IN LAKE FLOATING TREATMENT WETLANDS COULD PROVIDE ALGAE CONTROL THROUGH UNSUSPECTED MECHANISMS

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ABSTRACT

Cultural eutrophication has been negatively impacting both artificial and natural water bodies by triggering (at times harmful) algal blooms. These blooms are typically controlled by algaecides, which can collaterally affect aquatic organisms thus further damaging the hydrosystem. Preventing point and non-point nutrient sources from entering hydrosystems may not be sufficient to prevent cultural eutrophication since internal nutrient loading is generally high. Internal loading can however be reduced, often at great expense, through i) demucking, ii) chemical treatment, and iii) hypolimnetic aeration. Floating Treatment Wetlands (FTWs) are a new type of phytoremediation being used to reduce the impact of eutrophication. FTWs consist of plants grown hydroponically on a floating mat which uptake water nutrients from their roots. The nutrients become tied up in the tissues of the young growing plants, which tissues are then harvested before fully mature to remove the excess nutrients from the system to reduce external and internal loading. Nutrient removal performed by these FTWs is limited unless they cover 5-10% of the surface of the water body. However, most hydrosystems use a lower, e.g. 3% cover rate with often great algal control. Thus, poorly understood or identified mechanisms must be at play. A 2-year investigation was therefore led in three subtropical manmade urban ponds (Pond A, Livingston Pond and Collier Pond) of about an acre and located within the City of Naples, FL to examine whether the dense root network from FTWs' plants, *Juncus effusus* and *Canna flaccida*, would i) have allelochemicals able to control algae, ii) offer diurnal zooplankton protection against predation so that nocturnal grazing would be intensified and iii) harbor beneficial bacteria able to remove water nutrients. For the allelopathy experiments, two methods were used: a liquid culture assay as well as an agar

diffusion assay, both in which wells containing algae were inoculated with methanolic extracts from the roots potentially containing allelochemicals. Zooplankton presence/absence studies were completed by performing vertical tows underneath the FTW and in the open water column both during the day and night to explore their nycthemeral horizontal migrations. *J. effusus* and *C. flaccida* root microbial communities were characterized by extracting DNA from the biofilm living on root samples using the phenol-chloroform extraction method. The purified DNA was used for 16S rRNA gene high-throughput sequencing to compare community structure. Results showed that chemicals present in the two plants examined could either control algae (especially Cyanophyceae) but in some cases also enhance algae growth (especially Chlorophyceae). When inhibition was found, the agar diffusion assay displayed stronger inhibition than the liquid culture assay in which allelopathic compounds were diluted in liquid medium. In Livingston Pond and Collier Pond, it was determined that zooplankton did not utilize the root systems of FTWs, which is typical of subtropical systems. The bacterial community of Pond A FTWs was typical of oxic as well as anoxic and even anaerobic environments despite the presence of dissolved oxygen in the water underneath the FTWs. Based on the microbial community composition, it is hypothesized that the microbial biofilm growing on the root changed from oxic, to anoxic and anaerobic from its surface to its basal layer. Thus, FTW biofilms provide an environment in which major biologically mediated reactions could potentially occur (e.g. nitrification, denitrification, and sulfate reduction). Based on the results of this study it appears that FTWs have potential mechanisms to control algae growth aside from nutrient uptake. Allelopathy and a microenvironment prone to degradation were such mechanisms but more investigation should be conducted to fully understand the net algal

control and impact on nutrient cycling. Future research should include the investigation of these parameters on FTWs in different hydrosystems to determine if the findings of this study are common for all FTWs, or if each FTW provides its own unique environment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

All hydrosystems increase their trophic status (i.e. level of productivity) through a natural increase in nutrients often referred to as eutrophication. However, this natural process has largely been increased through cultural eutrophication where nutrient loading is enhanced by human activities. Cultural eutrophication, in freshwater in particular, has become an increasingly serious and universal problem accounting for half of the impaired lakes (as designated by the EPA's Clean Water Act) in the United States (Carpenter et al. 1999). Cultural eutrophication generally arises from non-point pollution sources, or those that are not easily discerned e.g. land runoff, drainage, seepage, and precipitation. Agricultural runoff appears to be a major cause of eutrophication (Imboden 1974). Chemical fertilizers containing phosphorus and nitrogen, mainly used for agriculture, turf, and other ornamental plants enter water bodies through runoff from sheet flow across the landscape or percolate down through soil into groundwater sources (Imboden 1974). Additionally, chemical fertilizers used for landscaping in suburban areas (Bennion et al. 2007) and sewage effluent that is not properly treated also contribute to the cultural eutrophication problem (Bachmann et al. 1999). Phosphorus is generally the most problematic nutrient (Van der Does et al. 1992) because it mainly limits primary production in limnetic systems.

Cultural eutrophication in freshwater evokes proliferation of algae and aquatic plants, which can cause subsequent problems. Increased plant growth and algal blooms may lead to decreased water transparency (Carpenter et al. 1999) which negatively affect sessile phototrophs, especially periphyton (i.e. a community dominated by attached algae) and plants due to the reduction of light penetration. Eventually, dead plant materials and

planktonic algae that cycle through the water body, are found under the water mixing zone, and then settle at the bottom of the system where they are decomposed by bacteria. This leads to a significant amount of dissolved oxygen depletion in the vicinity of the hypolimnion which can expand to the entire water column, especially at night when respiration prevails. Fish kills may occur in this condition (Carpenter et al. 1999). The overall result that persists for decades even when external nutrient loading is controlled is a recurrent internal nutrient loading (or cycling) where nutrients from decomposing plant matter trigger repeated algae blooms within the euphotic zone (Carpenter et al. 1999). Internal nutrient loading thus prevents or, to the best case, delays lake recovery from cultural eutrophication (Sondergaard et al. 2007).

The algal blooms caused by these excess nutrients can contain harmful species, typically Cyanophyceae (blue-green algae), which are a cause for concern for many researchers and environmental agencies because of their ability to release cyanotoxins (Codd et al. 1999; Briand et al. 2003; Codd et al. 2006). The contamination of cyanotoxins in water bodies used for drinking water or recreational purposes pose a threat to both animal and human health (Codd et al 1999; Briand et al. 2003; Codd et al. 2006). Cyanotoxic events have been recorded in at least 54 countries, and from 27 states within the United States alone (Codd et al. 1999). Not all cyanotoxins have the same detrimental effect on the surrounding organisms. Some toxins affect the skin, liver, or the nervous system (Codd et al. 1999; Briand et al. 2003; Codd et al. 2006) but most toxins can affect multiple organs. Furthermore, cyanotoxins can target competitive algae through allelopathy and prevent grazing since some can make the algae unpalatable (DeMott et al. 1991; Kirk & Gilbert 1992). Exposure to cyanotoxins may occur through skin contact, inhalation, haemodialysis,

and ingestion (Codd et al. 1999; Falconer 2001; Briand et al. 2003; Codd et al. 2006). Exposure to these toxins can result in adverse health effects that can range from mild irritation to death. The risk of exposure to these toxins may increase due to global climate change. Recently, based on geological records, paleobiological evidence, and physiological and ecological studies, researchers have suggested that Cyanophyceae are likely to benefit from increases in ultraviolet (UV) radiation (Paul 2008).

Cultural eutrophication can impact some systems more than others and this is especially true for stormwater manmade urban ponds. These manmade hydrosystems were constructed in response to an increasing amount of impervious surfaces (e.g. streets, driveways, parking lots) from urban development (Karouna-Renier & Sparling 2001; Karlsson et al. 2010). During precipitation events, contaminants were washed into natural water bodies, such as streams, lakes and wetlands thus altering their ecology. As a result, stormwater manmade urban ponds were built to limit such an alteration and were designed to intercept these pollutants before they enter natural bodies of water (Karouna-Renier & Sparling 2001; Karlsson et al. 2010). Stormwater ponds were designed to act as a sink for pollutants by allowing them to be absorbed by the biota or physically settle and accumulate in the sediments (Karouna-Renier & Sparling 2001; Karlsson et al. 2010). Stormwater ponds designed in the South Florida Water Management District were estimated to achieve 40-45% total nitrogen (TN) removal and 80-85% total phosphorus (TP) removal on an annual basis (Harper & Baker 2007).

However, it appears stormwater ponds often exhibit negative pollutant removal efficiencies and thus act a source rather than a sink for pollutants, including excess nutrients (Hogan & Walbridge 2007). Stormwater ponds are subject to strong anthropogenic

pressures, therefore these ponds accumulate pollutants of stormwater runoff across impervious surfaces at higher rates than what they have the ability to process. As a result, stormwater ponds are thought to promote symptoms of eutrophication e.g. harmful algal blooms and fish kills (Lewitus et al. 2008), which can lessen the ecological value of these hydrosystems.

There is a need for economically feasible and environmentally safe ways to reduce eutrophication in freshwater systems. Remediation of eutrophic systems is possible, and the reduction of external and internal nutrient loading is a key issue (Van der Does et al. 1992). Currently, algal blooms associated with eutrophic hydrosystems are mainly treated with copper-based algaecides, such as copper sulfate, copper oxychloride, chelated copper and cuprous oxide, because they are inexpensive, easy to apply, and relatively safe to humans (Garcia-Villada et al. 2004). However, these copper-based algaecides persist in the environment, and have been shown to negatively affect other organisms like zooplankton and fishes. Studies on copper toxicity have shown that Cladocerans, *Daphnia magna*, *Daphnia pulex*, and *Daphnia similis*, are highly susceptible to copper toxicity (Cyrino de Oliveira-Filho et al. 2004). The elimination of zooplankton from copper based algaecides in aquatic systems may promote algal blooms due to a lack of top-down control via grazing from these predators (e.g. Cyrino de Oliveira-Filho et al. 2004). Fishes, especially developing juveniles, are also susceptible to copper toxicity (Hanson & Stefan 1984; Straus & Tucker 1993; Karan et al. 1998). Research also points out the increase of copper tolerance in Cyanophyceae (Hanson & Stefan 1984; Garcia-Villada et al. 2004). The use of algaecides does not cure the problem of eutrophication, rather, it merely treats a symptom: thus different remediation options must be explored.

A simple way to control eutrophication is *via* hypolimnetic oxygenation (Carpenter et al. 1999). This strategy uses regular compressed air to reduce anoxia in the hypolimnion, which can bind certain nutrients e.g., phosphorus to the metal in the sediment (e.g. ferric iron) and reduce the likelihood of fish kills. Unfortunately, oxygenation is only a temporary solution because the nutrients are not removed from the system, unless used in combination with a water outfall, which allows water to reach a certain level in the hydrosystem before emptying the excess water into a drainage system. Oxygenation also tends to be more successful when used in conjunction with plants or algae that can be removed from the system after uptaking resuspended nutrients i.e. nitrate (Liboriussen et al. 2009). Another potential solution is lake dredging, which involves the removal of the nutrient-rich fine-grained organic sediment layer of the lake bed (Kleeberg & Khol 1999). This technique greatly reduces the internal loading of nutrients because the nutrients are physically removed from the system (Van der Does et al. 1992). However, it is expensive to implement, and the disposal of the sediment is costly especially when such a sediment is classified as toxic waste.

Reduction of external loading can be achieved through practices, such as biomanipulation. Ecological engineers have designed green technologies such as constructed wetlands, which utilize natural processes to improve water quality (Habrel et al. 2003). Wetlands are a transitional zone between land and water, so runoff from land must first pass through wetlands before entering the water body. Constructed wetlands essentially act as a natural filter; the macrophytes present in the system have the ability to remove excess nutrients or pollutants before they enter the water body (Haberl et al. 2003). This practice is sustainable and inexpensive, however, constructed wetlands do require

substantial surface area to be effective, which can be a limiting factor in an urban setting. They also do not reduce the internal loading of nutrients, which drives recurrent algae blooms. A more advanced way to thus engage wetland filtration involves the implementation of constructed wetlands on the water body itself.

FTWs, sometimes referred to as artificial floating islands, floating vegetation mats or eco-islands, are a simple and innovative way to remediate eutrophication in aquatic systems in which the littoral area alone does not provide sufficient filtration. This green technology consists of a floating mat upon which potted macrophytes, usually native terrestrial or riparian species, are grown hydroponically, with their roots dangling into the water through openings in the mat. The nutrient removal efficiencies of FTWs can be quite high due to the fact they have the ability to directly uptake nutrients from the water column. This eco-friendly technology has been utilized in remediating contaminated stormwater, sewage effluent, acid mine drainage, piggery effluent, poultry processing wastewater, and water supply reservoirs (Headley & Tanner 2006). It can be used in various aquatic systems e.g. rivers (Zhu et al. 2011; Zhao et al. 2012), lakes (Yang et al. 2008; Hu et al. 2010), lagoons (Hubbard et al. 2004) and manmade ponds (Chang et al. 2012a; Borne et al. 2013; Chang et al. 2013; Winston et al. 2013).

FTW platforms are composed from a wide range of construction materials and macrophytes. The materials that are used to create the platform upon which the macrophytes grow must be durable, buoyant, flexible, and have the ability to be anchored. Some materials that are often used include polyester matrix, sealed PVC pipes, polystyrene sheets, bamboo, inflatable vinyl pillows, timber frames, foam mats, and coconut fibers (Kerr-Upal et al. 2000, Smith & Kalin 2000, Hubbard et al. 2004, Van Acker et al. 2005,

Headley & Tanner 2006). The macrophytes chosen to grow on the FTWs will depend on the region in which the FTW is located, and also the time of year as some macrophytes have more efficient nutrient uptake during different seasons. Many different species of macrophytes have been grown on FTWs (summarized in Table 1). The composition of an FTW should be tailored to the system in which it is being placed as well as the region of its location.

Many studies have highlighted the successful and sometimes substantial overall water nutrient removal rates of FTWs. Removal rates for water TN have shown to be as high as 77% (Zhou & Wang 2010), and removal rates for water TP have been shown to be as high as 64% (Tanner & Headley 2011). Table 2 summarizes the removal rates for several FTW systems for mesocosm experiments and FTWs placed in river systems. There has been little investigation on the amount of FTW surface area coverage for successful nutrient concentration reduction. A study by Winston et al. (2013) investigated the effectiveness of 9% surface area coverage compared to 18% surface area coverage. The results of this study suggest a greater percent of FTW surface area coverage resulted in improved nutrient removal. The plants on FTWs have also been shown to remove other harmful contaminants such as Cu (Van de Moortel 2010; Tanner & Headley 2011; Zhao et al. 2012; Borne et al. 2013), Ni (Van de Moortel et al. 2010), Mn (Van de Moortel et al. 2010), Pb (Van de Moortel et al. 2010; Zhao et al. 2012), As (Zhao et al. 2012), Cd (Zhao et al. 2012), Hg (Zhao et al. 2012), Cr (Zhao et al. 2012), Benzene (Chen et al. 2012), and methyl *tert*-butyl ether (Chen et al. 2012).

FTWs may seem conceptually sound, but very often the nutrient removal rate is not sufficient to prevent recurrent algal blooms or very small compared to the nutrients

additionally implemented by external nutrient loading. Nutrient sampling performed in a one acre pond with 5% FTW coverage achieved no reduction in concentration; 0.31 ± 0.03 mg/L TN was measured at the hydrosystem inflow and 0.30 ± 0.05 mg/L TN was measured at the outflow while 0.06 ± 0.02 mg/L TP was measured at both the inflow and outflow of the hydrosystem. However, some aquatic systems equipped with FTWs remain paradoxically free of algal blooms despite apparently insufficient nutrient removal rates. Aside from nutrient removal, it is suspected there are several other processes which may confer additional functions for FTWs.

It is hypothesized that (1) allelopathy of macrophytes to phytoplankton, (2) grazer habitat provisioning and (3) enhancement of microbial mediated nutrient processing, could complement the success of FTWs. This gap in knowledge thus warrants further investigation of FTWs.

First, the roots of macrophytes have the ability to release allelopathic chemicals to limit the growth of potential competitors. The macrophytes that are chosen to be placed on FTWs would normally be rooted in a natural soil environment: therefore, when allelochemicals are released, it is in order to suppress the growth of other rooted macrophyte competitors at a distance (Blum et al. 1999). Since the macrophytes are grown hydroponically, the roots are exposed to water column and their release of allelochemicals may suppress the growth of periphyton and phytoplankton (summarized in Table 3). The algae may not be the original target of the macrophyte's allelochemicals, but there is still a possibility that algal growth could be inhibited or altered. In fact, research has shown that some lakes classified as eutrophic have been able to maintain a clear-water state due

to the dominance of allelopathically active submerged aquatic plants (Phillips et al. 1978; Wang et al. 2011; Pakdel et al. 2013).

Second, the root systems of FTWs may be utilized by zooplankton as a shelter. Zooplankton typically seek refuge in underwater macrophytes during the day, and leave this refuge to forage at night as a predator avoidance behavior (Lampert 1989; Ohman 1990; Bollens & Frost 1991; Loose & Dawidowicz 1994; Wojtal et al. 2003; Meerhoff et al. 2006). Zooplankton mainly feed on algae (Knisely & Gellar 1986; Kretzschmar et al. 1993), and some species of zooplankton have the ability to consume harmful algae species (Schoenberg & Carlson 1984; Haney 1987; Sarnelle 1992; Christoffersen et al. 1993; Sellner 1993; Hairston et al. 2001; Work & Havens 2003; Gyllstrom et al. 2005; Leonard & Paerl 2005; Sarnelle & Wilson 2005; Gobler et al. 2007). It is possible that zooplankton have begun utilizing the roots of FTWs for refuge in stormwater ponds that may be deprived of littoral submerged rooted vegetation. This may, in turn, promote a healthy zooplankton community to facilitate in the reduction of algae present in the system through predation. The reduction of algal biomass through zooplankton grazing could cause an increase in water transparency, which would allow macrophytes to compete with phytoplankton; potentially changing a phytoplankton dominated system back to a macrophyte dominated system (Theiss et al. 1990).

Finally, as previously mentioned, the roots of FTWs may provide a significant amount of surface area for microbes present in the system. Microbial processes such as nitrification followed by anaerobic denitrification play an integral part in reducing the nitrogen pool (Breen 1990; Morgan et al. 2008; Hu et al. 2010; Masters 2010; Zhu et al. 2011; Chang et al. 2012b). Researchers believe that increasing substrate surface for

microbial attachment in a hydrosystem can potentially enhance nutrient removal rates (Morgan et al. 2008; Stewart et al. 2008; Chang et al. 2012; Headley & Tanner 2012), which is a benefit of placing FTWs within a hydrosystem (Stewart et al. 2008; White et al. 2009; Hu et al. 2010; Masters 2010; Headley & Tanner 2012; Chang et al. 2013; Wang et al. 2015). The roots of the FTWs also trap suspended solids to create substrates (Kymbadde et al. 2005) as well as exude organic carbon which benefits microbial growth especially when they balance the carbon with the bioavailable phosphorus and nitrogen (Osem et al. 2007; Morgan et al. 2008; Hu et al. 2010; Masters 2010; Xiao et al. 2010). Microbial uptake and transformation of nutrients by the microbial communities present on the FTWs are thus important mechanisms for nutrient reduction in eutrophic hydrosystems.

In order to gain further knowledge and understanding of these potential mechanisms in subtropical stormwater ponds the objectives of this study were to:

1. Determine whether two species of macrophytes commonly grown on FTWs (i.e. *Juncus effusus* and *Canna flaccida*) synthesize allelochemicals that have the ability to suppress harmful algae, such as Cyanophyceae and other Chlorophyceae, which often bloom in (hyper-) eutrophic systems.
2. Examine if the dangling roots under FTWs provide additional refuge that would harbor enhanced abundances of herbivorous zooplankton relative to open water, and if there are horizontal diurnal migrations of zooplankton between the open water and FTW roots.
3. Characterize the microbial community on the submerged portions of an FTW to determine if any beneficial (e.g. denitrifying) bacteria are harbored

on the submerged portions of the FTWs and to elucidate the origin of plant root microbiomes deployed on FTWs.

METHODS

Study Sites

Pond A: The first study site, "Pond A" is a manmade stormwater urban pond adjacent to 7th Ave N in Naples, FL (UTM coordinates Easting: 419811 m Northing: 2893114 m, Figure 1). This stormwater pond was used for physicochemical profiling, the allelopathy study and the microbial community characterization. The north side of the pond ran adjacent to a road while the rest of the pond was surrounded by houses. Outfall boxes could be found in the northeast corner and southeast corner of the pond. At the time of investigation, the shoreline vegetation of the pond was well established. The most dominant species of shoreline vegetation included: *Typha* sp. (cattail), *Juncus effusus* (soft rush), *Pontederia cordata* (pickerelweed), *Saggitaria lancifolia* (arrowhead), *Salix caroliniana* (Carolina willow), and *Ludwigia repens* (primrose willow). Pond A contained two FTWs; one FTW was located in the northeast corner of the pond while the other FTW was centrally located. The two FTWs on this pond were approximately 2.5 m x 1.5 m in size. The plants present on these islands included *J. effusus* and *Canna flaccida* (golden canna).

