.029	1.000	-.119	.101
	Week 12	.088*	.022	.039	.003	.173
Week 12	Week 2	.270*	.034	.000	.141	.399
	Week 4	-.522*	.034	.000	-.653	-.391
	Week 6	-.128	.043	.201	-.293	.036
	Week 8	-.097	.027	.079	-.201	.008
	Week 10	-.088*	.022	.039	-.173	-.003

Richness

Table A.56: Shapiro-Wilk's Test of Normality for richness data of aerated and non-aerated systems, α = 0.05.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.991	6	.991
	NA	.963	6	.845
Week 4	A	.893	6	.332
	NA	.898	6	.364
Week 6	A	.926	6	.550
	NA	.896	6	.353
Week 8	A	.998	6	1.000
	NA	.812	6	.075
Week 10	A	.932	6	.598
	NA	.955	6	.782
Week 12	A	.944	6	.693
	NA	.915	6	.472

Table A.57: Mauchly's Test of Sphericity for richness data of aerated and non-aerated systems, α = 0.05.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.067	21.912	14	.092

Table A.58: Levene's Test of Equality of Error Variances for richness data of aerated and non-aerated systems, α = 0.05.

	F	df1	df2	Sig.
Week 2	.134	1	10	.722
Week 4	.000	1	10	1.000
Week 6	2.480	1	10	.146
Week 8	1.672	1	10	.225
Week 10	.470	1	10	.508
Week 12	6.494	1	10	.029

Table A.59: Pairwise Comparisons from repeated measures ANOVA for richness data of aerated and non-aerated systems, α = 0.05. Significant weeks are marked with an asterisk (*).

(I) time	(J) time		Std. Error	Sig.	95% Confidence Interval for Difference

		Mean Difference (I-J)			Lower Bound	Upper Bound
Week 2	Week 4	-13.358*	.776	.000	-16.327	-10.389
	Week 6	-8.217*	.702	.000	-10.903	-5.531
	Week 8	-6.825*	.755	.000	-9.716	-3.934
	Week 10	-7.300*	.836	.000	-10.499	-4.101
	Week 12	-5.808*	.896	.001	-9.236	-2.381
Week 4	Week 2	13.358*	.776	.000	10.389	16.327
	Week 6	5.142*	.528	.000	3.122	7.161
	Week 8	6.533*	.460	.000	4.771	8.295
	Week 10	6.058*	.292	.000	4.941	7.176
	Week 12	7.550*	.539	.000	5.487	9.613
Week 6	Week 2	8.217*	.702	.000	5.531	10.903
	Week 4	-5.142*	.528	.000	-7.161	-3.122
	Week 8	1.392	.615	.708	-.963	3.747
	Week 10	.917	.552	1.000	-1.197	3.031
	Week 12	2.408	.862	.284	-.890	5.706
Week 8	Week 2	6.825*	.755	.000	3.934	9.716
	Week 4	-6.533*	.460	.000	-8.295	-4.771
	Week 6	-1.392	.615	.708	-3.747	.963
	Week 10	-.475	.388	1.000	-1.959	1.009
	Week 12	1.017	.457	.752	-.731	2.764
Week 10	Week 2	7.300*	.836	.000	4.101	10.499
	Week 4	-6.058*	.292	.000	-7.176	-4.941
	Week 6	-.917	.552	1.000	-3.031	1.197
	Week 8	.475	.388	1.000	-1.009	1.959
	Week 12	1.492	.476	.159	-.329	3.312
Week 12	Week 2	5.808*	.896	.001	2.381	9.236
	Week 4	-7.550*	.539	.000	-9.613	-5.487
	Week 6	-2.408	.862	.284	-5.706	.890
	Week 8	-1.017	.457	.752	-2.764	.731
	Week 10	-1.492	.476	.159	-3.312	.329

Carbon Source Guilds – Carbohydrates

Table A.60: Shapiro-Wilk's Test of Normality for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.891	6	.325
	NA	.839	6	.129
Week 4	A	.812	6	.075
	NA	.896	6	.350

Week 6	A	.969	6	.888
	NA	.880	6	.267
Week 8	A	.893	6	.335
	NA	.897	6	.355
Week 10	A	.965	6	.856
	NA	.882	6	.281
Week 12	A	.736	6	.014
	NA	.967	6	.870

Table A.61: Mauchly's Test of Sphericity for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.346	8.604	14	.864

Table A.62: Levene's Test of Equality of Error Variances for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 2	10.354	1	10	.009
Week 4	1.798	1	10	.210
Week 6	5.571	1	10	.040
Week 8	11.699	1	10	.007
Week 10	.436	1	10	.524
Week 12	4.082	1	10	.071

Table A.63: Pairwise Comparisons from repeated measures ANOVA for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 4	-.325*	.019	.000	-.397	-.253
	Week 6	-.204*	.028	.000	-.309	-.098
	Week 8	-.135*	.022	.001	-.218	-.053
	Week 10	-.118*	.022	.005	-.202	-.033
	Week 12	-.121*	.026	.014	-.222	-.021
Week 4	Week 2	.325*	.019	.000	.253	.397
	Week 6	.122*	.020	.002	.044	.199
	Week 8	.190*	.018	.000	.122	.258
	Week 10	.207*	.014	.000	.154	.261

	Week 12	.204*	.020	.000	.127	.281
Week 6	Week 2	.204*	.028	.000	.098	.309
	Week 4	-.122*	.020	.002	-.199	-.044
	Week 8	.068	.020	.088	-.007	.143
	Week 10	.086*	.017	.008	.020	.151
	Week 12	.082*	.021	.047	.001	.164
Week 8	Week 2	.135*	.022	.001	.053	.218
	Week 4	-.190*	.018	.000	-.258	-.122
	Week 6	-.068	.020	.088	-.143	.007
	Week 10	.018	.014	1.000	-.036	.071
	Week 12	.014	.021	1.000	-.065	.093
Week 10	Week 2	.118*	.022	.005	.033	.202
	Week 4	-.207*	.014	.000	-.261	-.154
	Week 6	-.086*	.017	.008	-.151	-.020
	Week 8	-.018	.014	1.000	-.071	.036
	eek 12	-.004	.015	1.000	-.061	.054
Week 12	Week 2	.121*	.026	.014	.021	.222
	Week 4	-.204*	.020	.000	-.281	-.127
	Week 6	-.082*	.021	.047	-.164	-.001
	Week 8	-.014	.021	1.000	-.093	.065
	Week 10	.004	.015	1.000	-.054	.061

Carbon Source Guilds – Polymers

Table A.64: Shapiro-Wilk's Test of Normality for polymers data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.952	6	.758
	NA	.966	6	.863
Week 4	A	.981	6	.955
	NA	.931	6	.587
Week 6	A	.923	6	.529
	NA	.940	6	.659
Week 8	A	.942	6	.673
	NA	.957	6	.798
Week 10	A	.839	6	.127
	NA	.935	6	.622
Week 12	A	.765	6	.028
	NA	.932	6	.595

Table A.65: Mauchly's Test of Sphericity for polymers data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.155	15.117	14	.391

Table A.66: Levene's Test of Equality of Error Variances for polymers data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 2	.627	1	10	.447
Week 4	1.604	1	10	.234
Week 6	.028	1	10	.871
Week 8	.022	1	10	.884
Week 10	1.562	1	10	.240
Week 12	2.233	1	10	.166

Table A.67: Pairwise Comparisons from repeated measures ANOVA for polymers data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 4	-.073*	.008	.000	-.104	-.043
	Week 6	-.026	.009	.284	-.062	.010
	Week 8	-.028	.008	.078	-.059	.002
	Week 10	-.029*	.005	.001	-.046	-.012
	Week 12	-.043*	.006	.001	-.068	-.019
Week 4	Week 2	.073*	.008	.000	.043	.104
	Week 6	.047*	.008	.003	.015	.079
	Week 8	.045*	.010	.012	.009	.082
	Week 10	.044*	.008	.002	.015	.073
	Week 12	.030	.011	.281	-.011	.071
Week 6	Week 2	.026	.009	.284	-.010	.062
	Week 4	-.047*	.008	.003	-.079	-.015
	Week 6	-.002	.006	1.000	-.027	.023
	Week 10	-.003	.007	1.000	-.030	.025
	Week 12	-.017	.009	1.000	-.053	.019
Week 8	Week 2	.028	.008	.078	-.002	.059
	Week 4	-.045*	.010	.012	-.082	-.009
	Week 6	.002	.006	1.000	-.023	.027

	Week 10	-.001	.007	1.000	-.027	.026
	Week 12	-.015	.008	1.000	-.046	.016
Week 10	Week 2	.029*	.005	.001	.012	.046
	Week 4	-.044*	.008	.002	-.073	-.015
	Week 6	.003	.007	1.000	-.025	.030
	Week 8	.001	.007	1.000	-.026	.027
	Week 12	-.014	.005	.353	-.035	.006
Week 12	Week 2	.043*	.006	.001	.019	.068
	Week 4	-.030	.011	.281	-.071	.011
	Week 6	.017	.009	1.000	-.019	.053
	Week 8	.015	.008	1.000	-.016	.046
	Week 10	.014	.005	.353	-.006	.035

Carbon Source Guilds – Carboxylic and Acetic Acids

Table A.68: Shapiro-Wilk's Test of Normality for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.956	6	.785
	NA	.814	6	.078
Week 4	A	.874	6	.243
	NA	.954	6	.771
Week 6	A	.955	6	.779
	NA	.986	6	.979
Week 8	A	.957	6	.796
	NA	.887	6	.303
Week 10	A	.878	6	.261
	NA	.855	6	.172
Week 12	A	.966	6	.865
	NA	.925	6	.544

Table A.69: Mauchly's Test of Sphericity for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.240	11.549	14	.659

Table A.70: Levene's Test of Equality of Error Variances for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.

Week 2	8.713	1	10	.014
Week 4	.388	1	10	.547
Week 6	.555	1	10	.473
Week 8	4.695	1	10	.055
Week 10	1.600	1	10	.235
Week 12	.901	1	10	.365

Table A.71: Pairwise Comparisons from repeated measures ANOVA for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 4	-.186*	.017	.000	-.252	-.121
	Week 6	-.076*	.012	.001	-.122	-.030
	Week 8	-.091*	.013	.001	-.141	-.041
	Week 10	-.095*	.016	.002	-.157	-.033
	Week 12	-.046*	.012	.041	-.091	-.001
Week 4	Week 2	.186*	.017	.000	.121	.252
	Week 6	.110*	.014	.000	.057	.164
	Week 8	.095*	.010	.000	.058	.133
	Week 10	.091*	.013	.001	.041	.141
	Week 12	.140*	.015	.000	.081	.199
Week 6	Week 2	.076*	.012	.001	.030	.122
	Week 4	-.110*	.014	.000	-.164	-.057
	Week 6	-.015	.013	1.000	-.063	.033
	Week 10	-.019	.010	1.000	-.057	.019
	Week 12	.030	.015	1.000	-.029	.088
Week 8	Week 2	.091*	.013	.001	.041	.141
	Week 4	-.095*	.010	.000	-.133	-.058
	Week 6	.015	.013	1.000	-.033	.063
	Week 10	-.004	.012	1.000	-.051	.043
	Week 12	.045	.012	.065	-.002	.091
Week 10	Week 2	.095*	.016	.002	.033	.157
	Week 4	-.091*	.013	.001	-.141	-.041
	Week 6	.019	.010	1.000	-.019	.057
	Week 8	.004	.012	1.000	-.043	.051
	Week 12	.049	.015	.116	-.007	.105
Week 12	Week 2	.046*	.012	.041	.001	.091
	Week 4	-.140*	.015	.000	-.199	-.081
	Week 6	-.030	.015	1.000	-.088	.029

	Week 8	-.045	.012	.065	-.091	.002
	Week 10	-.049	.015	.116	-.105	.007

Carbo Source Guilds – Amino Acids

Table A.72: Shapiro-Wilk's Test of Normality for amino acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.865	6	.208
	NA	.897	6	.356
Week 4	A	.863	6	.201
	NA	.932	6	.593
Week 6	A	.980	6	.952
	NA	.875	6	.248
Week 8	A	.937	6	.635
	NA	.942	6	.676
Week 10	A	.895	6	.343
	NA	.899	6	.370
Week 12	A	.958	6	.804
	NA	.811	6	.073

Table A.73: Mauchly's Test of Sphericity for amino acids data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.084	20.108	14	.142

Table A.74: Levene's Test of Equality of Error Variances for amino acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 2	.000	1	10	.996
Week 4	4.607	1	10	.057
Week 6	.347	1	10	.569
Week 8	3.501	1	10	.091
Week 10	1.364	1	10	.270
Week 12	2.180	1	10	.171

Table A.75: Pairwise Comparisons from repeated measures ANOVA for amino acids data of aerated and non-aerated systems, α = 0.05. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 4	-.139*	.011	.000	-.182	-.096
	Week 6	-.065*	.008	.000	-.096	-.034
	Week 8	-.081*	.012	.001	-.129	-.033
	Week 10	-.085*	.011	.000	-.128	-.042
	Week 12	-.049*	.011	.016	-.090	-.008
Week 4	Week 2	.139*	.011	.000	.096	.182
	Week 6	.074*	.009	.000	.040	.108
	Week 8	.058*	.008	.000	.029	.087
	Week 10	.054*	.004	.000	.038	.071
	Week 12	.090*	.004	.000	.073	.107
Week 6	Week 2	.065*	.008	.000	.034	.096
	Week 4	-.074*	.009	.000	-.108	-.040
	Week 6	-.016	.009	1.000	-.051	.019
	Week 10	-.020	.010	1.000	-.059	.019
	Week 12	.016	.009	1.000	-.019	.052
Week 8	Week 2	.081*	.012	.001	.033	.129
	Week 4	-.058*	.008	.000	-.087	-.029
	Week 6	.016	.009	1.000	-.019	.051
	Week 10	-.003	.009	1.000	-.040	.033
	Week 12	.032	.009	.096	-.004	.068
Week 10	Week 2	.085*	.011	.000	.042	.128
	Week 4	-.054*	.004	.000	-.071	-.038
	Week 6	.020	.010	1.000	-.019	.059
	Week 8	.003	.009	1.000	-.033	.040
	Week 12	.036*	.005	.001	.016	.055
Week 12	Week 2	.049*	.011	.016	.008	.090
	Week 4	-.090*	.004	.000	-.107	-.073
	Week 6	-.016	.009	1.000	-.052	.019
	Week 8	-.032	.009	.096	-.068	.004
	Week 10	-.036*	.005	.001	-.055	-.016

Carbon Source Guilds – Amines/Amides

Table A.76: Shapiro-Wilk's Test of Normality for amines and amides data of aerated and non-aerated systems, α = 0.05.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.

Week 2	A	.825	6	.097
	NA	.911	6	.444
Week 4	A	.876	6	.252
	NA	.956	6	.785
Week 6	A	.945	6	.701
	NA	.892	6	.331
Week 8	A	.936	6	.624
	NA	.994	6	.996
Week 10	A	.974	6	.920
	NA	.902	6	.387
Week 12	A	.946	6	.710
	NA	.820	6	.089

Table A.77: Mauchly's Test of Sphericity for amines and amides data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.149	15.444	14	.369

Table A.78: Levene's Test of Equality of Error Variances for amines and amides data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 2	.017	1	10	.900
Week 4	4.520	1	10	.059
Week 6	1.785	1	10	.211
Week 8	1.785	1	10	.211
Week 10	5.599	1	10	.040
Week 12	2.400	1	10	.152

Table A.79: Pairwise Comparisons from repeated measures ANOVA for amines and amides data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 4	-.047*	.004	.000	-.063	-.031
	Week 6	-.022*	.004	.002	-.036	-.008
	Week 8	-.025*	.005	.014	-.046	-.004
	Week 10	-.025*	.004	.001	-.039	-.010

	Week 12	-.005	.004	1.000	-.021	.011
Week 4	Week 2	.047*	.004	.000	.031	.063
	Week 6	.025*	.002	.000	.017	.032
	Week 8	.022*	.004	.004	.007	.037
	Week 10	.023*	.003	.000	.012	.033
	Week 12	.042*	.004	.000	.025	.059
Week 6	Week 2	.022*	.004	.002	.008	.036
	Week 4	-.025*	.002	.000	-.032	-.017
	Week 6	-.003	.004	1.000	-.020	.014
	Week 10	-.002	.002	1.000	-.011	.006
	Week 12	.017*	.004	.032	.001	.033
Week 8	Week 2	.025*	.005	.014	.004	.046
	Week 4	-.022*	.004	.004	-.037	-.007
	Week 6	.003	.004	1.000	-.014	.020
	Week 10	.001	.004	1.000	-.013	.015
	Week 12	.020*	.005	.035	.001	.039
Week 10	Week 2	.025*	.004	.001	.010	.039
	Week 4	-.023*	.003	.000	-.033	-.012
	Week 6	.002	.002	1.000	-.006	.011
	Week 8	-.001	.004	1.000	-.015	.013
	Week 12	.019*	.005	.029	.002	.037
Week 12	Week 2	.005	.004	1.000	-.011	.021
	Week 4	-.042*	.004	.000	-.059	-.025
	Week 6	-.017*	.004	.032	-.033	-.001
	Week 8	-.020*	.005	.035	-.039	-.001
	Week 10	-.019*	.005	.029	-.037	-.002

Root Exudates

Table A.80: Shapiro-Wilk's Test of Normality for root exudates data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 2	A	.961	6	.828
	NA	.840	6	.130
Week 4	A	.921	6	.513
	NA	.906	6	.412
Week 6	A	.954	6	.771
	NA	.938	6	.644
Week 8	A	.913	6	.457
	NA	.954	6	.774
Week 10	A	.981	6	.955
	NA	.750	6	.020
Week 12	A	.901	6	.377

	NA	.967	6	.874

Table A.81: Mauchly's Test of Sphericity for root exudates data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.233	11.815	14	.639

Table A.82: Levene's Test of Equality of Error Variances for root exudates data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 2	1.911	1	10	.197
Week 4	.410	1	10	.536
Week 6	.174	1	10	.686
Week 8	4.147	1	10	.069
Week 10	6.419	1	10	.030
Week 12	1.786	1	10	.211

Table A.83: Pairwise Comparisons from repeated measures ANOVA for root exudates data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 4	-.279*	.023	.000	-.365	-.192
	Week 6	-.115*	.019	.002	-.188	-.042
	Week 8	-.149*	.023	.001	-.237	-.061
	Week 10	-.154*	.022	.001	-.239	-.068
	Week 12	-.078	.021	.069	-.160	.004
Week 4	Week 2	.279*	.023	.000	.192	.365
	Week 6	.164*	.017	.000	.100	.227
	Week 8	.129*	.014	.000	.076	.183
	Week 10	.125*	.012	.000	.081	.169
	Week 12	.201*	.015	.000	.144	.258
Week 6	Week 2	.115*	.019	.002	.042	.188
	Week 4	-.164*	.017	.000	-.227	-.100
	Week 6	-.034	.017	1.000	-.101	.032
	Week 10	-.039	.018	.874	-.108	.030
	Week 12	.037	.020	1.000	-.040	.114

Week 8	Week 2	.149*	.023	.001	.061	.237
	Week 4	-.129*	.014	.000	-.183	-.076
	Week 6	.034	.017	1.000	-.032	.101
	Week 10	-.004	.012	1.000	-.051	.042
	Week 12	.072*	.014	.008	.017	.126
Week 10	Week 2	.154*	.022	.001	.068	.239
	Week 4	-.125*	.012	.000	-.169	-.081
	Week 6	.039	.018	.874	-.030	.108
	Week 8	.004	.012	1.000	-.042	.051
	Week 12	.076*	.010	.000	.037	.115
Week 12	Week 2	.078	.021	.069	-.004	.160
	Week 4	-.201*	.015	.000	-.258	-.144
	Week 6	-.037	.020	1.000	-.114	.040
	Week 8	-.072*	.014	.008	-.126	-.017
	Week 10	-.076*	.010	.000	-.115	-.037

FDA

Table A.84: Shapiro-Wilk's Test of Normality for FDA data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 3	A	.853	6	.165
	NA	.924	6	.533
Week 4	A	.964	6	.854
	NA	.873	6	.239
Week 6	A	.845	6	.143
	NA	.948	6	.725
Week 7	A	.814	6	.079
	NA	.813	6	.077
Week 9	A	.812	6	.075
	NA	.902	6	.387
Week11	A	.862	6	.198
	NA	.951	6	.750

Table A.85: Mauchly's Test of Sphericity for FDA data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.233	11.815	14	.639

Table A.86: Levene's Test of Equality of Error Variances for FDA data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 3	1.911	1	10	.197
Week 4	.410	1	10	.536
Week 6	.174	1	10	.686
Week 7	4.147	1	10	.069
Week 9	6.419	1	10	.030
Week 11	1.786	1	10	.211

Table A.87: Pairwise Comparisons from repeated measures ANOVA for FDA data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 3	Week 4	-.279*	.023	.000	-.365	-.192
	Week 6	-.115*	.019	.002	-.188	-.042
	Week 7	-.149*	.023	.001	-.237	-.061
	Week 9	-.154*	.022	.001	-.239	-.068
	Week 11	-.078	.021	.069	-.160	.004
Week 4	Week 3	.279*	.023	.000	.192	.365
	Week 6	.164*	.017	.000	.100	.227
	Week 7	.129*	.014	.000	.076	.183
	Week 9	.125*	.012	.000	.081	.169
	Week 11	.201*	.015	.000	.144	.258
Week 6	Week 3	.115*	.019	.002	.042	.188
	Week 4	-.164*	.017	.000	-.227	-.100
	Week 7	-.034	.017	1.000	-.101	.032
	Week 9	-.039	.018	.874	-.108	.030
	Week 11	.037	.020	1.000	-.040	.114
Week 7	Week 3	.149*	.023	.001	.061	.237
	Week 4	-.129*	.014	.000	-.183	-.076
	Week 6	.034	.017	1.000	-.032	.101
	Week 9	-.004	.012	1.000	-.051	.042
	Week 11	.072*	.014	.008	.017	.126
Week 9	Week 3	.154*	.022	.001	.068	.239
	Week 4	-.125*	.012	.000	-.169	-.081
	Week 6	.039	.018	.874	-.030	.108
	Week 7	.004	.012	1.000	-.042	.051
	Week 11	.076*	.010	.000	.037	.115

Week 11	Week3	.078	.021	.069	-.004	.160
	Week 4	-.201*	.015	.000	-.258	-.144
	Week 6	-.037	.020	1.000	-.114	.040
	Week 7	-.072*	.014	.008	-.126	-.017
	Week 9	-.076*	.010	.000	-.115	-.037

B. APPENDIX B

Supplementary Information for Chapter 4: EFFECTS OF PLANT ESTABLISHMENT ON MICROBIAL COMMUNITIES, WATER CHEMISTRY, AND HYDROLOGY IN AERATED AND NON-AERATED CONSTRUCTED WETLAND MESOCOSMS

Statistical Tables for Chapter 4: **Table B.1** to **Table B.93.**

Student's t-test p-values, Shapiro-Wilk's Test for Normality, Mauchly's Test of Sphericity, Levene's Test of Equality Error Variances, Repeated Measures ANOVA Pairwise Comparison.

Statistical Tables for Chapter 4: WETLAND START-UP MONITORING OF AERATED AND NON-AERATED MESOCOSM CONSTRUCTED WETLANDS

Water Chemistry

Table B.1: Student's t-test p-values for water chemistry data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Temperature	pH	Conductivity	Dissolved Oxygen	Ammonium	Nitrate	ORP
1	0.05157 8428	1.32558 E-09*	0.00357 1659*	1.52916 E-06*	9.42792 E-08*	1.00541 E-06*	
2	0.00476 1859*	7.40086 E-10*	0.00161 5791*	4.02553 E-06*	3.08578 E-07*	1.5314E -08*	2.71873 E-11*
3	0.11123 0204	8.76375 E-11*	0.00622 4147*	1.07718 E-06*	4.77731 E-06*	5.78532 E-07*	4.31677 E-07*
4	0.74407 8259	1.81179 E-07*	0.92317 1608	4.62013 E-06*	1.01863 E-06*	9.47064 E-09*	1.76972 E-06*
5	0.02026 5105*	2.52004 E-09*	0.75856 8549	3.62625 E-07*	2.76354 E-07*	1.88157 E-09*	5.63387 E-07*
6	0.02154 3326*	7.51907 E-08*	0.02292 592*	1.50217 E-07*	1.27753 E-08*	1.32972 E-09*	8.45533 E-08*
7	0.00868 2592*	4.46917 E-10*	0.05927 5344	4.08981 E-07*	2.60728 E-07*	7.15202 E-10*	3.85303 E-08*
8	0.10141 2869	2.04266 E-08*	0.02193 1913*	5.57673 E-08*	1.26499 E-05*	6.14003 E-09*	1.52796 E-08*
9	0.06387 2997	8.33922 E-08*	0.01528 8704*	9.40748 E-07*	3.70338 E-05*	5.84637 E-14*	2.20171 E-12*
10	0.94950 4851	3.72843 E-09*	0.09116 3987	1.14327 E-06*	0.00018 6946*	8.65054 E-10*	3.82313 E-09*
11	0.41444 9346	5.26361 E-08*	0.60559 615	2.88994 E-06*			2.47816 E-09*
12	0.28689 6846	4.88069 E-06*	0.00749 3349*	1.52651 E-07*			8.24792 E-11*
13	0.63104 626	2.8005E -10*	0.01924 6669*	3.81573 E-07*			9.52406 E-09*
14	0.88609 4845	2.09475 E-06*		8.34677 E-07*			2.61127 E-08*
15	0.33912 2481	1.46758 E-06*	0.11367 4554	5.81515 E-06*			3.47401 E-07*

Temperature

Table B.2: Shapiro-Wilk's Test of Normality for temperature data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.912	6	.452
	NA	.951	6	.752
WEEK 2	A	.876	6	.252
	NA	.814	6	.078
WEEK 3	A	.815	6	.080
	NA	.974	6	.918
WEEK 4	A	.909	6	.433
	NA	.651	6	.002
WEEK 5	A	.878	6	.261
	NA	.799	6	.057
WEEK 6	A	.915	6	.473
	NA	.879	6	.266
WEEK 7	A	.983	6	.964
	NA	.958	6	.804
WEEK 8	A	.849	6	.154
	NA	.982	6	.961
WEEK 9	A	.912	6	.452
	NA	.921	6	.514
WEEK 10	A	.766	6	.029
	NA	.877	6	.258
WEEK 11	A	.859	6	.184
	NA	.960	6	.820
WEEK 12	A	.775	6	.035
	NA	.927	6	.554
WEEK 13	A	.824	6	.096
	NA	.640	6	.001
WEEK 14	A	.908	6	.421
	NA	.866	6	.212
WEEK 15	A	.828	6	.102
	NA	.822	6	.091

Table B.3: Mauchly's Test of Sphericity for temperature data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.