Livingston Pond and Collier Pond: Zooplankton sampling was initially performed on Pond A, however zooplankton were absent from all samples which may be a result of past copper algaecide usage in the hydrosystem. Due to these unforeseen circumstances, Pond A could not be used for zooplankton sampling, therefore two additional manmade stormwater urban ponds were chosen to carry out the sampling for zooplankton. The first pond, "Livingston Pond", (Figure 1) is a stormwater pond located at the intersection of

Pine Ridge Rd and Livingston Rd. in Naples, FL. The north and east sides of the pond run adjacent to the road while the south and west sides of the pond run adjacent to natural area. Outfall boxes can be found in the northeast corner of the pond as well as the eastern portion of the pond. At the time of the study, the shoreline vegetation in the pond was minimal aside from one stand of *Juncus effusus* located on the southwest portion of the pond. The Livingston pond contained two centrally located FTWs (FTW 1 and FTW 2). The two FTWs on this pond were approximately 2.5 m x 1.5 m in size. The plants hydroponically grown on these islands included *Canna flaccida* (golden canna), *Pontederia cordata* (pickerelweed), and *Sagittaria lancifolia* (arrowhead). These FTWs were also colonized by other plants found around the pond, and these plants included *Salix caroliniana* (Carolina willow), *Ludwigia repens* (primrose willow), and *Eupatorium capillifolium* (dog fennel). The second pond, "Collier Pond", (Figure 1) is a stormwater manmade urban pond located at the intersection of Collier Blvd and 7th Ave NW in Naples, FL. The north and east sides of the pond run adjacent to the road while the south and west sides of the pond are flanked by residential houses. An outfall box can be found in the northeast corner of the pond. When studied, the shoreline vegetation was composed mostly of *J. effusus*, and the lake bed was mostly covered in *Hydrilla verticillata* (hydrilla). This pond contains one FTW (FTW 3) that is located on the eastern portion of the pond. The FTW on this pond was approximately 3 m x 1.5 m. This FTW consisted of two plant species: *J. effusus* (softrush) and *Agrostis alba*. The FTW was also colonized by other plants from the surrounding environment, and these plants included *E. capillifolium* (dog fennel) and *Cyperus distinctus* (marshland flat sedge).

Physicochemical Profiling

The FTW sampled for this project was centrally located in the pond. The sampling occurred in early March at approximately 12:00 p.m. eastern standard time. At the sampling site a YSI 650MDS coupled with a sonde 6600 was used to capture water column profiles of temperature (°C), conductivity (μS/cm), dissolved oxygen (%), dissolved oxygen (mg/L), pH (a.u.), and ORP (mV) underneath the FTW and in the open water approximately 5 m away from the FTW to compare environmental conditions. A LICOR 1400 coupled with a LI-193 4π QUANTUM type sensor was used to create light profiles for both sites as well. To create water profiles underneath the FTW for a certain parameter or set of parameters, a plant pot was removed from the center of the FTW and the sonde was lowered down through the opening. Subsurface nutrient water samples were collected underneath the FTW, and approximately 1 m away from the FTW in the open water.

Zooplankton Sampling

The sampling for this event took place from 03/11/2014 – 03/12/2014. Zooplankton were captured with a homemade net (Figure 2) which was composed of 270 μm mesh with a diameter of 50 cm. The zooplankton net was then attached to a fishing net with an aperture of equal size. A 2 m long section of PVC pipe was connected to the arm of the fishing net. A catch was constructed from plastic bottles to capture organisms as they were filtered through the net. This device will be referred to as the zooplankton vertical tow net.

At each FTW, the depth extent of the roots was first measured by lowering an underwater Aquaview camera into the water column. Once the root maximum depth was determined, the length of the roots of each FTW was marked on the arm of the adapted zooplankton vertical tow net using a zip tie (FTW 1 = red, FTW 2 = blue, FTW 3 = green). To sample underneath each FTW, the vertical tow net was pushed down into the water

column until it reached the extent of the roots marked by the corresponding zip tie color depending on the FTW being sampled. The net was then slid horizontally underneath the FTW. From there, the net was lifted up vertically until it reached the top of the island, and it was subsequently pulled horizontally back to the boat. The net was sprayed down with DI water to ensure that any organisms caught in the net mesh would be filtered down into the bottle catch. The bottle catch was removed, and its contents were emptied into a graduated cylinder to record the initial volume. The sample was homogenized, and a 100 mL subsample was taken from the initial volume. The subsample was preserved with 10% chloroform. This method was performed in triplicate underneath each FTW within 30 minutes at random non overlapping locations. Control samples were taken in the open water in triplicate in the same way the roots were sampled. This method was performed once during the day and once during the night to obtain a snapshot of zooplankton nycthemeral migrations.

Biofilm Sampling for Microbial Community Analysis

***Field Sampling*:** On the surface of the FTW, a transect measuring 140 cm, was run through the center to the edge of the FTW (Figure 3). Plant root samples as well as biofilm samples were collected from this transect at equidistant locations: 0 cm, 80 cm, and 140 cm. The plant roots sampled were harvested from *J. effusus* and *C. flaccida*. The entire length of the root was sampled, i.e. from the base of the shoot to the root tip. Two samples were taken for each plant species i.e. *Juncus* 1, *Juncus* 2, *Canna* 1 and *Canna* 2. Biofilm samples were collected not only from the foam mat the FTW was constructed with but also from the plastic pots receiving the plants. All samples were placed in a plastic bag with lake water filtered through 0.2 μm syringe filter. Subsurface nutrient water samples were collected underneath the FTW, and approximately 1 m away from the FTW in the open

water. A subsurface microbial water sample was collected approximately 1 m away from the FTW in the open water. All samples were chilled on ice in a cooler for transportation back to the lab. Upon return to the lab, the length and the plant roots weight were measured. Close-up pictures of the plant roots, foam mat, and plastic pot were then taken while viewed underneath a Nikon 580038 dissecting microscope (Figure 4).

Allelopathy Assays

Root harvest: The roots of *J. effusus* and *C. flaccida* were harvested from the FTW centrally located in the Pond A. This FTW was selected because research has shown that younger plants have a stronger inhibitory effect on algae (Burks et al. 2006; Mulderij et al. 2007; Hu & Hong 2008) and this FTW had only been present in the pond for approximately one month compared to seven months for the other FTW of the same pond.

Roots were harvested from multiple random plants of each species, and placed in plastic bags. In the laboratory, the roots were weighed (i.e. fresh weight), were washed five times with tap water, and once with deionized water to remove -as much as possible- organisms or substances that may have been present on the roots. A thorough microscopic examination after washing using a Nikon 580038 dissecting microscope confirmed that most of the materials had been removed. Additionally, pictures of the roots were taken using an Olympus Tough TG-1 iHS F2.0 as evidence of the thorough cleaning. The roots were then freeze dried in a Labconco freeze dry system for 24 hours, and ground into a fine powder using a mortar and pestle.

Allelochemical Extraction: The allelochemical extraction method chosen for this study used the techniques developed by Erhard & Gross (2006) and Mulderij et al. (2007). The chemicals from the root materials were extracted with 100% methanol (1 mL solvent per 10 mg root freeze-dry mass) for two hours at room temperature (about 21ºC) in a 500 mL

Erlenmeyer flask under continuous stirring on a Corning Laboratory stirrer plate. The methanolic extracts were then filtered onto 47 mm diameter Whatman GF/F filters (0.7 μm nominal pore size) to filter out any particles. The filtered methanolic extract was then placed in a Caliper Life Sciences TurboVap 500 and evaporated to dryness. The dry residue was resuspended with 100% methanol to yield a final concentration of 100 mg extracted dry weight (DW) per mL. All manipulations were performed under a Thermo Scientific Hamilton SafeAire II fume hood to prevent air contamination. Extracts were finally stored in glass vials at -20°C until being used.

Algal Cultures: The Cyanophyceae and Chlorophyceae cultures were issued from Dr. M. Gantar, Department of Biological Sciences of Florida International University, Miami, FL. The cultures included ten Cyanophyceae: *Microcystis* sp. (strain 22-6), *Aphanothece* sp. (strain 30-12a), *Pseudanabaena* sp. (strain 12-9-3), *Lyngbya* sp. (strain 15-2), *Microcystis* sp. (strain 36-1), *Anabaena* sp. (strain 66-2), *Microcystis* sp. (strain 46-2), *Microcystis* sp. (strain 81-11), *Limnothrix* sp. (strain 37-2-1), and *Cylindrospermopsis* sp. (strain 121-1); and two Chlorophyceae: *Chlorella* sp. (strain 2-4) and *Scenedesmus* sp. (strain 145-2). The cultures were grown in BG11 freshwater medium (Sigma-Aldrich) under 12h of 30 μE $m^{-2}s^{-1}$ of PAR at ambient laboratory temperature (approximately 21°C) in 50 mL borosilicate test tubes. The growth rates of each culture were monitored every other day using a Turner Designs 10-AU fluorometer to determine the amount of time it takes to reach their log growth phase. The log growth phase is an ideal phase for performing experiments because it is when the algae are in their healthiest point of dynamic growth (Li & Hu 2005; Mulderij et al. 2005; Wang et al. 2011; Zuo et al. 2015).

***Allelopathy Liquid Culture Bioassay*:** Prior to the start of the experiment, all cultures were diluted to 15-20 $\mu g/L^{-1}$ total Chlorophyll to simulate eutrophic conditions (based on the Trophic Status Index, Carlson 1977). The determination of the total chlorophyll was accomplished with a PHYTO PAM Pulse Amplitude Modulation tetrafluorometer equipped with the PHYTO emitting light diode (ED attachment).

The liquid culture bioassay technique was slightly modified from Erhard & Gross (2006) and Mulderij (2006). This bioassay was conducted in sterile 24-well plates. The experiment consisted of three replicates which received an aliquot of the methanolic extract from the plant roots and one control that received absolute methanol. The experiment was performed in triplicate for each culture. Each well was filled with 2 mL of culture and 1 mL of 0.05 mg DW/mL of methanolic crude extract from the root of either *J. effusus* or *C. flaccida*. The control was inoculated with 1 mL of 100% methanol. All manipulations were performed under the aforementioned Thermo Scientific Hamilton SafeAire II fume hood to prevent any air contamination.

The well plates were then placed in a Fisher Scientific Isotemp growth chamber for 5 days at 28°C under a constant illumination of 13 $\mu E\ m^{-2}s^{-1}$. The PAR of the aforementioned growth chamber was measured with a WALZ Quantum US-SQS/L PAR sensor coupled with a LICOR LI-1400 meter prior to the start of the experiment to ensure light was uniform throughout the chamber. The optical density of each well plate was measured at 650 nm (Schrader et al. 1997) at 24-hour intervals using a Tecan GENios Pro plate reader.

Allelopathy Agar Diffusion Bioassay: The agar diffusion bioassay was slightly modified from Gantar et al. (2008). This bioassay was conducted in sterile 100x15 mm agar plates.

The agar gel was composed of 20 mL of N+ (BG11 solution), 200 mL of deionized water, 0.5 g of MES buffer, and 3.72 g of Bacto® Agar set to a pH of 7.0. The agar gel was poured into the agar plates, and allowed to dry. The agar plates were split into six sextiles; five sextiles contained treatments and one sextile contained the control (Figure 5). Wells in the agar were made using a sterile glass tube (7mm in diameter). The treatment wells were filled with 50 μL of extract, and the control well was filled with 50 μL of 100% methanol. The methanol was left to evaporate from the wells under a Thermo Scientific Hamilton SafeAire II fume hood. The culture biomass was harvested by centrifugation at 3000 rpm for 15 minutes at ambient laboratory temperature (21°C), and 2 mL of concentrated culture was spread over the agar plate. The well plates were incubated in a Fisher Scientific Isotemp growth chamber for 4 days at 28°C under a constant illumination of 13 μE $m^{-2}s^{-1}$. After 4 days, all plates were photographed and inhibition zones were examined.

Microbial Community Characterization

DNA Extraction: Duplicated DNA samples were prepared for the microbial biofilms formed on the roots of *J. effusus* and *C. flaccida*. Plant root samples were sonicated in zip lock plastic bags for 40 min with 50 mL of 0.9% NaCl solution. The supernatant was transferred into a 50 mL centrifuge tube and 5 mL (*Canna* 1 and *Juncus* 1) or 15 mL (*Canna* 2 and *Juncus* 2) of suspension was filtered through 0.2 μm polycarbonate filter (25 mm diameter, Millipore). The DNA extraction was carried out using a Powerwater® DNA isolation Kit (www.MoBio.com) following manufacturer's instructions. Plastic samples from the plant pot and foam were incubated in a 50 mL centrifuge tube at 60°C with proteinase K (final concentration, 50 μg/mL) for 3 hours. The tube's content was mixed by hand and centrifuged at 8000x g for 1 min. The supernatant was transferred into 2 mL

plastic tubes. The DNA extraction of each sample was carried out using the phenol-chloroform extraction method as described by Urakawa et al. (2010). For the DNA extraction directly from water pond samples, pond water was separated into two fractions. One fraction was filtered onto a 0.2 μm filter directly. The other fraction was pre-filtered with P8 filter paper (particle size retention 20 to 25μm, ThermoFisher Scientific) before the filtration onto a 0.2 μm filter. The filters were then treated by bead beating and phenol-chloroform extraction method as described in Urakawa et al. (2010). The DNA purity was determined using a NanoDrop Spectrophotometer (Thermo Fisher Scientific).

High Throughput DNA Sequencing Analysis: The microbial communities collected from plant root, biofilm and water samples were analyzed using high-throughput sequencing to determine the microbial community present in the samples. DNA was sequenced for the V1-V3 region of 16S rRNA using the 28F and 519R primer set and a Roche 454 FLX/ FLX+ platform with titanium chemistry (Research and Testing Laboratory, Lubbock, Texas). Denoising and chimera checking were performed on all the reads for each region of data using USEARCH and UCHIIME, respectively. Individual 454 reads were further annotated using BLASTN+, RDP pipeline (Cole et al. 2009) and MG-RAST (Meyer et al. 2008).

Data Analysis

Physicochemical Environment: Microsoft excel was used to create the water profiles, highlighting physicochemical properties underneath the FTW, next to the FTW, and in the open water. The light attenuation coefficient "k" was calculated using the following equation: $Ln(I_0/I_z) = k\ z\ Ln(I_0/I_z)$ and was plotted as a function of depth (z) to assess the slope "k", i.e. the light extinction coefficient. I_0, I_z, and z denote the subsurface irradiance,

the irradiance at depth z respectively and Ln is the natural logarithm. An atmospheric light simulator (http://clearskycalculator.com/quantumsensor.htm) was used to determine the hourly PAR change the day the light profiles were made. A light profile was then generated every hour using the light extinction equation above and the determined "k", characteristic of the pond water. All irradiance profiles were then averaged for the period there was daylight and also for the entire day. The average irradiance of the entire water column when there is daylight and during the entire day (i.e. night included) could then be calculated.

The Trophic state index (TSI) was calculated using the modified equations from Carlson (1977) for Florida developed by Brezonik (1984) and accounting for the nutrient limitation and the TSI based on the mass TN/TP ratio. SPSS was used to highlight any significant differences amongst the various water profile parameters between the different sampling sites. Prior to running an ANOVA, data normality and homogeneity of variance (i.e. homoscedasticity) were checked for each parameter. When differences were found a post-hoc Tukey test was performed, and when homoscedasticity was not met, a Dunnett's T3 post hoc test was used to make multiple pairwise comparisons.

Allelopathy Assays: For the liquid culture assay, SPSS was used to graph the readings obtained from the Tecan GENios Pro plate reader in a scatter plot. When growth inhibition was found, a Mann-Whitney U test with independent samples was run to highlight any statistical differences from the control. The agar diffusion assay was evaluated qualitatively. Growth inhibition was ranked in three categories 0, 1, and 2. The rank "0" indicated no inhibition or growth within the well was found. The rank "1" indicated a weak inhibition or a noticeable clearing zone around the well. The rank "2" indicated a strong inhibition or no growth present within the sextile.

Zooplankton Sampling: To calculate the amount of zooplankton per liter of lake water the following formula was used: (total zooplankton × initial volume)/tow volume = zooplankton/liter. An Independent T-Test was performed to highlight any significant differences between the amount of zooplankton collected underneath the FTW compared to the amount of zooplankton collected in the open water for the samples taken during the day as well as the samples taken at night.

Microbial Community Characterization: For the microbial community, a species count, the Shannon index (1948) $H' = -\sum_{i=1}^{s} p_i Ln(p_i)$, (where s is the total number of species in the community, p_i is the proportion of the i^{th} species compared to the total number of all individuals in the community and Ln is the natural logarithm) and the Pielou evenness index (Pielou 1977) were run for each sample. A rank abundance graph was a created to further illustrate species richness and evenness for each sample. Primer 6 was utilized to perform multivariate analyses on distribution and abundance of the microbial communities. Multidimensional scaling (MDS) ordinations based on Bray-Curtis similarities and analysis of similarity were performed to determine similarities amongst the microbial communities on the various samples.

RESULTS

Physicochemical Properties

In Pond A, the depth of the water column was approximately 141 cm (± 1). The Secchi disk depth in the open water was 60 cm. The average temperature, conductivity, dissolved oxygen, pH, and ORP underneath the FTW were 16.5 °C (± 0.04), 621.0 μS/cm (± 0.9), 9.5 mg/L (± 0.4), 8.1 a.u. (± 0.02) and 166.8 mV (± 1.2), respectively. The average temperature, conductivity, dissolved oxygen, pH, and ORP in the open water were 16.6 °C (± 0.1), 622.5 μS/cm (± 1.6), 9.1 mg/L (± 0.3), 7.9 a.u. (± 0.01) and 94.4 mV (± 1.5), respectively. Temperature underneath the FTW was slightly lower than the open water, and dissolved oxygen was lower in the open water than underneath the FTW. Conductivity, pH, and ORP were higher underneath the FTW compared to the open water. The ORP probe was not properly functioning: although differences between the profiles do exist, the scale displayed on the graph is incorrect. PAR showed that there was minimal, if any, light penetrating the center of the FTW. Light attenuation underneath the FTW and on the side of the FTW could not be properly calculated because of a shadow effect from the FTW. Light extinction coefficient "k" in the open water was 3.06 m^{-1}, and the depth of the euphotic zone was 1.51 m. The average light penetrating the water column during the daylight hours was 240.5 μmol photons $m^{-2}s^{-1}$ and 120.3μmol photons $m^{-2}s^{-1}$, for the entire day. Table 4 displays the results of the water profile ANOVAs. In the open water total nitrogen (TN) and total phosphorus (TP) was 47.7 μmol/L (± 3.3), or 0.7 mg/L and 3.5 μmol/L (± 1.6), or 108.5 μg/L respectively. TN was comprised of TON, 43.6 μmol/L (± 6.3) or 0.6 mg/L, and TIN, 4.1 μmol/L (± 2.9) or 0.06 mg/L. TIN was further separated into ammonium (NH_4^+), 3.6 μmol/L (± 2.9) or 0.05 mg/L, nitrite (NO_2^-), 0.2 μmol/L

(± 0.04) or 0.003 mg/L, and nitrate (NO_3^-), 0.3 μmol/L (± 0.0) or 0.004 mg/L. Soluble reactive phosphorus (SRP) was 1.9 μmol/L (± 0.5) or 58.9 μg/L. Underneath the FTW, TN and TP were 51.2 μmol/L (± 0.9) or 0.7 mg/L and 3.5 μmol/L (± 1.6), or 108.5 μg/L respectively. TN was comprised of TON, 46.6 μmol/L (± 4.6) or 0.7 mg/L, and TIN, 4.5 μmol/L (± 3.7) or 0.06 mg/L. TIN was further separated into ammonium (NH_4^+), 4.1 μmol/L (± 3.7) or 0.06 mg/L, nitrite (NO_2^-), 0.1 μmol/L (± 0.07) or 0.001 mg/L, and nitrate (NO_3^-), 0.3 μmol/L (± 0.08) or 0.004 mg/L. Soluble reactive phosphorus (SRP) was 2.0 μmol/L (± 0.6) or 61.9 μg/L. The mass nitrogen to phosphorus ratio (TN:TP) was approximately 8:1 and the TSI value for this hydrosystem was 88.

Allelopathy Assays

Liquid Culture Assay: The results of the *Juncus effusus* liquid culture assay exhibited growth inhibition after Day 2 in six out of the ten cultures, however, only two were statistically significant. Growth inhibition could be seen in six of the eight Cyanophyceae cultures, but the two Chlorophyceae cultures showed no inhibition.

Microcystis sp. (strain 22-6) showed a decline in growth after Day 2, and continued to decline over the rest of the experiment, however, the decline in growth was not statistically significant (P = 0.064) relative to the control. *Aphanothece* sp. (strain 30-12a) showed a decline in growth after Day 2, and continued to decline until Day 4 when growth was once again exhibited. Growth inhibition at Day 2 was not statistically significant (P = 0.164) compared to the control. *Pseudanabaena* sp. (strain 12-9-3) displayed a decline in growth at Day 2, but Day 3 showed a return to positive growth. Growth inhibition at Day 2 was not statistically significant (P = 1.0) relative to the control. *Lyngbya* sp. (strain 15-2) showed growth inhibition at Day 2, which continued throughout the rest of the

experiment. The decline in growth at Day 2 was statistically significant (P = 0.009) compared to the control. *Microcystis* sp. (strain 36-1) and *Anabaena* sp. (strain 66-2) did not show growth inhibition, instead, growth enhancement was displayed relative to the controls. *Microcystis* sp. (strain 46-2) displayed a decline in growth at Day 2, however, an increase in growth could be seen for the rest of the experiment. Growth inhibition at Day 2 was statistically significant (P = 0.009) compared to the control. *Cylindrospermopsis* sp. (strain 121-1) showed growth inhibition at Day 2, and growth continued to decline throughout the rest of the experiment, however, growth inhibition at Day 2 was not significantly significant (P = 1.0) relative to the control. *Chlorella* sp. (strain 2-4) and *Scenedesmus* sp. (strain 145-2) did not show growth inhibition, rather, growth enhancement was displayed compared to the controls.

The results of the *Canna flaccida* liquid culture assay exhibited growth inhibition after for five of the ten cultures. A decreased growth could be in seen in three cultures after Day 2, one culture after Day 3, and one culture after Day 4. Of the cultures that exhibited growth inhibition, only two of the five cultures were statistically significant. Growth inhibition could be seen in five of the eight Cyanophyceae cultures, but the two Chlorophyceae cultures showed no inhibition.

Microcystis sp. (strain 22-6) did not show any growth inhibition during the experiment. *Aphanothece* sp. (strain 30-12a) exhibited a decline in growth at Day 2, however, the culture displayed growth throughout the rest of the experiment. Growth inhibition at Day 2 was statistically significant (P = 0.009). *Pseudanabaena* sp. (strain 12-9-3) experienced growth inhibition at Day 3, which continued to decline for the remainder of the experiment, however, the growth decline at Day 3 was not statistically

significant (P = 0.864) relative to the control. *Lyngbya* sp. (strain 15-2) did not show growth inhibition for the duration of the experiment. *Microcystis* sp. (strain 36-1) exhibited growth inhibition at Day 2, but continued to grow for the rest of the experiment. The growth decline seen at Day 3 was statistically significant (P = 0.018) compared to the control. *Anabaena* sp. (strain 66-2) displayed growth inhibition at Day 2, but growth once again increased the following day. The decline in growth at Day 2 was not statistically significant (P = 0.209) when tested against the control. *Microcystis* sp. (strain 46-2) experienced a decline in growth on Day 4 of the experiment, but it was not statistically significant (P = 0.1) relative to the control. *Cylindrospermopsis* sp. (strain 121-1) displayed growth inhibition on Day 4 of the experiment, however, it was not tested for statistical significance because the control experienced a similar pattern in growth. *Chlorella* sp. (strain 2-4) exhibited a decline in growth at Day 4 of the experiment. This culture was also not tested for statistical significance because the control showed a similar pattern of decline. *Scenedesmus* sp. (strain 145-2) did not experience any growth inhibition during the experiment.

The results for the liquid culture assay treatments followed three patterns of growth: initial growth then continuous inhibition (IGCI Figure 10); initial growth, inhibition, and recovery (IGIR Figure 11); and continuous growth (CG Figure 12). IGCI was exhibited in two cultures in the *J. effusus* assay: *Microcystis* sp. (strain 22-6) and *Lyngbya* sp. (strain 15-2), and one culture in the *C. flaccida* assay: *Pseudanabaena* sp. (strain 12-9-3). IGIR was displayed in four cultures in the *J. effusus* assay: *Aphanothece* sp. (strain 30-12a), *Pseudanabaena* sp. (strain 12-9-3), *Microcystis* sp. (strain 46-2), and *Cylindrospermopsis* sp. (strain 121-1), and in three cultures in the *C. flaccida* assay: *Aphanothece* sp. (strain

30-12a), *Microcystis* sp. (strain 36-1), and *Anabaena* sp. (strain 66-2). CG was exhibited in four cultures in the *J. effusus* assay: *Microcystis* sp. (strain 36-1), *Anabaena* sp. (strain 66-2), *Chlorella* sp. (strain 2-4), and *Scenedesmus* sp. (strain 145-2), and in six cultures in the *C. flaccida* assay: *Microcystis* sp. (strain 22-6), *Lyngbya* sp. (strain 15-2), *Microcystis* sp. (strain 36-1), *Cylindrospermopsis* sp. (strain 121-1), *Chlorella* sp. (strain 2-4), and *Scenedesmus* sp. (strain 145-2).

Agar Diffusion Assay: The results of the *Juncus effusus* and *Canna flaccida* agar diffusion assay revealed growth inhibition in all cultures assayed. Growth inhibition was ranked in three categories, and is summarized in Table 5. *J. effusus* extracts showed strong inhibition in 26 of the treatment wells, weak inhibition in six of the treatments, and no inhibition in three of the treatment wells. Only one culture, *Cylindrospermopsis* sp. (strain 121-1), exhibited no growth in the control well and thus the results are inconclusive because the culture did not grow well in the agar. The Cyanophyceae culture, *Anabaena* sp. (strain 66-2), and the Chlorophyceae culture, *Scenedesmus* sp. (strain 145-2), showed the most resilience to the addition of *J. effusus* root extracts. *C. flaccida* extracts showed strong inhibition in 20 of the treatment wells, weak inhibition in 13 of the treatment wells, and no inhibition in two of the treatment wells. Only one culture, *Cylindrospermopsis* sp. (strain 121-1), displayed no growth in the control well thus the results were inconclusive because the culture did not grow well in the agar. The Cyanophyceae culture, *Microcystis* sp. (strain 81-11), and the Chlorophyceae culture, *Scenedesmus* sp. (strain 145-2), showed the most resilience to the addition of *C. flaccida* root extracts.

Zooplankton Sampling

Pond A was the original pond sampled for zooplankton presence/absence, however, no zooplankton were found in any of the samples collected. Thus Collier pond and Livingston

were chosen for presence/absence sampling. The statistical comparisons showed there was no statistical significance between the overall amount of zooplankton underneath the FTW and in the open water during the day. Similar comparisons of the five major taxonomic groups (i.e. Ceriodaphnia, Calanoida, Cyclopidae, Sididae and Rotifera) during the day time also did not reveal any statistical significance However, two of the three sampling sites, Collier 1 and Livingston 1, displayed statistical significance between the overall amount of zooplankton underneath the FTW compared to the open water at night. There were more zooplankton found in the open water relative to the FTW. The Ceriodaphnia and Calanoida night counts at the Livingston 1 site showed the same statistical significance. Although, all other night counts showed no statistical significance, there is no case in which there was significantly more zooplankton underneath the FTW compared to the open water. Table 7 shows the Independent T-test results for day vs. night comparisons for the FTW and the open water control.

Microbial Community Characterization

A total of 22,956 high-throughput sequences were analyzed including 11,998 from FTW plant roots, 4,006 from plastic samples and 6,952 from water samples. The investigation revealed the identification of 22 phyla in total with 12 to 17 phyla in the plant root samples, 14 to 16 phyla in the water samples and 1 to 4 samples in the plastic samples. The number of phyla identified for the plant root samples and the water samples did not differ significantly, however, the number of phyla found in the plastic samples was significantly lower. For class level characterization, *Canna* samples were dominated by Alphaproteobacteria (37.6% ± 0.4) followed by Cyanophyceae (22.3% ± 3.2). *Juncus* samples were dominated by Cyanophyceae (34.0% ± 2.4) followed by Alphaproteobacteria (26.7% ± 6.4). Actinobacteria (38.0% ± 2.1) and Cyanophyceae (31.0% ± 0.3) were the

most common classes of microbes for water and pre-filtered water samples, respectively. For species level characterization, *Canna* 1 had the greatest number of species (325), followed by *Juncus* 1 (322), *Canna* 2 (285), *Juncus* 2 (280), pre-filtered water (205), water (155), plastic pot (45), and finally foam (29) (Table 8). The most abundant microbe on the plastic pot biofilm was *Pseudomonas* spp., which represented 75.8% of the community and a representative denitrifying bacteria (Gamble et al. 1977). *Pseudomonas* spp. also dominated the foam biofilm making up 90% of the microbial community. For both *Juncus* samples, *Anabaena* spp. had the highest microbe representation (23.9% ± 5.2), followed by *Clostridium* spp. (5.6% ± 0.8). *Anabaena* (15.9% ± 3.5) was also the most abundant microorganism for both *Canna* samples; followed by *Rhodobacter* spp. (5.9% ± 0.5). For the water samples *Clavibacter* spp. (17.2%), *Anabaena* spp. (12.3%), and *Streptomyces* spp. (11.4%) comprised 41.0% of the unfiltered water sample community, and *Clavibacter* spp. (18.8%), *Anabaena* spp. (11.9%), and *Synechoccocus* spp. comprised 41.7% of the prefiltered water sample community. The rank abundance curve (Figure 17) gives a visual representation of species richness and species evenness of each sample in the FTW rhizosphere. The Shannon index shows that *Canna* (4.1 ± 0.4) and *Juncus* (3.5 ± 0.01) samples had the highest measures of species richness and evenness while the Pielou evenness index shows that *Canna* (0.7 ± 0.1) and *Juncus* (0.6 ± 0.01) samples also had the greatest species evenness. The samples with the least species richness and evenness, according to the Shannon Index was the foam biofilm (1.53) and pot biofilm (1.52), and the Pielou evenness index shows the foam biofilm (0.45) and pot biofilm (0.40) also had the least species evenness.

The similarity tests show the water sample has the highest ranked similarity with the pre-filtered water on both a species and genus level assessment: 64.9% and 67.8%, respectively. The plant roots which showed the highest similarity on the species and genus level were *Juncus* 1 and *Juncus* 2: 57.6% and 62.8%, respectively. The composition of the root microbial community more closely resembled to the water and pre-filtered water communities, than the plastic pot biofilm and foam biofilm did. The plastic pot biofilm and foam biofilm had the lowest similarity when compared to all the other groups (Table 9). Primer MDS ordination (Figure 18) and cluster analysis (Figure 19) provides a visual representation of the similarity amongst sample groups for species level comparisons.

DISCUSSION

Physicochemical Properties

The water column profiles show the environment underneath the FTW was different when compared to the open water column. Thus biotic or abiotic processes that occur in the open water may not occur at all or in the same way they do in the area below an FTW suggesting the FTW created a microclimate within the hydrosystem. Temperature and dissolved oxygen were well-mixed in this hydrosystem. The lower temperature underneath the FTW relative to the open water was likely due to shading from the FTW itself. Higher dissolved oxygen underneath the FTW is a result of root aeration; a process by which O_2 diffuses from root aerenchyma into the rhizosphere which is important for plant growth in water logged soils (Colmer 2003). The pH of Pond A was alkaline, which is typical of southwest Florida urban ponds dug through the limestone bedrock. Further, based on the TSI, the pond is hypereutrophic, this resulted in an excess production in dissolved oxygen via algal photosynthesis which drove the pH up. Half a mole of dissolved oxygen indeed reacts with 2 hydrogen ions (H+) to generate water. The pH is also enhanced because the creation of dissolved oxygen via photosynthesis equates to the use of carbon dioxide which tends to lower the pH when present (e.g. carbonic acid being decreased). The higher pH beneath the FTW compared to the open water is likely due to the photosynthesis performed by the plants on the FTW which roots bring dissolved oxygen underneath the FTW. Although little to no light was present directly beneath the FTW, the light penetrating the water adjacent to the FTW (240.5 $\mu E\ m^{-2}s^{-1}$/day on average during the daylight hours) suggests that some photosynthesis could occur on the periphery of the FTW during the middle of

the day and very likely when the sun is not at its zenithal position. The mass TN:TP ratio below 10 indicates Pond A was nitrogen limited and thus nitrogen fixers would have an advantage in such a system. The soluble reactive phosphorus was readily available as long as it could be balanced with nitrogen. Ammonium constituted a higher percentage of TN than nitrate, which is unusual in well oxygenated waters. In oxidized systems, such as Pond A, nitrate should typically be the predominate form on nitrogen, however this was not the case. The predominance of ammonium suggests anoxic environments may be present (Dodds & Whiles 2010). Readily available phosphorus was present in the form of soluble reactive phosphorus, and constituted more than half of the TP in Pond A. This high concentration of readily available nutrients would allow for the proliferation of plants and algae, however, this hypereutrophic hydrosystem would likely favor the dominance of algae (Scheffer et al. 2001; Körner & Nicklish 2002; Hilt et al. 2006). A phytoplankton dominated system causes macrophytes to more vigorously compete for resources such as light and nutrients, and such a competition can elicit the production of inhibitory compounds that are released into the environment to suppress potential competitors through allelopathy (Pakdel et al. 2013).

Allelopathy Assays

Liquid Culture Assay: Differences in algal sensitivities were observed between the two groups, and amongst the Cyanophyceae cultures. The Cyanophyceae cultures experienced the strongest inhibitory effects, but they also experienced weak inhibition or no inhibition to the allelochemicals. The Chlorophyceae cultures experienced no allelopathic inhibition. Studies show Cyanophyceae are more sensitive to the effects of allelochemicals compared to Chlorophyceae (Van Donk & Van de Bund 2002; Erhard & Gross 2006; Mulderij et al.

2007; Hilt & Gross 2008), which was apparent in the results of this study. However, research has shown that Chlorophyceae are not completely resistant, and it is possible that their growth can be altered by allelopathic compounds (Nakai et al. 2001; Körner & Nicklish 2002; Leu et al. 2002; Li & Hu 2005; Mulderij et al. 2005; Hu & Hong 2008; Jiang et al. 2014; Wang et al. 2011). This study also exhibited disparities in sensitivity between the Cyanophyceae cultures. Several *Microcystis* cultures were assayed, and the results showed some were strongly inhibited, while others were weakly inhibited or experienced no inhibition at all. Hilt & Gross (2008) report findings in which toxic strains of *Microcystis* were more sensitive to the effects of allelochemicals than non-toxic *Microcystis* which may be due to differences in energy allocation. The differences in sensitivity amongst various algal species can also be due to the structure of the cell wall, uptake mechanisms, photosystem structures, and differences in physiological processes (Hilt & Gross 2008; Zhu et al. 2010).

Differences in algal sensitivities in allelopathy assays are well documented in the literature (Van Donk & Van de Bund 2002; Mulderij et al. 2005; Mulderij et al. 2007; Erhard & Gross 2008; Hilt & Gross 2008; Zhu et al. 2010; Gao et al. 2011; Wang et al. 2011; Chang et al. 2012c; Laue et al. 2014; Nakai et al. 2014), and are apparent in the present study. According to Hu & Hong (2008), the factors that influence these sensitivities can be biological or non-biological. The biological factors include the algal species (Van Donk & Van de Bund 2002; Mulderij et al. 2007; Hilt & Gross 2008; Hu & Hong 2008; Zhu et al. 2010; Laue et al. 2014), growth stage of algae (Hu & Hong 2008; Pakdel et al. 2013), types of aquatic plants (Mulderij et al. 2005; Hu & Hong 2008; Pakdel et al. 2013), and presence of other organisms (Gross 2003; Gross et al. 2007; Hilt & Gross

2008; Chang et al. 2012c; Pakdel et al. 2013). The non-biological factors include nutrients (Mulderij et al. 2007; Hu & Hong 2008; Pakdel et al. 2013), temperature (Hu & Hong 2008; Nakai et al. 2014), and light (Gross et al. 2007; Gao et al. 2011; Wang et al. 2011; Nakai et al. 2014).

IGCI can be attributed to allelopathic effects of the root extracts because the controls did not experience inhibition. All cultures that displayed this growth pattern were Cyanophyceae. The literature shows that Cyanophyceae species have exhibited allelopathic inhibition when exposed to macrophyte allelochemicals. *Microcystis* displayed growth inhibition in the present study, and has been inhibited by the allelochemicals of submerged aquatic vegetation in past studies (Korner & Nicklisch 2002; Li & Hu 2005; Xian et al. 2005; Wu et al. 2007). Allelochemicals were successful in limiting the growth of *Microcystis* in whole plant bioassays (Korner & Nicklisch 2002; Li & Hu 2005; Xian et al. 2005) as well as co-culture experiments in which *Microcystis* were exposed to live plants in an aquarium (Wu et al. 2007). Erhard & Gross (2006) discovered the Cyanophyceae *Pseudanabaena* could be strongly inhibited by *Elodea canadensis* and *Elodea nuttallii* whole plant extracts which coincides with the results of this study.

The growth inhibition seen in the cultures that exhibited IGIR is likely due to allelopathic activity for five of the seven cultures. All of the cultures that experienced this pattern of growth were Cyanophyceae species. The controls of the cultures *Pseudanabaena* sp. (strain 12-9-3) and *Cylindrospermopsis* sp. (strain 121-1) from the *J. effusus* assay displayed a decline in growth. Thus, the lack of growth that can be seen in the treatments may be attributed to experimental conditions such as light, temperature, or nutrients rather than allelopathic activity. The controls of the five other controls showed

steady growth, therefore, the period of inhibition followed by recovery may be a weak allelopathic effect. The weak allelopathic effect is likely due to the one time application of root extracts. Pakdel et al. (2013) obtained similar results, and suggested the allelochemicals may be degraded or metabolized over time. Furthermore, a study by Laue et al. (2014) suggests that the weakening of inhibitory effect could be due to acclimation or inactivation of the allelochemicals. The literature suggests continuous addition of allelochemicals to the treatment wells may have resulted in a stronger inhibitory effect (Gross 1999: Nakai et al. 1999; Pakdel et al. 2013).

Several cultures did not exhibit any growth inhibition during experimentation; two Cyanophyceae and two Chlorophyceae cultures in the *J. effusus* assay, and four Cyanophyceae and two Chlorophyceae cultures in the *C. flaccida* assay. Although, three cultures did experience a growth decline on the last day of the experiment, *Microcystis* sp. (strain 46-2), *Chlorella* sp. (strain 2-4), and *Scenedesmus* sp. (strain 145-2); the controls for each of these cultures displayed the same decline. The growth decline may be attributed to i) the typical life cycle of the cultures or ii) the decrease in the availability of resources at the end of the experiment (Pakdel et al. 2013). Stimulatory effects of allelochemicals have been noted in experiments (Mulderij et al. 2005; Erhard & Gross 2006; Mulderij et al. 2007; Hu & Hong 2008), including the current study, suggesting they may benefit from growth enhancement compounds more than they suffer from allelochemicals present in the root extracts (Erhard & Gross 2006). Mulderij et al. (2007) believed their root extracts may have acted as a phosphorus source, which caused growth stimulation in their experiments. However, in this study the medium in which the algae were cultured and assayed contained an excess of phosphorus. Therefore it was unlikely additional

phosphorus from the extracts would stimulate growth in conditions in which there was already a phosphorus surplus. Thus the extracts must contain some other growth enhancing compound such as micronutrients or vitamins. Hu & Hong (2008) report *Eleocharis microcarpa* produced 3-hydroxy-cyclopentenone octadecenoic acids and 3-hydroxy-cyclopentyl eicosapentaenoic acids which enhanced Cyanophyceae growth in low concentrations.

The cultures of this study were assayed during exponential growth, when the algae are in a healthy phase of growth. This is a common practice of most assays in which phytoplankton are involved (Li & Hu 2005; Mulderij et al. 2005; Wang et al. 2011; Zuo et al. 2015). Conflicts in the results of assays may be evident if algae are not in their dynamic phase of growth. Pakdel et al. (2013) believed an initial growth decline experienced by Cyanophyceae in their assay was a result of using an older culture that had passed its exponential growth phase. Furthermore, Hu & Hong (2008) report the allelochemicals of *Phragmites communis* showed stronger inhibition on *Microcystis aeruginosa* in the lag phase than in the log phase. The growth of the cultures in this study were closely monitored, and all algae were tested during their log growth phase, therefore, growth phase stage is not believed to be the cause of the differences in algal sensitivities.

The allelochemicals of different species of aquatic plants, from whole plant extracts to exudates of live plants, have been shown to have diverse effects on phytoplankton species (Mulderij et al. 2005; Hu & Hong 2008; Pakdel et al. 2013). For example, *Microcystis* sp. (strain 22-6) and *Lyngbya* sp. (strain 15-2) displayed strong inhibition in the *J. effusus* assay, however, these two cultures did not exhibit any inhibition in the *C. flaccida* assay. A study by Pakdel et al. (2013) showed *Chara australis* has stronger

allelopathic effects on phytoplankton compared to *Potamogeton crispus*. The differences (summarized in Table 11) in allelopathic effects of *J. effusus* and *C. flaccida* is likely due to the various allelochemicals in the composition of each extract or the chance that the allelochemicals of one macrophyte species may degrade at a faster rate (Pakdel et al. 2013).

The presence of another organism in a culture being assayed could produce differences in algal sensitivities (Gross 2003; Gross et al. 2007; Hilt & Gross 2008; Chang et al. 2012c; Pakdel et al. 2013). Chang et al. (2012c) found that *Microcystis aeruginosa* was inhibited by the addition of macrophyte allelochemicals. They assayed *M. aeruginosa* in the presence of green algae, and growth of the Cyanophyceae was actually enhanced (Chang et al. 2012c). The algal cultures in this study were monospecific, so differences in algal sensitivities were not due to the presence of another phytoplankton species. However, despite extreme care, the cultures of this study were not axenic, and the presence of bacteria may have brought about differential sensitivities (Gross et al. 2007; Hilt & Gross 2008). Bacteria are thought to reduce the effectiveness of allelochemicals (Hilt & Gross 2008) because they may quicken the degradation of allelopathically active compounds (Gross 2003; Gross et al. 2007) and some bacteria can also excrete allelochemicals. Gross (2003) report allelochemicals of *Myriophyllum spicatum* became less effective overtime during algal assays, suggesting heterotrophic bacterial degradation. Several assays in this study experienced a point of inhibition followed by an increase in growth, which may be due to the microbial degradation of allelochemicals. However, some researchers do not believe such bacteria have a strong influence on the results of bioassays. Pakdel et al. (2013) state the presence of heterotrophic bacteria in their assays did not have large impact, and the effects on phytoplankton growth were most likely insignificant.