Time	.000	79.908	54	.045

Table B.4: Levene's Test of Equality of Error Variances for temperature data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.470	1	10	.508
Week 2	2.000	1	10	.188
Week 3	.000	1	10	1.000
Week 4	4.186	1	10	.068
Week 5	.222	1	10	.648
Week 6	.015	1	10	.904
Week 7	.804	1	10	.391
Week 8	1.250	1	10	.290
Week 9	1.586	1	10	.237
Week 10	.018	1	10	.895
Week 11	6.180	1	10	.032
Week 12	2.222	1	10	.167
Week 13	13.272	1	10	.005
Week 14	7.721	1	10	.019
Week 15	2.410	1	10	.152

Table B.5: Pairwise Comparisons from repeated measures ANOVA for temperature data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	-.233*	.029	.001	-.370	-.097
	Week 7	-1.692*	.033	.000	-1.844	-1.540
	Week 8	-.917*	.036	.000	-1.083	-.750
	Week 9	-1.483*	.021	.000	-1.581	-1.385
	Week 10	-1.192*	.053	.000	-1.440	-.944
	Week 11	.408*	.037	.000	.234	.582
	Week 12	.733*	.029	.000	.597	.870
	Week 13	3.292*	.037	.000	3.121	3.462
	Week 14	3.192*	.040	.000	3.007	3.376
	Week 15	3.800*	.057	.000	3.533	4.067
Week 2	Week 1	.233*	.029	.001	.097	.370
	Week 7	-1.458*	.029	.000	-1.594	-1.323
	Week 8	-.683*	.041	.000	-.875	-.492
	Week 9	-1.250*	.025	.000	-1.368	-1.132
	Week 10	-.958*	.051	.000	-1.194	-.723

	Week 11	.642*	.035	.000	.478	.805
	Week 12	.967*	.032	.000	.816	1.118
	Week 13	3.525*	.026	.000	3.404	3.646
	Week 14	3.425*	.034	.000	3.265	3.585
	Week 15	4.033*	.058	.000	3.763	4.304
Week 7	Week 1	1.692*	.033	.000	1.540	1.844
	Week 2	1.458*	.029	.000	1.323	1.594
	Week 8	.775*	.029	.000	.640	.910
	Week 9	.208*	.030	.002	.069	.348
	Week 10	.500*	.061	.001	.216	.784
	Week 11	2.100*	.041	.000	1.907	2.293
	Week 12	2.425*	.036	.000	2.258	2.592
	Week 13	4.983*	.037	.000	4.812	5.155
	Week 14	4.883*	.038	.000	4.707	5.060
	Week 15	5.492*	.068	.000	5.174	5.810
Week 8	Week 1	.917*	.036	.000	.750	1.083
	Week 2	.683*	.041	.000	.492	.875
	Week 7	-.775*	.029	.000	-.910	-.640
	Week 9	-.567*	.027	.000	-.692	-.442
	Week 10	-.275	.059	.051	-.551	.001
	Week 11	1.325*	.031	.000	1.181	1.469
	Week 12	1.650*	.031	.000	1.505	1.795
	Week 13	4.208*	.035	.000	4.045	4.372
	Week 14	4.108*	.037	.000	3.938	4.279
	Week 15	4.717*	.073	.000	4.376	5.057
Week 9	Week 1	1.483*	.021	.000	1.385	1.581
	Week 2	1.250*	.025	.000	1.132	1.368
	Week 7	-.208*	.030	.002	-.348	-.069
	Week 8	.567*	.027	.000	.442	.692
	Week 10	.292*	.052	.013	.048	.535
	Week 11	1.892*	.026	.000	1.770	2.013
	Week 12	2.217*	.019	.000	2.128	2.305
	Week 13	4.775*	.026	.000	4.654	4.896
	Week 14	4.675*	.034	.000	4.519	4.831
	Week 15	5.283*	.062	.000	4.994	5.572
Week 10	Week 1	1.192*	.053	.000	.944	1.440
	Week 2	.958*	.051	.000	.723	1.194
	Week 7	-.500*	.061	.001	-.784	-.216
	Week 8	.275	.059	.051	-.001	.551
	Week 9	-.292*	.052	.013	-.535	-.048
	Week 11	1.600*	.051	.000	1.365	1.835
	Week 12	1.925*	.052	.000	1.684	2.166
	Week 13	4.483*	.055	.000	4.230	4.737
	Week 14	4.383*	.060	.000	4.104	4.663

	Week 15	4.992*	.081	.000	4.615	5.368
Week 11	Week 1	-.408*	.037	.000	-.582	-.234
	Week 2	-.642*	.035	.000	-.805	-.478
	Week 7	-2.100*	.041	.000	-2.293	-1.907
	Week 8	-1.325*	.031	.000	-1.469	-1.181
	Week 9	-1.892*	.026	.000	-2.013	-1.770
	Week 10	-1.600*	.051	.000	-1.835	-1.365
	Week 12	.325*	.019	.000	.238	.412
	Week 13	2.883*	.019	.000	2.795	2.972
	Week 14	2.783*	.030	.000	2.645	2.922
	Week 15	3.392*	.065	.000	3.089	3.694
Week 12	Week 1	-.733*	.029	.000	-.870	-.597
	Week 2	-.967*	.032	.000	-1.118	-.816
	Week 7	-2.425*	.036	.000	-2.592	-2.258
	Week 8	-1.650*	.031	.000	-1.795	-1.505
	Week 9	-2.217*	.019	.000	-2.305	-2.128
	Week 10	-1.925*	.052	.000	-2.166	-1.684
	Week 11	-.325*	.019	.000	-.412	-.238
	Week 13	2.558*	.027	.000	2.432	2.684
	Week 14	2.458*	.037	.000	2.284	2.632
	Week 15	3.067*	.069	.000	2.747	3.386
Week 13	Week 1	-3.292*	.037	.000	-3.462	-3.121
	Week 2	-3.525*	.026	.000	-3.646	-3.404
	Week 7	-4.983*	.037	.000	-5.155	-4.812
	Week 8	-4.208*	.035	.000	-4.372	-4.045
	Week 9	-4.775*	.026	.000	-4.896	-4.654
	Week 10	-4.483*	.055	.000	-4.737	-4.230
	Week 11	-2.883*	.019	.000	-2.972	-2.795
	Week 12	-2.558*	.027	.000	-2.684	-2.432
	Week 14	-.100*	.017	.011	-.181	-.019
	Week 15	.508*	.052	.000	.265	.752
Week 14	Week 1	-3.192*	.040	.000	-3.376	-3.007
	Week 2	-3.425*	.034	.000	-3.585	-3.265
	Week 7	-4.883*	.038	.000	-5.060	-4.707
	Week 8	-4.108*	.037	.000	-4.279	-3.938
	Week 9	-4.675*	.034	.000	-4.831	-4.519
	Week 10	-4.383*	.060	.000	-4.663	-4.104
	Week 11	-2.783*	.030	.000	-2.922	-2.645
	Week 12	-2.458*	.037	.000	-2.632	-2.284
	Week 13	.100*	.017	.011	.019	.181
	Week 15	.608*	.045	.000	.397	.820
Week 15	Week 1	-3.800*	.057	.000	-4.067	-3.533
	Week 2	-4.033*	.058	.000	-4.304	-3.763
	Week 7	-5.492*	.068	.000	-5.810	-5.174

	Week 8	-4.717*	.073	.000	-5.057	-4.376
	Week 9	-5.283*	.062	.000	-5.572	-4.994
	Week 10	-4.992*	.081	.000	-5.368	-4.615
	Week 11	-3.392*	.065	.000	-3.694	-3.089
	Week 12	-3.067*	.069	.000	-3.386	-2.747
	Week 13	-.508*	.052	.000	-.752	-.265
	Week 14	-.608*	.045	.000	-.820	-.397

pH

Table B.6: Shapiro-Wilk's Test of Normality for pH data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.953	6	.763
	NA	.889	6	.315
WEEK2	A	.925	6	.542
	NA	.853	6	.167
WEEK 3	A	.944	6	.688
	NA	.945	6	.698
WEEK 4	A	.974	6	.918
	NA	.885	6	.292
WEEK 5	A	.854	6	.170
	NA	.774	6	.034
WEEK 6	A	.894	6	.342
	NA	.664	6	.003
WEEK 7	A	.793	6	.051
	NA	.874	6	.245
WEEK 8	A	.929	6	.573
	NA	.888	6	.310
WEEK 9	A	.872	6	.233
	NA	.914	6	.464
WEEK 10	A	.909	6	.428
	NA	.797	6	.055
WEEK 11	A	.962	6	.836
	NA	.844	6	.140
WEEK 12	A	.907	6	.415
	NA	.712	6	.008
WEEK 13	A	.941	6	.670
	NA	.949	6	.728
WEEK 14	A	.902	6	.387
	NA	.930	6	.578
WEEK 15	A	.936	6	.630
	NA	.934	6	.614

Table B.7: Mauchly's Test of Sphericity for pH data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	150.637	54	.000

Table B.8: Levene's Test of Equality of Error Variances for pH data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.316	1	10	.587
Week 2	.298	1	10	.597
Week 3	.111	1	10	.745
Week 4	4.556	1	10	.059
Week 5	2.932	1	10	.118
Week 6	1.715	1	10	.220
Week 7	.330	1	10	.578
Week 8	.616	1	10	.451
Week 9	.437	1	10	.523
Week 10	.098	1	10	.761
Week 11	.074	1	10	.791
Week 12	2.472	1	10	.147
Week 13	.341	1	10	.572
Week 14	2.248	1	10	.165
Week 15	.379	1	10	.552

Table B.9: Pairwise Comparisons from repeated measures ANOVA for pH data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.045	.016	.984	-.029	.119
	Week 7	-.006	.017	1.000	-.084	.072
	Week 8	-.035	.022	1.000	-.138	.068
	Week 9	-.029	.026	1.000	-.148	.090
	Week 10	.006	.025	1.000	-.108	.120
	Week 11	-.049	.027	1.000	-.173	.075
	Week 12	-.063	.025	1.000	-.179	.053
	Week 13	-.013	.021	1.000	-.110	.085
	Week 14	-.086*	.018	.037	-.168	-.003

	Week 15	.074	.026	1.000	-.049	.197
Week 2	Week 1	-.045	.016	.984	-.119	.029
	Week 7	-.051	.011	.068	-.104	.002
	Week 8	-.080*	.011	.001	-.131	-.029
	Week 9	-.074	.016	.061	-.150	.002
	Week 10	-.039	.016	1.000	-.112	.033
	Week 11	-.094*	.013	.002	-.155	-.034
	Week 12	-.108*	.013	.000	-.169	-.047
	Week 13	-.058	.013	.065	-.117	.002
	Week 14	-.131*	.012	.000	-.186	-.076
	Week 15	.029	.015	1.000	-.039	.097
Week 7	Week 1	.006	.017	1.000	-.072	.084
	Week 2	.051	.011	.068	-.002	.104
	Week 8	-.029	.010	.724	-.074	.016
	Week 9	-.023	.010	1.000	-.071	.025
	Week 10	.012	.009	1.000	-.030	.053
	Week 11	-.043	.014	.704	-.110	.023
	Week 12	-.058*	.012	.035	-.112	-.003
	Week 13	-.007	.013	1.000	-.068	.054
	Week 14	-.080*	.007	.000	-.113	-.047
	Week 15	.080*	.016	.028	.006	.154
Week 8	Week 1	.035	.022	1.000	-.068	.138
	Week 2	.080*	.011	.001	.029	.131
	Week 7	.029	.010	.724	-.016	.074
	Week 9	.006	.011	1.000	-.043	.055
	Week 10	.041*	.008	.030	.003	.079
	Week 11	-.014	.007	1.000	-.048	.020
	Week 12	-.028	.010	1.000	-.075	.019
	Week 13	.022	.008	1.000	-.016	.061
	Week 14	-.051*	.010	.027	-.097	-.004
	Week 15	.109*	.010	.000	.063	.155
Week 9	Week 1	.029	.026	1.000	-.090	.148
	Week 2	.074	.016	.061	-.002	.150
	Week 7	.023	.010	1.000	-.025	.071
	Week 8	-.006	.011	1.000	-.055	.043
	Week 10	.035*	.006	.014	.005	.065
	Week 11	-.020	.010	1.000	-.068	.028
	Week 12	-.034	.012	.954	-.090	.022
	Week 13	.017	.016	1.000	-.059	.093
	Week 14	-.057*	.011	.027	-.109	-.005
	Week 15	.103*	.013	.001	.041	.166
Week 10	Week 1	-.006	.025	1.000	-.120	.108
	Week 2	.039	.016	1.000	-.033	.112
	Week 7	-.012	.009	1.000	-.053	.030

	Week 8	-.041*	.008	.030	-.079	-.003
	Week 9	-.035*	.006	.014	-.065	-.005
	Week 11	-.055*	.011	.030	-.106	-.004
	Week 12	-.069*	.011	.005	-.120	-.018
	Week 13	-.018	.014	1.000	-.085	.049
	Week 14	-.092*	.011	.001	-.145	-.038
	Week 15	.068	.015	.050	-5.779E-5	.137
Week 11	Week 1	.049	.027	1.000	-.075	.173
	Week 2	.094*	.013	.002	.034	.155
	Week 7	.043	.014	.704	-.023	.110
	Week 8	.014	.007	1.000	-.020	.048
	Week 9	.020	.010	1.000	-.028	.068
	Week 10	.055*	.011	.030	.004	.106
	Week 12	-.014	.012	1.000	-.068	.040
	Week 13	.037	.013	.832	-.022	.095
	Week 14	-.037	.014	1.000	-.102	.029
	Week 15	.123*	.007	.000	.091	.156
Week 12	Week 1	.063	.025	1.000	-.053	.179
	Week 2	.108*	.013	.000	.047	.169
	Week 7	.058*	.012	.035	.003	.112
	Week 8	.028	.010	1.000	-.019	.075
	Week 9	.034	.012	.954	-.022	.090
	Week 10	.069*	.011	.005	.018	.120
	Week 11	.014	.012	1.000	-.040	.068
	Week 13	.051	.017	.671	-.027	.128
	Week 14	-.023	.014	1.000	-.086	.041
	Week 15	.138*	.016	.000	.061	.214
Week 13	Week 1	.013	.021	1.000	-.085	.110
	Week 2	.058	.013	.065	-.002	.117
	Week 7	.007	.013	1.000	-.054	.068
	Week 8	-.022	.008	1.000	-.061	.016
	Week 9	-.017	.016	1.000	-.093	.059
	Week 10	.018	.014	1.000	-.049	.085
	Week 11	-.037	.013	.832	-.095	.022
	Week 12	-.051	.017	.671	-.128	.027
	Week 14	-.073*	.012	.007	-.130	-.016
	Week 15	.087*	.010	.000	.040	.133
Week 14	Week 1	.086*	.018	.037	.003	.168
	Week 2	.131*	.012	.000	.076	.186
	Week 7	.080*	.007	.000	.047	.113
	Week 8	.051*	.010	.027	.004	.097
	Week 9	.057*	.011	.027	.005	.109
	Week 10	.092*	.011	.001	.038	.145
	Week 11	.037	.014	1.000	-.029	.102

	Week 12	.023	.014	1.000	-.041	.086
	Week 13	.073*	.012	.007	.016	.130
	Week 15	.160*	.014	.000	.093	.227
Week 15	Week 1	-.074	.026	1.000	-.197	.049
	Week 2	-.029	.015	1.000	-.097	.039
	Week 7	-.080*	.016	.028	-.154	-.006
	Week 8	-.109*	.010	.000	-.155	-.063
	Week 9	-.103*	.013	.001	-.166	-.041
	Week 10	-.068	.015	.050	-.137	5.779E-5
	Week 11	-.123*	.007	.000	-.156	-.091
	Week 12	-.138*	.016	.000	-.214	-.061
	Week 13	-.087*	.010	.000	-.133	-.040
	Week 14	-.160*	.014	.000	-.227	-.093

Conductivity

Table B.10: Shapiro-Wilk's Test of Normality for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.876	6	.253
	NA	.825	6	.098
WEEK 2	A	.974	6	.919
	NA	.866	6	.211
WEEK 3	A	.832	6	.111
	NA	.941	6	.671
WEEK 4	A	.833	6	.113
	NA	.853	6	.167
WEEK 5	A	.919	6	.501
	NA	.948	6	.728
WEEK 6	A	.956	6	.790
	NA	.894	6	.338
WEEK 7	A	.912	6	.452
	NA	.857	6	.178
WEEK 8	A	.847	6	.148
	NA	.636	6	.001
WEEK 9	A	.970	6	.890
	NA	.744	6	.017
WEEK 10	A	.887	6	.300
	NA	.737	6	.015
WEEK 11	A	.822	6	.092
	NA	.866	6	.212
WEEK 12	A	.954	6	.772
	NA	.990	6	.990

WEEK 13	A	.819	6	.087
	NA	.844	6	.140
WEEK 14	A	.941	6	.668
	NA	.936	6	.630
WEEK 15	A	.955	6	.780
	NA	.817	6	.083

Table B.11: Mauchly's Test of Sphericity for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	114.214	54	.000

Table B.12: Levene's Test of Equality of Error Variances for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.230	1	10	.642
Week 2	.825	1	10	.385
Week 3	.248	1	10	.629
Week 4	1.032	1	10	.334
Week 5	.241	1	10	.634
Week 6	1.615	1	10	.232
Week 7	.559	1	10	.472
Week 8	.028	1	10	.871
Week 9	.036	1	10	.853
Week 10	.055	1	10	.820
Week 11	.297	1	10	.598
Week 12	.016	1	10	.902
Week 13	1.248	1	10	.290
Week 14	.005	1	10	.946
Week 15	6.578	1	10	.028

Table B.13: Pairwise Comparisons from repeated measures ANOVA for conductivity data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	-5.500	1.767	.606	-13.715	2.715

	Week 7	4.917	2.656	1.000	-7.434	17.267
	Week 8	10.583	3.745	.988	-6.827	27.994
	Week 9	4.750	2.870	1.000	-8.592	18.092
	Week 10	-16.750*	3.398	.033	-32.548	-.952
	Week 11	.250	3.495	1.000	-15.997	16.497
	Week 12	-46.583*	3.916	.000	-64.790	-28.377
	Week 13	28.500*	5.079	.012	4.889	52.111
	Week 14	-259.750*	6.515	.000	-290.042	-229.458
	Week 15	-58.583*	6.827	.000	-90.321	-26.845
Week 2	Week 1	5.500	1.767	.606	-2.715	13.715
	Week 7	10.417	2.429	.088	-.878	21.711
	Week 8	16.083*	3.457	.050	.011	32.156
	Week 9	10.250	2.814	.248	-2.833	23.333
	Week 10	-11.250	2.875	.160	-24.619	2.119
	Week 11	5.750	2.748	1.000	-7.026	18.526
	Week 12	-41.083*	2.672	.000	-53.507	-28.660
	Week 13	34.000*	4.620	.001	12.519	55.481
	Week 14	-254.250*	5.312	.000	-278.947	-229.553
	Week 15	-53.083*	6.581	.001	-83.679	-22.488
Week 7	Week 1	-4.917	2.656	1.000	-17.267	7.434
	Week 2	-10.417	2.429	.088	-21.711	.878
	Week 8	5.667	2.432	1.000	-5.640	16.973
	Week 9	-.167	2.246	1.000	-10.609	10.275
	Week 10	-21.667*	3.274	.003	-36.889	-6.445
	Week 11	-4.667	2.288	1.000	-15.305	5.972
	Week 12	-51.500*	2.744	.000	-64.258	-38.742
	Week 13	23.583*	3.465	.003	7.473	39.693
	Week 14	-264.667*	5.581	.000	-290.613	-238.721
	Week 15	-63.500*	5.571	.000	-89.399	-37.601
Week 8	Week 1	-10.583	3.745	.988	-27.994	6.827
	Week 2	-16.083*	3.457	.050	-32.156	-.011
	Week 7	-5.667	2.432	1.000	-16.973	5.640
	Week 9	-5.833	1.771	.445	-14.067	2.400
	Week 10	-27.333*	3.586	.001	-44.007	-10.660
	Week 11	-10.333	2.522	.119	-22.059	1.393
	Week 12	-57.167*	3.265	.000	-72.347	-41.986
	Week 13	17.917	4.675	.182	-3.819	39.653
	Week 14	-270.333*	6.501	.000	-300.560	-240.106
	Week 15	-69.167*	6.460	.000	-99.199	-39.134
Week 9	Week 1	-4.750	2.870	1.000	-18.092	8.592
	Week 2	-10.250	2.814	.248	-23.333	2.833
	Week 7	.167	2.246	1.000	-10.275	10.609
	Week 8	5.833	1.771	.445	-2.400	14.067
	Week 10	-21.500*	2.792	.001	-34.482	-8.518

	Week 11	-4.500	1.632	1.000	-12.088	3.088
	Week 12	-51.333*	2.916	.000	-64.890	-37.776
	Week 13	23.750*	4.395	.016	3.316	44.184
	Week 14	-264.500*	6.473	.000	-294.595	-234.405
	Week 15	-63.333*	6.125	.000	-91.811	-34.855
Week 10	Week 1	16.750*	3.398	.033	.952	32.548
	Week 2	11.250	2.875	.160	-2.119	24.619
	Week 7	21.667*	3.274	.003	6.445	36.889
	Week 8	27.333*	3.586	.001	10.660	44.007
	Week 9	21.500*	2.792	.001	8.518	34.482
	Week 11	17.000*	2.352	.002	6.064	27.936
	Week 12	-29.833*	3.595	.000	-46.546	-13.120
	Week 13	45.250*	4.975	.000	22.122	68.378
	Week 14	-243.000*	5.323	.000	-267.746	-218.254
	Week 15	-41.833*	6.583	.005	-72.440	-11.226
Week 11	Week 1	-.250	3.495	1.000	-16.497	15.997
	Week 2	-5.750	2.748	1.000	-18.526	7.026
	Week 7	4.667	2.288	1.000	-5.972	15.305
	Week 8	10.333	2.522	.119	-1.393	22.059
	Week 9	4.500	1.632	1.000	-3.088	12.088
	Week 10	-17.000*	2.352	.002	-27.936	-6.064
	Week 12	-46.833*	2.339	.000	-57.709	-35.957
	Week 13	28.250*	3.607	.001	11.479	45.021
	Week 14	-260.000*	5.318	.000	-284.724	-235.276
	Week 15	-58.833*	5.412	.000	-83.995	-33.672
Week 12	Week 1	46.583*	3.916	.000	28.377	64.790
	Week 2	41.083*	2.672	.000	28.660	53.507
	Week 7	51.500*	2.744	.000	38.742	64.258
	Week 8	57.167*	3.265	.000	41.986	72.347
	Week 9	51.333*	2.916	.000	37.776	64.890
	Week 10	29.833*	3.595	.000	13.120	46.546
	Week 11	46.833*	2.339	.000	35.957	57.709
	Week 13	75.083*	4.087	.000	56.083	94.084
	Week 14	-213.167*	5.318	.000	-237.894	-188.440
	Week 15	-12.000	6.293	1.000	-41.257	17.257
Week 13	Week 1	-28.500*	5.079	.012	-52.111	-4.889
	Week 2	-34.000*	4.620	.001	-55.481	-12.519
	Week 7	-23.583*	3.465	.003	-39.693	-7.473
	Week 8	-17.917	4.675	.182	-39.653	3.819
	Week 9	-23.750*	4.395	.016	-44.184	-3.316
	Week 10	-45.250*	4.975	.000	-68.378	-22.122
	Week 11	-28.250*	3.607	.001	-45.021	-11.479
	Week 12	-75.083*	4.087	.000	-94.084	-56.083
	Week 14	-288.250*	5.675	.000	-314.633	-261.867

	Week 15	-87.083*	4.167	.000	-106.459	-67.708
Week 14	Week 1	259.750*	6.515	.000	229.458	290.042
	Week 2	254.250*	5.312	.000	229.553	278.947
	Week 7	264.667*	5.581	.000	238.721	290.613
	Week 8	270.333*	6.501	.000	240.106	300.560
	Week 9	264.500*	6.473	.000	234.405	294.595
	Week 10	243.000*	5.323	.000	218.254	267.746
	Week 11	260.000*	5.318	.000	235.276	284.724
	Week 12	213.167*	5.318	.000	188.440	237.894
	Week 13	288.250*	5.675	.000	261.867	314.633
	Week 15	201.167*	6.840	.000	169.366	232.968
Week 15	Week 1	58.583*	6.827	.000	26.845	90.321
	Week 2	53.083*	6.581	.001	22.488	83.679
	Week 7	63.500*	5.571	.000	37.601	89.399
	Week 8	69.167*	6.460	.000	39.134	99.199
	Week 9	63.333*	6.125	.000	34.855	91.811
	Week 10	41.833*	6.583	.005	11.226	72.440
	Week 11	58.833*	5.412	.000	33.672	83.995
	Week 12	12.000	6.293	1.000	-17.257	41.257
	Week 13	87.083*	4.167	.000	67.708	106.459
	Week 14	-201.167*	6.840	.000	-232.968	-169.366

Dissolved Oxygen

Table B.14: Shapiro-Wilk's Test of Normality for dissolved oxygen data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.953	6	.763
	NA	.889	6	.315
WEEK 2	A	.925	6	.542
	NA	.853	6	.167
WEEK 3	A	.944	6	.688
	NA	.945	6	.698
WEEK 4	A	.974	6	.918
	NA	.885	6	.292
WEEK 5	A	.854	6	.170
	NA	.774	6	.034
WEEK 6	A	.894	6	.342
	NA	.664	6	.003
WEEK 7	A	.793	6	.051
	NA	.874	6	.245
WEEK 8	A	.929	6	.573
	NA	.888	6	.310

WEEK 9	A	.872	6	.233
	NA	.914	6	.464
WEEK 10	A	.909	6	.428
	NA	.797	6	.055
WEEK 11	A	.962	6	.836
	NA	.844	6	.140
WEEK 12	A	.907	6	.415
	NA	.712	6	.008
WEEK 13	A	.941	6	.670
	NA	.949	6	.728
WEEK 14	A	.902	6	.387
	NA	.930	6	.578
WEEK 15	A	.936	6	.630
	NA	.934	6	.614

Table B.15: Mauchly's Test of Sphericity for dissolved oxygen data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	226.095	54	.000

Table B.16: Levene's Test of Equality of Error Variances for dissolved oxygen data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	17.102	1	10	.002
Week 2	9.287	1	10	.012
Week 3	13.589	1	10	.004
Week 4	20.223	1	10	.001
Week 5	48.459	1	10	.000
Week 6	12.341	1	10	.006
Week 7	28.017	1	10	.000
Week 8	6.846	1	10	.026
Week 9	11.091	1	10	.008
Week 10	12.531	1	10	.005
Week 11	1.380	1	10	.267
Week 12	11.938	1	10	.006
Week 13	17.113	1	10	.002
Week 14	34.282	1	10	.000
Week 15	8.718	1	10	.014

Table B.17: Pairwise Comparisons from repeated measures ANOVA for dissolved oxygen data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	-.294	.108	1.000	-.795	.206
	Week 7	-.118	.115	1.000	-.655	.418
	Week 8	-.122	.103	1.000	-.602	.359
	Week 9	.022	.143	1.000	-.643	.686
	Week 10	.357	.135	1.000	-.272	.987
	Week 11	.517	.218	1.000	-.497	1.531
	Week 12	.338	.132	1.000	-.274	.949
	Week 13	-.675*	.113	.008	-1.202	-.148
	Week 14	-.478	.108	.071	-.981	.024
	Week 15	-.964*	.176	.015	-1.782	-.146
Week 2	Week 1	.294	.108	1.000	-.206	.795
	Week 7	.176	.135	1.000	-.451	.802
	Week 8	.173	.099	1.000	-.287	.632
	Week 9	.316	.164	1.000	-.448	1.080
	Week 10	.652	.168	.171	-.131	1.434
	Week 11	.811	.252	.506	-.360	1.982
	Week 12	.632	.145	.077	-.041	1.304
	Week 13	-.381	.098	.167	-.837	.075
	Week 14	-.184	.100	1.000	-.648	.280
	Week 15	-.670*	.093	.002	-1.101	-.239
Week 7	Week 1	.118	.115	1.000	-.418	.655
	Week 2	-.176	.135	1.000	-.802	.451
	Week 8	-.003	.059	1.000	-.276	.269
	Week 9	.140	.034	.124	-.020	.300
	Week 10	.476*	.037	.000	.306	.646
	Week 11	.635	.180	.302	-.203	1.473
	Week 12	.456*	.051	.000	.217	.695
	Week 13	-.557*	.051	.000	-.794	-.319
	Week 14	-.360*	.052	.002	-.601	-.119
	Week 15	-.846*	.156	.016	-1.571	-.121
Week 8	Week 1	.122	.103	1.000	-.359	.602
	Week 2	-.173	.099	1.000	-.632	.287
	Week 7	.003	.059	1.000	-.269	.276
	Week 9	.143	.087	1.000	-.259	.546
	Week 10	.479*	.089	.018	.063	.895
	Week 11	.638	.175	.248	-.176	1.453
	Week 12	.459*	.058	.001	.190	.729
	Week 13	-.553*	.046	.000	-.767	-.339

	Week 14	-.357*	.060	.008	-.636	-.078
	Week 15	-.843*	.138	.006	-1.485	-.200
Week 9	Week 1	-.022	.143	1.000	-.686	.643
	Week 2	-.316	.164	1.000	-1.080	.448
	Week 7	-.140	.034	.124	-.300	.020
	Week 8	-.143	.087	1.000	-.546	.259
	Week 10	.336*	.030	.000	.196	.475
	Week 11	.495	.178	1.000	-.333	1.323
	Week 12	.316*	.059	.018	.041	.591
	Week 13	-.697*	.077	.000	-1.053	-.341
	Week 14	-.500*	.077	.004	-.858	-.142
	Week 15	-.986*	.175	.012	-1.801	-.170
Week 10	Week 1	-.357	.135	1.000	-.987	.272
	Week 2	-.652	.168	.171	-1.434	.131
	Week 7	-.476*	.037	.000	-.646	-.306
	Week 8	-.479*	.089	.018	-.895	-.063
	Week 9	-.336*	.030	.000	-.475	-.196
	Week 11	.159	.181	1.000	-.681	.999
	Week 12	-.020	.064	1.000	-.318	.278
	Week 13	-1.032*	.083	.000	-1.418	-.647
	Week 14	-.836*	.081	.000	-1.212	-.460
	Week 15	-1.322*	.186	.002	-2.186	-.457
Week 11	Week 1	-.517	.218	1.000	-1.531	.497
	Week 2	-.811	.252	.506	-1.982	.360
	Week 7	-.635	.180	.302	-1.473	.203
	Week 8	-.638	.175	.248	-1.453	.176
	Week 9	-.495	.178	1.000	-1.323	.333
	Week 10	-.159	.181	1.000	-.999	.681
	Week 12	-.179	.178	1.000	-1.008	.649
	Week 13	-1.192*	.186	.004	-2.058	-.326
	Week 14	-.995*	.196	.026	-1.904	-.086
	Week 15	-1.481*	.267	.014	-2.723	-.239
Week 12	Week 1	-.338	.132	1.000	-.949	.274
	Week 2	-.632	.145	.077	-1.304	.041
	Week 7	-.456*	.051	.000	-.695	-.217
	Week 8	-.459*	.058	.001	-.729	-.190
	Week 9	-.316*	.059	.018	-.591	-.041
	Week 10	.020	.064	1.000	-.278	.318
	Week 11	.179	.178	1.000	-.649	1.008
	Week 13	-1.013*	.076	.000	-1.364	-.661
	Week 14	-.816*	.084	.000	-1.207	-.424
	Week 15	-1.302*	.173	.001	-2.108	-.495
Week 13	Week 1	.675*	.113	.008	.148	1.202
	Week 2	.381	.098	.167	-.075	.837

	Week 7	.557*	.051	.000	.319	.794
	Week 8	.553*	.046	.000	.339	.767
	Week 9	.697*	.077	.000	.341	1.053
	Week 10	1.032*	.083	.000	.647	1.418
	Week 11	1.192*	.186	.004	.326	2.058
	Week 12	1.013*	.076	.000	.661	1.364
	Week 14	.197*	.019	.000	.107	.286
	Week 15	-.289	.109	1.000	-.797	.218
Week 14	Week 1	.478	.108	.071	-.024	.981
	Week 2	.184	.100	1.000	-.280	.648
	Week 7	.360*	.052	.002	.119	.601
	Week 8	.357*	.060	.008	.078	.636
	Week 9	.500*	.077	.004	.142	.858
	Week 10	.836*	.081	.000	.460	1.212
	Week 11	.995*	.196	.026	.086	1.904
	Week 12	.816*	.084	.000	.424	1.207
	Week 13	-.197*	.019	.000	-.286	-.107
	Week 15	-.486	.109	.066	-.991	.020
Week 15	Week 1	.964*	.176	.015	.146	1.782
	Week 2	.670*	.093	.002	.239	1.101
	Week 7	.846*	.156	.016	.121	1.571
	Week 8	.843*	.138	.006	.200	1.485
	Week 9	.986*	.175	.012	.170	1.801
	Week 10	1.322*	.186	.002	.457	2.186
	Week 11	1.481*	.267	.014	.239	2.723
	Week 12	1.302*	.173	.001	.495	2.108
	Week 13	.289	.109	1.000	-.218	.797
	Week 14	.486	.109	.066	-.020	.991

Ammonium

Table B.18: Shapiro-Wilk's Test of Normality for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.776	6	.035
	NA	.828	6	.104
WEEK 2	A	.927	6	.553
	NA	.976	6	.928
WEEK 3	A	.960	6	.819
	NA	.958	6	.805
WEEK 4	A	.956	6	.791
	NA	.973	6	.911
WEEK 5	A	.962	6	.833

	NA	.921	6	.516
WEEK 6	A	.974	6	.916
	NA	.917	6	.486
WEEK 7	A	.979	6	.948
	NA	.801	6	.061
WEEK 8	A	.947	6	.714
	NA	.844	6	.142
WEEK 9	A	.877	6	.256
	NA	.850	6	.157
WEEK 10	A	.930	6	.576
	NA	.927	6	.558

Table B.19: Mauchly's Test of Sphericity for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	130.871	44	.000

Table B.20: Levene's Test of Equality of Error Variances for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	3.044	1	10	.112
Week 2	4.031	1	10	.072
Week 3	7.762	1	10	.019
Week 4	4.383	1	10	.063
Week 5	10.534	1	10	.009
Week 6	7.837	1	10	.019
Week 7	13.726	1	10	.004
Week 8	4.599	1	10	.058
Week 9	3.958	1	10	.075
Week 10	9.345	1	10	.012

Table B.21: Pairwise Comparisons from repeated measures ANOVA for ammonium data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound

Week 1	Week 2	.177	.106	1.000	-.302	.655
	Week 3	.413	.154	1.000	-.282	1.109
	Week 4	.509	.172	.650	-.270	1.288
	Week 5	.772*	.141	.012	.136	1.407
	Week 6	1.686*	.142	.000	1.046	2.326
	Week 7	1.709*	.138	.000	1.085	2.333
	Week 8	2.034*	.140	.000	1.401	2.667
	Week 9	1.727*	.146	.000	1.066	2.387
	Week 10	2.180*	.179	.000	1.372	2.988
Week 2	Week 1	-.177	.106	1.000	-.655	.302
	Week 3	.237	.082	.732	-.134	.607
	Week 4	.332	.084	.122	-.048	.713
	Week 5	.595*	.057	.000	.338	.852
	Week 6	1.509*	.063	.000	1.224	1.794
	Week 7	1.533*	.062	.000	1.251	1.814
	Week 8	1.858*	.079	.000	1.501	2.214
	Week 9	1.550*	.101	.000	1.093	2.007
	Week 10	2.003*	.141	.000	1.368	2.639
Week 3	Week 1	-.413	.154	1.000	-1.109	.282
	Week 2	-.237	.082	.732	-.607	.134
	Week 4	.096	.047	1.000	-.116	.308
	Week 5	.358*	.062	.008	.079	.637
	Week 6	1.273*	.093	.000	.854	1.691
	Week 7	1.296*	.089	.000	.892	1.700
	Week 8	1.621*	.108	.000	1.135	2.107
	Week 9	1.313*	.134	.000	.710	1.917
	Week 10	1.767*	.169	.000	1.003	2.531
Week 4	Week 1	-.509	.172	.650	-1.288	.270
	Week 2	-.332	.084	.122	-.713	.048
	Week 3	-.096	.047	1.000	-.308	.116
	Week 5	.263*	.044	.006	.063	.462
	Week 6	1.177*	.070	.000	.862	1.491
	Week 7	1.200*	.077	.000	.852	1.548
	Week 8	1.525*	.112	.000	1.019	2.031
	Week 9	1.218*	.149	.000	.546	1.889
	Week 10	1.671*	.186	.000	.828	2.513
Week 5	Week 1	-.772*	.141	.012	-1.407	-.136
	Week 2	-.595*	.057	.000	-.852	-.338
	Week 3	-.358*	.062	.008	-.637	-.079
	Week 4	-.263*	.044	.006	-.462	-.063
	Week 6	.914*	.041	.000	.731	1.097
	Week 7	.938*	.041	.000	.753	1.122
	Week 8	1.262*	.084	.000	.884	1.641
	Week 9	.955*	.125	.001	.392	1.518

	Week 10	1.408*	.168	.000	.648	2.169
Week 6	Week 1	-1.686*	.142	.000	-2.326	-1.046
	Week 2	-1.509*	.063	.000	-1.794	-1.224
	Week 3	-1.273*	.093	.000	-1.691	-.854
	Week 4	-1.177*	.070	.000	-1.491	-.862
	Week 5	-.914*	.041	.000	-1.097	-.731
	Week 7	.023	.043	1.000	-.172	.218
	Week 8	.348	.095	.201	-.083	.780
	Week 9	.041	.138	1.000	-.583	.664
	Week 10	.494	.182	.973	-.327	1.315
Week 7	Week 1	-1.709*	.138	.000	-2.333	-1.085
	Week 2	-1.533*	.062	.000	-1.814	-1.251
	Week 3	-1.296*	.089	.000	-1.700	-.892
	Week 4	-1.200*	.077	.000	-1.548	-.852
	Week 5	-.938*	.041	.000	-1.122	-.753
	Week 6	-.023	.043	1.000	-.218	.172
	Week 8	.325*	.058	.010	.063	.587
	Week 9	.018	.107	1.000	-.467	.502
	Week 10	.471	.156	.583	-.234	1.176
Week 8	Week 1	-2.034*	.140	.000	-2.667	-1.401
	Week 2	-1.858*	.079	.000	-2.214	-1.501
	Week 3	-1.621*	.108	.000	-2.107	-1.135
	Week 4	-1.525*	.112	.000	-2.031	-1.019
	Week 5	-1.262*	.084	.000	-1.641	-.884
	Week 6	-.348	.095	.201	-.780	.083
	Week 7	-.325*	.058	.010	-.587	-.063
	Week 9	-.307*	.059	.017	-.573	-.042
	Week 10	.146	.113	1.000	-.366	.658
Week 9	Week 1	-1.727*	.146	.000	-2.387	-1.066
	Week 2	-1.550*	.101	.000	-2.007	-1.093
	Week 3	-1.313*	.134	.000	-1.917	-.710
	Week 4	-1.218*	.149	.000	-1.889	-.546
	Week 5	-.955*	.125	.001	-1.518	-.392
	Week 6	-.041	.138	1.000	-.664	.583
	Week 7	-.018	.107	1.000	-.502	.467
	Week 8	.307*	.059	.017	.042	.573
	Week 10	.453*	.058	.001	.189	.717
Week 10	Week 1	-2.180*	.179	.000	-2.988	-1.372
	Week 2	-2.003*	.141	.000	-2.639	-1.368
	Week 3	-1.767*	.169	.000	-2.531	-1.003
	Week 4	-1.671*	.186	.000	-2.513	-.828
	Week 5	-1.408*	.168	.000	-2.169	-.648
	Week 6	-.494	.182	.973	-1.315	.327
	Week 7	-.471	.156	.583	-1.176	.234

	Week 8	-.146	.113	1.000	-.658	.366
	Week 9	-.453*	.058	.001	-.717	-.189

Nitrate

Table B.22: Shapiro-Wilk's Test of Normality for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.929	6	.569
	NA	.928	6	.564
WEEK 2	A	.938	6	.646
	NA	.912	6	.449
WEEK 3	A	.948	6	.720
	NA	.950	6	.738
WEEK 4	A	.788	6	.046
	NA	.931	6	.589
WEEK 5	A	.926	6	.551
	NA	.786	6	.044
WEEK 6	A	.902	6	.384
	NA	.796	6	.055
WEEK 7	A	.971	6	.899
	NA	.875	6	.248
WEEK 8	A	.899	6	.369
	NA	.970	6	.894
WEEK 9	A	.974	6	.917
	NA	.910	6	.438
WEEK 10	A	.918	6	.493
	NA	.867	6	.216

Table B.23: Mauchly's Test of Sphericity for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	121.883	44	.000

Table B.24: Levene's Test of Equality of Error Variances for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.

Week 1	12.000	1	10	.006
Week 2	14.409	1	10	.004
Week 3	7.598	1	10	.020
Week 4	1.355	1	10	.271
Week 5	6.577	1	10	.028
Week 6	10.267	1	10	.009
Week 7	4.619	1	10	.057
Week 8	16.972	1	10	.002
Week 9	2.109	1	10	.177
Week 10	6.585	1	10	.028

Table B.25: Pairwise Comparisons from repeated measures ANOVA for nitrate data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	-4.868*	.551	.000	-7.357	-2.378
	Week 3	-1.977	.675	.679	-5.027	1.073
	Week 4	-.207	.733	1.000	-3.519	3.105
	Week 5	-15.678*	.979	.000	-20.100	-11.255
	Week 6	-4.346	1.141	.154	-9.500	.809
	Week 7	3.758	1.164	.407	-1.501	9.017
	Week 8	5.045*	1.096	.044	.091	9.999
	Week 9	1.755	1.260	1.000	-3.938	7.448
	Week 10	2.161	1.586	1.000	-5.005	9.327
Week 2	Week 1	4.868*	.551	.000	2.378	7.357
	Week 3	2.891	.776	.178	-.617	6.398
	Week 4	4.661*	.548	.000	2.187	7.135
	Week 5	-10.810*	.886	.000	-14.812	-6.808
	Week 6	.522	.693	1.000	-2.610	3.653
	Week 7	8.626*	.669	.000	5.604	11.648
	Week 8	9.913*	.609	.000	7.163	12.662
	Week 9	6.623*	.830	.001	2.873	10.372
	Week 10	7.028*	1.161	.006	1.784	12.272
Week 3	Week 1	1.977	.675	.679	-1.073	5.027
	Week 2	-2.891	.776	.178	-6.398	.617
	Week 4	1.770	.840	1.000	-2.027	5.567
	Week 5	-13.701*	.741	.000	-17.048	-10.354
	Week 6	-2.369	1.205	1.000	-7.815	3.077
	Week 7	5.735*	1.264	.049	.022	11.448
	Week 8	7.022*	1.217	.008	1.522	12.522

	Week 9	3.732	1.239	.588	-1.867	9.330
	Week 10	4.138	1.588	1.000	-3.038	11.313
Week 4	Week 1	.207	.733	1.000	-3.105	3.519
	Week 2	-4.661*	.548	.000	-7.135	-2.187
	Week 3	-1.770	.840	1.000	-5.567	2.027
	Week 5	-15.471*	.697	.000	-18.620	-12.322
	Week 6	-4.139*	.785	.016	-7.686	-.592
	Week 7	3.965*	.797	.025	.363	7.567
	Week 8	5.252*	.781	.002	1.724	8.780
	Week 9	1.962	.927	1.000	-2.229	6.152
	Week 10	2.367	1.232	1.000	-3.198	7.933
Week 5	Week 1	15.678*	.979	.000	11.255	20.100
	Week 2	10.810*	.886	.000	6.808	14.812
	Week 3	13.701*	.741	.000	10.354	17.048
	Week 4	15.471*	.697	.000	12.322	18.620
	Week 6	11.332*	.988	.000	6.869	15.794
	Week 7	19.436*	1.105	.000	14.443	24.429
	Week 8	20.723*	1.132	.000	15.608	25.837
	Week 9	17.433*	1.138	.000	12.291	22.574
	Week 10	17.838*	1.387	.000	11.573	24.104
Week 6	Week 1	4.346	1.141	.154	-.809	9.500
	Week 2	-.522	.693	1.000	-3.653	2.610
	Week 3	2.369	1.205	1.000	-3.077	7.815
	Week 4	4.139*	.785	.016	.592	7.686
	Week 5	-11.332*	.988	.000	-15.794	-6.869
	Week 7	8.104*	.321	.000	6.653	9.556
	Week 8	9.391*	.393	.000	7.614	11.167
	Week 9	6.101*	.401	.000	4.289	7.913
	Week 10	6.507*	.654	.000	3.552	9.461
Week 7	Week 1	-3.758	1.164	.407	-9.017	1.501
	Week 2	-8.626*	.669	.000	-11.648	-5.604
	Week 3	-5.735*	1.264	.049	-11.448	-.022
	Week 4	-3.965*	.797	.025	-7.567	-.363
	Week 5	-19.436*	1.105	.000	-24.429	-14.443
	Week 6	-8.104*	.321	.000	-9.556	-6.653
	Week 8	1.287*	.139	.000	.657	1.916
	Week 9	-2.003*	.421	.035	-3.908	-.099
	Week 10	-1.597	.663	1.000	-4.592	1.397
Week 8	Week 1	-5.045*	1.096	.044	-9.999	-.091
	Week 2	-9.913*	.609	.000	-12.662	-7.163
	Week 3	-7.022*	1.217	.008	-12.522	-1.522
	Week 4	-5.252*	.781	.002	-8.780	-1.724
	Week 5	-20.723*	1.132	.000	-25.837	-15.608
	Week 6	-9.391*	.393	.000	-11.167	-7.614

	Week 7	-1.287*	.139	.000	-1.916	-.657
	Week 9	-3.290*	.458	.001	-5.358	-1.222
	Week 10	-2.884	.739	.133	-6.225	.457
Week 9	Week 1	-1.755	1.260	1.000	-7.448	3.938
	Week 2	-6.623*	.830	.001	-10.372	-2.873
	Week 3	-3.732	1.239	.588	-9.330	1.867
	Week 4	-1.962	.927	1.000	-6.152	2.229
	Week 5	-17.433*	1.138	.000	-22.574	-12.291
	Week 6	-6.101*	.401	.000	-7.913	-4.289
	Week 7	2.003*	.421	.035	.099	3.908
	Week 8	3.290*	.458	.001	1.222	5.358
	Week 10	.406	.424	1.000	-1.509	2.321
Week 10	Week 1	-2.161	1.586	1.000	-9.327	5.005
	Week 2	-7.028*	1.161	.006	-12.272	-1.784
	Week 3	-4.138	1.588	1.000	-11.313	3.038
	Week 4	-2.367	1.232	1.000	-7.933	3.198
	Week 5	-17.838*	1.387	.000	-24.104	-11.573
	Week 6	-6.507*	.654	.000	-9.461	-3.552
	Week 7	1.597	.663	1.000	-1.397	4.592
	Week 8	2.884	.739	.133	-.457	6.225
	Week 9	-.406	.424	1.000	-2.321	1.509

ORP

Table B.26: Shapiro-Wilk's Test of Normality for ORP data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 2	A	.955	6	.777
	NA	.763	6	.027
WEEK 3	A	.871	6	.229
	NA	.729	6	.012
WEEK 4	A	.874	6	.241
	NA	.808	6	.069
WEEK 5	A	.950	6	.743
	NA	.661	6	.002
WEEK 6	A	.929	6	.576
	NA	.696	6	.006
WEEK 7	A	.919	6	.498
	NA	.785	6	.043
WEEK 8	A	.913	6	.455
	NA	.695	6	.006
WEEK 9	A	.950	6	.737
	NA	.877	6	.255

WEEK 10	A	.928	6	.564
	NA	.686	6	.004
WEEK 11	A	.909	6	.427
	NA	.776	6	.035
WEEK 12	A	.948	6	.727
	NA	.914	6	.467
WEEK 13	A	.854	6	.168
	NA	.893	6	.332
WEEK 14	A	.846	6	.146
	NA	.882	6	.281
WEEK 15	A	.930	6	.579
	NA	.967	6	.869

Table B.27: Mauchly's Test of Sphericity for ORP data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	208.809	54	.000

Table B.28: Levene's Test of Equality of Error Variances for ORP data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 2	.085	1	10	.777
Week 3	4.885	1	10	.052
Week 4	23.531	1	10	.001
Week 5	4.506	1	10	.060
Week 6	3.925	1	10	.076
Week 7	5.821	1	10	.037
Week 8	3.372	1	10	.096
Week 9	2.013	1	10	.186
Week 10	4.079	1	10	.071
Week 11	2.933	1	10	.118
Week 12	9.239	1	10	.012
Week 13	14.400	1	10	.004
Week 14	16.275	1	10	.002
Week 15	4.831	1	10	.053

Table B.29: Pairwise Comparisons from repeated measures ANOVA for ORP data of aerated and non-aerated systems, α = 0.05. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 2	Week 6	-.282	2.720	1.000	-12.925	12.362
	Week 7	-1.313	4.085	1.000	-20.307	17.680
	Week 8	3.840	3.181	1.000	-10.949	18.629
	Week 9	4.428	2.938	1.000	-9.233	18.088
	Week 10	.247	3.221	1.000	-14.726	15.220
	Week 11	.635	3.182	1.000	-14.157	15.427
	Week 12	.098	3.027	1.000	-13.976	14.171
	Week 13	3.731	3.162	1.000	-10.968	18.430
	Week 14	-2.114	3.180	1.000	-16.901	12.672
	Week 15	-12.676	3.308	.182	-28.057	2.706
Week 6	Week 2	.282	2.720	1.000	-12.362	12.925
	Week 7	-1.032	3.029	1.000	-15.114	13.050
	Week 8	4.122*	.757	.015	.604	7.639
	Week 9	4.709	1.028	.055	-.070	9.488
	Week 10	.528	1.248	1.000	-5.272	6.329
	Week 11	.917	1.271	1.000	-4.991	6.825
	Week 12	.379	1.744	1.000	-7.731	8.489
	Week 13	4.013	1.245	.502	-1.776	9.801
	Week 14	-1.832	1.449	1.000	-8.569	4.904
	Week 15	-12.394*	2.257	.015	-22.885	-1.903
Week 7	Week 2	1.313	4.085	1.000	-17.680	20.307
	Week 6	1.032	3.029	1.000	-13.050	15.114
	Week 8	5.153	2.999	1.000	-8.788	19.095
	Week 9	5.741	2.963	1.000	-8.034	19.515
	Week 10	1.560	2.940	1.000	-12.109	15.229
	Week 11	1.948	2.745	1.000	-10.813	14.709
	Week 12	1.411	2.962	1.000	-12.360	15.182
	Week 13	5.044	2.665	1.000	-7.348	17.436
	Week 14	-.801	2.699	1.000	-13.349	11.748
	Week 15	-11.362	2.899	.158	-24.840	2.115
Week 8	Week 2	-3.840	3.181	1.000	-18.629	10.949
	Week 6	-4.122*	.757	.015	-7.639	-.604
	Week 7	-5.153	2.999	1.000	-19.095	8.788
	Week 9	.587	.793	1.000	-3.099	4.274
	Week 10	-3.593	.860	.104	-7.593	.407
	Week 11	-3.205	.866	.225	-7.229	.819
	Week 12	-3.743	1.635	1.000	-11.345	3.860

	Week 13	-.109	.865	1.000	-4.129	3.910
	Week 14	-5.954*	1.003	.008	-10.620	-1.289
	Week 15	-16.516*	2.223	.001	-26.852	-6.179
Week 9	Week 2	-4.428	2.938	1.000	-18.088	9.233
	Week 6	-4.709	1.028	.055	-9.488	.070
	Week 7	-5.741	2.963	1.000	-19.515	8.034
	Week 8	-.587	.793	1.000	-4.274	3.099
	Week 10	-4.181*	.500	.000	-6.504	-1.858
	Week 11	-3.793*	.593	.004	-6.547	-1.038
	Week 12	-4.330	.971	.067	-8.845	.185
	Week 13	-.697	.534	1.000	-3.177	1.784
	Week 14	-6.542*	.752	.000	-10.039	-3.044
	Week 15	-17.103*	1.620	.000	-24.635	-9.572
Week 10	Week 2	-.247	3.221	1.000	-15.220	14.726
	Week 6	-.528	1.248	1.000	-6.329	5.272
	Week 7	-1.560	2.940	1.000	-15.229	12.109
	Week 8	3.593	.860	.104	-.407	7.593
	Week 9	4.181*	.500	.000	1.858	6.504
	Week 11	.388	.600	1.000	-2.402	3.179
	Week 12	-.149	.974	1.000	-4.678	4.380
	Week 13	3.484*	.494	.002	1.189	5.779
	Week 14	-2.361	.714	.437	-5.682	.960
	Week 15	-12.922*	1.554	.000	-20.146	-5.699
Week 11	Week 2	-.635	3.182	1.000	-15.427	14.157
	Week 6	-.917	1.271	1.000	-6.825	4.991
	Week 7	-1.948	2.745	1.000	-14.709	10.813
	Week 8	3.205	.866	.225	-.819	7.229
	Week 9	3.793*	.593	.004	1.038	6.547
	Week 10	-.388	.600	1.000	-3.179	2.402
	Week 12	-.537	.946	1.000	-4.934	3.859
	Week 13	3.096*	.200	.000	2.166	4.025
	Week 14	-2.749*	.276	.000	-4.033	-1.465
	Week 15	-13.311*	1.621	.001	-20.845	-5.777
Week 12	Week 2	-.098	3.027	1.000	-14.171	13.976
	Week 6	-.379	1.744	1.000	-8.489	7.731
	Week 7	-1.411	2.962	1.000	-15.182	12.360
	Week 8	3.743	1.635	1.000	-3.860	11.345
	Week 9	4.330	.971	.067	-.185	8.845
	Week 10	.149	.974	1.000	-4.380	4.678
	Week 11	.537	.946	1.000	-3.859	4.934
	Week 13	3.633	.913	.143	-.611	7.878
	Week 14	-2.212	1.016	1.000	-6.937	2.513
	Week 15	-12.773*	.895	.000	-16.934	-8.613
Week 13	Week 2	-3.731	3.162	1.000	-18.430	10.968

	Week 6	-4.013	1.245	.502	-9.801	1.776
	Week 7	-5.044	2.665	1.000	-17.436	7.348
	Week 8	.109	.865	1.000	-3.910	4.129
	Week 9	.697	.534	1.000	-1.784	3.177
	Week 10	-3.484*	.494	.002	-5.779	-1.189
	Week 11	-3.096*	.200	.000	-4.025	-2.166
	Week 12	-3.633	.913	.143	-7.878	.611
	Week 14	-5.845*	.340	.000	-7.427	-4.263
	Week 15	-16.407*	1.568	.000	-23.698	-9.115
Week 14	Week 2	2.114	3.180	1.000	-12.672	16.901
	Week 6	1.832	1.449	1.000	-4.904	8.569
	Week 7	.801	2.699	1.000	-11.748	13.349
	Week 8	5.954*	1.003	.008	1.289	10.620
	Week 9	6.542*	.752	.000	3.044	10.039
	Week 10	2.361	.714	.437	-.960	5.682
	Week 11	2.749*	.276	.000	1.465	4.033
	Week 12	2.212	1.016	1.000	-2.513	6.937
	Week 13	5.845*	.340	.000	4.263	7.427
	Week 15	-10.562*	1.687	.005	-18.407	-2.717
Week 15	Week 2	12.676	3.308	.182	-2.706	28.057
	Week 6	12.394*	2.257	.015	1.903	22.885
	Week 7	11.362	2.899	.158	-2.115	24.840
	Week 8	16.516*	2.223	.001	6.179	26.852
	Week 9	17.103*	1.620	.000	9.572	24.635
	Week 10	12.922*	1.554	.000	5.699	20.146
	Week 11	13.311*	1.621	.001	5.777	20.845
	Week 12	12.773*	.895	.000	8.613	16.934
	Week 13	16.407*	1.568	.000	9.115	23.698
	Week 14	10.562*	1.687	.005	2.717	18.407

Wastewater Treatment

Table B.30: Student's t-test p-values for wastewater treatment data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Total Organic Carbon Removal Rate	Total Nitrogen Removal Rate
4	2.79203E-05*	1.38471E-05*
5	0.0002007*	1.57621E-08*
6	0.000551776*	2.13697E-07*
7	0.000225977*	4.59188E-08*
8	1.77611E-07*	1.98904E-08*
9	8.73508E-06*	6.43226E-10*
10	4.96289E-05*	8.34404E-12*
11	0.008662263*	5.29388E-08*
12	0.042704888*	2.88609E-06*
13	0.000110941*	1.81118E-07*
14	0.001507523*	
15	0.056410375	2.35227E-08*

Total Organic Carbon Removal Rate

Table B.31: Shapiro-Wilk's Test of Normality for total organic carbon removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 4	A	.753	5	.032
	NA	.939	6	.648
WEEK 5	A	.901	5	.418
	NA	.917	6	.482
WEEK 6	A	.946	5	.706
	NA	.934	6	.614
WEEK 7	A	.865	5	.247
	NA	.899	6	.366
WEEK 8	A	.669	5	.004
	NA	.850	6	.158
WEEK 9	A	.913	5	.489
	NA	.804	6	.064
WEEK 10	A	.821	5	.119
	NA	.764	6	.027
WEEK 11	A	.903	5	.424
	NA	.769	6	.031
WEEK 12	A	.979	5	.932

	NA	.945	6	.698
WEEK 13	A	.981	5	.941
	NA	.923	6	.525
WEEK 14	A	.820	5	.117
	NA	.856	6	.176
WEEK 15	A	.912	5	.480
	NA	.973	6	.909

Table B.32: Mauchly's Test of Sphericity for total organic carbon removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	111.431	44	.000

Table B.33: Levene's Test of Equality of Error Variances for total organic carbon removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 4	5.915	1	9	.038
Week 5	6.171	1	9	.035
Week 6	4.333	1	9	.067
Week 7	5.874	1	9	.038
Week 8	.097	1	9	.763
Week 9	15.592	1	9	.003
Week 10	.287	1	9	.605
Week 11	3.038	1	9	.115
Week 12	12.859	1	9	.006
Week 13	.580	1	9	.466
Week 14	12.350	1	9	.007
Week 15	.552	1	9	.476

Table B.34: Pairwise Comparisons from repeated measures ANOVA for total organic carbon removal rate data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 4	Week 5	-3.835*	.256	.000	-5.039	-2.631
	Week 8	-2.867*	.334	.001	-4.437	-1.296

	Week 9	4.083*	.450	.000	1.966	6.200
	Week 10	-2.761*	.385	.002	-4.572	-.950
	Week 11	-1.115	.864	1.000	-5.179	2.950
	Week 12	-3.449*	.508	.004	-5.837	-1.060
	Week 13	-1.947*	.412	.048	-3.883	-.010
	Week 14	7.944*	.930	.001	3.567	12.320
	Week 15	-3.804*	.325	.000	-5.333	-2.276
Week 5	Week 4	3.835*	.256	.000	2.631	5.039
	Week 8	.968*	.186	.025	.092	1.845
	Week 9	7.918*	.385	.000	6.107	9.729
	Week 10	1.074	.252	.094	-.110	2.258
	Week 11	2.720	.849	.485	-1.276	6.716
	Week 12	.386	.378	1.000	-1.393	2.166
	Week 13	1.888*	.280	.004	.571	3.206
	Week 14	11.778*	.955	.000	7.284	16.273
	Week 15	.031	.231	1.000	-1.057	1.118
Week 8	Week 4	2.867*	.334	.001	1.296	4.437
	Week 5	-.968*	.186	.025	-1.845	-.092
	Week 9	6.950*	.347	.000	5.317	8.582
	Week 10	.106	.241	1.000	-1.027	1.238
	Week 11	1.752	.791	1.000	-1.973	5.476
	Week 12	-.582	.337	1.000	-2.170	1.006
	Week 13	.920	.311	.717	-.542	2.382
	Week 14	10.810*	1.119	.000	5.547	16.073
	Week 15	-.938*	.160	.011	-1.690	-.186
Week 9	Week 4	-4.083*	.450	.000	-6.200	-1.966
	Week 5	-7.918*	.385	.000	-9.729	-6.107
	Week 8	-6.950*	.347	.000	-8.582	-5.317
	Week 10	-6.844*	.382	.000	-8.640	-5.048
	Week 11	-5.198*	.631	.001	-8.168	-2.228
	Week 12	-7.532*	.573	.000	-10.226	-4.837
	Week 13	-6.030*	.500	.000	-8.384	-3.675
	Week 14	3.860	1.100	.298	-1.314	9.035
	Week 15	-7.888*	.450	.000	-10.004	-5.771
Week 10	Week 4	2.761*	.385	.002	.950	4.572
	Week 5	-1.074	.252	.094	-2.258	.110
	Week 8	-.106	.241	1.000	-1.238	1.027
	Week 9	6.844*	.382	.000	5.048	8.640
	Week 11	1.646	.936	1.000	-2.758	6.050
	Week 12	-.688	.286	1.000	-2.032	.657
	Week 13	.814	.403	1.000	-1.080	2.709
	Week 14	10.704*	1.013	.000	5.937	15.472
	Week 15	-1.043	.371	.917	-2.791	.704
Week 11	Week 4	1.115	.864	1.000	-2.950	5.179

	Week 5	-2.720	.849	.485	-6.716	1.276
	Week 8	-1.752	.791	1.000	-5.476	1.973
	Week 9	5.198*	.631	.001	2.228	8.168
	Week 10	-1.646	.936	1.000	-6.050	2.758
	Week 12	-2.334	1.095	1.000	-7.485	2.817
	Week 13	-.832	.862	1.000	-4.890	3.226
	Week 14	9.058*	1.459	.007	2.194	15.923
	Week 15	-2.690	.788	.348	-6.400	1.021
Week 12	Week 4	3.449*	.508	.004	1.060	5.837
	Week 5	-.386	.378	1.000	-2.166	1.393
	Week 8	.582	.337	1.000	-1.006	2.170
	Week 9	7.532*	.573	.000	4.837	10.226
	Week 10	.688	.286	1.000	-.657	2.032
	Week 11	2.334	1.095	1.000	-2.817	7.485
	Week 13	1.502	.530	.881	-.991	3.995
	Week 14	11.392*	1.180	.000	5.838	16.946
	Week 15	-.356	.415	1.000	-2.308	1.596
Week 13	Week 4	1.947*	.412	.048	.010	3.883
	Week 5	-1.888*	.280	.004	-3.206	-.571
	Week 8	-.920	.311	.717	-2.382	.542
	Week 9	6.030*	.500	.000	3.675	8.384
	Week 10	-.814	.403	1.000	-2.709	1.080
	Week 11	.832	.862	1.000	-3.226	4.890
	Week 12	-1.502	.530	.881	-3.995	.991
	Week 14	9.890*	1.001	.000	5.179	14.601
	Week 15	-1.858*	.331	.015	-3.417	-.299
Week 14	Week 4	-7.944*	.930	.001	-12.320	-3.567
	Week 5	-11.778*	.955	.000	-16.273	-7.284
	Week 8	-10.810*	1.119	.000	-16.073	-5.547
	Week 9	-3.860	1.100	.298	-9.035	1.314
	Week 10	-10.704*	1.013	.000	-15.472	-5.937
	Week 11	-9.058*	1.459	.007	-15.923	-2.194
	Week 12	-11.392*	1.180	.000	-16.946	-5.838
	Week 13	-9.890*	1.001	.000	-14.601	-5.179
	Week 15	-11.748*	1.139	.000	-17.108	-6.388
Week 15	Week 4	3.804*	.325	.000	2.276	5.333
	Week 5	-.031	.231	1.000	-1.118	1.057
	Week 8	.938*	.160	.011	.186	1.690
	Week 9	7.888*	.450	.000	5.771	10.004
	Week 10	1.043	.371	.917	-.704	2.791
	Week 11	2.690	.788	.348	-1.021	6.400
	Week 12	.356	.415	1.000	-1.596	2.308
	Week 13	1.858*	.331	.015	.299	3.417
	Week 14	11.748*	1.139	.000	6.388	17.108

Total Nitrogen Removal Rate

Table B.35: Shapiro-Wilk's Test of Normality for total nitrogen removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 4	A	.716	6	.009
	NA	.990	6	.990
WEEK 5	A	.980	6	.950
	NA	.908	6	.423
WEEK 6	A	.995	6	.997
	NA	.735	6	.014
WEEK 7	A	.909	6	.433
	NA	.842	6	.135
WEEK 8	A	.828	6	.104
	NA	.659	6	.002
WEEK 9	A	.903	6	.392
	NA	.758	6	.024
WEEK 10	A	.900	6	.373
	NA	.869	6	.223
WEEK 11	A	.793	6	.050
	NA	.961	6	.830
WEEK 12	A	.942	6	.675
	NA	.799	6	.058
WEEK 13	A	.799	6	.058
	NA	.918	6	.493
WEEK 14	A	.962	6	.838
	NA	.973	6	.909
WEEK 15	A	.927	6	.557
	NA	.973	6	.909

Table B.36: Mauchly's Test of Sphericity for total nitrogen removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	142.438	54	.000

Table B.37: Levene's Test of Equality of Error Variances for total nitrogen removal rate data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.