The availability of nutrients in an experiment also impacts algal sensitivities (Mulderij et al. 2007; Hu & Hong 2008; Pakdel et al. 2013). Algae grown in a nitrogen limited media displayed stronger inhibitory effects during allelochemicals assays, while algae cultured in phosphorus limited media did not show any inhibition (Hu & Hong 2008). Mulderij et al. (2007) observed a decrease in allelochemical sensitivity for *Synechococcus elongates* and *Scenedesmus obliquus* when grown in a phosphorus limited environment. The differences in algal sensitivities exhibited in this study may be due to varying nutrient availability throughout the experiment. However, nutrients were not measured prior to the start of the experiment or after, so it is unknown whether nutrient availability played a role in the different algal sensitivities.

Temperature has also been shown to produce different algal sensitivities (Hu & Hong 2008; Nakai et al. 2014). Research reveals the allelochemicals of *Myriophyllum spicatum* were most effective at inhibiting algal growth when assayed in a lower temperature range: 20-30°C (Nakai et al. 2014). Hu & Hong (2008) report *Eichornia crassipes* was only able to inhibit algal growth in a certain temperature range, and the ability to deter growth was lost at lower temperatures. Higher temperatures (>30°C) may increase the degradation of allelochemicals, while lower temperatures (<20°C) may reduce protein synthesis within the algal cell (Nakai et al. 2014). In this study, the algae were assayed at the temperature of 28°C, which is within the effective temperature range determined by Nakai et al. (2014). If the algae had been assayed at a different temperature it is possible that different results would have been produced.

Light may also elicit differences in algal sensitivities (Gross et al. 2007; Gao et al. 2011; Wang et al. 2011; Nakai et al. 2014). Nakai et al. (2014) found that lower light

conditions (25 μE $m^{-2}s^{-1}$) produced stronger allelopathic effects in phytoplankton assays compared to high light conditions (75 μE $m^{-2}s^{-1}$). Furthermore, *Stratiotes* allelochemicals showed greater inhibition in low light conditions (35 μE $m^{-2}s^{-1}$) compared to high light conditions (105 μE $m^{-2}s^{-1}$) (Gross et al. 2007). High light conditions are believed to increase the rate of allelochemical photodegradation. However, research does show that reactive oxygen species were produced at higher rates in light allelopathy assays compared to dark allelopathy assays (Gao et al. 2011; Wang et al. 2011), suggesting light is needed to produce stronger inhibitory effects. Low light conditions, 13 μE $m^{-2}s^{-1}$, were used in the current study. Higher light conditions in the assay may have degraded the allelochemicals at an increased rate, which could have generated weaker growth inhibition in the algal assays. At the time of root harvest, the amount of light underneath the center of the FTW was not strong enough to produce inhibitory effects. However, based on the simulated light profiles, the average light conditions in the water column should cause allelochemicals to degrade quickly, especially at distance of the FTW.

Agar Diffusion Assay: The agar diffusion assay exhibited very strong inhibitory results, suggesting allelopathy. Strong inhibition was displayed most often in the treatment wells, and only a few wells overall displayed no inhibition. The results of the agar diffusion assay produced much stronger allelopathic inhibition compared to the liquid culture assay. The group Chlorophyceae did not exhibit any growth inhibition in the liquid culture assay, however, inhibition was seen in the agar diffusion assay. For example, *Chlorella* sp. (2-4) experienced growth enhancement in the liquid culture assay, however, the algae was strongly inhibited in the agar diffusion assay. There were no cases in which inhibition was seen in the liquid culture assay, and not the agar diffusion assay. Unlike the liquid culture

assay, the agar diffusion assay displayed some form of inhibition in all cultures tested with the exception of *Cylindrospermopsis* sp. (121-1) which did not grow well in the agar. This is likely due to the nature of the root extracts. In the liquid culture assay, the root extracts were more diluted than for the agar diffusion assay. Although the agar diffusion assay generated more contrasting inhibitory results, it is unlikely allelochemicals would be produced in such a high concentration in nature unless algae are in direct contact with the plant root. When allelochemicals are released as exudates by plant roots in an aquatic system, the concentration will be diluted because of the watery environment in which the plants live. The liquid culture assay is better representation of allelochemical interactions in an aquatic environment.

The plants used on the FTWs in this study do appear to have allelopathic potential. The allelochemicals manufactured in the roots of *J. effusus* and *C. flaccida* did have the ability to inhibit the growth of algae, thus they may be able to reduce algae occurring in a hydrosystem. However, allelopathic compounds may not be the only factor leading to a reduction in the algal biomass. Grazing performed by herbivorous zooplankton may further decrease algal biomass within a hydrosystem, and is the focus of the next section.

Zooplankton Presence/Absence

Zooplankton typically seek refuge (i.e. vegetative structures) during the day to avoid the visual detection of zooplanktivores. The results of this study suggest zooplankton do not rely on submerged FTW roots as a refuge since there was no significant difference in the overall amount of zooplankton underneath the FTW compared to the control during the day. Even though the T-tests did not show much statistical significance, at almost all the sites there was more zooplankton in the open water than hiding amongst the roots. It seems more zooplankton were found in the open water, which implies they are possibly seeking

shelter elsewhere in the hydrosystem. These findings are consistent with previous research in that zooplankton have a tendency to avoid floating macrophytes for two major reasons: chemical repellents released by plants (Lauridson & Lodge 1996; Burks et al. 2000; Burks et al. 2001; Van Donk & Van de Bund 2002; Meerhoff et al. 2003; Meerhoff 2006) and increased predation (Burks et al. 2001; Van de Meutter et al. 2005; Meerhoff et al. 2003; Meerhoff 2006; Iglesias et al. 2007).

Researchers believe the allelopathic chemicals released by floating aquatic plants to deter algal growth may indirectly repel zooplankton causing them to refrain from utilizing these vegetative structures as shelter (Lauridson & Lodge 1996; Burks et al. 2000; Burks et al. 2001; Van Donk & Van de Bund 2002; Meerhoff et al. 2003; Meerhoff 2006). A study by Meerhoff (2006) resulted in Daphnia having a strong avoidance of free-floating macrophytes compared to submerged plants despite the cues of an increased risk of predation. Zooplankton have been observed avoiding real and plastic plants in an experimental tank that did not contain fish, suggesting chemical repellency by macrophytes (Burks et al. 2001). Meerhoff (2006) believes allelopathic compounds exuded by plants may negatively affect reproduction and development of zooplankton.

Previous research has also shown zooplankton are at increased risk of predation in floating macrophyte beds (Burks et al. 2001; Van de Meutter et al. 2005; Meerhoff et al. 2003; Meerhoff 2006; Iglesias et al. 2007). Zooplanktivorous fishes are known to seek refuge in macrophyte stands from predatory fish (Iglesias et al. 2007), and studies in subtropical lakes note that free floating vegetation is an integral nursery habitat for many juvenile fish species (Meerhoff 2006). Freshwater shrimp are also thought to be an important predator in subtropic habitats (Iglesias et al. 2007), and in this study

Palaemonetes paludosus (freshwater grass shrimp) were present in samples taken underneath the FTW. Certain species of fish (e.g. *Perca fluvialitis*) are very successful when foraging in dense macrophyte habitat. Burks et al. (2001) report *P. fluvialitis* foraging in dense plant beds had the ability to consume 80% of the Daphnia. Furthermore, the structure of macrophyte beds may interfere with zooplankton locomotion, which in turn can further increase their risk of predation (Lauridson & Lodge 1996; Meerhoff 2006).

Unfortunately, the mesh size of the vertical tow was too large to capture smaller microzooplankton e.g. ciliates and flagellates. Microzooplankton have been known to have a large impact on overall phytoplankton grazing (Weisse et al. 1990) including cyanobacteria (Burkhill et al. 1993). Microzooplankton also graze on bacteria, which could impact bacterial biomass and nutrient cycling. Ciliates and flagellates have the ability to migrate when searching for prey items, so it may be possible these organisms may be migrating to and from the FTWs. Due to the lack of microzooplankton sampling data FTW migration is only a speculation.

Thus, zooplankton are most likely seeking refuge in the hypolimnetic zone or the littoral zone of these subtropical hydrosystems. Not relevant to the set goals of this thesis, further investigations were made to determine the preferred refuge of zooplankton. Vertical tows of the water column performed during the day and night confirmed zooplankton were performing diel vertical migration from the hypolimnetic zone. FTWs did not appear to be a preferred refuge for herbivorous zooplankton in the hydrosystems of this study; therefore FTWs did not increase top-down algal control. However, it is possible the hydrosystems are experiencing bottom-up algal control through microbial mediated nutrient cycles.

Microbial Community Characterization

The microbial community study shows the rhizosphere of FTWs was inhabited by a wide array of organisms that belong to many different functional groups. In the FTW rhizosphere, various known functional groups (e.g. photoautotrophs, nitrogen-fixing bacteria, nitrifying bacteria, autotrophic sulfur-oxidizing bacteria, purple non-sulfur bacteria, sulfur- and sulfate-reducing bacteria, methylotrophic/methanotrophic bacteria) of microbes were present. Although denitrifiers were not a functional group identified through 16S rRNA gene sequencing, a closer look at the functions of the individual microbes revealed denitrifying bacteria were present in the biofilm samples (i.e. *Pseudomonas* Gamble et al. 1977). All of these aforementioned organisms play a role in nutrient cycling within the hydrosystem. These organisms likely have an impact on nitrogen cycling, phosphorus cycling, and sulfur cycling.

Photoautotrophs, nitrogen-fixing bacteria, nitrifying bacteria, and denitrifying bacteria affect the cycling of nitrogen. Photoautotrophs will uptake forms of nitrogen, such as nitrate (NO_3^-), to satisfy metabolic functions; and some of the photoautotrophs (e.g. *Nostoc* and *Anabaena*) perform nitrogen fixation converting nitrogen gas (N_2) into organic nitrogen. The nitrogen-fixing bacteria (e.g. *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Azovibria* and *Azospria*) also convert nitrogen gas (N_2) into organic nitrogen, thus photoautotrophs and nitrogen-fixing bacteria are both capable of increasing the nitrogen pool within the hydrosystems. Nitrifying bacteria assimilate ammonium (NH_4^+) to nitrite (NO_2^-) and nitrite to nitrate (NO_3^-), which converts nitrogen into a usable form for other organisms. The plant roots contained both *Nitrosomonas* and *Nitrosospira*; bacteria that are responsible for ammonium (NH_4^+) oxidation. Members of the genus *Nitrospira* are also known to perform nitrite oxidation, which suggests the inclusion of the entire

nitrification cycle in the root rhizosphere of FTWs. Furthermore, denitrifying bacteria were present within the microbial community of the FTW. These denitrifiers use nitrate (NO_3^-) as an electron acceptor for the oxidation of carbon, and this function yields nitrous oxide (N_2O) and nitrogen gas (N_2), which drives the loss of nitrogen in the hydrosystem. Thus, the entire nitrogen cycle is potentially performed beneath the FTWs.

Phosphorus present in the hydrosystem will be consumed by the organisms of all the functional groups to meet their physiological needs, thus reducing the phosphorus pool. On the contrary, organisms will excrete phosphorus when it is in excess in the form of orthophosphate (i.e. labile) or dissolved organic phosphorus (i.e. refractory P). Dissolved organic phosphorus can be converted to phosphate through the cleavage of phosphatase enzymes released by microbial organisms.

Autotrophic sulfur-oxidizing bacteria, purple non-sulfur bacteria, and sulfur and sulfate reducing bacteria have an impact on the sulfur cycle. Autotrophic sulfur-oxidizing bacteria and purple non-sulfur bacteria oxidize sulfide (S^{2-}) to sulfur (S^0) to thiosulfate ($S_2O_3^{2-}$) to sulfate (SO_4^{2-}). These groups also use various forms of sulfur as an electron receptor for processes such as anoxygenic photosynthesis (S^{2-} or S^0 converted to SO_4^{2-}) or to respire organic carbon. Sulfur- and sulfate-reducing bacteria perform assimilation processes in which SO_4^{2-} is reduced to $S_2O_3^{2-}$ and S^0 is reduced to S^{2-}. The presence of these microbial groups suggests the existence of the complete sulfur cycle in the FTW rhizosphere.

The microbial community present on the root biofilm had a low level of similarity when compared to the microbial community in the water column. However, they do have an overlap of microbial organisms, which suggests a portion of the root biofilm microbes

most likely originated from the water column communities. The plants growing on the FTW did not start their life cycle in the pond in which the FTW is present. The plants were cultivated in a nursery, and then transported to the pond later. The portion of the root biofilm microbial communities that is different from the water column communities may be the microbial communities that were inoculated in the nursery environment. If so, beneficial bacteria could be inoculated into the nursery water before the young plants are transported and placed on FTWs in a different hydrosystem. The biofilm communities on the plastic pot and foam were very dissimilar from the root and water communtities. The genus *Pseudomonas*, known to perform denitrification, constituted a majority of the plastic pot and foam microbial community thus the biofilm communities present on the inorganic portions of the FTWs may contribute significantly in the reduction of the nitrogen pool.

Based on the dissolved oxygen levels in the water column the environment underneath the FTW was aerobic, however anaerobic processes were still occurring. For all of these processes to occur simultaneously underneath the FTW there has to be variation in the environmental conditions (i.e. oxic, anoxic, and anaerobic) present in the rhizosphere. Oxic refers to an environment in which oxygen is present, anoxic refers to environments in which molecular oxygen is present (i.e. NO_3^-) and anaerobic environments are totally devoid of oxygen. The presence of a wide variety of functional groups that require very different environments to perform their tasks is proof of this. For example: sulfur-oxidizing bacteria require the boarder of oxic/anoxic environments while sulfur-reducing bacteria usually require anaerobic environments. Findings such as these have been documented in other rhizosphere research in which functional groups with contrasting environmental needs have been found in close proximity (Stewart et al. 2008; Cunningham

et al. 2010). Previous research has elucidated the existence of "micro sites" (minute areas of varying conditions) within rhizospheres (Billore et al. 2008; Morgan et al. 2008). These micro sites allow for both aerobic and anaerobic processes to occur, which can amplify the microbial diversity in an FTW rhizosphere. The existence of these micro sites suggests the possibility of a redox potential gradient occurring on a microscopic scale within the biofilm in the FTW rhizosphere. Past research has discovered clearly defined stratification within biofilms; aerobic oxidation occurred near the surface and sulfate reduction occurred in anoxic/anaerobic zone found within the deeper layers of the biofilm (Yu & Bishop 1998; Bishop & Yu 1999). According to Yu & Bishop (2001), redox potential stratification of the biofilm in which they measured occurred within a 50 μm depth. Such stratification may also be occurring within the various FTW biofilms.

CONCLUSIONS

There is still much research to be done to truly understand the dynamics of allelopathy, and its potential use as a natural biocide for the two plant species chosen in this study. The next step should involve separating the crude extracts into different chemical fractions to determine which compounds are the most effective in inhibiting algal growth. Once the most allelopathically active compounds are identified, their effectiveness should be compared against synthetic herbicides that inhibit photosynthesis, such as DCMU (a well-known herbicide also known commercially as Diuron^{TM}) and create LC_{50} curves with the different strains of algae used in this thesis responding negatively to the crude extract exposure. This would help in the determination of the effective dose of allelochemicals on a naturally occurring Cyanophyceae blooms. Furthermore, research should also aim to uncover any damaging effects related to the use of allelochemicals, which may be incurred on other organisms (zooplankton, fishes, macrophytes, etc.) present in the system. Research of this nature would help determine if the use of allelochemicals as a replacement for synthetic algaecides is feasible; the use of extracted allelochemicals would need to solve the problem at an equitable cost and in a reasonable amount of time. The knowledge scientists gain through the investigation of allelopathy will hopefully help the scientific community progress towards a more sustainable and environmentally friendly future with the goal of public safety as a top priority.

The zooplankton results of this research, like the other parameters, are only a snapshot in time. Zooplankton were sampled during a particular month of the year, so the findings of this study may not replicate during different times of the year. Also, only three

FTWs in subtropical lakes were sampled in this study. FTWs in different hydrosystems in other subtropical regions as well as temperate regions may be an ideal refuge for zooplankton. The plant composition of FTWs could have also played a role in the low numbers of zooplankton in FTW roots; some plants will have stronger allelopathic repellency than others. Therefore, it is possible the plants present on the FTWs used in this study exuded strong allelochemicals. Furthermore, the tow net used in this study had a larger mesh size that may have missed part of the zooplankton community i.e. microzooplankton. Future research into the presence of zooplankton in the roots of FTWs should take these parameters into consideration to determine if the results of this study are true for all FTWs.

The FTW was able to create an oxygen gradient at a microscale near the roots, and this finding was indirectly proved by existence of a wide array of functional groups contained within the biofilms. Although denitrifiers were not one of the identified functional groups based on the 16S rRNA gene sequencing, an examination of the microbes on a species level confirms the presence of denitrifiers. Even though the water column beneath the FTW was oxygenated, the conditions in the biofilm (i.e. anaerobic micro sites) do allow for denitrification processes to occur. The roots sampled were from young plants during the month of March, therefore a more mature root system or perhaps a particular time of the year could yield more denitrifying bacteria leading to greater rates of denitrification. Goals of further research should include microbial characterization during different seasons and during different stages of FTW rhizosphere maturation, and the use of microelectrodes to characterize the redox potentials of the biofilms found underneath

the FTW. Investigations such as these would help to further the understanding of microbial interactions in the FTW rhizosphere.

This investigation shows FTWs contain more benefits than just nutrient uptake. FTWs have the potential to directly control algae through allelopathic inhibition, and also indirectly through reductions in the nitrogen pool via denitrification. It appears FTWs are more valuable than once thought. FTWs may potentially be an effective method in remediating symptoms of eutrophication, however, they could be even more successful when used in conjunction with other remediation approaches such as shoreline vegetation plantings or constructed wetlands. These findings will hopefully guide management decisions dealing with cultural eutrophication towards a more eco-friendly solution.

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TABLES

Table 1. Macrophyte species which have been utilized on FTWs.

Macrophyte Species	Study Citation
Acorus calamus	Hu et al. 2010
Acorus gramineus	Winston et al. 2013
Calla palustris	Zhao et al. 2012
Canna flaccida	Sun et al. 2009; Chang et al. 2012b; White & Cousins 2013
Canna indica	Zhao et al. 2012
Carex virgata	Van de Moortel 2008; Van de Moortel et al. 2010; De Stefani et al. 2011; Tanner et al. 2011; Tanner & Headley 2011; Borne et al. 2013
Carex stricta	Winston et al. 2013
Cyperus ustulatus	Tanner et al. 2011; Tanner & Headley 2011
Dracaena sanderiana	Zhu et al. 2011
Eichornia crassipes	Zhao et al. 2012
Glyceria maxima	De Stefani et al. 2011
Hibiscus moscheutos	Winston et al. 2013
Hydrocharis dubia	Zhao et al. 2012
Hydrocotyle verticillata	Zhao et al. 2012
Ipomoea aquatica	Li et al. 2007; Li et al. 2010
Iris pseudacorus	DeStefani et al. 2011
Juncus effusus	Hubbard et al. 2004; Strosnider & Nairn 2010; Van de Moortel et al. 2010; De Stefani et al. 2011; Chang et al. 2012b; Chang et al. 2013; White & Cousins 2013; Winston et al. 2013
Juncus edgariae	Tanner et al. 2011; Tanner & Headley 2011
Jussiaea repens	Zhao et al. 2012
Lythrum salicaria	Van de Moortel et al. 2010; Yao et al. 2011
Myriophyllum aquaticum	Zhao et al. 2012
Oenanthe javanica	Yang et al. 2008; Zhou & Wang 2010; Zhu et al. 2011
Panicum hematomon	Hubbard et al. 2004
Peltandra virginica	Winston et al. 2013
Phragmites australis	Garbett 2004; Van de Moortel et al. 2010; De Stefani et al. 2011; Chen et al. 2012
Pisitia stratiotes	Zhao et al. 2012
Pontederia cordata	Chang et al. 2012b; Zhao et al. 2012; Chang et al. 2013; Winston et al. 2013
Rohdea japonica	Zhu et al. 2011
Salix babylonica	Zhu et al. 2011
Schoenoplectus tabernaemontani	Tanner et al. 2011; Tanner & Headley 2011
Scirpus validus	Yao et al. 2011
Spartina pectinata	Winston et al. 2013
Typha latifolia	Hubbard et al. 2004; Strosnider & Nairn 2010; De Stefani et al. 2011
Typha minima	Yao et al. 2011

Table 2. Removal rates for water TN and TP for various FTW systems.

Study Citation	Water TN Removal	Study Citation	Water TP Removal
Tanner et al. 2011	77.00%	DeStefani et al. 2011	64.00%
Sun et al. 2009	72.70%	Chang et al. 2012b	53.00%
Chang et al. 2012b	61.00%	Li et al. 2010	47.30%
Li et al. 2010	45.10%	Zhao et al. 2012	43.30%
Van de Moortel et al. 2010	42.30%	Tanner et al. 2011	35.00%
Zhao et al. 2012	36.90%	Van de Moortel et al. 2010	22.10%
DeStefani et al. 2011	22.00%	Sun et al. 2009	n/a

Table 3. Algal species that have been inhibited by macrophyte allelochemicals.