Week 4	4.524	1	10	.059
Week 5	7.929	1	10	.018
Week 6	6.239	1	10	.032
Week 7	1.646	1	10	.228
Week 8	9.433	1	10	.012
Week 9	1.713	1	10	.220
Week 10	.072	1	10	.794
Week 11	10.989	1	10	.008
Week 12	16.345	1	10	.002
Week 13	47.406	1	10	.000
Week 15	15.513	1	10	.003

Table B.38: Pairwise Comparisons from repeated measures ANOVA for total nitrogen removal rate data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 4	Week 5	-1.869	1.948	1.000	-10.925	7.186
	Week 6	-4.741	2.160	1.000	-14.782	5.301
	Week 7	-8.142*	1.704	.041	-16.066	-.219
	Week 8	-8.232	1.969	.104	-17.388	.924
	Week 9	9.984*	1.545	.004	2.802	17.167
	Week 10	-1.913	1.871	1.000	-10.613	6.787
	Week 11	-10.845*	1.845	.009	-19.421	-2.269
	Week 12	-17.803*	1.547	.000	-24.993	-10.613
	Week 13	-8.984*	1.750	.024	-17.120	-.848
	Week 15	2.460	1.740	1.000	-5.630	10.550
Week 5	Week 4	1.869	1.948	1.000	-7.186	10.925
	Week 6	-2.872	1.070	1.000	-7.847	2.103
	Week 7	-6.273*	.625	.000	-9.177	-3.369
	Week 8	-6.363*	.911	.002	-10.596	-2.129
	Week 9	11.853*	1.114	.000	6.672	17.034
	Week 10	-.044	.973	1.000	-4.568	4.480
	Week 11	-8.976*	.825	.000	-12.812	-5.140
	Week 12	-15.934*	.817	.000	-19.731	-12.137
	Week 13	-7.115*	.604	.000	-9.925	-4.305
	Week 15	4.329*	.601	.002	1.536	7.122
Week 6	Week 4	4.741	2.160	1.000	-5.301	14.782
	Week 5	2.872	1.070	1.000	-2.103	7.847
	Week 7	-3.402	.732	.050	-6.806	.002
	Week 8	-3.491	1.343	1.000	-9.734	2.753

	Week 9	14.725*	1.341	.000	8.492	20.958
	Week 10	2.827	1.124	1.000	-2.397	8.052
	Week 11	-6.104	1.507	.127	-13.109	.901
	Week 12	-13.063*	1.603	.001	-20.517	-5.608
	Week 13	-4.243	1.354	.583	-10.537	2.050
	Week 15	7.201*	1.223	.008	1.514	12.888
Week 7	Week 4	8.142*	1.704	.041	.219	16.066
	Week 5	6.273*	.625	.000	3.369	9.177
	Week 6	3.402	.732	.050	-.002	6.806
	Week 8	-.089	.853	1.000	-4.056	3.878
	Week 9	18.127*	.795	.000	14.433	21.821
	Week 10	6.229*	.750	.000	2.744	9.714
	Week 11	-2.703	.938	.901	-7.065	1.660
	Week 12	-9.661*	.907	.000	-13.879	-5.442
	Week 13	-.842	.836	1.000	-4.726	3.043
	Week 15	10.602*	.807	.000	6.850	14.355
Week 8	Week 4	8.232	1.969	.104	-.924	17.388
	Week 5	6.363*	.911	.002	2.129	10.596
	Week 6	3.491	1.343	1.000	-2.753	9.734
	Week 7	.089	.853	1.000	-3.878	4.056
	Week 9	18.216*	.687	.000	15.022	21.410
	Week 10	6.318*	.744	.000	2.860	9.776
	Week 11	-2.613*	.463	.012	-4.766	-.461
	Week 12	-9.572*	.865	.000	-13.593	-5.550
	Week 13	-.752	.795	1.000	-4.448	2.943
	Week 15	10.692*	.824	.000	6.858	14.525
Week 9	Week 4	-9.984*	1.545	.004	-17.167	-2.802
	Week 5	-11.853*	1.114	.000	-17.034	-6.672
	Week 6	-14.725*	1.341	.000	-20.958	-8.492
	Week 6	-18.127*	.795	.000	-21.821	-14.433
	Week 7	-18.216*	.687	.000	-21.410	-15.022
	Week 10	-11.898*	.553	.000	-14.470	-9.325
	Week 11	-20.829*	.751	.000	-24.322	-17.337
	Week 12	-27.788*	.884	.000	-31.898	-23.677
	Week 13	-18.968*	1.016	.000	-23.691	-14.246
	Week 15	-7.524*	1.103	.003	-12.654	-2.395
Week 10	Week 4	1.913	1.871	1.000	-6.787	10.613
	Week 5	.044	.973	1.000	-4.480	4.568
	Week 6	-2.827	1.124	1.000	-8.052	2.397
	Week 7	-6.229*	.750	.000	-9.714	-2.744
	Week 8	-6.318*	.744	.000	-9.776	-2.860
	Week 9	11.898*	.553	.000	9.325	14.470
	Week 11	-8.932*	.757	.000	-12.452	-5.412
	Week 12	-15.890*	1.110	.000	-21.049	-10.731

	Week 13	-7.071*	1.121	.005	-12.283	-1.858
	Week 15	4.373	1.056	.110	-.535	9.282
Week 11	Week 4	10.845*	1.845	.009	2.269	19.421
	Week 5	8.976*	.825	.000	5.140	12.812
	Week 6	6.104	1.507	.127	-.901	13.109
	Week 7	2.703	.938	.901	-1.660	7.065
	Week 8	2.613*	.463	.012	.461	4.766
	Week 9	20.829*	.751	.000	17.337	24.322
	Week 10	8.932*	.757	.000	5.412	12.452
	Week 12	-6.958*	.704	.000	-10.233	-3.684
	Week 13	1.861	.776	1.000	-1.749	5.470
	Week 15	13.305*	.764	.000	9.752	16.858
Week 12	Week 4	17.803*	1.547	.000	10.613	24.993
	Week 5	15.934*	.817	.000	12.137	19.731
	Week 6	13.063*	1.603	.001	5.608	20.517
	Week 7	9.661*	.907	.000	5.442	13.879
	Week 8	9.572*	.865	.000	5.550	13.593
	Week 9	27.788*	.884	.000	23.677	31.898
	Week 10	15.890*	1.110	.000	10.731	21.049
	Week 11	6.958*	.704	.000	3.684	10.233
	Week 13	8.819*	.581	.000	6.118	11.520
	Week 15	20.263*	.825	.000	16.427	24.100
Week 13	Week 4	8.984*	1.750	.024	.848	17.120
	Week 5	7.115*	.604	.000	4.305	9.925
	Week 6	4.243	1.354	.583	-2.050	10.537
	Week 7	.842	.836	1.000	-3.043	4.726
	Week 8	.752	.795	1.000	-2.943	4.448
	Week 9	18.968*	1.016	.000	14.246	23.691
	Week 10	7.071*	1.121	.005	1.858	12.283
	Week 11	-1.861	.776	1.000	-5.470	1.749
	Week 12	-8.819*	.581	.000	-11.520	-6.118
	Week 15	11.444*	.532	.000	8.971	13.917
Week 15	Week 4	-2.460	1.740	1.000	-10.550	5.630
	Week 5	-4.329*	.601	.002	-7.122	-1.536
	Week 6	-7.201*	1.223	.008	-12.888	-1.514
	Week 7	-10.602*	.807	.000	-14.355	-6.850
	Week 8	-10.692*	.824	.000	-14.525	-6.858
	Week 9	7.524*	1.103	.003	2.395	12.654
	Week 10	-4.373	1.056	.110	-9.282	.535
	Week 11	-13.305*	.764	.000	-16.858	-9.752
	Week 12	-20.263*	.825	.000	-24.100	-16.427
	Week 13	-11.444*	.532	.000	-13.917	-8.971

Hydrological Parameters

Table B.39: Student's t-test p-values for porosity and evapotranspiration data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Porosity	Evapotranspiration
1	0.830433435	0.049347266*
2	0.368425963	0.888852631
3	0.079186715	0.152285912
4	0.002468184*	0.004716626*
5	0.001676238*	0.001297294*
6	0.01897459*	0.005216474
7	0.387631827	0.003226759*
8	0.027497843*	0.98293792
9	0.001323074*	0.106825078
10	0.04915325*	0.305090417
11	0.00123703*	0.015266234*
12	0.064919598	0.028764033*
13	0.028519234*	0.00266762*
14	0.046899886*	0.004051084*
15	0.01342342*	0.000264327*

Porosity

Table B.40: Shapiro-Wilk's Test of Normality for porosity data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.969	6	.887
	NA	.872	6	.233
WEEK 2	A	.904	6	.401
	NA	.915	6	.472
WEEK 3	A	.940	6	.659
	NA	.963	6	.845
WEEK 4	A	.817	6	.084
	NA	.845	6	.142
WEEK 5	A	.904	6	.399
	NA	.916	6	.477
WEEK 6	A	.944	6	.690
	NA	.901	6	.381
WEEK 7	A	.890	6	.317
	NA	.787	6	.045
WEEK 8	A	.884	6	.288
	NA	.867	6	.215

WEEK 9	A	.913	6	.459
	NA	.939	6	.651
WEEK 10	A	.933	6	.600
	NA	.915	6	.473
WEEK 11	A	.927	6	.555
	NA	.971	6	.897
WEEK 12	A	.908	6	.423
	NA	.882	6	.278
WEEK 13	A	.947	6	.715
	NA	.978	6	.943
WEEK 14	A	.988	6	.983
	NA	.943	6	.682
WEEK 15	A	.943	6	.687
	NA	.860	6	.191

Table B.41: Mauchly's Test of Sphericity for porosity data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	107.166	54	.000

Table B.42: Levene's Test of Equality of Error Variances for porosity data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.334	1	10	.576
Week 2	.479	1	10	.505
Week 3	.603	1	10	.455
Week 4	.039	1	10	.847
Week 5	.120	1	10	.736
Week 6	.537	1	10	.481
Week 7	.443	1	10	.521
Week 8	.291	1	10	.601
Week 9	2.431	1	10	.150
Week 10	1.895	1	10	.199
Week 11	.538	1	10	.480
Week 12	1.622	1	10	.232
Week 13	1.552	1	10	.241
Week 14	.742	1	10	.409
Week 15	3.408	1	10	.095

Table B.43: Pairwise Comparisons from repeated measures ANOVA for porosity data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.004	.002	1.000	-.007	.015
	Week 7	-.003	.002	1.000	-.012	.006
	Week 8	-.007	.002	.457	-.017	.003
	Week 9	-.010*	.002	.043	-.021	.000
	Week 10	-.012	.003	.108	-.025	.001
	Week 11	-.008	.003	.478	-.020	.004
	Week 12	-.004	.002	1.000	-.014	.007
	Week 13	-.001	.003	1.000	-.013	.011
	Week 14	-.001	.003	1.000	-.013	.010
	Week 15	-.001	.003	1.000	-.016	.014
Week 2	Week 1	-.004	.002	1.000	-.015	.007
	Week 7	-.007	.002	.369	-.017	.003
	Week 8	-.011*	.002	.018	-.020	-.001
	Week 9	-.014*	.001	.000	-.019	-.010
	Week 10	-.016*	.002	.000	-.024	-.008
	Week 11	-.012*	.002	.002	-.021	-.004
	Week 12	-.008	.002	.650	-.019	.004
	Week 13	-.005	.002	.854	-.013	.003
	Week 14	-.005	.002	.736	-.013	.003
	Week 15	-.005	.002	1.000	-.017	.006
Week 7	Week 1	.003	.002	1.000	-.006	.012
	Week 2	.007	.002	.369	-.003	.017
	Week 8	-.004	.002	1.000	-.014	.007
	Week 9	-.007	.002	.061	-.014	.000
	Week 10	-.008	.002	.112	-.018	.001
	Week 11	-.005	.002	1.000	-.015	.005
	Week 12	.000	.002	1.000	-.010	.010
	Week 13	.002	.002	1.000	-.006	.010
	Week 14	.002	.002	1.000	-.005	.010
	Week 15	.002	.002	1.000	-.009	.013
Week 8	Week 1	.007	.002	.457	-.003	.017
	Week 2	.011*	.002	.018	.001	.020
	Week 7	.004	.002	1.000	-.007	.014
	Week 9	-.003	.002	1.000	-.013	.006
	Week 10	-.005	.002	1.000	-.016	.006
	Week 11	-.001	.002	1.000	-.010	.007
	Week 12	.003	.002	1.000	-.007	.014
	Week 13	.006	.002	.975	-.004	.016

	Week 14	.006	.002	.184	-.001	.013
	Week 15	.006	.002	1.000	-.005	.016
Week 9	Week 1	.010*	.002	.043	.000	.021
	Week 2	.014*	.001	.000	.010	.019
	Week 7	.007	.002	.061	.000	.014
	Week 8	.003	.002	1.000	-.006	.013
	Week 10	-.001	.001	1.000	-.008	.005
	Week 11	.002	.002	1.000	-.006	.010
	Week 12	.007	.002	.471	-.003	.016
	Week 13	.009*	.002	.007	.002	.017
	Week 14	.009*	.001	.002	.003	.015
	Week 15	.009	.002	.053	-7.395E-5	.018
Week 10	Week 1	.012	.003	.108	-.001	.025
	Week 2	.016*	.002	.000	.008	.024
	Week 7	.008	.002	.112	-.001	.018
	Week 8	.005	.002	1.000	-.006	.016
	Week 9	.001	.001	1.000	-.005	.008
	Week 11	.004	.002	1.000	-.008	.015
	Week 12	.008	.002	.187	-.002	.018
	Week 13	.011*	.002	.011	.002	.020
	Week 14	.011*	.002	.008	.002	.019
	Week 15	.011*	.002	.045	.000	.021
Week 11	Week 1	.008	.003	.478	-.004	.020
	Week 2	.012*	.002	.002	.004	.021
	Week 7	.005	.002	1.000	-.005	.015
	Week 8	.001	.002	1.000	-.007	.010
	Week 9	-.002	.002	1.000	-.010	.006
	Week 10	-.004	.002	1.000	-.015	.008
	Week 12	.005	.002	1.000	-.006	.015
	Week 13	.007*	.001	.025	.001	.014
	Week 14	.007	.002	.128	-.001	.015
	Week 15	.007	.003	1.000	-.005	.019
Week 12	Week 1	.004	.002	1.000	-.007	.014
	Week 2	.008	.002	.650	-.004	.019
	Week 7	.000	.002	1.000	-.010	.010
	Week 8	-.003	.002	1.000	-.014	.007
	Week 9	-.007	.002	.471	-.016	.003
	Week 10	-.008	.002	.187	-.018	.002
	Week 11	-.005	.002	1.000	-.015	.006
	Week 13	.003	.002	1.000	-.005	.011
	Week 14	.002	.002	1.000	-.007	.012
	Week 15	.003	.002	1.000	-.008	.013
Week 13	Week 1	.001	.003	1.000	-.011	.013
	Week 2	.005	.002	.854	-.003	.013

	Week 7	-.002	.002	1.000	-.010	.006
	Week 8	-.006	.002	.975	-.016	.004
	Week 9	-.009*	.002	.007	-.017	-.002
	Week 10	-.011*	.002	.011	-.020	-.002
	Week 11	-.007*	.001	.025	-.014	-.001
	Week 12	-.003	.002	1.000	-.011	.005
	Week 14	.000	.001	1.000	-.007	.006
	Week 15	.000	.002	1.000	-.009	.009
Week 14	Week 1	.001	.003	1.000	-.010	.013
	Week 2	.005	.002	.736	-.003	.013
	Week 7	-.002	.002	1.000	-.010	.005
	Week 8	-.006	.002	.184	-.013	.001
	Week 9	-.009*	.001	.002	-.015	-.003
	Week 10	-.011*	.002	.008	-.019	-.002
	Week 11	-.007	.002	.128	-.015	.001
	Week 12	-.002	.002	1.000	-.012	.007
	Week 13	.000	.001	1.000	-.006	.007
	Week 15	3.333E-5	.001	1.000	-.005	.005
Week 15	Week 1	.001	.003	1.000	-.014	.016
	Week 2	.005	.002	1.000	-.006	.017
	Week 7	-.002	.002	1.000	-.013	.009
	Week 8	-.006	.002	1.000	-.016	.005
	Week 9	-.009	.002	.053	-.018	7.395E-5
	Week 10	-.011*	.002	.045	-.021	.000
	Week 11	-.007	.003	1.000	-.019	.005
	Week 12	-.003	.002	1.000	-.013	.008
	Week 13	.000	.002	1.000	-.009	.009
	Week 14	-3.333E-5	.001	1.000	-.005	.005

Evapotranspiration

Table B.44: Shapiro-Wilk's Test of Normality for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 1	A	.947	6	.712
	NA	.958	6	.807
WEEK 2	A	.976	6	.927
	NA	.898	6	.360
WEEK 3	A	.981	6	.957
	NA	.845	6	.143
WEEK 4	A	.924	6	.538
	NA	.876	6	.251
WEEK 5	A	.965	6	.858

	NA	.949	6	.735
WEEK 6	A	.901	6	.379
	NA	.854	6	.169
WEEK 7	A	.988	6	.985
	NA	.863	6	.199
WEEK 8	A	.917	6	.486
	NA	.784	6	.042
WEEK 9	A	.898	6	.362
	NA	.908	6	.420
WEEK 10	A	.971	6	.902
	NA	.826	6	.100
WEEK 11	A	.859	6	.186
	NA	.991	6	.991
WEEK 12	A	.941	6	.667
	NA	.833	6	.114
WEEK 13	A	.884	6	.288
	NA	.902	6	.388
WEEK 14	A	.962	6	.832
	NA	.966	6	.866
WEEK 15	A	.969	6	.886
	NA	.942	6	.678

Table B.45: Mauchly's Test of Sphericity for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	120.729	54	.000

Table B.46: Levene's Test of Equality of Error Variances for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	1.281	1	10	.284
Week 2	.002	1	10	.969
Week 3	.012	1	10	.915
Week 4	.788	1	10	.395
Week 5	.372	1	10	.555
Week 6	.224	1	10	.646
Week 7	.606	1	10	.454
Week 8	2.126	1	10	.175
Week 9	2.824	1	10	.124

Week 10	4.485	1	10	.060
Week 11	4.507	1	10	.060
Week 12	2.704	1	10	.131
Week 13	3.365	1	10	.096
Week 14	3.135	1	10	.107
Week 15	.716	1	10	.417

Table B.47: Pairwise Comparisons from repeated measures ANOVA for evapotranspiration data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	-.004	.006	1.000	-.030	.022
	Week 7	.022	.007	.472	-.009	.054
	Week 8	.005	.010	1.000	-.042	.051
	Week 9	-.061	.015	.125	-.130	.009
	Week 10	-.025	.010	1.000	-.070	.020
	Week 11	-.120*	.014	.000	-.184	-.056
	Week 12	-.140*	.020	.002	-.232	-.047
	Week 13	-.085*	.012	.002	-.143	-.028
	Week 14	-.058*	.010	.010	-.105	-.011
	Week 15	-.186*	.017	.000	-.263	-.108
Week 2	Week 1	.004	.006	1.000	-.022	.030
	Week 7	.026	.010	1.000	-.022	.075
	Week 8	.009	.013	1.000	-.050	.067
	Week 9	-.057	.016	.250	-.129	.016
	Week 10	-.021	.012	1.000	-.076	.034
	Week 11	-.116*	.015	.001	-.187	-.045
	Week 12	-.136*	.020	.002	-.227	-.044
	Week 13	-.082*	.013	.006	-.143	-.020
	Week 14	-.054*	.011	.029	-.105	-.004
	Week 15	-.182*	.017	.000	-.262	-.102
Week 7	Week 1	-.022	.007	.472	-.054	.009
	Week 2	-.026	.010	1.000	-.075	.022
	Week 8	-.018	.006	.549	-.043	.008
	Week 9	-.083*	.012	.003	-.140	-.026
	Week 10	-.047*	.008	.011	-.085	-.009
	Week 11	-.142*	.010	.000	-.190	-.094
	Week 12	-.162*	.018	.000	-.246	-.078
	Week 13	-.108*	.010	.000	-.154	-.062
	Week 14	-.081*	.009	.000	-.122	-.040
	Week 15	-.208*	.016	.000	-.281	-.135

Week 8	Week 1	-.005	.010	1.000	-.051	.042
	Week 2	-.009	.013	1.000	-.067	.050
	Week 7	.018	.006	.549	-.008	.043
	Week 9	-.065*	.012	.012	-.119	-.012
	Week 10	-.029	.007	.068	-.060	.001
	Week 11	-.125*	.009	.000	-.168	-.082
	Week 12	-.144*	.017	.000	-.224	-.064
	Week 13	-.090*	.010	.000	-.138	-.042
	Week 14	-.063*	.009	.002	-.105	-.021
	Week 15	-.190*	.018	.000	-.276	-.105
Week 9	Week 1	.061	.015	.125	-.009	.130
	Week 2	.057	.016	.250	-.016	.129
	Week 7	.083*	.012	.003	.026	.140
	Week 8	.065*	.012	.012	.012	.119
	Week 10	.036	.010	.230	-.009	.081
	Week 11	-.059*	.005	.000	-.081	-.037
	Week 12	-.079*	.007	.000	-.112	-.046
	Week 13	-.025	.006	.065	-.050	.001
	Week 14	.002	.006	1.000	-.026	.031
	Week 15	-.125*	.014	.000	-.190	-.060
Week 10	Week 1	.025	.010	1.000	-.020	.070
	Week 2	.021	.012	1.000	-.034	.076
	Week 7	.047*	.008	.011	.009	.085
	Week 8	.029	.007	.068	-.001	.060
	Week 9	-.036	.010	.230	-.081	.009
	Week 11	-.095*	.007	.000	-.128	-.062
	Week 12	-.115*	.014	.001	-.181	-.049
	Week 13	-.061*	.009	.003	-.103	-.019
	Week 14	-.034*	.007	.045	-.067	.000
	Week 15	-.161*	.018	.000	-.244	-.078
Week 11	Week 1	.120*	.014	.000	.056	.184
	Week 2	.116*	.015	.001	.045	.187
	Week 7	.142*	.010	.000	.094	.190
	Week 8	.125*	.009	.000	.082	.168
	Week 9	.059*	.005	.000	.037	.081
	Week 10	.095*	.007	.000	.062	.128
	Week 12	-.020	.009	1.000	-.061	.022
	Week 13	.034*	.006	.006	.009	.060
	Week 14	.062*	.006	.000	.033	.090
	Week 15	-.066	.015	.067	-.134	.003
Week 12	Week 1	.140*	.020	.002	.047	.232
	Week 2	.136*	.020	.002	.044	.227
	Week 7	.162*	.018	.000	.078	.246
	Week 8	.144*	.017	.000	.064	.224

	Week 9	.079*	.007	.000	.046	.112
	Week 10	.115*	.014	.001	.049	.181
	Week 11	.020	.009	1.000	-.022	.061
	Week 13	.054*	.010	.018	.007	.101
	Week 14	.081*	.011	.001	.030	.132
	Week 15	-.046	.017	1.000	-.126	.034
Week 13	Week 1	.085*	.012	.002	.028	.143
	Week 2	.082*	.013	.006	.020	.143
	Week 7	.108*	.010	.000	.062	.154
	Week 8	.090*	.010	.000	.042	.138
	Week 9	.025	.006	.065	-.001	.050
	Week 10	.061*	.009	.003	.019	.103
	Week 11	-.034*	.006	.006	-.060	-.009
	Week 12	-.054*	.010	.018	-.101	-.007
	Week 14	.027*	.003	.000	.012	.042
	Week 15	-.100*	.012	.000	-.155	-.046
Week 14	Week 1	.058*	.010	.010	.011	.105
	Week 2	.054*	.011	.029	.004	.105
	Week 7	.081*	.009	.000	.040	.122
	Week 8	.063*	.009	.002	.021	.105
	Week 9	-.002	.006	1.000	-.031	.026
	Week 10	.034*	.007	.045	.000	.067
	Week 11	-.062*	.006	.000	-.090	-.033
	Week 12	-.081*	.011	.001	-.132	-.030
	Week 13	-.027*	.003	.000	-.042	-.012
	Week 15	-.127*	.013	.000	-.188	-.067
Week 15	Week 1	.186*	.017	.000	.108	.263
	Week 2	.182*	.017	.000	.102	.262
	Week 7	.208*	.016	.000	.135	.281
	Week 8	.190*	.018	.000	.105	.276
	Week 9	.125*	.014	.000	.060	.190
	Week 10	.161*	.018	.000	.078	.244
	Week 11	.066	.015	.067	-.003	.134
	Week 12	.046	.017	1.000	-.034	.126
	Week 13	.100*	.012	.000	.046	.155
	Week 14	.127*	.013	.000	.067	.188

Plant Growth

Table B.48: Student's t-test p-values for plant growth data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

Week	Plant Stems	Plant Height
4	0.067283526	0.192485491
5	0.070779142	0.019336508*
6	0.092485703	0.539243582
7	0.00595946*	0.004240571*
8	0.013280801*	7.18136E-06*
9	0.010629023*	1.7295E-05*
10	0.018073997*	0.000437283*
11	0.012339405*	9.62498E-05*
12	0.000725815*	0.00080405*
13		
14	0.001339643*	8.1152E-06*
15	0.000234926*	0.000184969*

Plant Stems

Table B.49: Shapiro-Wilk's Test of Normality for plant stem data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 4	A	.922	6	.522
	NA	.967	6	.869
Week 5	A	.963	6	.843
	NA	.965	6	.861
Week 6	A	.908	6	.424
	NA	.829	6	.105
Week 7	A	.892	6	.331
	NA	.983	6	.966
Week 8	A	.930	6	.578
	NA	.918	6	.489
Week 9	A	.958	6	.801
	NA	.934	6	.615
Week 10	A	.945	6	.702
	NA	.867	6	.216
Week 11	A	.886	6	.296
	NA	.939	6	.655
Week 12	A	.814	6	.078
	NA	.883	6	.281
Week 14	A	.922	6	.520

	NA	.890	6	.316
Week 15	A	.919	6	.500
	NA	.756	6	.023

Table B.50: Mauchly's Test of Sphericity for plant stem data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	112.167	54	.000

Table B.51: Levene's Test of Equality of Error Variances for plant stem data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 4	1.263	1	10	.287
Week 5	4.820	1	10	.053
Week 6	1.388	1	10	.266
Week 7	4.072	1	10	.071
Week 8	3.296	1	10	.100
Week 9	7.670	1	10	.020
Week 10	.238	1	10	.636
Week 11	4.714	1	10	.055
Week 12	.283	1	10	.607
Week 14	.504	1	10	.494
Week 15	.242	1	10	.633

Table B.52: Pairwise Comparisons from repeated measures ANOVA for plant stem data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 4	Week 5	-20.500*	1.981	.000	-29.711	-11.289
	Week 6	-26.500*	3.097	.000	-40.897	-12.103
	Week 7	-21.167*	1.479	.000	-28.041	-14.293
	Week 8	-15.917*	1.690	.000	-23.775	-8.058
	Week 9	-17.667*	1.706	.000	-25.599	-9.734
	Week 10	-8.750*	1.580	.014	-16.095	-1.405
	Week 11	-15.333*	1.539	.000	-22.490	-8.177
	Week 12	-22.667*	2.415	.000	-33.893	-11.440

	Week 14	-20.667*	1.491	.000	-27.597	-13.736
	Week 15	-20.417*	1.835	.000	-28.949	-11.884
Week 5	Week 4	20.500*	1.981	.000	11.289	29.711
	Week 6	-6.000	3.341	1.000	-21.534	9.534
	Week 7	-.667	2.312	1.000	-11.415	10.081
	Week 8	4.583	2.226	1.000	-5.768	14.934
	Week 9	2.833	2.328	1.000	-7.990	13.657
	Week 10	11.750	2.626	.065	-.459	23.959
	Week 11	5.167	2.246	1.000	-5.275	15.609
	Week 12	-2.167	3.982	1.000	-20.680	16.346
	Week 14	-.167	2.935	1.000	-13.812	13.479
	Week 15	.083	3.438	1.000	-15.900	16.066
Week 6	Week 4	26.500*	3.097	.000	12.103	40.897
	Week 5	6.000	3.341	1.000	-9.534	21.534
	Week 7	5.333	2.583	1.000	-6.673	17.340
	Week 8	10.583*	2.051	.023	1.047	20.119
	Week 7	8.833	2.344	.202	-2.065	19.731
	Week 10	17.750*	2.605	.003	5.640	29.860
	Week 11	11.167*	2.381	.047	.097	22.237
	Week 12	3.833	3.455	1.000	-12.229	19.896
	Week 14	5.833	3.494	1.000	-10.413	22.080
	Week 15	6.083	3.597	1.000	-10.641	22.808
Week 7	Week 4	21.167*	1.479	.000	14.293	28.041
	Week 5	.667	2.312	1.000	-10.081	11.415
	Week 6	-5.333	2.583	1.000	-17.340	6.673
	Week 8	5.250	1.436	.243	-1.427	11.927
	Week 9	3.500	1.661	1.000	-4.222	11.222
	Week 10	12.417*	1.690	.001	4.558	20.275
	Week 11	5.833*	.778	.001	2.215	9.451
	Week 12	-1.500	2.570	1.000	-13.449	10.449
	Week 14	.500	1.970	1.000	-8.659	9.659
	Week 15	.750	2.281	1.000	-9.853	11.353
Week 8	Week 4	15.917*	1.690	.000	8.058	23.775
	Week 5	-4.583	2.226	1.000	-14.934	5.768
	Week 6	-10.583*	2.051	.023	-20.119	-1.047
	Week 6	-5.250	1.436	.243	-11.927	1.427
	Week 9	-1.750	1.023	1.000	-6.505	3.005
	Week 10	7.167	1.555	.053	-.065	14.398
	Week 11	.583	1.057	1.000	-4.333	5.499
	Week 12	-6.750	2.724	1.000	-19.413	5.913
	Week 14	-4.750	2.306	1.000	-15.472	5.972
	Week 15	-4.500	2.614	1.000	-16.651	7.651
Week 9	Week 4	17.667*	1.706	.000	9.734	25.599
	Week 5	-2.833	2.328	1.000	-13.657	7.990

	Week 6	-8.833	2.344	.202	-19.731	2.065
	Week 7	-3.500	1.661	1.000	-11.222	4.222
	Week 8	1.750	1.023	1.000	-3.005	6.505
	Week 10	8.917*	1.535	.009	1.779	16.054
	Week 11	2.333	1.182	1.000	-3.162	7.829
	Week 12	-5.000	2.565	1.000	-16.927	6.927
	Week 14	-3.000	1.877	1.000	-11.726	5.726
	Week 15	-2.750	2.184	1.000	-12.902	7.402
Week 10	Week 4	8.750*	1.580	.014	1.405	16.095
	Week 5	-11.750	2.626	.065	-23.959	.459
	Week 6	-17.750*	2.605	.003	-29.860	-5.640
	Week 7	-12.417*	1.690	.001	-20.275	-4.558
	Week 8	-7.167	1.555	.053	-14.398	.065
	Week 9	-8.917*	1.535	.009	-16.054	-1.779
	Week 11	-6.583*	1.228	.017	-12.291	-.876
	Week 12	-13.917*	2.066	.003	-23.522	-4.312
	Week 14	-11.917*	1.962	.007	-21.041	-2.793
	Week 15	-11.667*	2.222	.021	-21.999	-1.334
Week 11	Week 4	15.333*	1.539	.000	8.177	22.490
	Week 5	-5.167	2.246	1.000	-15.609	5.275
	Week 6	-11.167*	2.381	.047	-22.237	-.097
	Week 7	-5.833*	.778	.001	-9.451	-2.215
	Week 8	-.583	1.057	1.000	-5.499	4.333
	Week 9	-2.333	1.182	1.000	-7.829	3.162
	Week 10	6.583*	1.228	.017	.876	12.291
	Week 12	-7.333	2.529	.871	-19.090	4.423
	Week 14	-5.333	1.916	1.000	-14.239	3.573
	Week 15	-5.083	2.219	1.000	-15.400	5.233
Week 12	Week 4	22.667*	2.415	.000	11.440	33.893
	Week 5	2.167	3.982	1.000	-16.346	20.680
	Week 6	-3.833	3.455	1.000	-19.896	12.229
	Week 7	1.500	2.570	1.000	-10.449	13.449
	Week 8	6.750	2.724	1.000	-5.913	19.413
	Week 9	5.000	2.565	1.000	-6.927	16.927
	Week 10	13.917*	2.066	.003	4.312	23.522
	Week11	7.333	2.529	.871	-4.423	19.090
	Week 14	2.000	2.023	1.000	-7.404	11.404
	Week 15	2.250	2.140	1.000	-7.699	12.199
Week 14	Week 4	20.667*	1.491	.000	13.736	27.597
	Week 5	.167	2.935	1.000	-13.479	13.812
	Week 6	-5.833	3.494	1.000	-22.080	10.413
	Week 7	-.500	1.970	1.000	-9.659	8.659
	Week 8	4.750	2.306	1.000	-5.972	15.472
	Week 9	3.000	1.877	1.000	-5.726	11.726

	Week 10	11.917*	1.962	.007	2.793	21.041
	Week 11	5.333	1.916	1.000	-3.573	14.239
	Week 12	-2.000	2.023	1.000	-11.404	7.404
	Week 15	.250	.761	1.000	-3.288	3.788
Week 15	Week 4	20.417*	1.835	.000	11.884	28.949
	Week 5	-.083	3.438	1.000	-16.066	15.900
	Week 6	-6.083	3.597	1.000	-22.808	10.641
	Week 7	-.750	2.281	1.000	-11.353	9.853
	Week 8	4.500	2.614	1.000	-7.651	16.651
	Week 9	2.750	2.184	1.000	-7.402	12.902
	Week 10	11.667*	2.222	.021	1.334	21.999
	Week 11	5.083	2.219	1.000	-5.233	15.400
	Week 12	-2.250	2.140	1.000	-12.199	7.699
	Week 14	-.250	.761	1.000	-3.788	3.288

Plant Height

Table B.53: Shapiro-Wilk's Test of Normality for plant height data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
WEEK 4	A	.775	6	.035
	NA	.908	6	.421
WEEK 5	A	.908	6	.425
	NA	.866	6	.212
WEEK 6	A	.921	6	.514
	NA	.780	6	.039
WEEK 7	A	.838	6	.126
	NA	.957	6	.799
WEEK 8	A	.907	6	.414
	NA	.866	6	.212
WEEK 9	A	.819	6	.086
	NA	.906	6	.408
WEEK 10	A	.831	6	.109
	NA	.922	6	.522
WEEK 11	A	.918	6	.489
	NA	.898	6	.361
WEEK 12	A	.960	6	.821
	NA	.930	6	.583
WEEK 14	A	.975	6	.924
	NA	.963	6	.841
WEEK 15	A	.935	6	.620
	NA	.910	6	.434

Table B.54: Mauchly's Test of Sphericity plant height data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	130.236	54	.000

Table B.55: Levene's Test of Equality of Error Variances plant height data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 4	2.961	1	10	.116
Week 5	.000	1	10	1.000
Week 6	.013	1	10	.912
Week 7	.223	1	10	.647
Week 8	.135	1	10	.721
Week 9	.001	1	10	.975
Week 10	7.409	1	10	.021
Week 11	3.755	1	10	.081
Week 12	1.999	1	10	.188
Week 14	.416	1	10	.534
Week 15	1.200	1	10	.299

Table B.56: Pairwise Comparisons from repeated measures ANOVA for plant height data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 4	Week 5	-3.917*	.305	.000	-5.335	-2.498
	Week 6	-8.875*	.910	.000	-13.106	-4.644
	Week 7	-16.500*	1.666	.000	-24.248	-8.752
	Week 8	-20.167*	1.163	.000	-25.574	-14.759
	Week 9	-23.792*	1.446	.000	-30.516	-17.067
	Week 10	-22.792*	1.249	.000	-28.597	-16.987
	Week 11	-26.250*	1.310	.000	-32.342	-20.158
	Week 12	-27.075*	1.764	.000	-35.276	-18.874
	Week 14	-35.958*	1.216	.000	-41.613	-30.303
	Week 15	-36.958*	1.521	.000	-44.031	-29.886
Week 5	Week 4	3.917*	.305	.000	2.498	5.335
	Week 6	-4.958*	.809	.006	-8.720	-1.197

	Week 7	-12.583*	1.509	.000	-19.601	-5.565
	Week 8	-16.250*	1.011	.000	-20.951	-11.549
	Week 9	-19.875*	1.346	.000	-26.132	-13.618
	Week 10	-18.875*	1.277	.000	-24.811	-12.939
	Week 11	-22.333*	1.381	.000	-28.755	-15.912
	Week 12	-23.158*	1.719	.000	-31.149	-15.167
	Week 14	-32.042*	1.353	.000	-38.330	-25.753
	Week 15	-33.042*	1.570	.000	-40.340	-25.743
Week 6	Week 4	8.875*	.910	.000	4.644	13.106
	Week 5	4.958*	.809	.006	1.197	8.720
	Week 7	-7.625*	.877	.000	-11.704	-3.546
	Week 8	-11.292*	.582	.000	-14.000	-8.584
	Week 7	-14.917*	1.210	.000	-20.542	-9.291
	Week 10	-13.917*	1.078	.000	-18.929	-8.905
	Week 11	-17.375*	1.248	.000	-23.176	-11.574
	Week 12	-18.200*	1.635	.000	-25.803	-10.597
	Week 14	-27.083*	1.723	.000	-35.093	-19.074
	Week 15	-28.083*	1.886	.000	-36.852	-19.315
Week 7	Week 4	16.500*	1.666	.000	8.752	24.248
	Week 5	12.583*	1.509	.000	5.565	19.601
	Week 6	7.625*	.877	.000	3.546	11.704
	Week 8	-3.667*	.722	.026	-7.024	-.309
	Week 9	-7.292*	1.206	.007	-12.899	-1.685
	Week 10	-6.292	1.637	.178	-13.900	1.317
	Week 11	-9.750*	1.839	.019	-18.302	-1.198
	Week 12	-10.575*	2.039	.022	-20.053	-1.097
	Week 14	-19.458*	2.414	.001	-30.680	-8.237
	Week 15	-20.458*	2.489	.001	-32.033	-8.884
Week 8	Week 4	20.167*	1.163	.000	14.759	25.574
	Week 5	16.250*	1.011	.000	11.549	20.951
	Week 6	11.292*	.582	.000	8.584	14.000
	Week 6	3.667*	.722	.026	.309	7.024
	Week 9	-3.625	.842	.085	-7.539	.289
	Week 10	-2.625	1.590	1.000	-10.019	4.769
	Week 11	-6.083	1.576	.174	-13.410	1.243
	Week 12	-6.908	1.855	.217	-15.532	1.715
	Week 14	-15.792*	1.850	.000	-24.393	-7.190
	Week 15	-16.792*	2.213	.001	-27.081	-6.503
Week 9	Week 4	23.792*	1.446	.000	17.067	30.516
	Week 5	19.875*	1.346	.000	13.618	26.132
	Week 6	14.917*	1.210	.000	9.291	20.542
	Week 7	7.292*	1.206	.007	1.685	12.899
	Week 8	3.625	.842	.085	-.289	7.539
	Week 10	1.000	2.019	1.000	-8.387	10.387

	Week 11	-2.458	1.903	1.000	-11.308	6.391
	Week 12	-3.283	2.588	1.000	-15.315	8.749
	Week 14	-12.167*	2.111	.010	-21.980	-2.353
	Week 15	-13.167*	2.477	.019	-24.683	-1.650
Week 10	Week 4	22.792*	1.249	.000	16.987	28.597
	Week 5	18.875*	1.277	.000	12.939	24.811
	Week 6	13.917*	1.078	.000	8.905	18.929
	Week 7	6.292	1.637	.178	-1.317	13.900
	Week 8	2.625	1.590	1.000	-4.769	10.019
	Week 9	-1.000	2.019	1.000	-10.387	8.387
	Week 11	-3.458	.968	.279	-7.958	1.041
	Week 12	-4.283	1.806	1.000	-12.678	4.111
	Week 14	-13.167*	1.902	.002	-22.011	-4.322
	Week 15	-14.167*	1.275	.000	-20.094	-8.240
Week 11	Week 4	26.250*	1.310	.000	20.158	32.342
	Week 5	22.333*	1.381	.000	15.912	28.755
	Week 6	17.375*	1.248	.000	11.574	23.176
	Week 7	9.750*	1.839	.019	1.198	18.302
	Week 8	6.083	1.576	.174	-1.243	13.410
	Week 9	2.458	1.903	1.000	-6.391	11.308
	Week 10	3.458	.968	.279	-1.041	7.958
	Week 11	-.825	1.944	1.000	-9.865	8.215
	Week 14	-9.708*	1.513	.004	-16.741	-2.675
	Week 15	-10.708*	1.178	.000	-16.185	-5.232
Week 12	Week 4	27.075*	1.764	.000	18.874	35.276
	Week 5	23.158*	1.719	.000	15.167	31.149
	Week 6	18.200*	1.635	.000	10.597	25.803
	Week 7	10.575*	2.039	.022	1.097	20.053
	Week 8	6.908	1.855	.217	-1.715	15.532
	Week 9	3.283	2.588	1.000	-8.749	15.315
	Week 10	4.283	1.806	1.000	-4.111	12.678
	Week11	.825	1.944	1.000	-8.215	9.865
	Week 14	-8.883*	1.758	.027	-17.059	-.708
	Week 15	-9.883	2.159	.056	-19.920	.153
Week 14	Week 4	35.958*	1.216	.000	30.303	41.613
	Week 5	32.042*	1.353	.000	25.753	38.330
	Week 6	27.083*	1.723	.000	19.074	35.093
	Week 7	19.458*	2.414	.001	8.237	30.680
	Week 8	15.792*	1.850	.000	7.190	24.393
	Week 9	12.167*	2.111	.010	2.353	21.980
	Week 10	13.167*	1.902	.002	4.322	22.011
	Week 11	9.708*	1.513	.004	2.675	16.741
	Week 12	8.883*	1.758	.027	.708	17.059
	Week 15	-1.000	1.717	1.000	-8.982	6.982

Week 15	Week 4	36.958*	1.521	.000	29.886	44.031
	Week 5	33.042*	1.570	.000	25.743	40.340
	Week 6	28.083*	1.886	.000	19.315	36.852
	Week 7	20.458*	2.489	.001	8.884	32.033
	Week 8	16.792*	2.213	.001	6.503	27.081
	Week 9	13.167*	2.477	.019	1.650	24.683
	Week 10	14.167*	1.275	.000	8.240	20.094
	Week 11	10.708*	1.178	.000	5.232	16.185
	Week 12	9.883	2.159	.056	-.153	19.920
	Week 14	1.000	1.717	1.000	-6.982	8.982

Microbial Community Analysis

Table B.57: Student's t-test p-values for microbial community data between aerated and non-aerated mesocosm system replicates. Significant weeks for each parameter are marked with an asterisk (*), $\alpha = 0.05$. Empty cells represent weeks where data was not collected or analyzed.

			Carbon Source Guilds						
Week	AWCD	Richness	Carbohydrates	Polymers	Carboxylic and Acetic Acids	Amino Acids	Amines and Amides	Root Exudates	FDA
1	0.0496309 66*	0.0169417 17*	2.4569E-05*	0.0049350 4*	0.7651972 16	0.5935223 05	0.3515009 17	0.8154291 67	0.0047321 16*
2	0.1908310 62	0.3526089 75	0.0015172 16*	0.7112271 86	0.5390222 86	0.0896417 36	0.0174457 74*	0.2045894 62	0.0011095 99*
3	0.1280948 55	0.8398334 64	0.2387908 85	0.4808191 85	0.0024733 89*	0.1194387 31	0.0039581 18*	0.0424796 04*	0.0027498 17*
6	0.0409714 14*	0.0250886 64*	0.0026136 38*	0.0132940 93*	0.0742197 03*	0.5099187 41	0.0148491 96*	0.3847792 45	0.0214216 85*
									0.0033063 35*
9	0.0967780 58	0.0313754 98*	0.0792664 54	0.3370606 35	0.5647237 61	0.2933567 47	0.6661038 76	0.1011813 19	
10									0.0041905 59*
11	0.4857660 79	0.0514491 39	0.0483851 8*	0.4103609 18	0.2737456 32	0.6184647 31	0.2392049 94	0.9368906 66	0.6746043 21
13	0.1510808 43	0.0081948 49*	0.0102613 48*	0.2154621 02	0.0518096 8	0.9121908 35	0.0906274 92	0.1359200 87	1.02949E-05*
15	0.3115672 02	0.0432632 9*	0.0235892 56*	0.0714064 35	0.5136654 31	0.2123208 47	0.0263034 27*	0.7763417 92	0.0008010 42*

Average Well Colour Development

Table B.58: Shapiro-Wilk's Test of Normality for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week1	A	.895	6	.344
	NA	.962	6	.838
Week 2	A	.953	6	.762
	NA	.981	6	.957
Week 3	A	.851	6	.160
	NA	.703	6	.007
Week 6	A	.872	6	.235
	NA	.855	6	.173
Week 7	A	.912	6	.447
	NA	.897	6	.354
Week 9	A	.986	6	.978
	NA	.912	6	.448
Week 11	A	.881	6	.273
	NA	.997	6	.999
Week 13	A	.762	6	.026
	NA	.925	6	.543
Week 15	A	.971	6	.898
	NA	.915	6	.471

Table B.59: Mauchly's Test of Sphericity for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.010	33.117	35	.672

Table B.60: Levene's Test of Equality of Error Variances for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.000	1	10	1.000
Week 2	.406	1	10	.538
Week 3	6.997	1	10	.025
Week 6	10.383	1	10	.009
Week 7	8.825	1	10	.014
Week 9	1.343	1	10	.273

Week 11	.093	1	10	.767
Week 13	.657	1	10	.437
Week 15	.110	1	10	.747

Table B.61: Pairwise Comparisons from repeated measures ANOVA for average well colour development data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.071	.067	1.000	-.223	.364
	Week 3	-.354*	.070	.018	-.660	-.048
	Week 6	-.181	.070	.963	-.486	.124
	Week 7	.081	.069	1.000	-.219	.381
	Week 9	-.040	.069	1.000	-.340	.260
	Week 11	.012	.082	1.000	-.345	.370
	Week 13	.024	.093	1.000	-.383	.431
	Week 15	.028	.069	1.000	-.273	.329
Week 2	Week 1	-.071	.067	1.000	-.364	.223
	Week 3	-.425*	.065	.002	-.710	-.139
	Week 6	-.252	.070	.170	-.556	.053
	Week 7	.011	.063	1.000	-.264	.286
	Week 9	-.111	.061	1.000	-.376	.155
	Week 11	-.058	.078	1.000	-.400	.284
	Week 13	-.046	.074	1.000	-.369	.276
	Week 15	-.042	.060	1.000	-.305	.221
Week 3	Week 1	.354*	.070	.018	.048	.660
	Week 2	.425*	.065	.002	.139	.710
	Week 6	.173	.077	1.000	-.164	.510
	Week 7	.435*	.070	.004	.129	.741
	Week 9	.314*	.070	.040	.010	.618
	Week 11	.366*	.068	.011	.067	.666
	Week 13	.378*	.085	.043	.008	.749
	Week 15	.382*	.065	.006	.097	.668
Week 6	Week 1	.181	.070	.963	-.124	.486
	Week 2	.252	.070	.170	-.053	.556
	Week 3	-.173	.077	1.000	-.510	.164
	Week 7	.262*	.042	.003	.081	.444
	Week 9	.141*	.028	.020	.017	.265
	Week 11	.193	.048	.081	-.015	.401
	Week 13	.205	.053	.107	-.025	.436
	Week 15	.209*	.047	.042	.005	.413

Week 7	Week 1	-.081	.069	1.000	-.381	.219
	Week 2	-.011	.063	1.000	-.286	.264
	Week 3	-.435*	.070	.004	-.741	-.129
	Week 6	-.262*	.042	.003	-.444	-.081
	Week 9	-.121	.036	.267	-.280	.037
	Week 11	-.069	.054	1.000	-.305	.167
	Week 13	-.057	.058	1.000	-.312	.198
	Week 15	-.053	.046	1.000	-.252	.147
Week 9	Week 1	.040	.069	1.000	-.260	.340
	Week 2	.111	.061	1.000	-.155	.376
	Week 3	-.314*	.070	.040	-.618	-.010
	Week 6	-.141*	.028	.020	-.265	-.017
	Week 7	.121	.036	.267	-.037	.280
	Week 11	.052	.037	1.000	-.111	.216
	Week 13	.064	.038	1.000	-.102	.230
	Week 15	.068	.032	1.000	-.073	.210
Week 11	Week 1	-.012	.082	1.000	-.370	.345
	Week 2	.058	.078	1.000	-.284	.400
	Week 3	-.366*	.068	.011	-.666	-.067
	Week 6	-.193	.048	.081	-.401	.015
	Week 7	.069	.054	1.000	-.167	.305
	Week 9	-.052	.037	1.000	-.216	.111
	Week 13	.012	.047	1.000	-.193	.217
	Week 15	.016	.047	1.000	-.189	.221
Week 13	Week 1	-.024	.093	1.000	-.431	.383
	Week 2	.046	.074	1.000	-.276	.369
	Week 3	-.378*	.085	.043	-.749	-.008
	Week 6	-.205	.053	.107	-.436	.025
	Week 7	.057	.058	1.000	-.198	.312
	Week 9	-.064	.038	1.000	-.230	.102
	Week 11	-.012	.047	1.000	-.217	.193
	Week15	.004	.037	1.000	-.156	.164
Week 15	Week 1	-.028	.069	1.000	-.329	.273
	Week 2	.042	.060	1.000	-.221	.305
	Week 3	-.382*	.065	.006	-.668	-.097
	Week 6	-.209*	.047	.042	-.413	-.005
	Week 7	.053	.046	1.000	-.147	.252
	Week 9	-.068	.032	1.000	-.210	.073
	Week 11	-.016	.047	1.000	-.221	.189
	Week 13	-.004	.037	1.000	-.164	.156

Carbon Source Guilds – Carbohydrates

Table B.62: Shapiro-Wilk's Test of Normality for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.942	6	.673
	NA	.891	6	.325
Week 2	A	.791	6	.049
	NA	.897	6	.355
Week 3	A	.867	6	.214
	NA	.933	6	.607
Week 6	A	.890	6	.319
	NA	.855	6	.174
Week 7	A	.826	6	.099
	NA	.881	6	.273
Week 9	A	.860	6	.188
	NA	.924	6	.536
Week 11	A	.820	6	.088
	NA	.900	6	.373
Week 13	A	.690	6	.005
	NA	.907	6	.419
Week 15	A	.903	6	.394
	NA	.893	6	.334

Table B.63: Mauchly's Test of Sphericity for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.006	36.065	35	.538

Table B.64: Levene's Test of Equality of Error Variances for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	3.994	1	10	.074
Week 2	6.488	1	10	.029
Week 3	5.316	1	10	.044
Week 6	6.894	1	10	.025
Week 7	39.899	1	10	.000
Week 9	7.914	1	10	.018

Week 11	1.161	1	10	.307
Week 13	1.242	1	10	.291
Week 15	8.685	1	10	.015

Table B.65: Pairwise Comparisons from repeated measures ANOVA for carbohydrates data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.006	.027	1.000	-.115	.126
	Week 3	-.136*	.024	.007	-.239	-.033
	Week 6	-.099	.030	.287	-.231	.032
	Week 7	-.005	.032	1.000	-.146	.136
	Week 9	-.021	.031	1.000	-.155	.113
	Week 11	-.005	.034	1.000	-.153	.142
	Week 13	.001	.039	1.000	-.169	.172
	Week 15	-.004	.027	1.000	-.122	.113
Week 2	Week 1	-.006	.027	1.000	-.126	.115
	Week 3	-.141	.036	.097	-.298	.015
	Week 6	-.105	.039	.808	-.275	.065
	Week 7	-.011	.029	1.000	-.137	.116
	Week 9	-.026	.030	1.000	-.156	.103
	Week 11	-.011	.036	1.000	-.170	.148
	Week 13	-.004	.037	1.000	-.165	.156
	Week 15	-.010	.032	1.000	-.148	.128
Week 3	Week 1	.136*	.024	.007	.033	.239
	Week 2	.141	.036	.097	-.015	.298
	Week 6	.036	.038	1.000	-.131	.204
	Week 7	.131	.039	.253	-.039	.300
	Week 9	.115	.045	1.000	-.081	.311
	Week 11	.130	.036	.175	-.028	.289
	Week 13	.137	.047	.541	-.068	.342
	Week 15	.131	.038	.207	-.033	.296
Week 6	Week 1	.099	.030	.287	-.032	.231
	Week 2	.105	.039	.808	-.065	.275
	Week 3	-.036	.038	1.000	-.204	.131
	Week 7	.094	.031	.447	-.041	.230
	Week 9	.079	.025	.415	-.033	.190
	Week 11	.094*	.019	.022	.010	.178
	Week 13	.101*	.021	.026	.009	.193
	Week 15	.095*	.016	.006	.024	.166
Week 7	Week 1	.005	.032	1.000	-.136	.146