Algae allelopathically inhibited by macrophytes	**Study Citation**
Anabaena spp.	Leu et al. 2002; Körner & Nicklisch 2002; Erhard & Gross 2006; Mulderij et al. 2007; Pakdel et al. 2013
Chlamydomonas spp.	Leu et al. 2002
Chlorella spp.	Li & Hu 2005; Jiang et al. 2014
Limnothrix spp.	Körner & Nicklisch 2002
Microcystis spp.	Nakai et al. 1999; Nakai et al. 2001; Körner & Nicklisch 2002; Li & Hu 2005; Mulderij et al. 2005; Nakai et al. 2005; Xian et al. 2005;Mulderij et al. 2007; Zhu et al. 2010; Gao et al. 2011; Laue et al. 2014; Nakai et al. 2014
Planktothrix spp.	Körner & Nicklisch 2002
Pseudanabaena spp.	Erhard & Gross 2006
Scenedesmus spp.	Körner & Nicklisch 2002; Wu et al. 2007
Synechoccus spp.	Leu et al. 2002; Erhard & Gross 2006; Mulderij et al. 2007

Table 4. Water profile ANOVA results. Significant differences between sampling sites are marked with an asterisk (*).

Parameter	Site	Averages	Significance
Temperature (ºC)	FTW vs. FTW Side	16.5 vs. 16.5	0.109
Temperature (ºC)	FTW vs. Open Water	16.5 vs. 16.6	0.000*
Conductivity (µS/cm)	FTW vs. FTW Side	621.0 vs. 620.0	0.225
Conductivity (µS/cm)	FTW vs. Open Water	621.0 vs. 622.5	0.000*
Dissolved Oxygen (mg/L)	FTW vs. FTW Side	9.5 vs. 8.4	0.000*
Dissolved Oxygen (mg/L)	FTW vs. Open Water	9.5 vs. 9.1	0.000*
pH (a.u.)	FTW vs. FTW Side	8.1 vs. 7.9	0.000*
pH (a.u.)	FTW vs. Open Water	8.1 vs. 7.9	0.000*
ORP (mV)	FTW vs. FTW Side	166.8 vs. 143.7	0.000*
ORP (mV)	FTW vs. Open Water	166.8 vs. 94.4	0.000*

Table 5. *J. effusus* agar diffusion assay. The rank "0" indicates no inhibition, the rank "1" indicates weak inhibition, and the rank "2" indicates strong inhibition. NC = non conclusive.

J. effusus	***Microcystis* sp. (strain 81-11)**	***Anabaena* sp. (strain 66-2)**	***Limnothrix* sp. (strain 37-2-1)**	***Cylindrospermopsis* sp. (strain 121-1)**	***Microcystis* sp. (strain 36-1)**	***Scenedesmus* sp. (strain 145-2)**	***Chlorella* sp. (strain 2-4)**
Control	0	0	0	2	0	0	0
Treatment	2	1	2	NC	2	0	2
Treatment	2	1	2	NC	2	0	2
Treatment	2	1	2	NC	2	0	2
Treatment	1	1	2	NC	2	1	2
Treatment	1	2	2	NC	2	1	2

Table 6. *C. flaccida* agar diffusion assay. The rank "0" indicates no inhibition, the rank "1" indicates weak inhibition, and the rank "2" indicates strong inhibition. NC = non conclusive.

C. flaccida	***Microcystis* sp. (strain 81-11)**	***Anabaena* sp. (strain 66-2)**	***Limnothrix* sp. (strain 37-2-1)**	***Cylindrospermopsis* sp. (strain 121-1)**	***Microcystis* sp. (strain 36-1)**	***Scenedesmus* sp. (strain 145-2)**	***Chlorella* sp. (strain 2-4)**
Control	0	0	0	1	0	0	0
Treatment	0	1	2	NC	2	0	2
Treatment	1	2	2	NC	1	1	2
Treatment	1	2	2	NC	2	1	2
Treatment	1	2	1	NC	1	1	2
Treatment	1	2	1	NC	2	1	2

Table 7. Independent T-test results for the day vs. night comparisons for the FTW and the open water control. An asterisk (*) shows results with statistical significance. Cells of the table marked "n/a" denote zooplankton counts in which none of a particular taxonomic group zooplankton were present in the sample counts. Counts are expressed in individuals per liter.

Counts (individuals/L)	**FTW**	**Open Water**	**Livingston 1**	**FTW**	**Open Water**	**Livingston 2**	**FTW**	**Open Water**	**Collier 1**
Total Day	29.3	49.5	0.138	43.0	47.0	0.542	19.8	23.3	0.533
Total Night	20.7	89.7	0.001*	48.2	109.5	0.059	23.0	59.6	0.036*
Ceriodaphnia Day	2.5	14.6	0.211	6.0	6.3	0.965	6.2	4.9	0.731
Ceriodaphnia Night	0.0	34.2	0.029*	9.6	12.2	0.501	9.4	23.3	0.234
Calanoida Day	2.5	8.7	0.331	5.8	9.4	0.675	1.2	3.7	0.063
Calanoida Night	3.1	35.9	0.027*	9.6	28.0	0.282	1.2	14.7	0.555
Cyclopidae Day	0.0	0.0	n/a	3.1	3.1	1.00	1.2	1.2	1.00
Cyclopidae Night	0.0	3.0	0.374	3.2	28.1	0.077	4.4	12.1	0.406
Sididae Day	0.0	0.0	n/a	0.0	0.0	n/a	0.0	2.4	0.116
Sididae Night	0.0	0.0	n/a	3.2	17.5	0.540	1.1	5.5	0.148
Rotifera Day	0.0	3.7	0.057	8.7	9.4	0.725	0.0	0.0	n/a
Rotifera Night	0.0	1.2	0.867	3.2	5.8	0.581	8.0	8.7	0.955

Table 8. An overview of the number of reads, operational taxonomic units (OTUs) and diversity indices for each sample.

	Plant root				**Plastic**		**Water**	
	Canna 1	**Canna 2**	**Juncus 1**	**Juncus 2**	**Foam Biofilm**	**Pot Biofilm**	**Water**	**Pre-filtered Water**
Number of read	4505	1996	3063	2434	2051	1955	3221	3731
OTU (97%)	430	374	474	344	27	27	293	335
Mean G+C content (%)	53	53	52	52	54	54		
Species count	325	285	322	280	29	45	155	207
Shannon index	3.85	4.37	3.51	3.52	1.53	1.52	2.94	3.09
Pielou evenness index	0.67	0.77	0.61	0.62	0.45	0.4	0.58	0.58

Table 9. Primer analysis of similarity (ANOSIM) results for species and genus level comparisons. The percentage indicates the similarity of the microbial community structure when comparing samples. Each sample was compared to all of the other remaining samples.

Species Level Comparison	**Similarity**	**Genus Level Comparison**	**Similarity**
Water vs. Pre-filtered Water	64.94%	Water vs. Pre-filtered Water	67.84%
Juncus 1 vs. *Juncus* 2	57.62%	*Juncus* 1 vs. *Juncus* 2	62.82%
Canna 1 vs *Canna* 2	54.86%	*Canna* 1 vs *Canna* 2	60.70%
Juncus 1 vs. *Canna* 1	48.81%	*Juncus* 1 vs. *Canna* 1	56.64%
Juncus 2 vs *Canna* 2	49.74%	*Juncus* 2 vs *Canna* 2	55.13%
Juncus 1 vs. *Canna* 2	49.57%	*Juncus* 1 vs. *Canna* 2	53.52%
Juncus 2 vs. *Canna* 1	46.50%	*Juncus* 2 vs. *Canna* 1	53.35%
Juncus 1 vs. Pre-filtered Water	36.87%	*Juncus* 1 vs. Pre-filtered Water	41.79%
Canna 2 vs. Water	33.84%	*Canna* 2 vs. Water	41.73%
Canna 2 vs. Pre-filtered Water	34.73%	*Canna* 2 vs. Pre-filtered Water	41.34%
Juncus 2 vs. Pre-filtered Water	34.66%	*Juncus* 2 vs. Pre-filtered Water	41.13%
Juncus 1 vs. Water	33.83%	*Juncus* 1 vs. Water	38.43%
Canna 1 vs. Pre-filtered Water	31.47%	*Canna* 1 vs. Pre-filtered Water	38.42%
Juncus 2 vs. Water	31.90%	*Juncus* 2 vs. Water	37.42%
Canna 1 vs. Water	29.67%	*Canna* 1 vs. Water	36.05%
Pot Biofilm vs. Foam Biofilm	39.57%	Pot Biofilm vs. Foam Biofilm	27.13%
Pot Biofilm vs. *Canna* 2	7.71%	Pot Biofilm vs. *Canna* 2	10.27%
Pot Biofilm vs. *Juncus* 2	6.19%	Pot Biofilm vs. *Juncus* 2	9.45%
Pot Biofilm vs. *Canna* 1	8.07%	Pot Biofilm vs. *Canna* 1	9.16%
Pot Biofilm vs. Water	5.16%	Pot Biofilm vs. Water	8.61%
Pot Biofilm vs. Pre-filtered Water	6.36%	Pot Biofilm vs. Pre-filtered Water	7.08%
Pot Biofilm vs. *Juncus* 1	4.76%	Pot Biofilm vs. *Juncus* 1	6.43%
Foam Biofilm vs. Pre-filtered Water	6.34%	Foam Biofilm vs. Pre-filtered Water	5.24%
Foam Biofilm vs. Water	3.60%	Foam Biofilm vs. Water	4.89%
Foam Biofilm vs. *Juncus* 2	4.37%	Foam Biofilm vs. *Juncus* 2	4.15%
Foam Biofilm vs. *Canna* 1	5.37%	Foam Biofilm vs. *Canna* 1	4.14%
Foam Biofilm vs. *Canna* 2	3.57%	Foam Biofilm vs. *Canna* 2	3.88%
Foam Biofilm vs. *Juncus* 1	3.08%	Foam Biofilm vs. *Juncus* 1	2.76%

Table 10. The results of the liquid culture assay for *J. effusus* and *C. flaccida*. IGCI refers to a pattern of initial growth then continuous inhibition. IGIR refers to a pattern of initial growth, inhibition, and recovery. CG refers to a pattern of continuous growth with no inhibition.

Liquid Culture Assay	***J. effusus***	***C. flaccida***
Microcystis sp. (strain 22-6)	IGCI	CG
Aphanothece sp. (strain 30-12a)	IGIR	IGIR
Pseudanabaena sp. (strain 12-9-3)	IGIR	IGCI
Lyngbya sp. (strain 15-2)	IGCI	CG
Microcystis sp. (strain 36-1)	CG	IGIR
Anabaena sp. (strain 66-2)	CG	IGIR
Microcystis sp. (strain 46-2)	IGIR	CG
Cylindrospermopsis sp. (strain 121-1)	IGIR	CG
Chlorella sp. (strain 2-4)	CG	CG
Scenedesmus sp. (strain 145-2)	CG	CG

Table 11. Comparison of the results of the liquid culture assay (LCA) and the agar diffusion assay (ADA) for *J. effusus*.

J. effusus	**LCA**	**ADA**
Microcystis sp. (strain 36-1)	CG	Strong Inhibition
Anabaena sp. (strain 66-2)	CG	Strong Inhibition
Cylindrospermopsis sp. (strain 121-1)	IGIR	Strong Inhibition
Chlorella sp. (strain 2-4)	CG	Strong Inhibition
Scenedesmus sp. (strain 145-2)	CG	Weak Inhibition

Table 12. Comparison the results of the liquid culture assay (LCA) and the agar diffusion assay (ADA) for *C. flaccida*.

C. flaccida	**LCA**	**ADA**
Microcystis sp. (strain 36-1)	IGIR	Strong Inhibition
Anabaena sp. (strain 66-2)	IGIR	Strong Inhibition
Cylindrospermopsis sp. (strain 121-1)	CG	Strong Inhibition
Chlorella sp. (strain 2-4)	CG	Strong Inhibition
Scenedesmus sp. (strain 145-2)	CG	Weak Inhibition

Table 13. The relative abundance of high-throughput sequencing reads at the class level.

Class	*Canna* 1	*Canna* 2	*Juncus* 1	*Juncus* 2	Pot Biofilm	Foam Biofilm	Water	Pre-filtered Water
Acidobacteriia	0.59	0.21	1.51	1.74	0	0	0.03	0.08
Actinobacteria	3.9	2.22	3.96	3.3	0	0.63	36.5	39.5
Alphaproteobacteria	37.3	37.8	31.2	22.1	6.65	0	10.1	6.37
Anaerolineae	0.26	0.21	0.11	0.28	0	0	0	0.25
Aquificae	0	0.05	0.07	0.05	0	0	0	0
Bacilli	0.28	0.16	0.29	0.14	0	0	0.03	0.03
Bacteroidetes	0	0	0	0	0	0	0.1	0.03
Bacteroidia	0.12	0.32	0.32	0.64	0	0.05	0.29	0.11
Betaproteobacteria	9.13	12	5.25	8.21	11.7	2.93	5.63	4.7
Caldilineae	0.59	0.16	1.4	0.6	0	0	0	0
Chlamydiia	0.09	0	0	0	0	0	0	0
Chloroflexi	0.76	0.05	0.76	0.96	0	0	0.29	1.17
Clostridia	8.61	4.97	10.1	11.7	0	0.54	1.33	2.46
Cyanophyceae	20	24.5	32.3	35.7	0	0	30.8	31.2
Cytophagia	1.8	3.06	0.43	0.28	0	0	1.26	1.29
Dehalococcoidia	0	0	0	0.09	0	0	0	0
Deinococci	0	0	0.11	0.18	0	0	0	0
Deltaproteobacteria	0.97	0.69	0.9	0.55	0	0	0.19	0.34
Epsilonproteobacteria	0.05	0.16	0.07	0.28	0	0	0.06	0.08
Erysipelotrichia	0.59	0.21	0.4	0.28	0	0	0	0
Fibrobacteria	0	0	0	0	0	0	0.03	0
Flavobacteriia	4.56	4.75	1.73	1.83	0	0	2.85	3.97
Fusobacteriia	0.4	0.9	0.58	0.6	0	0	0.23	0.98
Gammaproteobacteria	5.01	3.12	4.39	6.65	81.6	95.9	0.55	1.82
Gemmatimonadetes	0	0	0	0	0	0	0.03	0.06
Gloeobacteria	0	0	0	0	0	0	0.1	0.08
Holophagae	0.31	0.11	0	0	0	0	0	0
Ignavibacteria	0	0	0	0	0	0	0	0.03
Lentisphaeria	0	0.21	0.25	0.05	0	0	0	0.03
Mollicutes	0	0.05	0	0.05	0	0	0	0
Nitrospira	0	0.16	0.11	0.05	0	0	0	0
Opitutae	0.12	0	0.14	0.18	0	0	1.13	0.22
Planctomycetia	1.23	0.37	0.14	0.23	0	0	0.19	0.5
Sphingobacteriia	1.65	1.32	1.11	1.28	0	0	7.37	3.27
Spirochaetia	0	0.05	0	0.14	0	0	0.13	0.22
Synergistia	0	0.05	0	0	0	0	0	0.03
Thermolithobacteria	0.24	0.05	0.22	0.32	0	0	0	0.06
Thermomicrobia	0	0.42	0.14	0.55	0	0	0.52	0.5
TM7	0.17	0.37	0.11	0.6	0	0	0.06	0.14
Verrucomicrobiae	1.21	1.27	1.94	0.37	0	0	0.16	0.48

Table 14. The number of sequences classified to be within known functional bacterial genera. Note: the table carries on over the next three pages.

	Relative abundance of sequences classified to be within known functional bacterial genera							
	Plant Root				Biofilm		Water	
	Canna 1	*Canna* 2	*Juncus* 1	*Juncus* 2	Pot Biofilm	Foam Biofilm	Water	Pre-filtered Water
Autotrophs								
Synechococcus	3.38	3.33	4.78	2.84	0	0	8.09	10.96
Nostoc	0.09	0.11	0.14	0.05	0	0	0	0.08
Nostochopsis	0.54	0.90	1.55	1.28	0	0	0.29	0.03
Anabaena	13.52	18.38	20.68	28.03	0	0	12.45	12.38
Microcystis	0.05	0.05	0.07	0	0	0	1.58	1.73
Cyanobacterium	0.09	0.16	0.11	0.05	0	0	0.29	0.28
Cyanothece	0.21	0.05	0.32	0.28	0	0	0.45	0.48
Synechocystis	0	0.16	0	0.28	0	0	0	0
Cyanobium	0.57	0.16	0.43	0.46	0	0	0.49	0.61
Oscillatoria	0.05	0	0.07	0.09	0	0	0.10	0.11
Spirulina	0	0	0	0	0	0	0.06	0
Nitrogen-fixing bacteria								
Rhizobium	13.76	10.51	3.06	3.99	0	0	0.16	0.25
Bradyrhizobium	0.26	0.32	0.07	0.09	0	0	0	0
Azorhizobium	0	0	0	0.05	0	0	0	0
Azovibrio	0.02	0	0	0	0	0	0	0
Azospira	0.02	0	0	0	0	0	0	0
Nitrifying bacteria								
Nitrosomonas	0.00	0.05	0	0.18	0	0	0	0
Nitrosovibrio	0.09	0	0	0.05	0	0	0	0
Nitrospira	0.00	0.16	0.11	0.05	0	0	0	0
Sulfur-oxidizing bacteria								
Thiobacillus	0.17	0.58	0	0	0	0	0	0.31
Thiobacter	0.00	0.11	0	0	0	0	0	0
Beggiatoa	0.02	0.11	0	0	0	0	0	0
Sulfitobacter	0.00	0.05	0	0	0	0	0	0
Thiothrix	0.02	0	0	0	0	0	0.03	0

	Relative abundance of sequences classified to be within known functional bacterial genera							
	Plant Root				Biofilm		Water	
	Canna 1	*Canna* 2	*Juncus* 1	*Juncus* 2	Pot Biofilm	Foam Biofilm	Water	Pre-filtered Water
Purple non-sulfur bacteria								
Thiococcus	0	0	0.07	0.09	0	0	0	0
Chromatium	0.09	0	0.04	0	0	0	0	0
Rhodovulum	0.02	0	0	0	0	0	0	0
Rhodobacter	6.71	8.45	9.35	5.96	0.82	0	0.16	0.31
Rhodoferax	0.14	0.79	0.07	0.05	0	0	0.03	0.06
Rhodomicrobium	0.45	1.37	0.18	0.14	0	0	0.10	0.03
Rhodoblastus	0.05	0.11	0.18	0	0	0	0	0
Roseobacter	0	0.42	0	0	0	0	0	0
Rhodoplanes	0.02	0.21	0	0	0	0	0	0
Rhodoluna	0	0	0.07	0	0	0	0	0.06
Rhodopseudomonas	0	0	0.04	0.05	0	0	0	0
Sulfur and sulfate reducing bacteria								
Geobacter	0.05	0.21	0.14	0	0	0	0.13	0.14
Desulfurobacterium	0	0.05	0.07	0.05	0	0	0	0
Desulfosarcina	0	0.05	0	0	0	0	0	0
Desulfobacterium	0	0	0	0.05	0	0	0	0.17
Desulforhabdus	0	0	0.04	0.05	0	0	0	0.03
Desulfobacca	0.09	0	0.11	0	0	0	0	0
Desulforegula	0.12	0	0.07	0	0	0	0	0
Desulfotomaculum	0.02	0	0	0	0	0	0	0
Desulfomonile	0.07	0	0	0	0	0	0	0
Desulfofustis	0	0	0.14	0	0	0	0	0
Desulfovibrio	0	0	0.07	0	0	0	0	0
	0.35	0.32	0.65	0.14	0	0	0.16	0.34
Methylotrophic bacteria								
Methylotenera	0.05	0.42	0	0	0	0	0	0
Methylophilus	0.02	0.21	0	0	0	0	0.13	0.25
Methylobacterium	0.26	0.26	0.97	0.18	0.92	0	0.39	0.14
Methylothermus	0	0.11	0.07	0.05	0	0	0	0.03
Methylobacillus	0	0	0	0	0	0	0	0.08
Methyloversatilis	0	0	0	0	0	0	0	0.03
Methylovorus	0	0	0	0	0	0	0	0.03
Methylovulum	0.12	0	0	0	0	0	0	0

	Relative abundance of sequences classified to be within known functional bacterial genera							
	Plant Root				Biofilm		Water	
	Canna 1	*Canna* 2	*Juncus* 1	*Juncus* 2	Pot Biofilm	Foam Biofilm	Water	Pre-filtered Water
Methanotrophic bacteria								
Methylomonas	0.02	0.05	0	0	0	0	0	0
Methylosinus	0.09	0.05	0	0.37	0	0	0	0
Methylomicrobium	0.02	0	0	0.00	0	0	0	0
Methylobacter	0.09	0	0	0.00	0	0	0	0
Methylococcus	0	0.11	0.07	0.00	0	0	0.03	0
Methylocystis	1.42	2.01	1.22	0.96	0	0	0.19	0.17
Methylocaldum	0.31	0.26	0.22	0.18	0	0	0.06	0.14
Methylocapsa	0.80	0.26	0.68	0.55	0	0	0	0
Agrobacterium	0.97	0.48	0.40	0.23	0	0	0	0

FIGURES

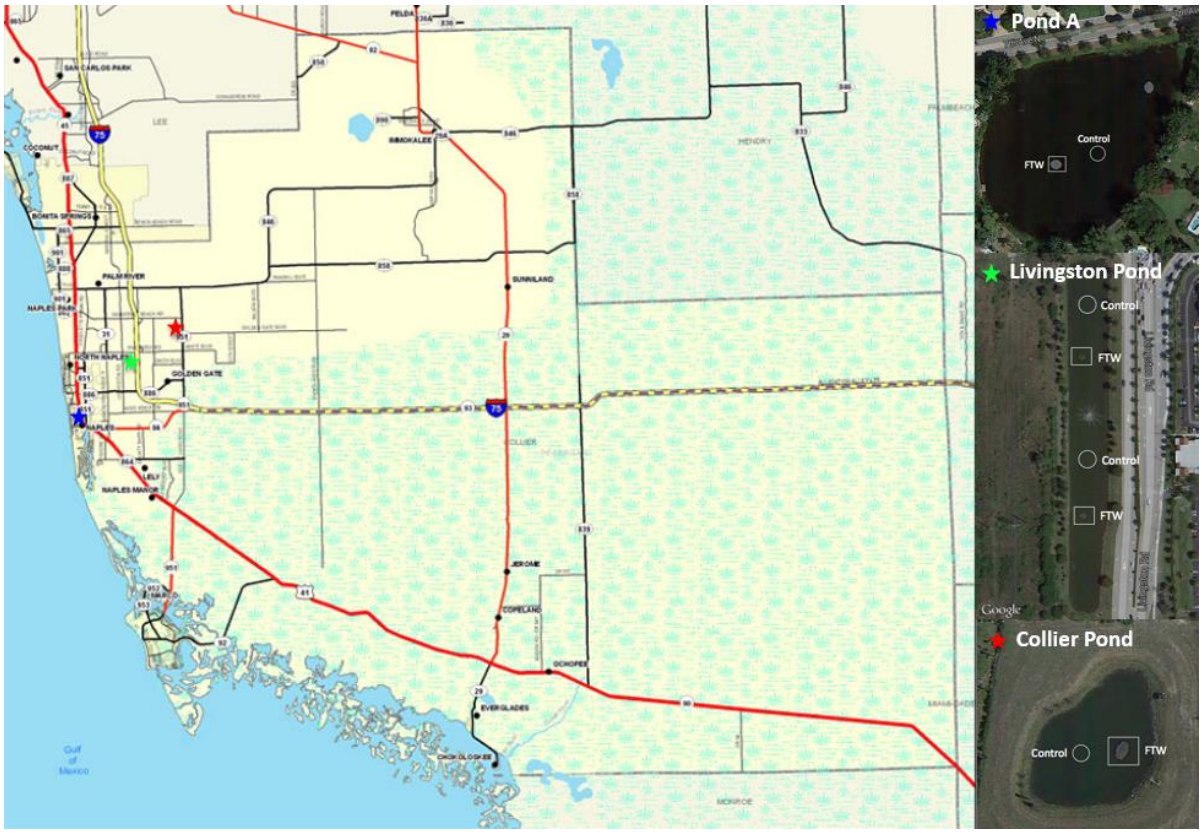


Figure 1. The study site locations. Pond A located on the 7th Ave N in Naples, FL. Easting: 419811 m Northing: 2893114 m. Livingston Pond located in Naples, FL. Easting: 042488 m Northing: 2899115 m. Livingston Pond is 2.96 acres with a perimeter of 680 m. Collier Pond located in Naples, FL. Easting: 043121 m Northing: 2902562 m. Collier Pond is 0.72 acres with a perimeter of 200 m.