	Week 2	.011	.029	1.000	-.116	.137
	Week 3	-.131	.039	.253	-.300	.039
	Week 6	-.094	.031	.447	-.230	.041
	Week 9	-.016	.030	1.000	-.145	.113
	Week 11	.000	.028	1.000	-.121	.120
	Week 13	.006	.031	1.000	-.131	.144
	Week 15	.001	.028	1.000	-.123	.124
Week 9	Week 1	.021	.031	1.000	-.113	.155
	Week 2	.026	.030	1.000	-.103	.156
	Week 3	-.115	.045	1.000	-.311	.081
	Week 6	-.079	.025	.415	-.190	.033
	Week 7	.016	.030	1.000	-.113	.145
	Week 11	.015	.026	1.000	-.099	.130
	Week 13	.022	.028	1.000	-.099	.143
	Week 15	.016	.023	1.000	-.082	.115
Week 11	Week 1	.005	.034	1.000	-.142	.153
	Week 2	.011	.036	1.000	-.148	.170
	Week 3	-.130	.036	.175	-.289	.028
	Week 6	-.094*	.019	.022	-.178	-.010
	Week 7	.000	.028	1.000	-.120	.121
	Week 9	-.015	.026	1.000	-.130	.099
	Week 13	.007	.023	1.000	-.095	.109
	Week 15	.001	.021	1.000	-.090	.092
Week 13	Week 1	-.001	.039	1.000	-.172	.169
	Week 2	.004	.037	1.000	-.156	.165
	Week 3	-.137	.047	.541	-.342	.068
	Week 6	-.101*	.021	.026	-.193	-.009
	Week 7	-.006	.031	1.000	-.144	.131
	Week 9	-.022	.028	1.000	-.143	.099
	Week 11	-.007	.023	1.000	-.109	.095
	Week15	-.006	.019	1.000	-.089	.077
Week 15	Week 1	.004	.027	1.000	-.113	.122
	Week 2	.010	.032	1.000	-.128	.148
	Week 3	-.131	.038	.207	-.296	.033
	Week 6	-.095*	.016	.006	-.166	-.024
	Week 7	-.001	.028	1.000	-.124	.123
	Week 9	-.016	.023	1.000	-.115	.082
	Week 11	-.001	.021	1.000	-.092	.090
	Week 13	.006	.019	1.000	-.077	.089

Carbon Source Guilds – Polymers

Table B.66: Shapiro-Wilk's Test of Normality for polymers data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.933	6	.601
	NA	.903	6	.392
Week 2	A	.911	6	.445
	NA	.834	6	.116
Week 3	A	.908	6	.423
	NA	.933	6	.607
Week 6	A	.922	6	.521
	NA	.927	6	.559
Week 7	A	.878	6	.259
	NA	.941	6	.668
Week 9	A	.890	6	.316
	NA	.931	6	.592
Week 11	A	.872	6	.235
	NA	.853	6	.166
Week 13	A	.827	6	.102
	NA	.888	6	.307
Week 15	A	.894	6	.342
	NA	.832	6	.112

Table B.67: Mauchly's Test of Sphericity for polymers data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	54.327	35	.041

Table B.68: Levene's Test of Equality of Error Variances for polymers data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.117	1	10	.739
Week 2	1.065	1	10	.326
Week 3	.190	1	10	.672
Week 6	1.579	1	10	.237
Week 7	5.686	1	10	.038
Week 9	6.945	1	10	.025

Week 11	1.441	1	10	.258
Week 13	.597	1	10	.458
Week 15	.125	1	10	.731

Table B.69: Pairwise Comparisons from repeated measures ANOVA for polymers data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.023	.007	.378	-.009	.055
	Week 3	-.052	.014	.124	-.111	.008
	Week 6	-.054*	.012	.036	-.105	-.002
	Week 7	-.032	.012	.741	-.084	.019
	Week 9	-.028	.013	1.000	-.087	.031
	Week 11	-.003	.015	1.000	-.070	.064
	Week 13	-.023	.013	1.000	-.079	.033
	Week 15	-.031	.012	.812	-.081	.019
Week 2	Week 1	-.023	.007	.378	-.055	.009
	Week 3	-.074*	.014	.010	-.134	-.015
	Week 6	-.076*	.010	.000	-.118	-.035
	Week 7	-.055*	.011	.024	-.105	-.006
	Week 9	-.051*	.009	.008	-.090	-.011
	Week 11	-.026	.013	1.000	-.083	.031
	Week 13	-.046	.015	.387	-.109	.018
	Week 15	-.054	.013	.065	-.110	.002
Week 3	Week 1	.052	.014	.124	-.008	.111
	Week 2	.074*	.014	.010	.015	.134
	Week 6	-.002	.018	1.000	-.080	.076
	Week 7	.019	.017	1.000	-.057	.095
	Week 9	.024	.018	1.000	-.057	.104
	Week 11	.049	.019	.922	-.032	.129
	Week 13	.029	.021	1.000	-.065	.122
	Week 15	.021	.018	1.000	-.057	.098
Week 6	Week 1	.054*	.012	.036	.002	.105
	Week 2	.076*	.010	.000	.035	.118
	Week 3	.002	.018	1.000	-.076	.080
	Week 7	.021	.005	.096	-.002	.045
	Week 9	.026	.008	.242	-.007	.059
	Week 11	.051*	.011	.047	.000	.101
	Week 13	.031	.013	1.000	-.027	.089
	Week 15	.023	.013	1.000	-.032	.077
Week 7	Week 1	.032	.012	.741	-.019	.084

	Week 2	.055*	.011	.024	.006	.105
	Week 3	-.019	.017	1.000	-.095	.057
	Week 6	-.021	.005	.096	-.045	.002
	Week 9	.004	.010	1.000	-.040	.049
	Week 11	.029	.012	1.000	-.024	.083
	Week 13	.010	.012	1.000	-.044	.064
	Week 15	.001	.010	1.000	-.041	.043
Week 9	Week 1	.028	.013	1.000	-.031	.087
	Week 2	.051*	.009	.008	.011	.090
	Week 3	-.024	.018	1.000	-.104	.057
	Week 6	-.026	.008	.242	-.059	.007
	Week 7	-.004	.010	1.000	-.049	.040
	Week 11	.025	.009	.821	-.016	.065
	Week 13	.005	.013	1.000	-.050	.060
	Week 15	-.003	.014	1.000	-.064	.057
Week 11	Week 1	.003	.015	1.000	-.064	.070
	Week 2	.026	.013	1.000	-.031	.083
	Week 3	-.049	.019	.922	-.129	.032
	Week 6	-.051*	.011	.047	-.101	.000
	Week 7	-.029	.012	1.000	-.083	.024
	Week 9	-.025	.009	.821	-.065	.016
	Week 13	-.020	.011	1.000	-.069	.030
	Week 15	-.028	.013	1.000	-.085	.029
Week 13	Week 1	.023	.013	1.000	-.033	.079
	Week 2	.046	.015	.387	-.018	.109
	Week 3	-.029	.021	1.000	-.122	.065
	Week 6	-.031	.013	1.000	-.089	.027
	Week 7	-.010	.012	1.000	-.064	.044
	Week 9	-.005	.013	1.000	-.060	.050
	Week 11	.020	.011	1.000	-.030	.069
	Week15	-.008	.010	1.000	-.052	.036
Week 15	Week 1	.031	.012	.812	-.019	.081
	Week 2	.054	.013	.065	-.002	.110
	Week 3	-.021	.018	1.000	-.098	.057
	Week 6	-.023	.013	1.000	-.077	.032
	Week 7	-.001	.010	1.000	-.043	.041
	Week 9	.003	.014	1.000	-.057	.064
	Week 11	.028	.013	1.000	-.029	.085
	Week 13	.008	.010	1.000	-.036	.052

Carbon Source Guilds – Carboxylic and Acetic Acids

Table B.70: Shapiro-Wilk's Test of Normality for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.985	6	.972
	NA	.942	6	.673
Week 2	A	.918	6	.491
	NA	.921	6	.516
Week 3	A	.906	6	.412
	NA	.921	6	.514
Week 6	A	.945	6	.699
	NA	.881	6	.275
Week 7	A	.873	6	.237
	NA	.796	6	.055
Week 9	A	.901	6	.380
	NA	.941	6	.670
Week 11	A	.727	6	.012
	NA	.918	6	.493
Week 13	A	.831	6	.109
	NA	.878	6	.258
Week 15	A	.929	6	.569
	NA	.941	6	.665

Table B.71: Mauchly's Test of Sphericity for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.003	41.730	35	.299

Table B.72: Levene's Test of Equality of Error Variances for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.313	1	10	.588
Week 2	.244	1	10	.632
Week 3	5.403	1	10	.042
Week 6	10.975	1	10	.008
Week 7	6.295	1	10	.031
Week 9	.404	1	10	.540

Week 11	.081	1	10	.782
Week 13	.258	1	10	.623
Week 15	.026	1	10	.876

Table B.73: Pairwise Comparisons from repeated measures ANOVA for carboxylic and acetic acids data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.001	.021	1.000	-.091	.092
	Week 3	-.084	.022	.108	-.178	.010
	Week 6	-.027	.018	1.000	-.108	.053
	Week 7	.054	.023	1.000	-.045	.153
	Week 9	.005	.022	1.000	-.090	.100
	Week 11	-.010	.021	1.000	-.103	.084
	Week 13	.001	.027	1.000	-.115	.117
	Week 15	.002	.017	1.000	-.074	.077
Week 2	Week 1	-.001	.021	1.000	-.092	.091
	Week 3	-.084*	.016	.014	-.156	-.013
	Week 6	-.028	.018	1.000	-.108	.052
	Week 7	.053	.020	.773	-.032	.139
	Week 9	.004	.024	1.000	-.099	.108
	Week 11	-.010	.024	1.000	-.117	.096
	Week 13	.001	.024	1.000	-.102	.103
	Week 15	.001	.022	1.000	-.096	.098
Week 3	Week 1	.084	.022	.108	-.010	.178
	Week 2	.084*	.016	.014	.013	.156
	Week 6	.056	.021	.802	-.035	.148
	Week 7	.138*	.011	.000	.091	.184
	Week 9	.089*	.015	.004	.025	.153
	Week 11	.074*	.017	.049	.000	.148
	Week 13	.085*	.017	.016	.012	.158
	Week 15	.085*	.019	.043	.002	.169
Week 6	Week 1	.027	.018	1.000	-.053	.108
	Week 2	.028	.018	1.000	-.052	.108
	Week 3	-.056	.021	.802	-.148	.035
	Week 7	.081*	.016	.020	.010	.153
	Week 9	.032	.018	1.000	-.044	.109
	Week 11	.018	.021	1.000	-.073	.109
	Week 13	.029	.023	1.000	-.071	.129
	Week 15	.029	.020	1.000	-.057	.115

Week 7	Week 1	-.054	.023	1.000	-.153	.045
	Week 2	-.053	.020	.773	-.139	.032
	Week 3	-.138*	.011	.000	-.184	-.091
	Week 6	-.081*	.016	.020	-.153	-.010
	Week 9	-.049*	.010	.027	-.094	-.004
	Week 11	-.064*	.013	.018	-.119	-.008
	Week 13	-.053	.015	.213	-.119	.014
	Week 15	-.052	.018	.567	-.131	.026
Week 9	Week 1	-.005	.022	1.000	-.100	.090
	Week 2	-.004	.024	1.000	-.108	.099
	Week 3	-.089*	.015	.004	-.153	-.025
	Week 6	-.032	.018	1.000	-.109	.044
	Week 7	.049*	.010	.027	.004	.094
	Week 11	-.015	.014	1.000	-.077	.048
	Week 13	-.004	.014	1.000	-.063	.055
	Week 15	-.003	.017	1.000	-.079	.073
Week 11	Week 1	.010	.021	1.000	-.084	.103
	Week 2	.010	.024	1.000	-.096	.117
	Week 3	-.074*	.017	.049	-.148	.000
	Week 6	-.018	.021	1.000	-.109	.073
	Week 7	.064*	.013	.018	.008	.119
	Week 9	.015	.014	1.000	-.048	.077
	Week 13	.011	.014	1.000	-.049	.071
	Week 15	.011	.014	1.000	-.051	.073
Week 13	Week 1	-.001	.027	1.000	-.117	.115
	Week 2	-.001	.024	1.000	-.103	.102
	Week 3	-.085*	.017	.016	-.158	-.012
	Week 6	-.029	.023	1.000	-.129	.071
	Week 7	.053	.015	.213	-.014	.119
	Week 9	.004	.014	1.000	-.055	.063
	Week 11	-.011	.014	1.000	-.071	.049
	Week15	.000	.016	1.000	-.069	.070
Week 15	Week 1	-.002	.017	1.000	-.077	.074
	Week 2	-.001	.022	1.000	-.098	.096
	Week 3	-.085*	.019	.043	-.169	-.002
	Week 6	-.029	.020	1.000	-.115	.057
	Week 7	.052	.018	.567	-.026	.131
	Week 9	.003	.017	1.000	-.073	.079
	Week 11	-.011	.014	1.000	-.073	.051
	Week 13	.000	.016	1.000	-.070	.069

Carbon Source Guilds – Amino Acids

Table B.74: Shapiro-Wilk's Test of Normality for amino acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.957	6	.798
	NA	.937	6	.636
Week 2	A	.899	6	.368
	NA	.984	6	.968
Week 3	A	.839	6	.127
	NA	.907	6	.420
Week 6	A	.867	6	.216
	NA	.744	6	.017
Week 7	A	.931	6	.590
	NA	.890	6	.319
Week 9	A	.904	6	.395
	NA	.954	6	.772
Week 11	A	.916	6	.474
	NA	.948	6	.727
Week 13	A	.977	6	.936
	NA	.877	6	.256
Week 15	A	.903	6	.395
	NA	.965	6	.856

Table B.75: Mauchly's Test of Sphericity for amino acids data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.002	46.145	35	.164

Table B.76: Levene's Test of Equality of Error Variances for amino acids data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.683	1	10	.428
Week 2	.552	1	10	.475
Week 3	3.719	1	10	.083
Week 6	1.955	1	10	.192
Week 7	.799	1	10	.392
Week 9	3.451	1	10	.093

Week 11	.690	1	10	.425
Week 13	2.164	1	10	.172
Week 15	7.303	1	10	.022

Table B.77: Pairwise Comparisons from repeated measures ANOVA for amino acids data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.030	.016	1.000	-.039	.099
	Week 3	-.044	.019	1.000	-.126	.039
	Week 6	-.006	.018	1.000	-.085	.073
	Week 7	.037	.018	1.000	-.041	.116
	Week 9	.007	.021	1.000	-.086	.100
	Week 11	.024	.024	1.000	-.082	.131
	Week 13	.025	.024	1.000	-.082	.132
	Week 15	.047	.022	1.000	-.049	.144
Week 2	Week 1	-.030	.016	1.000	-.099	.039
	Week 3	-.074*	.009	.000	-.115	-.033
	Week 6	-.036	.010	.186	-.080	.008
	Week 7	.007	.013	1.000	-.047	.062
	Week 9	-.023	.014	1.000	-.085	.039
	Week 11	-.006	.019	1.000	-.088	.077
	Week 13	-.005	.014	1.000	-.068	.058
	Week 15	.017	.012	1.000	-.033	.067
Week 3	Week 1	.044	.019	1.000	-.039	.126
	Week 2	.074*	.009	.000	.033	.115
	Week 6	.038	.011	.250	-.011	.087
	Week 7	.081*	.012	.002	.028	.134
	Week 9	.051	.017	.466	-.023	.125
	Week 11	.068	.017	.077	-.004	.141
	Week 13	.069	.017	.092	-.007	.144
	Week 15	.091*	.015	.004	.025	.157
Week 6	Week 1	.006	.018	1.000	-.073	.085
	Week 2	.036	.010	.186	-.008	.080
	Week 3	-.038	.011	.250	-.087	.011
	Week 7	.043*	.006	.001	.018	.069
	Week 9	.013	.011	1.000	-.034	.060
	Week 11	.030	.014	1.000	-.029	.090
	Week 13	.031	.013	1.000	-.026	.087
	Week 15	.053*	.010	.014	.009	.098
Week 7	Week 1	-.037	.018	1.000	-.116	.041

	Week 2	-.007	.013	1.000	-.062	.047
	Week 3	-.081*	.012	.002	-.134	-.028
	Week 6	-.043*	.006	.001	-.069	-.018
	Week 9	-.030	.011	.584	-.076	.016
	Week 11	-.013	.013	1.000	-.069	.043
	Week 13	-.012	.013	1.000	-.070	.045
	Week 15	.010	.012	1.000	-.042	.061
Week 9	Week 1	-.007	.021	1.000	-.100	.086
	Week 2	.023	.014	1.000	-.039	.085
	Week 3	-.051	.017	.466	-.125	.023
	Week 6	-.013	.011	1.000	-.060	.034
	Week 7	.030	.011	.584	-.016	.076
	Week 11	.017	.016	1.000	-.052	.086
	Week 13	.018	.012	1.000	-.033	.069
	Week 15	.040	.012	.213	-.010	.091
Week 11	Week 1	-.024	.024	1.000	-.131	.082
	Week 2	.006	.019	1.000	-.077	.088
	Week 3	-.068	.017	.077	-.141	.004
	Week 6	-.030	.014	1.000	-.090	.029
	Week 7	.013	.013	1.000	-.043	.069
	Week 9	-.017	.016	1.000	-.086	.052
	Week 13	.001	.013	1.000	-.058	.059
	Week 15	.023	.015	1.000	-.041	.087
Week 13	Week 1	-.025	.024	1.000	-.132	.082
	Week 2	.005	.014	1.000	-.058	.068
	Week 3	-.069	.017	.092	-.144	.007
	Week 6	-.031	.013	1.000	-.087	.026
	Week 7	.012	.013	1.000	-.045	.070
	Week 9	-.018	.012	1.000	-.069	.033
	Week 11	-.001	.013	1.000	-.059	.058
	Week15	.022	.008	.506	-.011	.055
Week 15	Week 1	-.047	.022	1.000	-.144	.049
	Week 2	-.017	.012	1.000	-.067	.033
	Week 3	-.091*	.015	.004	-.157	-.025
	Week 6	-.053*	.010	.014	-.098	-.009
	Week 7	-.010	.012	1.000	-.061	.042
	Week 9	-.040	.012	.213	-.091	.010
	Week 11	-.023	.015	1.000	-.087	.041
	Week 13	-.022	.008	.506	-.055	.011

Carbon Source Guilds – Amines/Amides

Table B.78: Shapiro-Wilk's Test of Normality for amines and amides data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.878	6	.260
	NA	.864	6	.205
Week 2	A	.848	6	.152
	NA	.939	6	.651
Week 3	A	.900	6	.372
	NA	.891	6	.325
Week 6	A	.900	6	.376
	NA	.821	6	.090
Week 7	A	.935	6	.620
	NA	.942	6	.675
Week 9	A	.983	6	.966
	NA	.902	6	.384
Week 11	A	.963	6	.841
	NA	.827	6	.101
Week 13	A	.828	6	.102
	NA	.816	6	.082
Week 15	A	.944	6	.690
	NA	.737	6	.015

Table B.79: Mauchly's Test of Sphericity for amines and amides data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.001	50.086	35	.088

Table B.80: Levene's Test of Equality of Error Variances for amines and amides data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.404	1	10	.539
Week 2	8.983	1	10	.013
Week 3	8.193	1	10	.017
Week 6	4.945	1	10	.050
Week 7	3.916	1	10	.076
Week 9	.010	1	10	.922

Week 11	.444	1	10	.520
Week 13	2.760	1	10	.128
Week 15	5.402	1	10	.042

Table B.81: Pairwise Comparisons from repeated measures ANOVA for amines and amides data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.007	.008	1.000	-.030	.044
	Week 3	-.022	.012	1.000	-.077	.032
	Week 6	.006	.011	1.000	-.041	.053
	Week 7	.022	.010	1.000	-.024	.067
	Week 9	-.005	.009	1.000	-.044	.035
	Week 11	.008	.010	1.000	-.036	.051
	Week 13	.018	.012	1.000	-.035	.071
	Week 15	.018	.012	1.000	-.033	.068
Week 2	Week 1	-.007	.008	1.000	-.044	.030
	Week 3	-.029*	.007	.044	-.058	-.001
	Week 6	-.001	.008	1.000	-.035	.032
	Week 7	.015	.006	1.000	-.012	.042
	Week 9	-.012	.008	1.000	-.045	.021
	Week 11	.001	.010	1.000	-.041	.042
	Week 13	.011	.010	1.000	-.033	.056
	Week 15	.011	.010	1.000	-.034	.055
Week 3	Week 1	.022	.012	1.000	-.032	.077
	Week 2	.029*	.007	.044	.001	.058
	Week 6	.028	.008	.275	-.009	.065
	Week 7	.044*	.007	.004	.013	.075
	Week 9	.018	.010	1.000	-.026	.061
	Week 11	.030	.009	.371	-.012	.071
	Week 13	.041*	.008	.025	.004	.077
	Week 15	.040	.009	.059	-.001	.080
Week 6	Week 1	-.006	.011	1.000	-.053	.041
	Week 2	.001	.008	1.000	-.032	.035
	Week 3	-.028	.008	.275	-.065	.009
	Week 7	.016	.005	.211	-.004	.036
	Week 9	-.010	.006	1.000	-.038	.018
	Week 11	.002	.008	1.000	-.034	.038
	Week 13	.013	.008	1.000	-.024	.049
	Week 15	.012	.009	1.000	-.029	.053
Week 7	Week 1	-.022	.010	1.000	-.067	.024

	Week 2	-.015	.006	1.000	-.042	.012
	Week 3	-.044*	.007	.004	-.075	-.013
	Week 6	-.016	.005	.211	-.036	.004
	Week 9	-.026	.007	.142	-.057	.005
	Week 11	-.014	.006	1.000	-.041	.013
	Week 13	-.003	.007	1.000	-.033	.027
	Week 15	-.004	.007	1.000	-.036	.028
Week 9	Week 1	.005	.009	1.000	-.035	.044
	Week 2	.012	.008	1.000	-.021	.045
	Week 3	-.018	.010	1.000	-.061	.026
	Week 6	.010	.006	1.000	-.018	.038
	Week 7	.026	.007	.142	-.005	.057
	Week 11	.012	.008	1.000	-.024	.048
	Week 13	.023	.010	1.000	-.022	.068
	Week 15	.022	.010	1.000	-.023	.067
Week 11	Week 1	-.008	.010	1.000	-.051	.036
	Week 2	-.001	.010	1.000	-.042	.041
	Week 3	-.030	.009	.371	-.071	.012
	Week 6	-.002	.008	1.000	-.038	.034
	Week 7	.014	.006	1.000	-.013	.041
	Week 9	-.012	.008	1.000	-.048	.024
	Week 13	.011	.005	1.000	-.011	.033
	Week 15	.010	.006	1.000	-.016	.036
Week 13	Week 1	-.018	.012	1.000	-.071	.035
	Week 2	-.011	.010	1.000	-.056	.033
	Week 3	-.041*	.008	.025	-.077	-.004
	Week 6	-.013	.008	1.000	-.049	.024
	Week 7	.003	.007	1.000	-.027	.033
	Week 9	-.023	.010	1.000	-.068	.022
	Week 11	-.011	.005	1.000	-.033	.011
	Week15	-.001	.004	1.000	-.018	.016
Week 15	Week 1	-.018	.012	1.000	-.068	.033
	Week 2	-.011	.010	1.000	-.055	.034
	Week 3	-.040	.009	.059	-.080	.001
	Week 6	-.012	.009	1.000	-.053	.029
	Week 7	.004	.007	1.000	-.028	.036
	Week 9	-.022	.010	1.000	-.067	.023
	Week 11	-.010	.006	1.000	-.036	.016
	Week 13	.001	.004	1.000	-.016	.018

Root Exudates

Table B.82: Shapiro-Wilk's Test of Normality for root exudates data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.892	6	.328
	NA	.971	6	.897
Week 2	A	.954	6	.771
	NA	.934	6	.613
Week 3	A	.811	6	.074
	NA	.898	6	.363
Week 6	A	.894	6	.342
	NA	.766	6	.029
Week 7	A	.964	6	.850
	NA	.857	6	.178
Week 9	A	.949	6	.729
	NA	.892	6	.330
Week 11	A	.960	6	.819
	NA	.962	6	.834
Week 13	A	.824	6	.095
	NA	.889	6	.315
Week 15	A	.884	6	.286
	NA	.919	6	.497

Table B.83: Mauchly's Test of Sphericity for root exudates data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	55.437	35	.033

Table B.84: Levene's Test of Equality of Error Variances for root exudates data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.625	1	10	.448
Week 2	.116	1	10	.740
Week 3	3.518	1	10	.090
Week 6	2.514	1	10	.144
Week 7	2.908	1	10	.119
Week 9	.000	1	10	.991

Week 11	.887	1	10	.368
Week 13	4.999	1	10	.049
Week 15	3.109	1	10	.108

Table B.85: Pairwise Comparisons from repeated measures ANOVA for root exudate data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	.037	.029	1.000	-.090	.164
	Week 3	-.104	.036	.553	-.261	.052
	Week 6	-.023	.034	1.000	-.171	.125
	Week 7	.062	.031	1.000	-.074	.199
	Week 9	-.008	.036	1.000	-.165	.149
	Week 11	.036	.035	1.000	-.118	.190
	Week 13	.046	.042	1.000	-.137	.230
	Week 15	.060	.040	1.000	-.116	.235
Week 2	Week 1	-.037	.029	1.000	-.164	.090
	Week 3	-.142*	.016	.000	-.213	-.070
	Week 6	-.060	.020	.477	-.147	.027
	Week 7	.025	.021	1.000	-.069	.119
	Week 9	-.045	.025	1.000	-.154	.063
	Week 11	-.001	.026	1.000	-.114	.111
	Week 13	.009	.026	1.000	-.106	.125
	Week 15	.023	.024	1.000	-.081	.126
Week 3	Week 1	.104	.036	.553	-.052	.261
	Week 2	.142*	.016	.000	.070	.213
	Week 6	.082*	.018	.046	.001	.162
	Week 7	.167*	.019	.000	.084	.249
	Week 9	.096	.024	.082	-.007	.200
	Week 11	.140*	.022	.003	.042	.239
	Week 13	.151*	.025	.005	.039	.262
	Week 15	.164*	.025	.002	.056	.272
Week 6	Week 1	.023	.034	1.000	-.125	.171
	Week 2	.060	.020	.477	-.027	.147
	Week 3	-.082*	.018	.046	-.162	-.001
	Week 7	.085*	.012	.001	.032	.139
	Week 9	.014	.014	1.000	-.047	.076
	Week 11	.059*	.012	.025	.006	.112
	Week 13	.069	.017	.094	-.007	.145
	Week 15	.082*	.015	.011	.015	.150
Week 7	Week 1	-.062	.031	1.000	-.199	.074

	Week 2	-.025	.021	1.000	-.119	.069
	Week 3	-.167*	.019	.000	-.249	-.084
	Week 6	-.085*	.012	.001	-.139	-.032
	Week 9	-.071*	.013	.010	-.127	-.014
	Week 11	-.026	.013	1.000	-.083	.030
	Week 13	-.016	.017	1.000	-.092	.060
	Week 15	-.003	.021	1.000	-.095	.089
Week 9	Week 1	.008	.036	1.000	-.149	.165
	Week 2	.045	.025	1.000	-.063	.154
	Week 3	-.096	.024	.082	-.200	.007
	Week 6	-.014	.014	1.000	-.076	.047
	Week 7	.071*	.013	.010	.014	.127
	Week 11	.044	.014	.352	-.017	.105
	Week 13	.055	.017	.284	-.018	.127
	Week 15	.068	.020	.253	-.020	.156
Week 11	Week 1	-.036	.035	1.000	-.190	.118
	Week 2	.001	.026	1.000	-.111	.114
	Week 3	-.140*	.022	.003	-.239	-.042
	Week 6	-.059*	.012	.025	-.112	-.006
	Week 7	.026	.013	1.000	-.030	.083
	Week 9	-.044	.014	.352	-.105	.017
	Week 13	.011	.013	1.000	-.047	.068
	Week 15	.024	.016	1.000	-.047	.095
Week 13	Week 1	-.046	.042	1.000	-.230	.137
	Week 2	-.009	.026	1.000	-.125	.106
	Week 3	-.151*	.025	.005	-.262	-.039
	Week 6	-.069	.017	.094	-.145	.007
	Week 7	.016	.017	1.000	-.060	.092
	Week 9	-.055	.017	.284	-.127	.018
	Week 11	-.011	.013	1.000	-.068	.047
	Week15	.013	.012	1.000	-.041	.067
Week 15	Week 1	-.060	.040	1.000	-.235	.116
	Week 2	-.023	.024	1.000	-.126	.081
	Week 3	-.164*	.025	.002	-.272	-.056
	Week 6	-.082*	.015	.011	-.150	-.015
	Week 7	.003	.021	1.000	-.089	.095
	Week 9	-.068	.020	.253	-.156	.020
	Week 11	-.024	.016	1.000	-.095	.047
	Week 13	-.013	.012	1.000	-.067	.041

Richness

Table B.86: Shapiro-Wilk's Test of Normality for richness data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.904	6	.396
	NA	.980	6	.951
Week 2	A	.871	6	.231
	NA	.860	6	.188
Week 3	A	.838	6	.125
	NA	.914	6	.466
Week 6	A	.915	6	.473
	NA	.657	6	.002
Week 7	A	.876	6	.252
	NA	.975	6	.925
Week 9	A	.955	6	.782
	NA	.925	6	.542
Week 11	A	.746	6	.018
	NA	.982	6	.962
Week13	A	.815	6	.080
	NA	.842	6	.135
Week15	A	.921	6	.513
	NA	.958	6	.804

Table B.87: Mauchly's Test of Sphericity for richness data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.014	30.632	35	.775

Table B.88: Levene's Test of Equality of Error Variances for richness data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.034	1	10	.857
Week 2	.870	1	10	.373
Week 3	4.601	1	10	.058
Week 6	2.131	1	10	.175
Week 7	4.541	1	10	.059
Week 9	3.962	1	10	.075

Week 11	.219	1	10	.650
Week 13	3.317	1	10	.099
Week 15	1.116	1	10	.316

Table B.89: Pairwise Comparisons from repeated measures ANOVA for richness data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	1.217	1.185	1.000	-3.968	6.402
	Week 3	-5.858*	.979	.005	-10.143	-1.574
	Week 6	-2.508	1.278	1.000	-8.099	3.082
	Week 7	1.708	1.320	1.000	-4.068	7.485
	Week 9	-.850	1.270	1.000	-6.404	4.704
	Week 11	.600	1.405	1.000	-5.546	6.746
	Week 13	.067	1.671	1.000	-7.245	7.379
	Week 15	.242	1.230	1.000	-5.141	5.625
Week 2	Week 1	-1.217	1.185	1.000	-6.402	3.968
	Week 3	-7.075*	.980	.001	-11.361	-2.789
	Week 6	-3.725	1.203	.408	-8.989	1.539
	Week 7	.492	1.188	1.000	-4.703	5.687
	Week 9	-2.067	1.205	1.000	-7.340	3.206
	Week 11	-.617	1.339	1.000	-6.474	5.241
	Week 13	-1.150	1.390	1.000	-7.232	4.932
	Week 15	-.975	1.354	1.000	-6.897	4.947
Week 3	Week 1	5.858*	.979	.005	1.574	10.143
	Week 2	7.075*	.980	.001	2.789	11.361
	Week 6	3.350	1.298	.987	-2.330	9.030
	Week 7	7.567*	1.168	.003	2.458	12.675
	Week 9	5.008	1.310	.121	-.724	10.741
	Week 11	6.458*	1.162	.009	1.377	11.540
	Week 13	5.925	1.420	.069	-.286	12.136
	Week 15	6.100*	1.102	.009	1.281	10.919
Week 6	Week 1	2.508	1.278	1.000	-3.082	8.099
	Week 2	3.725	1.203	.408	-1.539	8.989
	Week 3	-3.350	1.298	.987	-9.030	2.330
	Week 7	4.217*	.841	.019	.535	7.898
	Week 9	1.658	.423	.103	-.192	3.509
	Week 11	3.108	.906	.232	-.856	7.073
	Week 13	2.575	1.024	1.000	-1.905	7.055
	Week 15	2.750	.852	.326	-.977	6.477
Week 7	Week 1	-1.708	1.320	1.000	-7.485	4.068

	Week 2	-.492	1.188	1.000	-5.687	4.703
	Week 3	-7.567*	1.168	.003	-12.675	-2.458
	Week 6	-4.217*	.841	.019	-7.898	-.535
	Week 9	-2.558	.894	.609	-6.470	1.353
	Week 11	-1.108	.935	1.000	-5.197	2.980
	Week 13	-1.642	1.273	1.000	-7.210	3.926
	Week 15	-1.467	1.122	1.000	-6.374	3.441
Week 9	Week 1	.850	1.270	1.000	-4.704	6.404
	Week 2	2.067	1.205	1.000	-3.206	7.340
	Week 3	-5.008	1.310	.121	-10.741	.724
	Week 6	-1.658	.423	.103	-3.509	.192
	Week 7	2.558	.894	.609	-1.353	6.470
	Week 11	1.450	.822	1.000	-2.147	5.047
	Week 13	.917	.955	1.000	-3.262	5.096
	Week 15	1.092	.929	1.000	-2.972	5.155
Week 11	Week 1	-.600	1.405	1.000	-6.746	5.546
	Week 2	.617	1.339	1.000	-5.241	6.474
	Week 3	-6.458*	1.162	.009	-11.540	-1.377
	Week 6	-3.108	.906	.232	-7.073	.856
	Week 7	1.108	.935	1.000	-2.980	5.197
	Week 9	-1.450	.822	1.000	-5.047	2.147
	Week 13	-.533	1.003	1.000	-4.920	3.853
	Week 15	-.358	.920	1.000	-4.384	3.667
Week 13	Week 1	-.067	1.671	1.000	-7.379	7.245
	Week 2	1.150	1.390	1.000	-4.932	7.232
	Week 3	-5.925	1.420	.069	-12.136	.286
	Week 6	-2.575	1.024	1.000	-7.055	1.905
	Week 7	1.642	1.273	1.000	-3.926	7.210
	Week 9	-.917	.955	1.000	-5.096	3.262
	Week 11	.533	1.003	1.000	-3.853	4.920
	Week15	.175	.817	1.000	-3.398	3.748
Week 15	Week 1	-.242	1.230	1.000	-5.625	5.141
	Week 2	.975	1.354	1.000	-4.947	6.897
	Week 3	-6.100*	1.102	.009	-10.919	-1.281
	Week 6	-2.750	.852	.326	-6.477	.977
	Week 7	1.467	1.122	1.000	-3.441	6.374
	Week 9	-1.092	.929	1.000	-5.155	2.972
	Week 11	.358	.920	1.000	-3.667	4.384
	Week 13	-.175	.817	1.000	-3.748	3.398

FDA

Table B.90: Shapiro-Wilk's Test of Normality for FDA data of aerated and non-aerated systems, $\alpha = 0.05$.

	Aeration	Shapiro-Wilk		
		Statistic	df	Sig.
Week 1	A	.900	6	.376
	NA	.969	5	.868
Week 2	A	.778	6	.037
	NA	.974	5	.900
Week 3	A	.688	6	.005
	NA	.717	5	.014
Week 6	A	.830	6	.108
	NA	.968	5	.864
Week 7	A	.910	6	.438
	NA	.975	5	.907
Week 10	A	.820	6	.088
	NA	.945	5	.702
Week 11	A	.951	6	.749
	NA	.983	5	.952
Week 13	A	.881	6	.274
	NA	.749	5	.029
Week 15	A	.963	6	.839
	NA	.951	5	.747

Table B.91: Mauchly's Test of Sphericity for FDA data of aerated and non-aerated systems, $\alpha = 0.05$.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
Time	.000	57.163	35	.028

Table B.92: Levene's Test of Equality of Error Variances for FDA data of aerated and non-aerated systems, $\alpha = 0.05$.

	F	df1	df2	Sig.
Week 1	.154	1	9	.704
Week 2	1.022	1	9	.338
Week 3	.001	1	9	.979
Week 6	2.104	1	9	.181
Week 7	.007	1	9	.936
Week 10	.050	1	9	.827

Week 11	5.769	1	9	.040
Week 13	.037	1	9	.851
Week 15	.188	1	9	.675

Table B.93: Pairwise Comparisons from repeated measures ANOVA for FDA data of aerated and non-aerated systems, $\alpha = 0.05$. Significant weeks are marked with an asterisk (*).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Week 1	Week 2	-1.343	.551	1.000	-3.851	1.164
	Week 3	.789	.410	1.000	-1.075	2.653
	Week 6	-2.503	.569	.062	-5.093	.088
	Week 7	-2.246	.563	.114	-4.809	.316
	Week 10	-5.209*	.700	.001	-8.391	-2.027
	Week 11	-2.359	.983	1.000	-6.832	2.113
	Week 13	-1.740	.586	.567	-4.405	.926
	Week 15	-1.891	.633	.548	-4.770	.987
Week 2	Week 1	1.343	.551	1.000	-1.164	3.851
	Week 3	2.132*	.427	.027	.190	4.075
	Week 6	-1.159	.490	1.000	-3.387	1.069
	Week 7	-.903	.443	1.000	-2.917	1.111
	Week 10	-3.866*	.678	.011	-6.949	-.783
	Week 11	-1.016	.777	1.000	-4.552	2.520
	Week 13	-.396	.405	1.000	-2.236	1.444
	Week 15	-.548	.531	1.000	-2.961	1.865
Week 3	Week 1	-.789	.410	1.000	-2.653	1.075
	Week 2	-2.132*	.427	.027	-4.075	-.190
	Week 6	-3.292*	.289	.000	-4.605	-1.978
	Week 7	-3.035*	.214	.000	-4.011	-2.060
	Week 10	-5.998*	.754	.001	-9.428	-2.569
	Week 11	-3.149	.749	.083	-6.555	.258
	Week 13	-2.529*	.392	.004	-4.313	-.745
	Week 15	-2.680*	.463	.009	-4.785	-.576
Week 6	Week 1	2.503	.569	.062	-.088	5.093
	Week 2	1.159	.490	1.000	-1.069	3.387
	Week 3	3.292*	.289	.000	1.978	4.605
	Week 7	.256	.391	1.000	-1.520	2.033
	Week 10	-2.707	.843	.384	-6.542	1.129
	Week 11	.143	.753	1.000	-3.281	3.568
	Week 13	.763	.556	1.000	-1.764	3.290
	Week 15	.611	.535	1.000	-1.822	3.045
Week 7	Week 1	2.246	.563	.114	-.316	4.809

	Week 2	.903	.443	1.000	-1.111	2.917
	Week 3	3.035*	.214	.000	2.060	4.011
	Week 6	-.256	.391	1.000	-2.033	1.520
	Week 10	-2.963	.787	.160	-6.542	.616
	Week 11	-.113	.761	1.000	-3.576	3.349
	Week 13	.507	.350	1.000	-1.084	2.097
	Week 15	.355	.466	1.000	-1.764	2.474
Week 10	Week 1	5.209*	.700	.001	2.027	8.391
	Week 2	3.866*	.678	.011	.783	6.949
	Week 3	5.998*	.754	.001	2.569	9.428
	Week 6	2.707	.843	.384	-1.129	6.542
	Week 7	2.963	.787	.160	-.616	6.542
	Week 11	2.850	1.201	1.000	-2.614	8.314
	Week 13	3.469*	.644	.016	.542	6.397
	Week 15	3.318*	.471	.002	1.176	5.460
Week 11	Week 1	2.359	.983	1.000	-2.113	6.832
	Week 2	1.016	.777	1.000	-2.520	4.552
	Week 3	3.149	.749	.083	-.258	6.555
	Week 6	-.143	.753	1.000	-3.568	3.281
	Week 7	.113	.761	1.000	-3.349	3.576
	Week 10	-2.850	1.201	1.000	-8.314	2.614
	Week 13	.620	.747	1.000	-2.777	4.016
	Week 15	.468	.945	1.000	-3.831	4.767
Week 13	Week 1	1.740	.586	.567	-.926	4.405
	Week 2	.396	.405	1.000	-1.444	2.236
	Week 3	2.529*	.392	.004	.745	4.313
	Week 6	-.763	.556	1.000	-3.290	1.764
	Week 7	-.507	.350	1.000	-2.097	1.084
	Week 10	-3.469*	.644	.016	-6.397	-.542
	Week 11	-.620	.747	1.000	-4.016	2.777
	Week15	-.152	.350	1.000	-1.746	1.442
Week 15	Week 1	1.891	.633	.548	-.987	4.770
	Week 2	.548	.531	1.000	-1.865	2.961
	Week 3	2.680*	.463	.009	.576	4.785
	Week 6	-.611	.535	1.000	-3.045	1.822
	Week 7	-.355	.466	1.000	-2.474	1.764
	Week 10	-3.318*	.471	.002	-5.460	-1.176
	Week 11	-.468	.945	1.000	-4.767	3.831
	Week 13	.152	.350	1.000	-1.442	1.746

C.APPENDIX C

Supplementary Information and Raw Data for Chapter 6: EFFECTS OF SILVER NANOPARTICLES ON CONSTRUCTED WETLAND MICROBIAL COMMUNITIES

Table C.1: Ingredients in Tide Liquid Original Detergent. Reproduced from P&G Webpage (https://www.pg.com/productsafety/ingredients/household_care/laundary_fabric_care/Tide/Tide_Liquid_Original.pdf). Ingredients are listed along with their use within the laundry detergent.

Table C.2: Comparison of silver (Ag) concentrations in deionized water (dH2O) stock solutions with aerated (A) and non-aerated (NA) wetland interstitial water dosing solutions. Theoretical Ag concentrations (mg/L) are compared with the measured Ag (mg/L), ionic (Ag+) and % ionic silver at hour 0 of the experiment, and hour 40 of the experiment when ecotoxicity data was calculated. Grey squares represent data which is not available.

Table C.3: Total silver (Ag) concentrations in deionized water (dH2O) stock solutions and aerated (A) and non-aerated (NA) wetland interstitial water dosing solutions. Theoretical Ag concentrations (mg/L) are compared with the measured Ag (mg/L), ionic (mg/L) and the hour of sampling.

Table C.4: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for AWCD from the non-aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested.

Table C.5: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for richness from the non-aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested.

Table C.6: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for AWCD from the aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested. Dashes indicate treatments for which a post-hoc analysis could not be performed because there were too few treatments. In this case the results were not significantly different ($p<0.05$) between Ag+ and CMC treatments at concentrations between 0.005 and 0.05.

Table C.7: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for richness from the non-aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested. Dashes indicate treatments for which a post-hoc analysis could not be performed because there were

too few treatments. In this case the results were not significantly different ($p<0.05$) between Ag+ and CMC treatments at concentrations between 0.005 and 0.05. Forward slash indicates treatments for which a post-hoc analysis could not be performed because all values were zero.

Table C.8: Summary of Ag NP dose-response data for non-aerated microbial communities and associated p values from a 2-sided Dunnett's test comparing dose concentration response (0.1 - 10 mg/L) with the control response (0 mg/L). Average raw data is displayed in this table for average well colour development (AWCD) and Richness. Triplicate raw data from each BIOLOG EcoPlate™ was used to compute ANOVA and the subsequent 2-sided Dunnett's test. Grey squares are used to represent concentrations which were not tested.

Table C.9: Summary of Ag NP dose-response data for aerated microbial communities and associated *p* values from a 2-sided Dunnett's test comparing dose concentration response (0.005 - 10 mg/L) with the control response (0 mg/L). Average raw data is displayed in this table for average well colour development (AWCD) and Richness. Triplicate raw data from each BIOLOG EcoPlate™ was used to compute ANOVA and the subsequent 2-sided Dunnett's test. Grey squares are used to represent concentrations which were not tested.

Table C.10: Compiled CLPP raw data from *ex-situ* silver nanoparticle exposures on wetland interstitial water microbial communities. Average well colour development (AWCD) and richness and represented as a mean of the three-replicated carbon source utilization patterns (CSUPs) present on the BIOLOG EcoPlate™**.**

Figure C.1: Summary of EC50s for aerated (red) and non-aerated (blue) microbial communities treated with various types of silver and silver nanoparticles. EC50s were calculated by linear interpolation for richness. Grey bars = EC50 could not be calculated for concentrations in this studies dose-response curve as they were not high enough to create a 50% effect. EC50s were calculated using raw data.

Statistical Tables for Chapter 5: Table C.11 to Table C.35

Table C.1: Ingredients in Tide Liquid Original Detergent. Reproduced from P&G Webpage (https://www.pg.com/productsafety/ingredients/household_care/laundary_fabric_care/Tide/Tide_Liquid_Original.pdf). Ingredients are listed along with their use within the laundry detergent.

Ingredient	**Cleaning Technology**
water	process aid
alcoholethoxy sulfate	surfactant
linear alkylbenzene sulfonate	surfactant
propylene glycol	process aid
citric acid	captures soil
sodium hydroxide	pH neutralizer
borax	captures soil
ethanolamine	process aid
ethanol	process aid
alcohol sulfate	surfactant
polyethyleneimine ethoxylate	polymer
sodium fatty acids	surfactant
diquaternium ethoxysulfate	polymer
protease	enzyme
diethylene glycol	process aid
laureth-9	surfactant
alkyldimethylamine oxide	surfactant
fragrance	fragrance
amylase	enzyme
disodium diaminostilbene disulfonate	brightener
DTPA	captures soil
sodium formate	process aid
calcium formate	process aid
polyethylene glycol 4000	process aid
mannanase	enzyme
Liquitint™ Blue	colorant
dimethicone	process aid

Table C.2: Comparison of silver (Ag) concentrations in deionized water (dH2O) stock solutions with aerated (A) and non-aerated (NA) wetland interstitial water dosing solutions. Theoretical Ag concentrations (mg/L) are compared with the measured Ag (mg/L), ionic (Ag^+) and % ionic silver at hour 0 of the experiment, and hour 40 of the experiment when ecotoxicity data was calculated. Grey squares represent data which is not available.

			Hour 0			Hour 40		
Type of Silver	Condition	Theoretical Ag (mg/L)	Measured Ag (mg/L)	Ag^+ (mg/L)	% Ag^+	Measured Ag (mg/L)	Ag^+ (mg/L)	% Ag^+
Ag+	Deionized Water	200	131.90	128.12	97.13			
	Aerated Wetland Water	10	6.83	0.05	0.73	9.43	0.04	0.42
	Non-Aerated Wetland Water	10	11.29	0.01	0.09	9.25	0.02	0.22
CMC	Deionized Water	200	103.48	96.49	93.25			
	Aerated Wetland Water	10	14.73	0.01	0.07	9.87	0.04	0.41
	Non-Aerated Wetland Water	10	9.93	0.04	0.40	10.67	0.03	0.28
Citrate	Deionized Water	20	20.93	0.08	0.38			
	Aerated Wetland Water	1	1.27	0.11	8.66	0.67	0.00	0.00
	Non-Aerated Wetland Water	1	1.03	0.01	0.97	0.54	0.00	0.00
PVP	Deionized Water	200	98.58	0.23	0.23			
	Aerated Wetland Water	10	8.50	0.00	0.00	3.45	0.00	0.00
	Non-Aerated Wetland Water	10	6.76	0.01	0.15	3.52	0.00	0.00
Uncoated	Deionized Water	200	83.19	0.26	0.31			
	Aerated Wetland Water	20	4.60	0.00	0.00	15.63	0.00	0.00
	Non-Aerated Wetland Water	20	7.99	0.01	0.13	3.2	0.00	0.00
Sulphidized	Deionized Water	50	59.02	0.01	0.01			
	Aerated Wetland Water	5	3.14	0.00	0.00	1.978	0.001	0.05
	Non-Aerated Wetland Water	5	3.23	0.00	0.00	0.572	0.015	2.62
Sock Wash	Deionized Water	20	14.20	0.04	0.30			
	Aerated Wetland Water	5	2.83	0.01	0.42	30.71	0.002	0.01
	Non-Aerated Wetland Water	5	2.98	0.00	0.03	2.658	0.013	0.49

Table C.3: Total silver (Ag) concentrations in deionized water (dH2O) stock solutions and aerated (A) and non-aerated (NA) wetland interstitial water dosing solutions. Theoretical Ag concentrations (mg/L) are compared with the measured Ag (mg/L), ionic (mg/L) and the hour of sampling.

Experiment	Type of Silver	Water Type	Theoretical Ag Concentration (mg/L)	Total Ag (mg/L)	Ionic Ag (mg/L)	Hour
1	CMC Ag NPs	dH2O	200	156.61	79.87	0
2	CMC Ag NPs	dH2O	200	130.48	96.49	0
1	CMC Ag NPs	Aerated	10	7.16	0.05	0
2	CMC Ag NPs	Aerated	10	14.73	0.01	0
2	CMC Ag NPs	Aerated	10	9.87	0.03	40
2	CMC Ag NPs	Aerated	0.1	0.11		0
2	CMC Ag NPs	Aerated	0.1	0.11		0
2	CMC Ag NPs	Aerated	0.1	0.12		0
2	CMC Ag NPs	Aerated	0.05	0.03		0
2	CMC Ag NPs	Aerated	0.01	0.00		0
2	CMC Ag NPs	Aerated	0.025	0.02		0
2	CMC Ag NPs	Aerated	0.005	0.06		0
1	CMC Ag NPs	Non-Aerated	10	7.71	0.03	0
2	CMC Ag NPs	Non-Aerated	10	9.93	0.04	0
2	CMC Ag NPs	Non-Aerated	10	10.67	0.04	40
1	Ag+	dH2O	200	167.73		0
2	Ag+	dH2O	200	131.90	128.12	0
3	Ag+	dH2O	50	44.19	44.11	0
3	Ag+	dH2O	50	46.45	43.04	0
3	Ag+	dH2O	50	44.44	41.68	0
1	Ag+	Aerated	10	7.47		0
2	Ag+	Aerated	10	6.83	0.05	0
2	Ag+	Aerated	10	9.43	0.04	40
2	Ag+	Aerated	0.1	0.20		0
2	Ag +	Aerated	0.05	0.65		0
2	Ag+	Aerated	0.025	0.01		0
2	Ag+	Aerated	0.01	-0.01		0
2	Ag+	Aerated	0.005	-0.01		0
3	Ag+	Aerated	0.1	0.10		0
3	Ag+	Aerated	0.25	0.16		0
3	Ag+	Aerated	0.5	0.70		0
3	Ag+	Aerated	1	1.21		0
3	Ag+	Aerated	5	3.85	0.01	0

3	Ag+	Aerated	5	3.79		40
1	Ag+	Non-Aerated	10	9.44		0
2	Ag+	Non-Aerated	10	11.29	0.01	0
2	Ag+	Non-Aerated	10	9.25	0.02	40
2	Ag+	Non-Aerated	10	9.50		40
2	Ag+	Non-Aerated	10	9.02		40
3	Ag+	Non-Aerated	0.1	0.05		0
3	Ag+	Non-Aerated	0.25	0.01		0
3	Ag+	Non-Aerated	0.25	0.02		0
3	Ag+	Non-Aerated	0.25	0.18		0
3	Ag+	Non-Aerated	0.5	0.42		0
3	Ag+	Non-Aerated	1	1.14		0
3	Ag+	Non-Aerated	5	3.91	0.01	0
3	Ag+	Non-Aerated	5	2.99		40
1	citrate Ag NPs	dH2O	20	19.18	0.26	0
2	citrate Ag NPs	dH2O	20	20.93	0.08	0
1	citrate Ag NPs	Aerated	1	0.67	0.00	0
2	citrate Ag NPs	Aerated	1	1.27	0.11	0
2	citrate Ag NPs	Aerated	1	0.67	-0.01	40
1	citrate Ag NPs	Non-Aerated	1	0.88	0.00	0
2	citrate Ag NPs	Non-Aerated	1	0.99	0.01	0
2	citrate Ag NPs	Non-Aerated	1	1.05		0
2	citrate Ag NPs	Non-Aerated	1	1.07		0
2	citrate Ag NPs	Non-Aerated	1	0.54	-0.01	40
2	uncoated Ag NPs	dH2O	200	88.66	0.26	0
2	uncoated Ag NPs	dH2O	200	74.61		0
2	uncoated Ag NPs	dH2O	200	86.30		0
1	uncoated Ag NPs	dH2O	200	149.80	0.62	0
2	uncoated Ag NPs	Aerated	10	15.63	-0.02	40
2	uncoated Ag NPs	Aerated	10	4.60	0.00	0
1	uncoated Ag NPs	Aerated	10	4.44	0.00	0
1	uncoated Ag NPs	Aerated	10	4.20		0
1	uncoated Ag NPs	Non-Aerated	10	1.58	0.00	0
2	uncoated Ag NPs	Non-Aerated	10	3.20	-0.01	40
2	uncoated Ag NPs	Non-Aerated	10	7.99	-0.01	0
1	PVP Ag NPs	dH2O	200	119.59	0.49	0
2	PVP Ag NPs	dH2O	200	98.58	0.23	0
1	PVP Ag NPs	Aerated	10	3.20	0.00	0
2	PVP Ag NPs	Aerated	10	3.52	-0.01	40
2	PVP Ag NPs	Aerated	10	8.50	-0.01	0

1	PVP Ag NPs	Non-Aerated	10	6.19	0.00	0
2	PVP Ag NPs	Non-Aerated	10	6.76	0.01	0
2	PVP Ag NPs	Non-Aerated	10	3.36	-0.01	40
2	PVP Ag NPs	Non-Aerated	10	4.56		40
2	PVP Ag NPs	Non-Aerated	10	2.45		40
3	Ag2S Ag NPs	dH2O	50	56.55	0.00	0
3	Ag2S Ag NPs	dH2O	50	60.70		0
3	Ag2S Ag NPs	dH2O	50	59.80		0
3	Ag2S Ag NPs	Aerated	0.1	0.02		0
3	Ag2S Ag NPs	Aerated	0.25	0.04		0
3	Ag2S Ag NPs	Aerated	0.5	0.24		0
3	Ag2S Ag NPs	Aerated	1	0.92		0
3	Ag2S Ag NPs	Aerated	1	0.63		0
3	Ag2S Ag NPs	Aerated	1	0.75		0
3	Ag2S Ag NPs	Aerated	5	3.14	0.00	0
3	Ag2S Ag NPs	Aerated	5	1.98	0.00	40
3	Ag2S Ag NPs	Non-Aerated	0.1	0.04		0
3	Ag2S Ag NPs	Non-Aerated	0.25	0.11		0
3	Ag2S Ag NPs	Non-Aerated	0.5	0.25		0
3	Ag2S Ag NPs	Non-Aerated	0.5	0.24		0
3	Ag2S Ag NPs	Non-Aerated	0.5	0.18		0
3	Ag2S Ag NPs	Non-Aerated	1	1.32		0
3	Ag2S Ag NPs	Non-Aerated	5	3.23	0.00	0
3	Ag2S Ag NPs	Non-Aerated	5	0.57	0.02	40
3	Sock wash	dH2O	20	14.01	0.03	0
3	Sock wash	dH2O	20	14.51	0.05	0
3	Sock wash	dH2O	20	14.07		0
3	Sock wash	Aerated	0.1	0.05		0
3	Sock wash	Aerated	0.25	0.12		0
3	Sock wash	Aerated	0.5	0.30		0
3	Sock wash	Aerated	1	0.68		0
3	Sock wash	Aerated	1	0.65		0
3	Sock wash	Aerated	1	0.66		0
3	Sock wash	Aerated	5	2.83	0.00	0
3	Sock wash	Aerated	5	3.07	0.01	40
3	Sock wash	Non-Aerated	0.1	0.03		0
3	Sock wash	Non-Aerated	0.1	0.03		0
3	Sock wash	Non-Aerated	0.1	0.02		0
3	Sock wash	Non-Aerated	0.25	0.11		0
3	Sock wash	Non-Aerated	0.5	0.28		0

3	Sock wash	Non-Aerated	1	0.64		0
3	Sock wash	Non-Aerated	5	2.98	0.02	0
3	Sock wash	Non-Aerated	5	2.66	0.00	40
3	Soap Control	dH2O	0	0.03	0.00	0
3	Soap Control	Aerated	0	0.00		0
3	Soap Control	Aerated	0	0.01		0
3	Soap Control	Aerated	0	0.01		0
3	Soap Control	Aerated	0	0.00		0
3	Soap Control	Aerated	0	0.00	0.00	0
3	Soap Control	Aerated	0	0.00		40
3	Soap Control	Non-Aerated	0	0.00		0
3	Soap Control	Non-Aerated	0	0.00		0
3	Soap Control	Non-Aerated	0	0.00		0
3	Soap Control	Non-Aerated	0	0.00		0
3	Soap Control	Non-Aerated	0	0.01	0.00	0
3	Soap Control	Non-Aerated	0	0.02		40

Table C.4: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for AWCD from the non-aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested.

	Concentration of Silver (mg/L)					
Treatment	**0.1**	**0.25**	**0.5**	**1**	**5**	**10**
Ag+	CD	B	C	B	A	A
CMC	BC	BC	BC	B	A	A
Sock Wash	A	A	A	A	A	
Soap	A	A	A	A	A	
Citrate	D	C	E	C		
Uncoated	B	B	D	C	B	B
PVP	BC	BC	DE	C	B	B
Sulphidized	A	A	B	B	A	

Table C.5: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for richness from the non-aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested.

	Concentration of Silver (mg/L)					
Treatment	**0.1**	**0.25**	**0.5**	**1**	**5**	**10**
Ag+	CD	BC	B	BC	A	-
CMC	C	C	B	BC	A	-
Sock Wash	A	AB	A	A	A	
Soap	AB	A	A	B	A	
Citrate	D	C	C	D		
Uncoated	B	BC	C	D	C	A
PVP	C	BC	C	D	C	A
Sulphidized	A	A	B	C	B	

Table C.6: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for AWCD from the aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested. Dashes indicate treatments for which a post-hoc analysis could not be performed because there were too few treatments. In this case the results were not significantly different ($p<0.05$) between Ag+ and CMC treatments at concentrations between 0.005 and 0.05.

	Concentration of Silver (mg/L)									
Treatment	**0.005**	**0.01**	**0.025**	**0.05**	**0.1**	**0.25**	**0.5**	**1**	**5**	**10**
Ag+	-	-	-	-	A	A	A	A	A	A
CMC	-	-	-	-	A	A	A	A	A	A
Sock Wash					A	A	A	B	A	
Soap					B	B	A	B	A	
Citrate					B	AB	A	AB		
Uncoated					C	BC	B	B	A	A
PVP					C	C	B	B	A	A
Sulphidized					B	B	B	C	B	

Table C.7: Summary table for One-Way ANOVA ($p<0.05$) with post-hoc Tukey HSD performed to discern statically related groupings for silver treatments at each concentration. The results displayed here for richness from the non-aerated microbial communities. Values in each column of this table can only be compared to other values within the same column, not between columns. Grey boxes indicate concentrations which were not tested. Dashes indicate treatments for which a post-hoc analysis could not be performed because there were too few treatments. In this case the results were not significantly different ($p<0.05$) between Ag+ and CMC treatments at concentrations between 0.005 and 0.05. Forward slash indicates treatments for which a post-hoc analysis could not be performed because all values were zero.