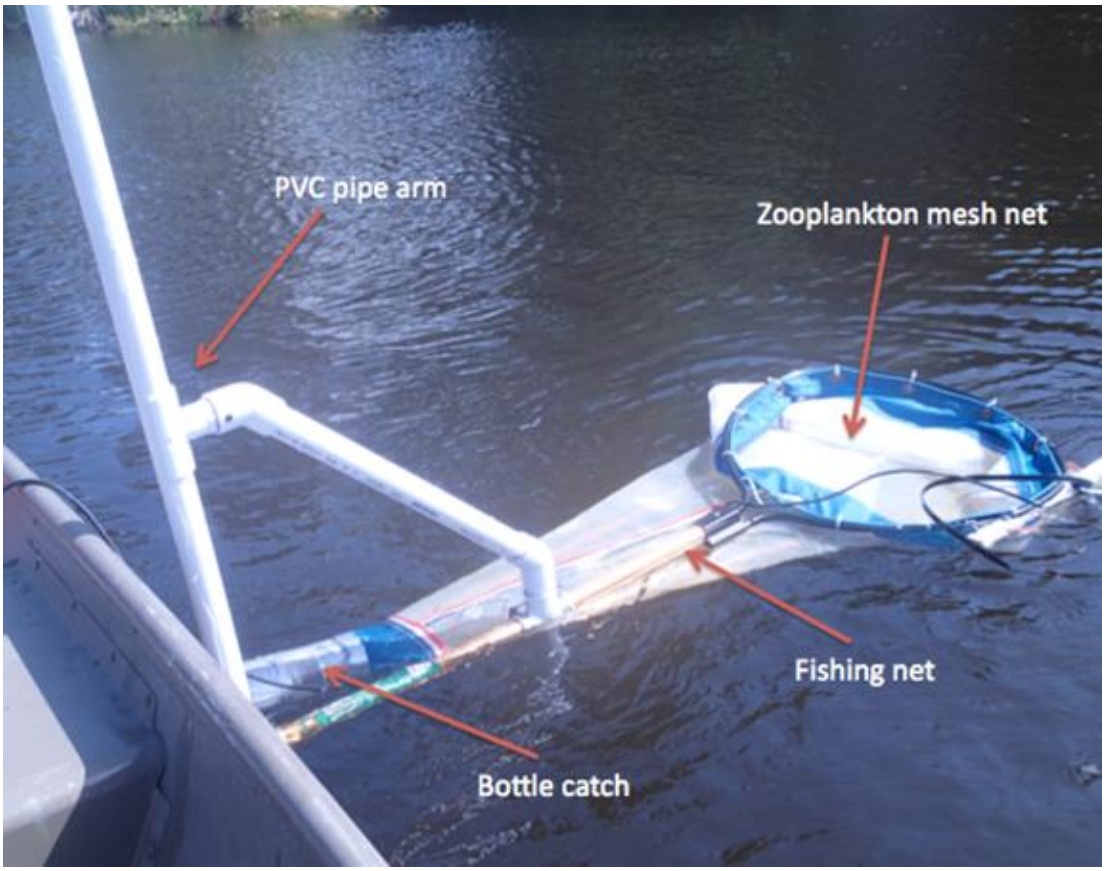


Figure 2. The design of the adapted zooplankton vertical tow net for the zooplankton presence/absence study.

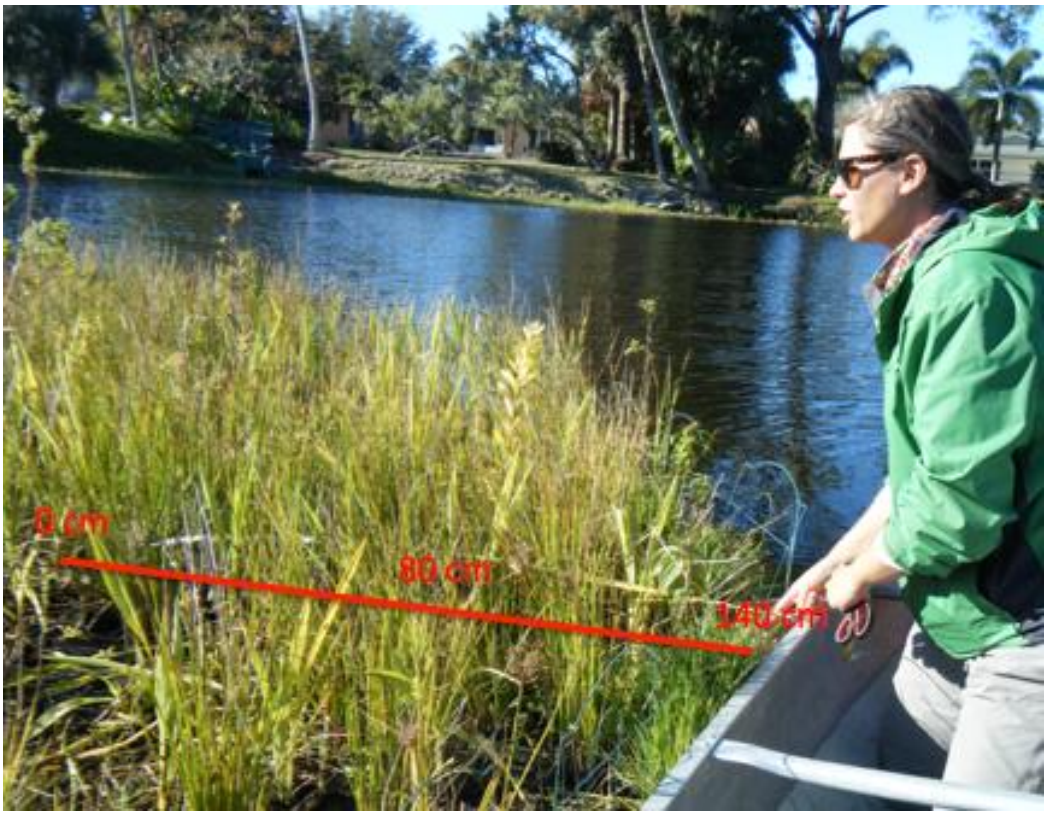


Figure 3. The transect run from the center to the edge of the FTW for microbial community characterization sampling. All samples were collected from the edge to the center at 0 cm, 80 cm, and 140 cm.

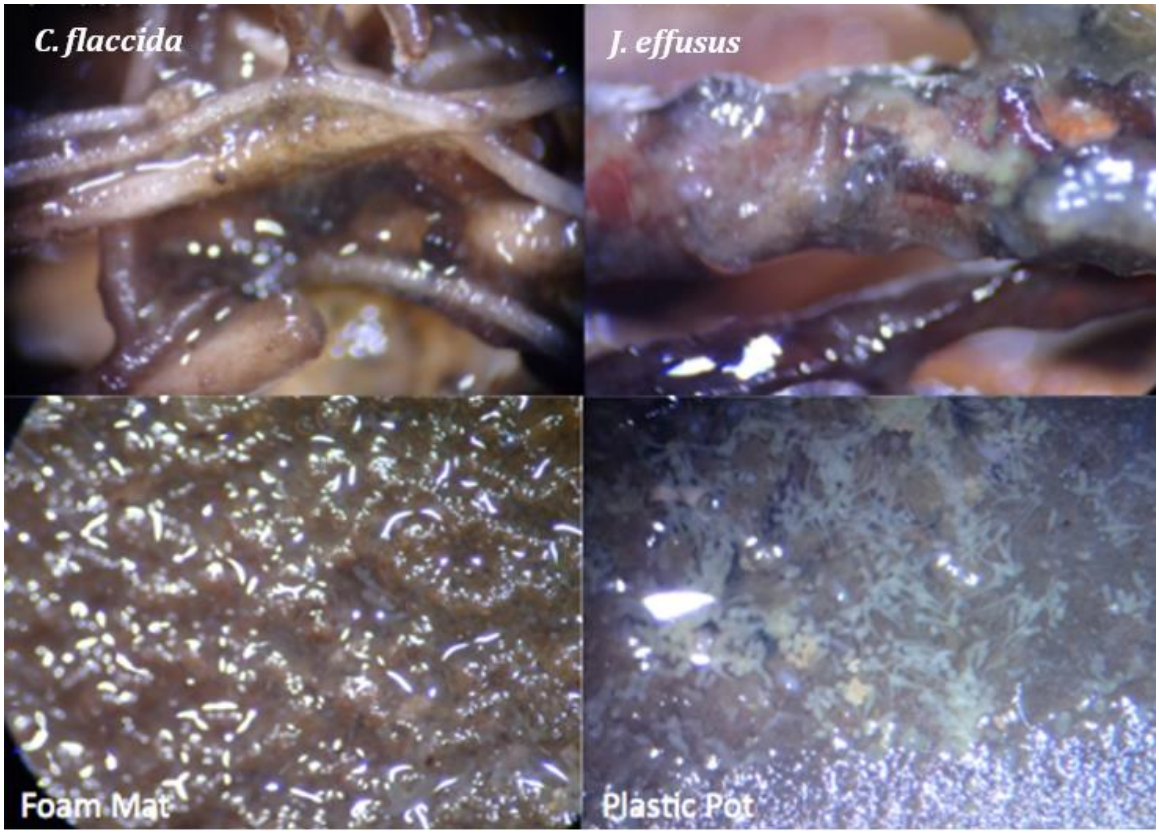


Figure 4. Biofilm formation on the various samples. Purple fringing are chromatic aberrations.

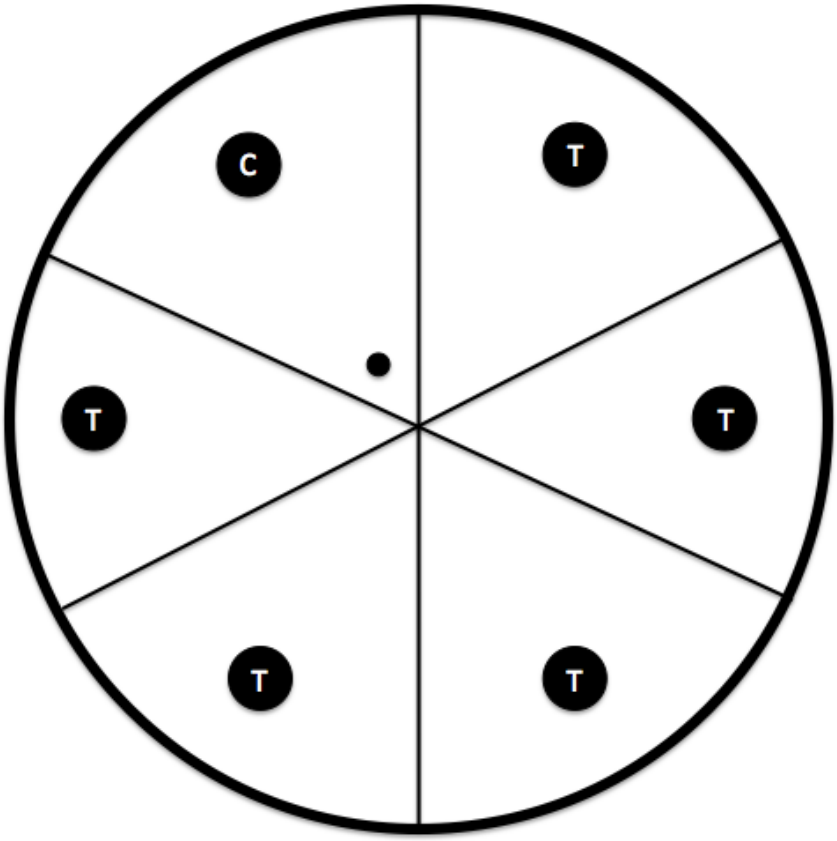


Figure 5. The setup of the agar plate for the agar diffusion assay. The circle marked "C" is the control well and the circles marked "T" are the treatment wells. The smaller black dot conveniently marked the control well during the experiment.

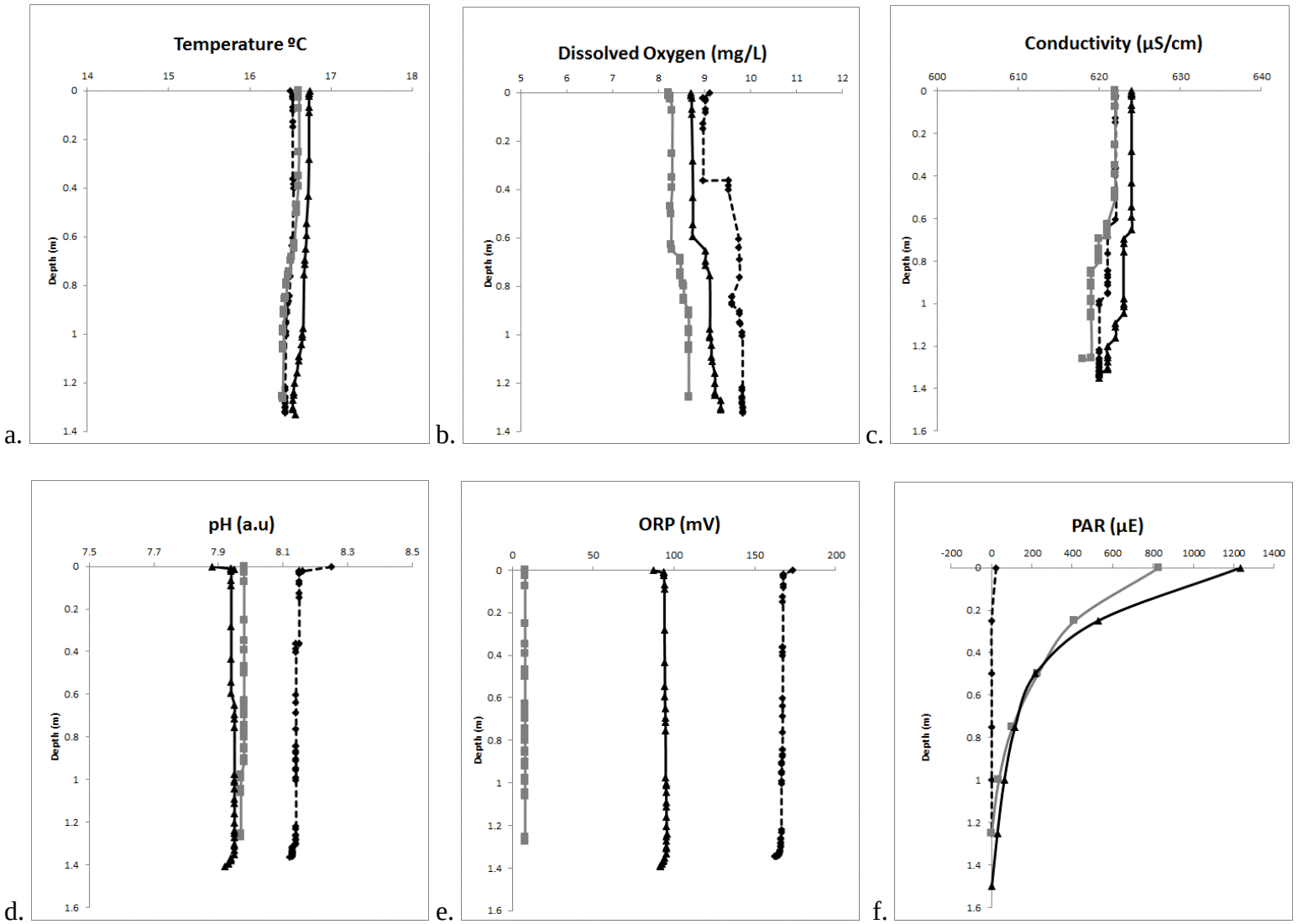
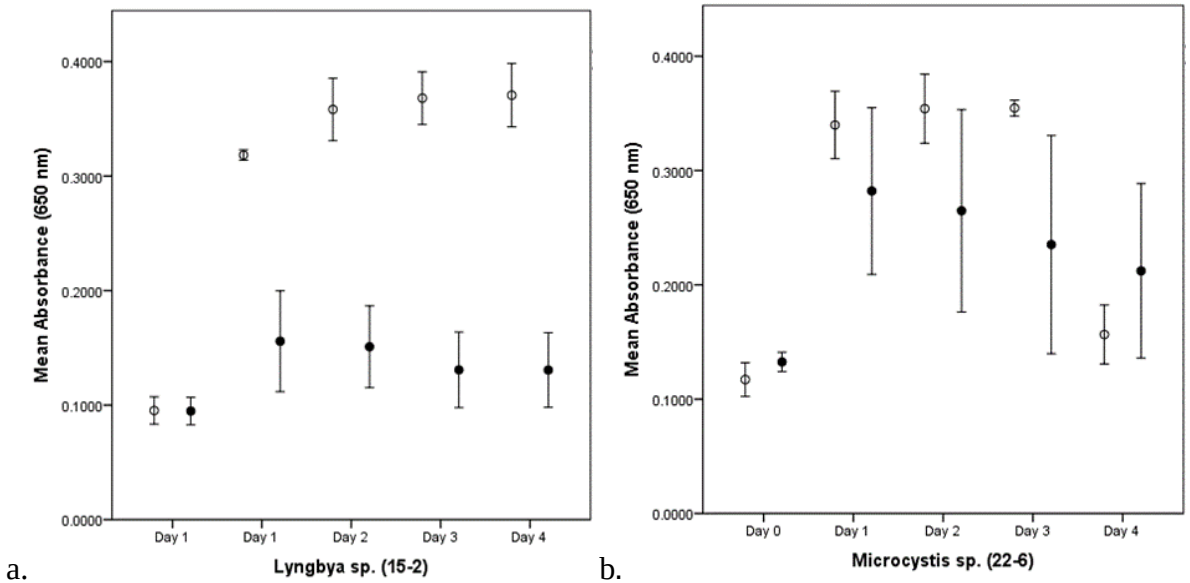


Figure 6. The water column profiles performed in Pond A at approximately 12:00 p.m (March 2014). The water column profiles were performed underneath the FTW, adjacent to the FTW and in the open water. The dashed line represents the measurements taken underneath the FTW, the gray line represents the measurements taken adjacent to the FTW and the black line represents measurements taken in the open water.



a. b.

Figure 7. Initial growth, continuous inhibition (IGCI) growth pattern of the *J. effusus* liquid culture assay. The growth of the treatment is represented by the black circles and the growth of the control is represented by the white circles. The error bars display ± 1 standard deviation.

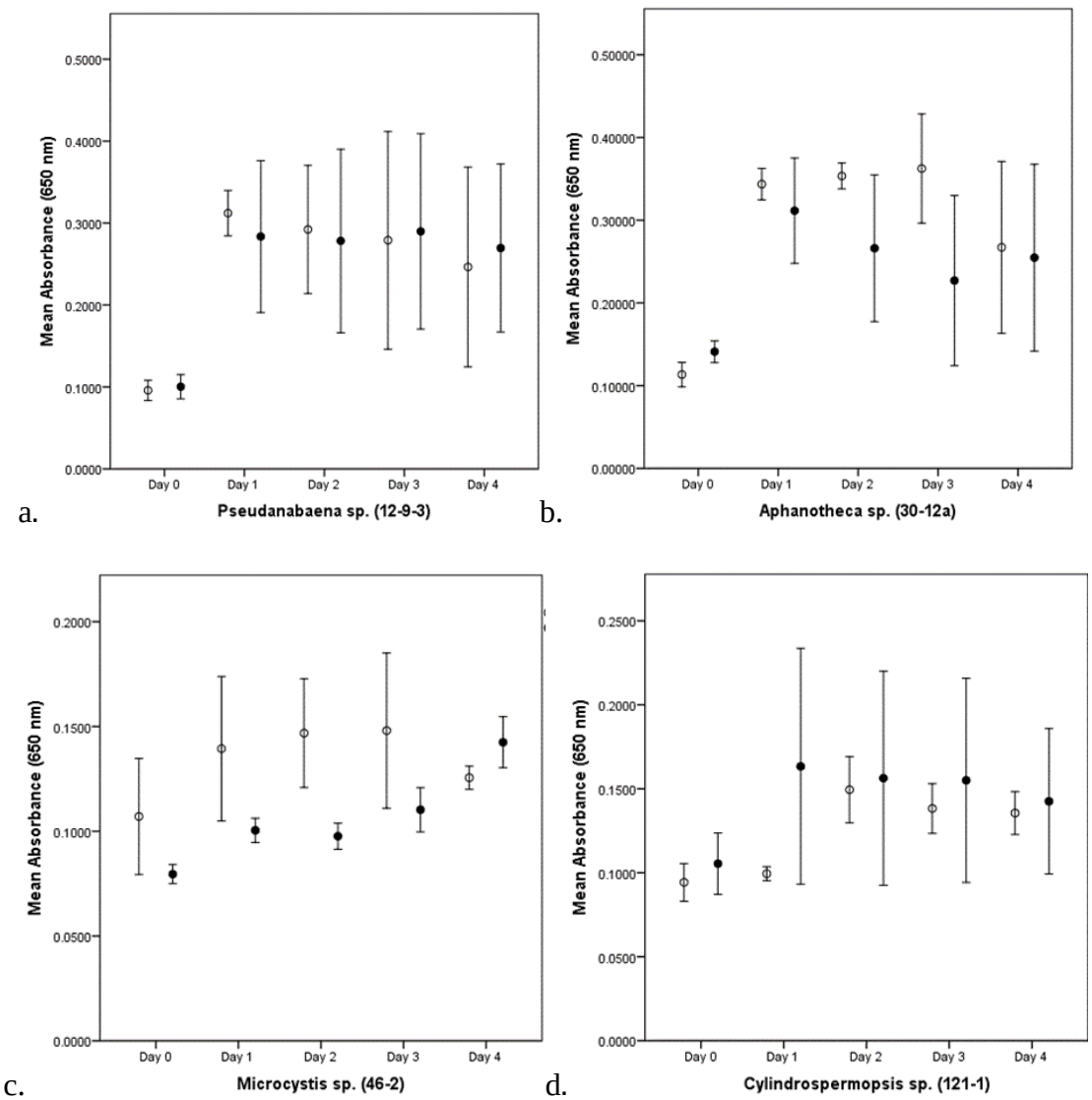


Figure 8. Initial growth, inhibition, recovery (IGCI) growth pattern of the *J. effusus* liquid culture assay. The growth of the treatment is represented by the black circles and the growth of the control is represented by the white circles. The error bars display ± 1 standard deviation.

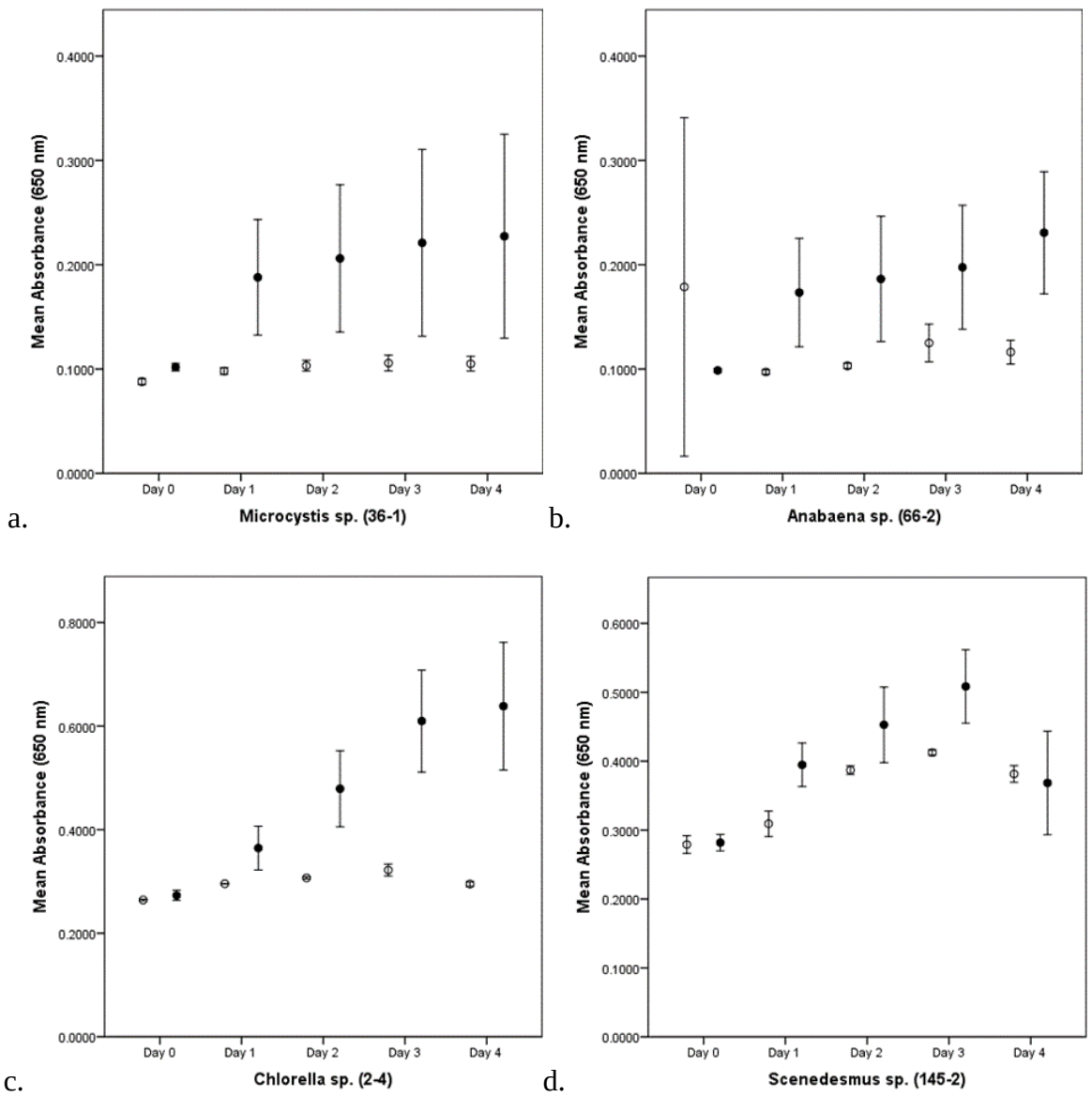


Figure 9. Continuous growth (CG) growth pattern of the *J. effusus* liquid culture assay. The growth of the treatment is represented by the black circles and the growth of the control is represented by the white circles. The error bars display ± 1 standard deviation.

a.

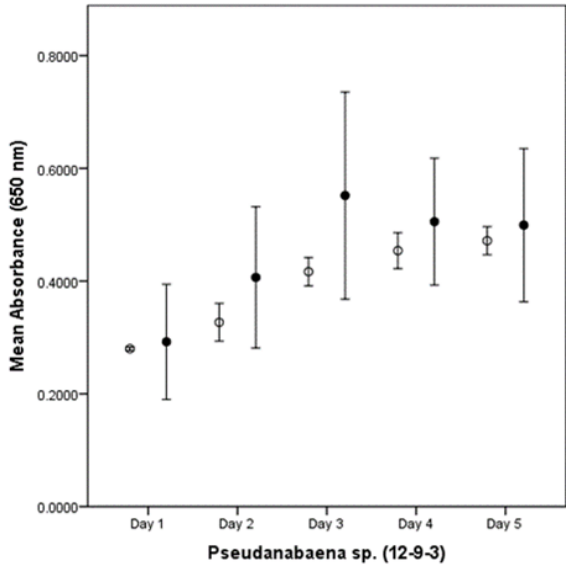
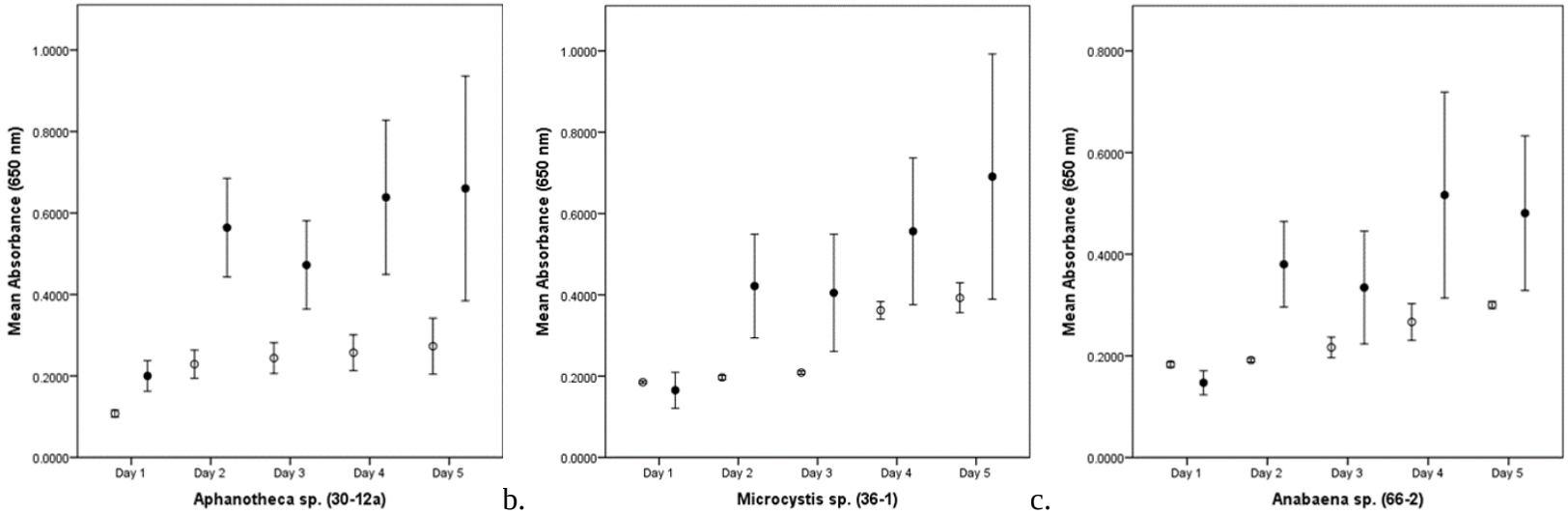


Figure 10. Initial growth, continuous inhibition (IGCI) growth pattern of the *C. flaccida* liquid culture assay. The growth of the treatment is represented by the black circles and the growth of the control is represented by the white circles. The error bars display ± 1 standard deviation.



a. b. c.

Figure 11. Initial growth, inhibition, recovery (IGCI) growth pattern of the *C. flaccida* liquid culture assay. The growth of the treatment is represented by the black circles and the growth of the control is represented by the white circles. The error bars display ± 1 standard deviation.

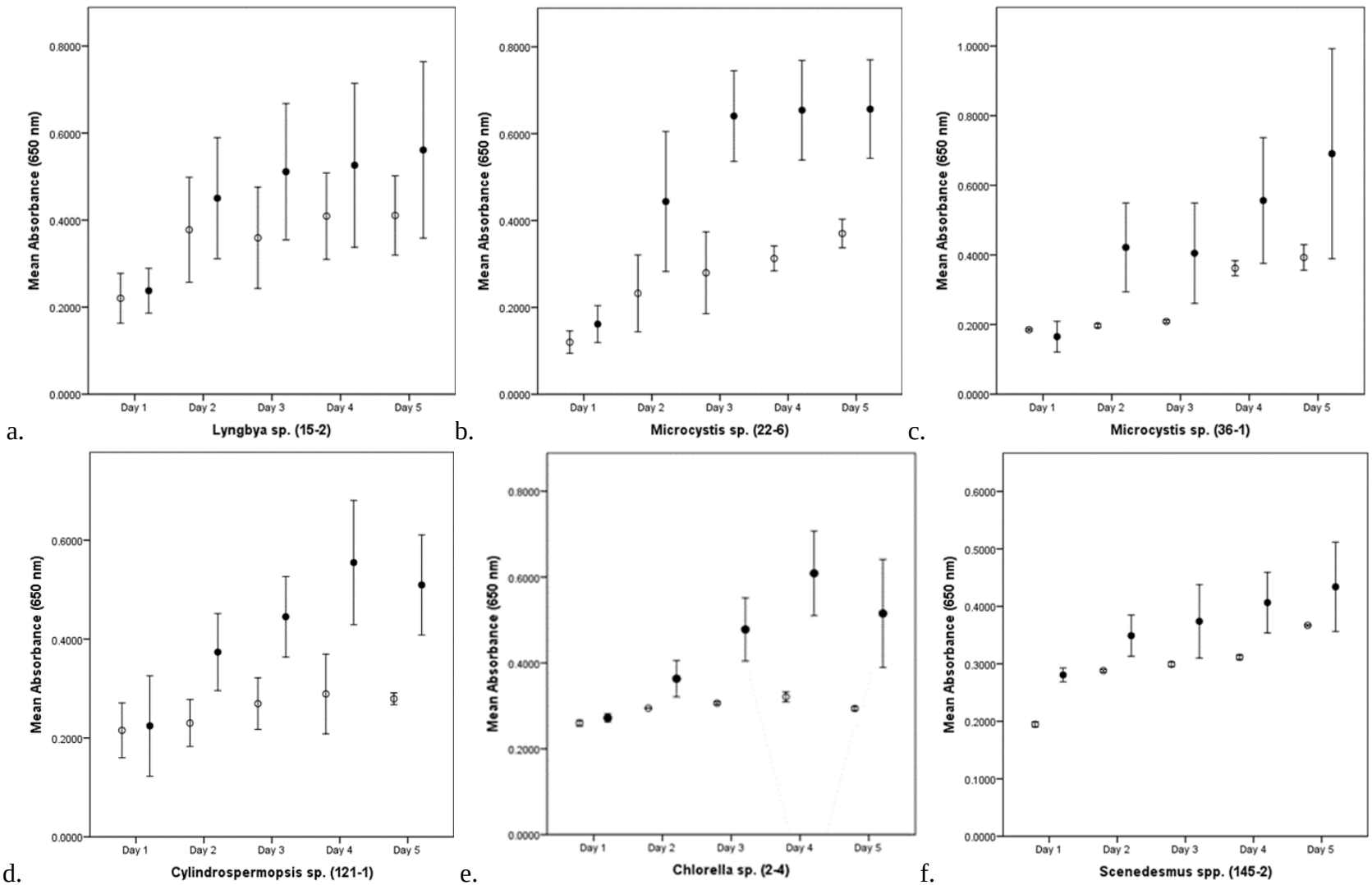


Figure 12. Continuous growth (CG) growth pattern of the *C. flaccida* liquid culture assay. The growth of the treatment is represented by the black circles and the growth of the control is represented by the white circles. The error bars display ± 1 standard deviation.

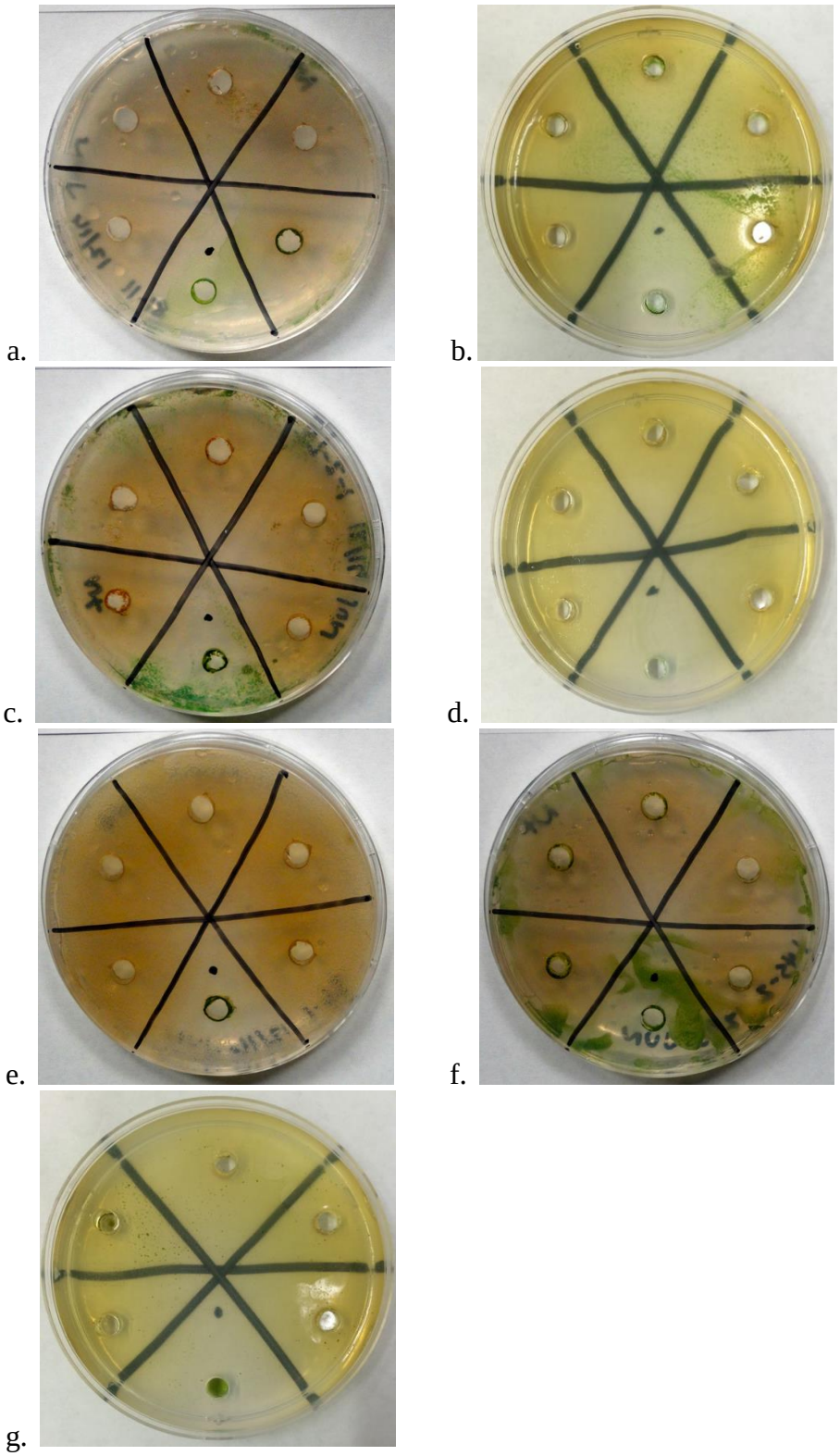


Figure 13. The results of the *J. effusus* agar diffusion assay in the following order: *Microcystis* sp. (strain 81-11), *Anabaena* sp. (strain 66-2), *Limnothrix* sp. (strain 37-2-1), *Cylindrospermopsis* sp. (strain 121-1), *Microcystis* sp. (strain 36-1), *Scenedesmus* sp. (strain 145-2) and *Chlorella* sp. (strain 2-4). Cf. text for more information.