	Concentration of Silver (mg/L)									
Treatment	**0.005**	**0.01**	**0.025**	**0.05**	**0.1**	**0.25**	**0.5**	**1**	**5**	**10**
Ag+	-	-	-	-	A	A	A	A	A	/
CMC	-	-	-	-	A	A	A	A	A	/
Sock Wash					A	A	A	AB	A	
Soap					B	C	A	AB	A	
Citrate					B	B	A	AB		
Uncoated					C	C	B	B	A	/
PVP					C	D	BC	B	A	/
Sulphidized					B	C	C	C	B	

Table C.8: Summary of Ag NP dose-response data for non-aerated microbial communities and associated p values from a 2-sided Dunnett's test comparing dose concentration response (0.1 - 10 mg/L) with the control response (0 mg/L). Average raw data is displayed in this table for average well colour development (AWCD) and Richness. Triplicate raw data from each BIOLOG EcoPlate™ was used to compute ANOVA and the subsequent 2-sided Dunnett's test. Grey squares are used to represent concentrations which were not tested.

	Dose (mg/L)							**MCLCEC (mg/L)**
	0	**0.1**	**0.25**	**0.5**	**1**	**5**	**10**	
Ag+								
AWCD	0.829	0.923	0.754	0.501	0.401	0.016	0.018	**0.5**
Dunnett *p* value	-	0.307	0.635	**0.0001**	**0.0001**	**0.0001**	**0.0001**	
Richness	21	21	19	12	11	0	0	**0.5**
Dunnett *p* value	-	1	0.144	**0.0001**	**0.0001**	**0.0001**	**0.0001**	
Citrate								
AWCD	0.829	1.035	0.994	0.969	0.948			**0.1[a]**
Dunnett *p* value	-	**0.048[a]**	0.131	0.243	0.205			
Richness	21	23	21	22	20			**N/A**
Dunnett *p* value	-	0.62	1	0.956	1			
Uncoated								
AWCD	0.829	0.710	0.777	0.792	0.795	0.828	0.799	**N/A**
Dunnett *p* value	-	0.077	0.788	0.953	0.971	1	0.812	
Richness	20	16	19	18	19	19	18	**0.1***
Dunnett *p* value	-	**0.015***	0.470	0.315	0.958	0.837	0.201	
PVP								
AWCD	0.829	0.862	0.802	0.819	0.855	0.814	0.790	**N/A**
Dunnett *p* value	-	0.999	1	1	1	1	0.997	
Richness	21	20	19	18	19	19	20	**N/A**
Dunnett *p* value	-	0.985	0.651	0.495	0.926	0.806	0.974	
CMC								
AWCD	0.829	0.847	0.839	0.423	0.377	0.026	0.034	**0.5**
Dunnett *p* value	-	1	1	**0.028**	**0.014**	**0.002**	**0.0001**	
Richness	21	20	20	12	10	0	0	**0.5**
Dunnett *p* value	-	0.795	0.968	**0.0001**	**0.0001**	**0.0001**	**0.0001**	
Sulphidized								
AWCD	0.431	0.367	0.363	0.340	0.343	0.372		**N/A**
Dunnett *p* value	-	0.312	0.267	0.082	0.097	0.395		
Richness	14	12	13	13	12	12		**N/A**
Dunnett *p* value	-	0.495	0.712	0.990	0.495	0.495		
Sock Wash								
AWCD	0.431	0.428	0.475	0.062	0.075	0.045		**0.5**
Dunnett *p* value	-	1	0.441	**0.0001**	**0.0001**	**0.0001**		
Richness	14	13	15	5	0	0		**0.5**
Dunnett *p* value	-	0.883	0.987	**0.0001**	**0.0001**	**0.0001**		

[a]The MCLCEC is reported for a significantly different positive effect.
*Only concentration at which there is a significant difference from the control in the series.
MCLCEC = minimum community level catabolic effect concentration. N/A = not applicable.

Table C.9: Summary of Ag NP dose-response data for aerated microbial communities and associated p values from a 2-sided Dunnett's test comparing dose concentration response (0.005 - 10 mg/L) with the control response (0 mg/L). Average raw data is displayed in this table for average well colour development (AWCD) and Richness. Triplicate raw data from each BIOLOG EcoPlate™ was used to compute ANOVA and the subsequent 2-sided Dunnett's test. Grey squares are used to represent concentrations which were not tested.

	Dose (mg/L)							**MCLCEC (mg/L)**
	0	**0.005**	**0.01**	**0.025**	**0.05**	**0.1**		
Ag+								
AWCD	0.290	0.145	0.070	0.020	0.013	0.013		**0.005**
Dunnett *p* value	-	**0.022**	**0.001**	**0.0001**	**0.0001**	**0.0001**		
Richness	7	4	1	0	0	0		**0.01**
Dunnett *p* value	-	**0.110**	**0.001**	**0.0003**	**0.0002**	**0.0002**		
CMC								
AWCD	0.290	0.013	0.025	0.008	0.015	0.007		**0.005**
Dunnett *p* value	-	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**		
Richness	7	0	0	0	0	0		**0.005**
Dunnett *p* value	-	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**		
	Dose (mg/L)							**MCLCEC (mg/L)**
	0	**0.1**	**0.25**	**0.5**	**1**	**5**	**10**	
Citrate								
AWCD	0.410	0.190	0.101	0.040	0.029			**0.1**
Dunnett *p* value	-	**0.002**	**0.0001**	**0.0001**	**0.0001**			
Richness	11	6	2	1	1			**0.1**
Dunnett *p* value	-	**0.015**	**0.0002**	**0.0001**	**0.0001**			
Uncoated								
AWCD	0.410	0.426	0.216	0.178	0.086	0.009	0.010	**0.25**
Dunnett *p* value	-	1	**0.009**	**0.002**	**0.0001**	**0.0001**	**0.0001**	
Richness	11	12	6	6	3	0	0	**0.25**
Dunnett *p* value		0.958	**0.003**	**0.002**	**0.0001**	**0.0001**	**0.0001**	
PVP								
AWCD	0.410	0.426	0.315	0.253	0.102	0.005	0.006	**1**
Dunnett *p* value	-	1	0.403	0.058	0.0003	**0.0001**	**0.0001**	
Richness	11	12	10	6	3	0		**0.5**
Dunnett *p* value	-	0.977	0.751	**0.019**	**0.0001**	**0.0001**	**0.0001**	
Sulphidized								
AWCD	0.229	0.183	0.194	0.227	0.220	0.207		**N/A**
Dunnett *p* value	-	0.544	0.566	1	1	0.98		
Richness	9	7	7	9	8	9		**N/A**
Dunnett *p* value	-	0.316	0.495	1	1	1		
Sock Wash								
AWCD	0.229	0.010	0.041	0.065	0.092	0.040		**0.1**
Dunnett *p* value	-	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**		
Richness	9	0	0	1	2	0		**0.1**
Dunnett *p* value	-	0.0001	0.0001	0.0001	0.0001	0.0001		

MCLCEC = minimum community level catabolic effect concentration. N/A = not applicable.

Table C.10: Compiled CLPP raw data from ex-situ silver nanoparticle exposures on wetland interstitial water microbial communities. Average well colour development (AWCD) and richness and represented as a mean of the three-replicated carbon source utilization patterns (CSUPs) present on the BIOLOG EcoPlate™.

				Mean		Standard Deviation	
Experiment	Type of Silver	Concentration (mg/L)	Water Type	AWCD	Richness	AWCD	Richness
1	Blank	0	Non-Aerated	0.83	20.67	0.06	1.53
1	Blank	0	Aerated	0.41	11.33	0.10	3.06
2	Blank	0	Aerated	0.29	6.67	0.05	0.58
3	Blank	0	Non-Aerated	0.43	14.00	0.05	1.00
3	Blank	0	Aerated	0.23	8.67	0.04	0.58
1	Ag+	0.1	Non-Aerated	0.93	20.67	0.09	0.58
1	Ag+	0.25	Non-Aerated	0.75	18.67	0.06	0.58
1	Ag+	0.5	Non-Aerated	0.50	11.67	0.04	1.15
1	Ag+	1	Non-Aerated	0.40	10.67	0.10	1.53
1	Ag+	5	Non-Aerated	0.02	0.00	0.00	0.00
1	Ag+	10	Non-Aerated	0.02	0.00	0.00	0.00
1	Ag+	0.1	Aerated	0.00	0.00	0.00	0.00
1	Ag+	0.25	Aerated	0.01	0.00	0.00	0.00
1	Ag+	0.5	Aerated	0.01	0.00	0.00	0.00
1	Ag+	1	Aerated	0.01	0.00	0.00	0.00
1	Ag+	5	Aerated	0.01	0.00	0.00	0.00
1	Ag+	10	Aerated	0.00	0.00	0.00	0.00
2	Ag+	0	Aerated	0.15	4.00	0.09	2.65
2	Ag+	0.005	Aerated	0.07	1.00	0.06	1.00
2	Ag+	0.025	Aerated	0.02	0.33	0.01	0.58
2	Ag+	0.05	Aerated	0.01	0.00	0.00	0.00
2	Ag+	0.1	Aerated	0.01	0.00	0.01	0.00
3	Ag+	0.1	Non-Aerated	0.43	12.67	0.04	2.08
3	Ag+	0.25	Non-Aerated	0.29	10.67	0.03	2.08
3	Ag+	0.5	Non-Aerated	0.13	4.33	0.01	0.58
3	Ag+	1	Non-Aerated	0.09	3.67	0.03	0.58
3	Ag+	5	Non-Aerated	0.01	0.00	0.00	0.00
3	Ag+	0.1	Aerated	0.00	0.00	0.00	0.00
3	Ag+	0.25	Aerated	0.00	0.00	0.00	0.00
3	Ag+	0.5	Aerated	0.00	0.00	0.00	0.00
3	Ag+	1	Aerated	0.00	0.00	0.00	0.00
3	Ag+	5	Aerated	0.00	0.00	0.00	0.00
1	CMC Ag NPs	0.1	Non-Aerated	0.85	19.67	0.03	1.15
1	CMC Ag NPs	0.25	Non-Aerated	0.84	20.00	0.07	1.00

1	CMC Ag NPs	0.5	Non-Aerated	0.42	12.33	0.04	0.58
1	CMC Ag NPs	1	Non-Aerated	0.38	10.00	0.02	1.00
1	CMC Ag NPs	5	Non-Aerated	0.03	0.00	0.01	0.00
1	CMC Ag NPs	10	Non-Aerated	0.03	0.00	0.01	0.00
1	CMC Ag NPs	0.1	Aerated	0.01	0.00	0.00	0.00
1	CMC Ag NPs	0.25	Aerated	0.01	0.00	0.00	0.00
1	CMC Ag NPs	0.5	Aerated	0.01	0.00	0.00	0.00
1	CMC Ag NPs	1	Aerated	0.00	0.00	0.00	0.00
1	CMC Ag NPs	5	Aerated	0.00	0.00	0.00	0.00
1	CMC Ag NPs	10	Aerated	0.02	0.00	0.00	0.00
2	CMC Ag NPs	0.005	Aerated	0.01	0.00	0.00	0.00
2	CMC Ag NPs	0.01	Aerated	0.02	0.00	0.01	0.00
2	CMC Ag NPs	0.025	Aerated	0.01	0.00	0.01	0.00
2	CMC Ag NPs	0.05	Aerated	0.02	0.00	0.01	0.00
2	CMC Ag NPs	0.1	Aerated	0.01	0.00	0.00	0.00
1	PVP Ag NPs	0.1	Non-Aerated	0.86	19.67	0.10	2.08
1	PVP Ag NPs	0.25	Non-Aerated	0.80	18.67	0.05	0.58
1	PVP Ag NPs	0.5	Non-Aerated	0.82	18.33	0.12	3.06
1	PVP Ag NPs	1	Non-Aerated	0.85	19.33	0.08	1.53
1	PVP Ag NPs	5	Non-Aerated	0.81	19.00	0.08	1.00
1	PVP Ag NPs	10	Non-Aerated	0.79	19.67	0.05	0.58
1	PVP Ag NPs	0.1	Aerated	0.43	12.33	0.06	1.53
1	PVP Ag NPs	0.25	Aerated	0.32	9.67	0.09	0.58
1	PVP Ag NPs	0.5	Aerated	0.25	6.67	0.06	2.08
1	PVP Ag NPs	1	Aerated	0.10	3.00	0.04	1.00
1	PVP Ag NPs	5	Aerated	0.00	0.00	0.00	0.00
1	PVP Ag NPs	10	Aerated	0.01	0.00	0.00	0.00
1	Uncoated Ag NPs	0.1	Non-Aerated	0.71	16.33	0.07	0.58
1	Uncoated Ag NPs	0.25	Non-Aerated	0.78	18.67	0.06	1.53
1	Uncoated Ag NPs	0.5	Non-Aerated	0.79	18.33	0.03	0.58
1	Uncoated Ag NPs	1	Non-Aerated	0.79	19.67	0.02	1.15
1	Uncoated Ag NPs	5	Non-Aerated	0.83	19.33	0.04	0.58
1	Uncoated Ag NPs	10	Non-Aerated	0.78	18.00	0.06	2.65
1	Uncoated Ag NPs	0.1	Aerated	0.43	12.33	0.09	1.53
1	Uncoated Ag NPs	0.25	Aerated	0.22	6.00	0.06	1.00
1	Uncoated Ag NPs	0.5	Aerated	0.18	5.67	0.06	0.58
1	Uncoated Ag NPs	1	Aerated	0.09	3.00	0.02	1.00
1	Uncoated Ag NPs	5	Aerated	0.01	0.00	0.00	0.00
1	Uncoated Ag NPs	10	Aerated	0.01	0.00	0.00	0.00
1	Citrate Ag NPs	0.1	Non-Aerated	1.03	23.00	0.03	1.00

1	Citrate Ag NPs	0.25	Non-Aerated	0.99	21.33	0.13	3.51
1	Citrate Ag NPs	0.5	Non-Aerated	0.97	22.00	0.06	1.00
1	Citrate Ag NPs	1	Non-Aerated	0.95	20.67	0.06	1.15
1	Citrate Ag NPs	0.1	Aerated	0.19	6.33	0.04	0.58
1	Citrate Ag NPs	0.25	Aerated	0.10	2.33	0.02	0.58
1	Citrate Ag NPs	0.5	Aerated	0.04	1.00	0.01	1.00
1	Citrate Ag NPs	1	Aerated	0.03	0.67	0.01	0.58
3	Ag_2S Ag NPs	0.1	Non-Aerated	0.37	12.33	0.02	1.15
3	Ag_2S Ag NPs	0.25	Non-Aerated	0.36	12.67	0.01	0.58
3	Ag_2S Ag NPs	0.5	Non-Aerated	0.34	13.33	0.03	1.15
3	Ag_2S Ag NPs	1	Non-Aerated	0.34	12.33	0.03	1.53
3	Ag_2S Ag NPs	5	Non-Aerated	0.37	12.33	0.06	1.53
3	Ag_2S Ag NPs	0.1	Aerated	0.18	6.67	0.01	0.58
3	Ag_2S Ag NPs	0.25	Aerated	0.19	7.00	0.02	1.00
3	Ag_2S Ag NPs	0.5	Aerated	0.23	8.67	0.04	0.58
3	Ag_2S Ag NPs	1	Aerated	0.22	8.33	0.04	2.08
3	Ag_2S Ag NPs	5	Aerated	0.21	8.67	0.04	1.53
3	Sock Wash	0.1	Non-Aerated	0.43	13.00	0.02	1.00
3	Sock Wash	0.25	Non-Aerated	0.48	14.67	0.02	2.08
3	Sock Wash	0.5	Non-Aerated	0.06	2.33	0.02	0.58
3	Sock Wash	1	Non-Aerated	0.08	1.67	0.03	0.58
3	Sock Wash	5	Non-Aerated	0.05	1.00	0.03	1.00
3	Sock Wash	0.1	Aerated	0.01	0.00	0.01	0.00
3	Sock Wash	0.25	Aerated	0.04	0.00	0.01	0.00
3	Sock Wash	0.5	Aerated	0.06	1.33	0.03	0.58
3	Sock Wash	1	Aerated	0.09	2.33	0.04	0.58
3	Sock Wash	5	Aerated	0.04	0.33	0.00	0.58
3	Soap Control	0.1	Non-Aerated	0.38	13.67	0.03	1.15
3	Soap Control	0.25	Non-Aerated	0.40	13.33	0.08	1.15
3	Soap Control	0.5	Non-Aerated	0.17	6.00	0.03	1.00
3	Soap Control	1	Non-Aerated	0.16	7.33	0.03	1.53
3	Soap Control	5	Non-Aerated	0.03	0.33	0.01	0.58
3	Soap Control	0.1	Aerated	0.16	7.00	0.04	0.00
3	Soap Control	0.25	Aerated	0.16	7.33	0.02	0.58
3	Soap Control	0.5	Aerated	0.06	1.33	0.02	0.58
3	Soap Control	1	Aerated	0.09	2.33	0.02	0.58
3	Soap Control	5	Aerated	0.02	0.33	0.01	0.58

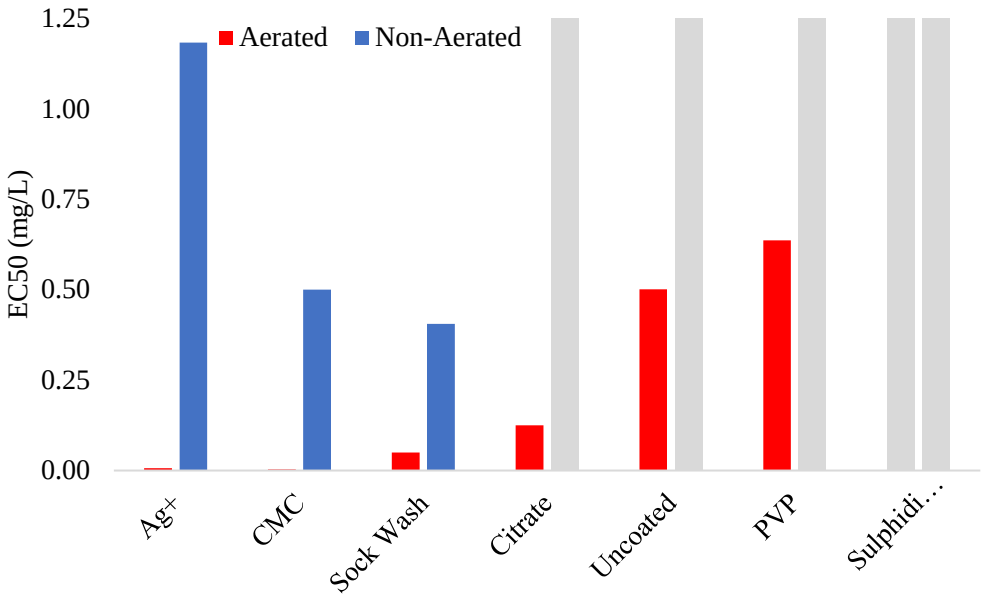

Figure C.1: Summary of EC50s for aerated (red) and non-aerated (blue) microbial communities treated with various types of silver and silver nanoparticles. EC_{50}s were calculated by linear interpolation for richness. Grey bars = EC_{50} could not be calculated for concentrations in this studies dose-response curve as they were not high enough to create a 50% effect. EC_{50}s were calculated using raw data.

Statistical Tables for Chapter 5: EFFECTS OF SILVER NANOPARTICLES ON CONSTRUCTED WETLAND MICROBIAL COMMUNITIES

Aerated – Average Well Colour Development

Table C.11: One-Way ANOVA for Differences Between Silver Treatments, Tests of Between-Subjects Effects for Ag+ and CMC Ag NPs. Only two silver treatments performed at these concentrations therefore no post-hoc Tukey pairings could be calculated.

Concentration of Ag (mg/L)	Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
0.005	Ag	.026	1	.026	6.278	.066	.611
0.01	Ag	.003	1	.003	2.007	.229	.334
0.025	Ag	.000	1	.000	3.216	.147	.446
0.05	Ag	1.350E-5	1	1.350E-5	.494	.521	.110

Table C.12: One-Way ANOVA post-hoc Tukey HSD groupings

0.1 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Ag	3	.00367		
	CMC	3	.00733		
	Sock	3	.01033		
	Soap	3		.16167	
	Ag2S	3		.18300	
	Citrate	3		.19000	
	Uncoated	3			.42633
	PVP	3			.42733
	Sig.		1.000	.991	1.000

Table C.13: One-Way ANOVA post-hoc Tukey HSD groupings

0.25 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Ag	3	.00600		
	CMC	3	.00800		
	Sock	3	.04133		
	Citrate	3	.10100	.10100	
	Soap	3		.16433	
	Ag2S	3		.18400	

	Uncoated	3		.21633	.21633
	PVP	3			.31500
	Sig.		.161	.055	.134

Table C.14: One-Way ANOVA post-hoc Tukey HSD groupings

0.5 mg/L				
	Ag Type	N	Subset	
			1	2
Tukey HSD	Ag	3	.00733	
	CMC	3	.00867	
	Citrate	3	.04033	
	Soap	3	.05633	
	Sock	3	.06467	
	Uncoated	3		.17867
	Ag2S	3		.22733
	PVP	3		.25267
	Sig.		.495	.219

Table C.15: One-Way ANOVA post-hoc Tukey HSD groupings

1 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	CMC	3	.00433		
	Ag	3	.00900		
	Citrate	3	.02900	.02900	
	Uncoated	3		.08600	
	Soap	3		.08767	
	Sock	3		.09200	
	PVP	3		.10200	
	Ag2S	3			.22000
	Sig.		.930	.051	1.000

Table C.16: One-Way ANOVA post-hoc Tukey HSD groupings

5 mg/L				
	Ag Type	N	Subset	
			1	2
Tukey HSD	CMC	3	.00400	

	PVP	3	.00467	
	Uncoated	3	.00867	
	Ag	3	.01133	
	Soap	3	.02133	
	Sock	3	.03967	
	Ag2S	3		.20767
	Sig.		.126	1.000

Table C.17: One-Way ANOVA post-hoc Tukey HSD groupings

10 mg/L			
	Ag Type	N	Subset
			1
Tukey HSD	Ag	3	.00433
	Uncoated	3	.00967
	CMC	3	.01433
	PVP	3	.02967
	Sig.		.523

Aerated – Richness

Table C.18: One-Way ANOVA for Differences Between Silver Treatments, Tests of Between-Subjects Effects for Ag+ and CMC Ag NPs. Only two silver treatments performed at these concentrations therefore no post-hoc Tukey pairings could be calculated.

Concentration of Ag (mg/L)	Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
0.005	Ag	24.000	1	24.000	6.857	.059	.632
0.01	Ag	1.500	1	1.500	3.000	.158	.429
0.025	Ag	.167	1	.167	1.000	.374	.200
0.05	Ag	.000	1	.000	.	.	.

Table C.19: One-Way ANOVA post-hoc Tukey HSD groupings

0.1 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Ag	3	.00000		
	CMC	3	.00000		
	Sock	3	.00000		
	Citrate	3		6.33333	
	Ag2S	3		6.66667	
	Soap	3		7.00000	

	PVP	3			12.3333 3
	Uncoated	3			12.3333 3
	Sig.		1.000	.968	1.000

Table C.20: One-Way ANOVA post-hoc Tukey HSD groupings

0.25 mg/L						
	Ag Type	N	Subset			
			1	2	3	4
Tukey HSD	Ag	3	.00000			
	CMC	3	.00000			
	Sock	3	.00000			
	Citrate	3		2.33333		
	Uncoated	3			6.00000	
	Ag2S	3			7.00000	
	Soap	3			7.33333	
	PVP	3				9.66667
	Sig.		1.000	1.000	.202	1.000

Table C.21: One-Way ANOVA post-hoc Tukey HSD groupings

0.5 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Ag	3	.00000		
	CMC	3	.00000		
	Citrate	3	1.00000		
	Soap	3	1.33333		
	Sock	3	1.33333		
	Uncoated	3		5.66667	
	PVP	3		6.66667	6.66667
	Ag2S	3			8.66667
	Sig.		.635	.870	.197

Table C.22: One-Way ANOVA post-hoc Tukey HSD groupings

1 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Ag	3	.00000		
	CMC	3	.00000		

	Citrate	3	.66667	.66667	
	Soap	3	2.33333	2.33333	
	Sock	3	2.33333	2.33333	
	PVP	3		3.00000	
	Uncoated	3		3.00000	
	Ag2S	3			8.33333
	Sig.		.119	.119	1.000

Table C.23: One-Way ANOVA post-hoc Tukey HSD groupings

5 mg/L				
	Ag Type	N	Subset	
			1	2
Tukey HSD	Ag	3	.00000	
	CMC	3	.00000	
	PVP	3	.00000	
	Uncoated	3	.00000	
	Soap	3	.33333	
	Sock	3	.33333	
	Ag2S	3		8.66667
	Sig.		.995	1.000

Non-Aerated – Average Well Colour Development

Table C.24: One-Way ANOVA post-hoc Tukey HSD groupings

0.1 mg/L						
	Ag Type	N	Subset			
			1	2	3	4
Tukey HSD	Ag2S	3	.36667			
	Soap	3	.37633			
	Sock	3	.42733			
	Uncoated	3		.70967		
	CMC	3		.84700	.84700	
	PVP	3		.86233	.86233	
	Ag	3			.93233	.93233
	Citrate	3				1.03467
	Sig.		.885	.070	.608	.400

Table C.25: One-Way ANOVA post-hoc Tukey HSD groupings

0.25 mg/L					
	Ag Type	N	Subset		
			1	2	3

Tukey HSD	Ag2S	3	.36333		
	Soap	3	.40033		
	Sock	3	.47500		
	Ag	3		.75367	
	Uncoated	3		.77733	
	PVP	3		.80233	.80233
	CMC	3		.83900	.83900
	Citrate	3			.99467
	Sig.		.549	.808	.064

Table C.26: One-Way ANOVA post-hoc Tukey HSD groupings

0.5 mg/L							
	Ag Type	N	Subset				
			1	2	3	4	5
Tukey HSD	Sock	3	.06233				
	Soap	3	.16800				
	Ag2S	3		.34000			
	CMC	3		.42267	.42267		
	Ag	3			.50133		
	Uncoated	3				.79200	
	PVP	3				.81900	.81900
	Citrate	3					.96867
	Sig.		.330	.609	.662	.998	.065

Table C.27: One-Way ANOVA post-hoc Tukey HSD groupings

1 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Sock	3	.07533		
	Soap	3	.16033		
	Ag2S	3		.34333	
	CMC	3		.37700	
	Ag	3		.40100	
	Uncoated	3			.79500
	PVP	3			.85500
	Citrate	3			.94833
	Sig.		.570	.891	.054

Table C.28: One-Way ANOVA post-hoc Tukey HSD groupings

5 mg/L				
	Ag Type	N	Subset	
			1	2
Tukey HSD	Ag	3	.01567	
	Soap	3	.03267	
	Sock	3	.04567	
	CMC	3	.24167	
	Ag2S	3	.37200	
	PVP	3		.81400
	Uncoated	3		.82800
	Sig.		.109	1.000

Table C.29: One-Way ANOVA post-hoc Tukey HSD groupings

10 mg/L				
	Ag Type	N	Subset	
			1	2
Tukey HSD	Ag	3	.01867	
	CMC	3	.03367	
	Uncoated	3		.77900
	PVP	3		.79033
	Sig.		.967	.985

Non-Aerated – Richness

Table C.30: One-Way ANOVA post-hoc Tukey HSD groupings

0.1 mg/L						
	Ag Type	N	Subset			
			1	2	3	4
Tukey HSD	Ag2S	3	12.33333			
	Sock	3	13.00000			
	Soap	3	13.66667	13.66667		
	Uncoated	3		16.33333		
	CMC	3			19.66667	
	PVP	3			19.66667	

	Ag	3			20.66667	20.66667
	Citrate	3				23.00000
	Sig.		.848	.167	.960	.288

Table C.31: One-Way ANOVA post-hoc Tukey HSD groupings

0.25 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Ag2S	3	12.66667		
	Soap	3	13.33333		
	Sock	3	14.66667	14.66667	
	Ag	3		18.66667	18.66667
	PVPA	3		18.66667	18.66667
	Uncoated	3		18.66667	18.66667
	CMC	3			20.00000
	Citrate	3			21.33333
	Sig.		.814	.130	.537

Table C.32: One-Way ANOVA post-hoc Tukey HSD groupings

0.5 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Sock	3	2.33333		
	Soap	3	6.00000		
	Ag	3		11.66667	
	CMC	3		12.33333	
	Ag2S	3		13.33333	
	PVP	3			18.33333

	Uncoated	3			18.33333
	Citrate	3			22.00000
	Sig.		.070	.802	.070

Table C.33: One-Way ANOVA post-hoc Tukey HSD groupings

1 mg/L						
	Ag Type	N	Subset			
			1	2	3	4
Tukey HSD	Sock	3	1.66667			
	Soap	3		7.33333		
	CMC	3		10.00000	10.00000	
	Ag	3		10.66667	10.66667	
	Ag2S	3			12.33333	
	PVP	3				19.33333
	Uncoated	3				19.66667
	Citrate	3				20.66667
	Sig.		1.000	.087	.393	.899

Table C.34: One-Way ANOVA post-hoc Tukey HSD groupings

5 mg/L					
	Ag Type	N	Subset		
			1	2	3
Tukey HSD	Ag	3	.00000		
	CMC	3	.00000		
	Soap	3	.33333		
	Sock	3	1.00000		
	Ag2S	3		12.33333	
	PVP	3			19.00000
	Uncoated	3			19.33333
	Sig.		.768	1.000	.999

Table C.35: One-Way ANOVA post-hoc Tukey HSD groupings

10 mg/L				
	Ag Type	N	Subset	
			1	2
Tukey HSD	Citrate	3	.00000	
	PVP	3	.00000	
	Ag2S	3		18.0000
	Soap	3		19.6666 7
	Sig.		1.000	.476