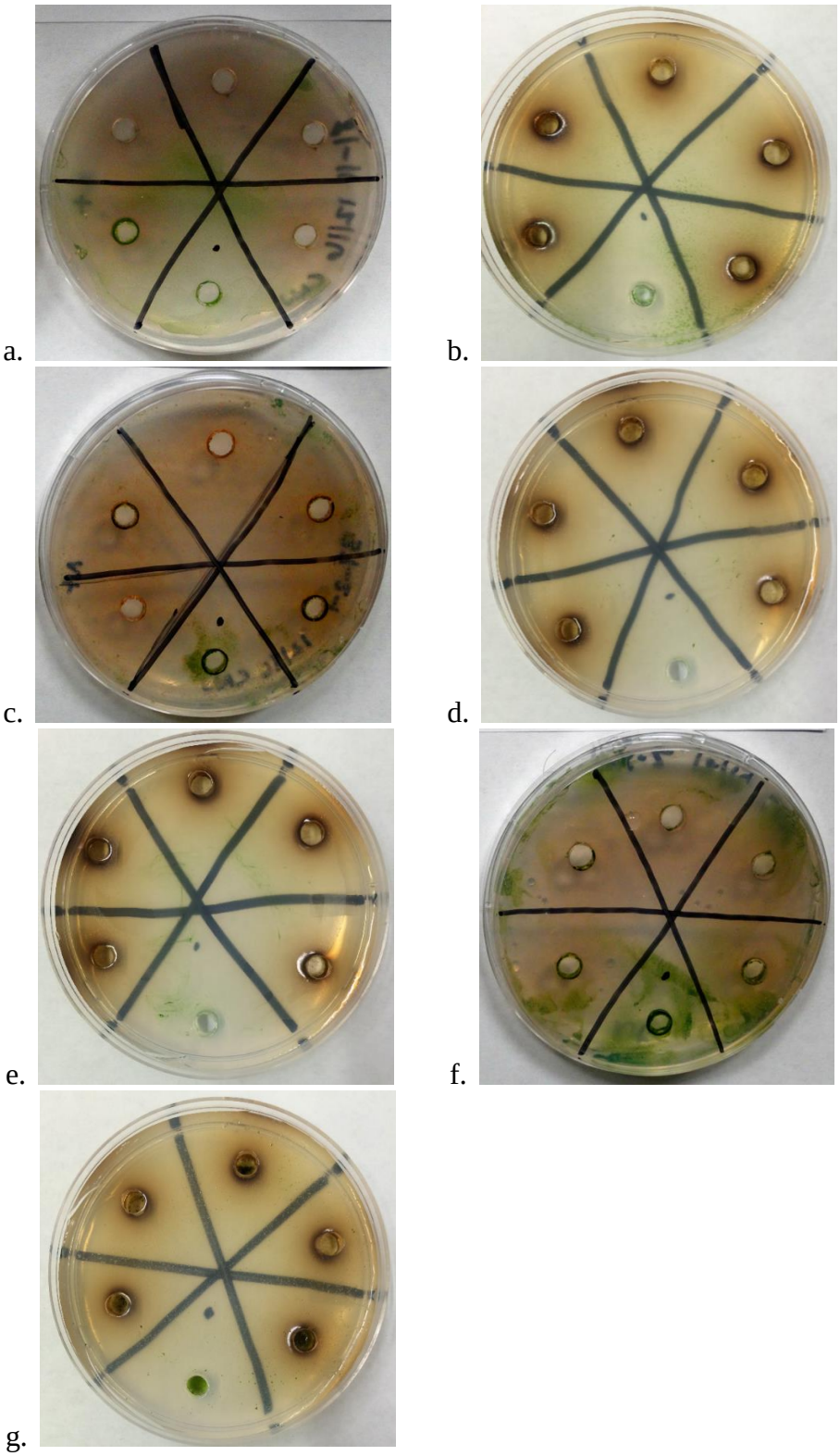


Figure 14. The results of the *C. flaccida* agar diffusion assay in the following order: *Microcystis* sp. (strain 81-11), *Anabaena* sp. (strain 66-2), *Limnothrix* sp. (strain 37-2-1), *Cylindrospermopsis* sp. (strain 121-1), *Microcystis* sp. (strain 36-1), *Scenedesmus* sp. (strain 145-2) and *Chlorella* sp. (strain 2-4). Cf. text for more information.

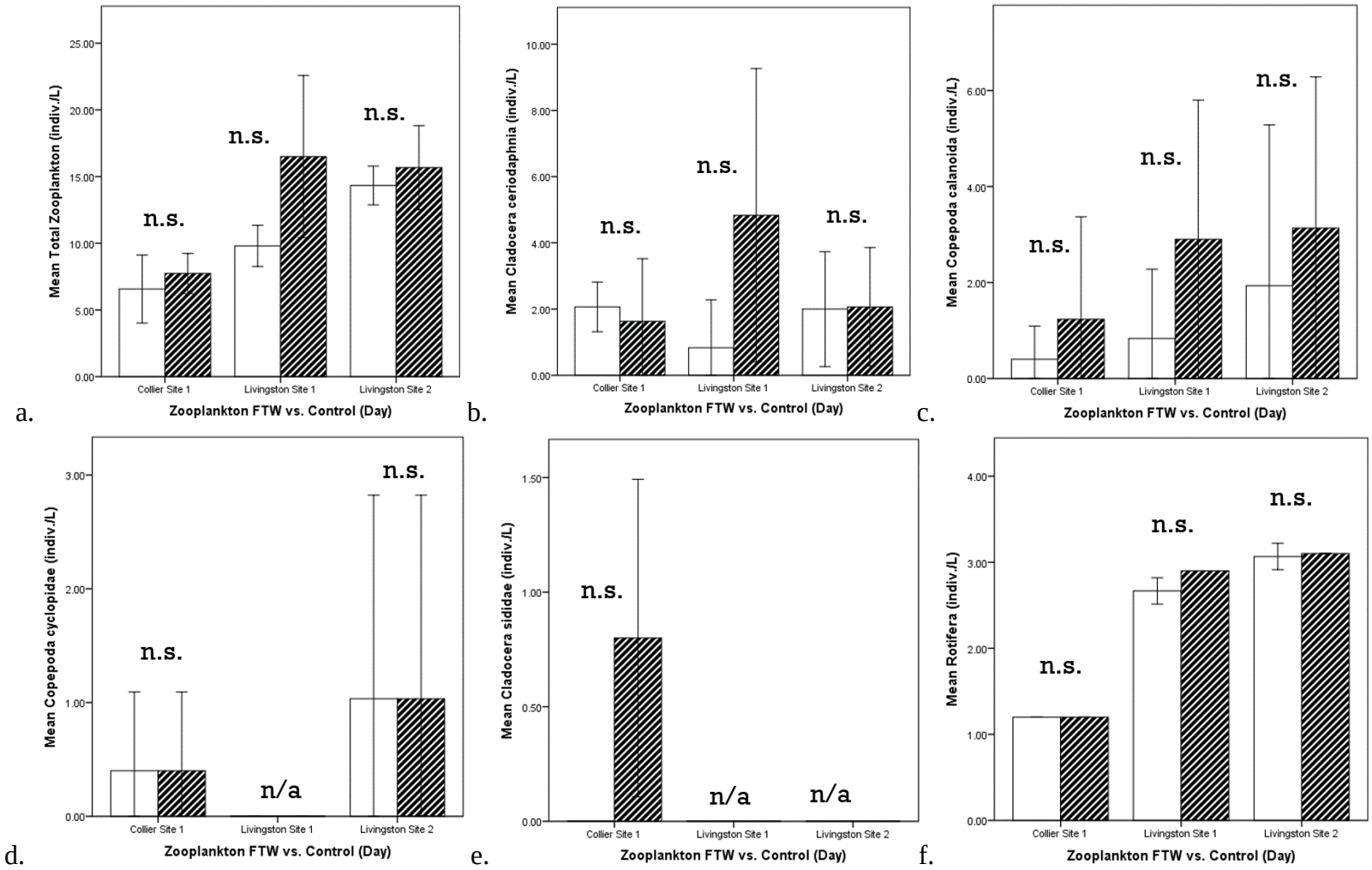


Figure 15. The amount of zooplankton present underneath the FTW and in the open water control during the day. The white bars represent the samples taken below the FTW and the striped bars represent the samples taken in the open water. The error bars display ± 1 standard deviation.

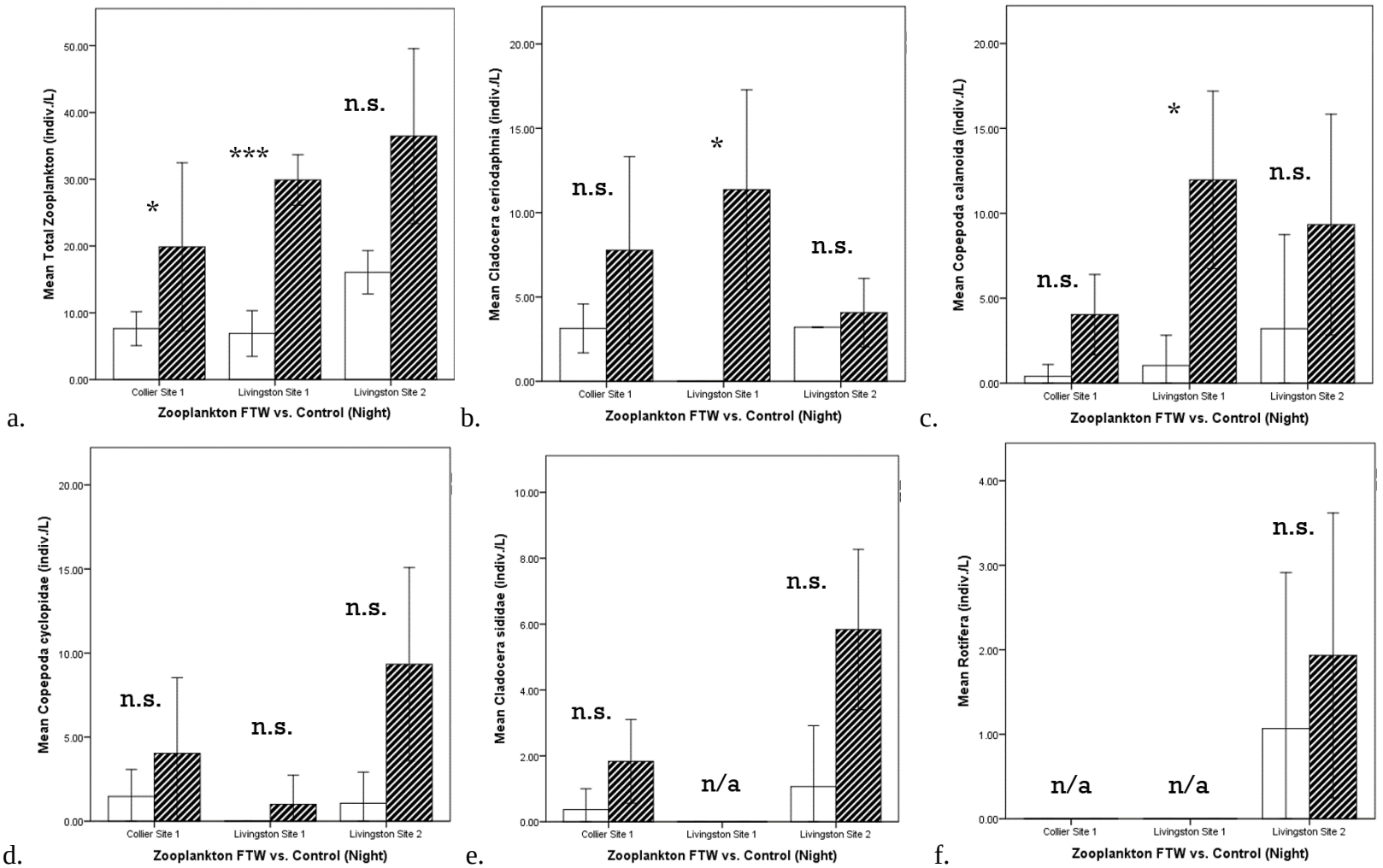


Figure 16. The amount of zooplankton present underneath the FTW and in the open water control at night. The white bars represent the samples taken below the FTW and the striped bars represent the samples taken in the open water. The error bars display ± 1 standard deviation.

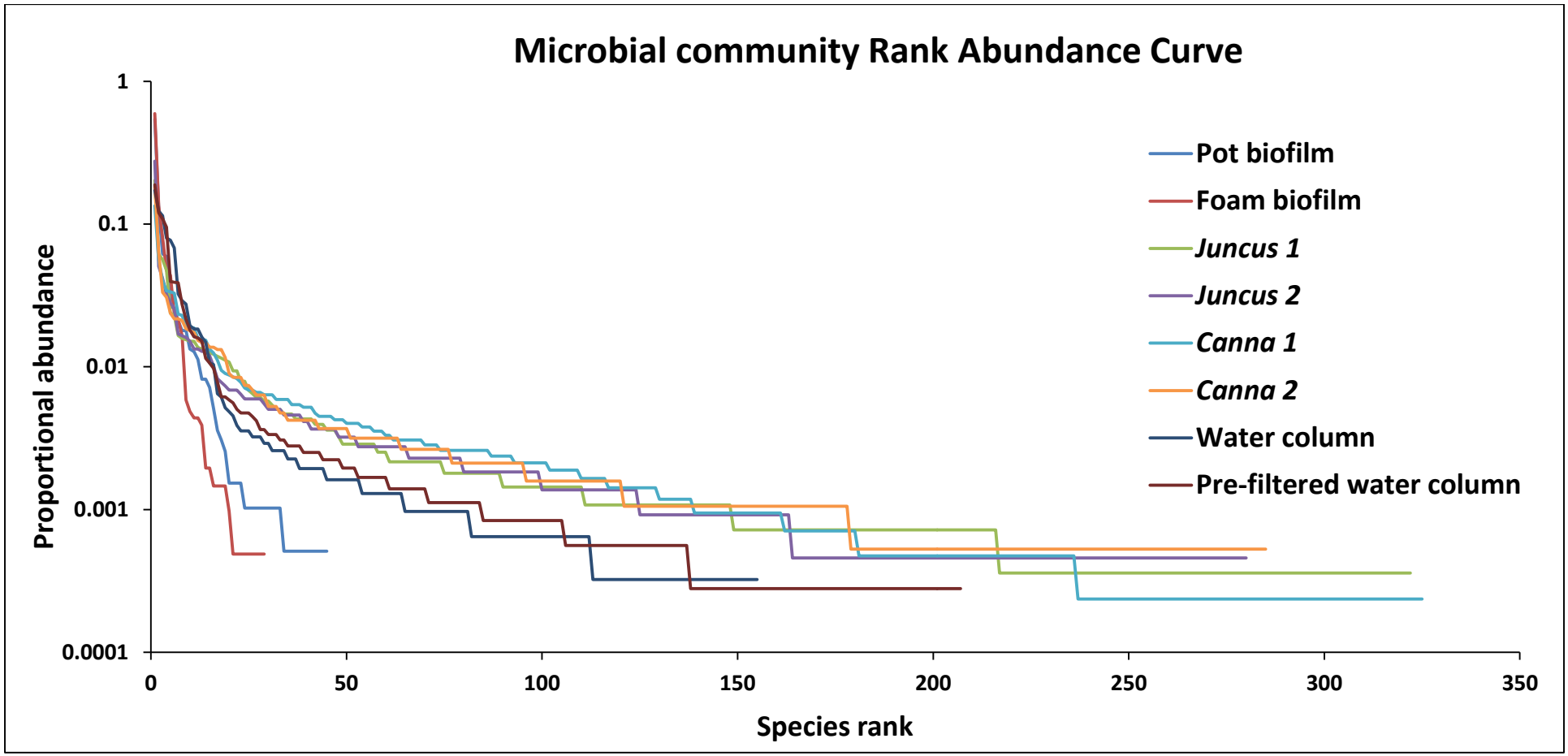


Figure 17. The rank abundance curve for each microbial community living within the FTW rhizosphere, the open water, the foam and the pot materials.

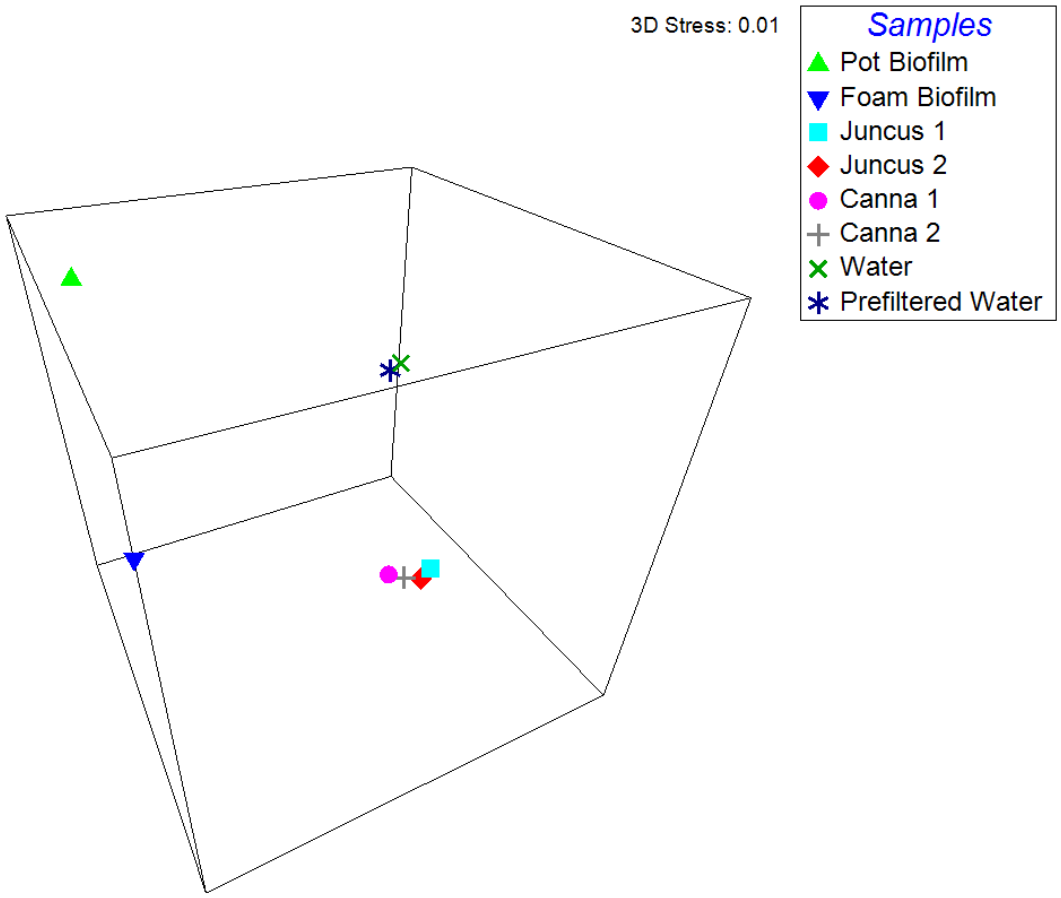


Figure 18. Multidimensional scaling (MDS) ordination of the microbial communities for a species level comparison.

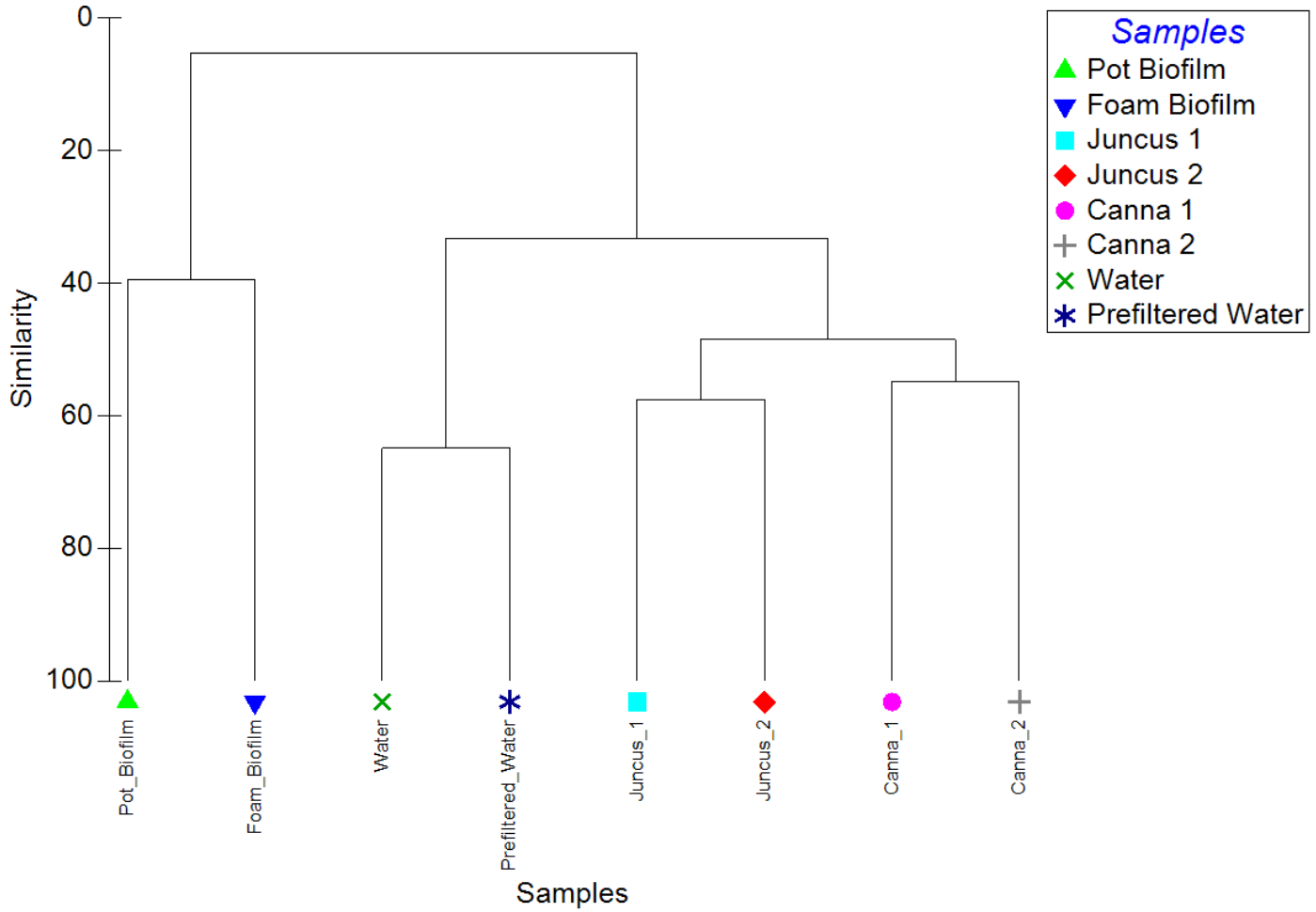


Figure 19. Cluster analysis of the microbial community on a species level.

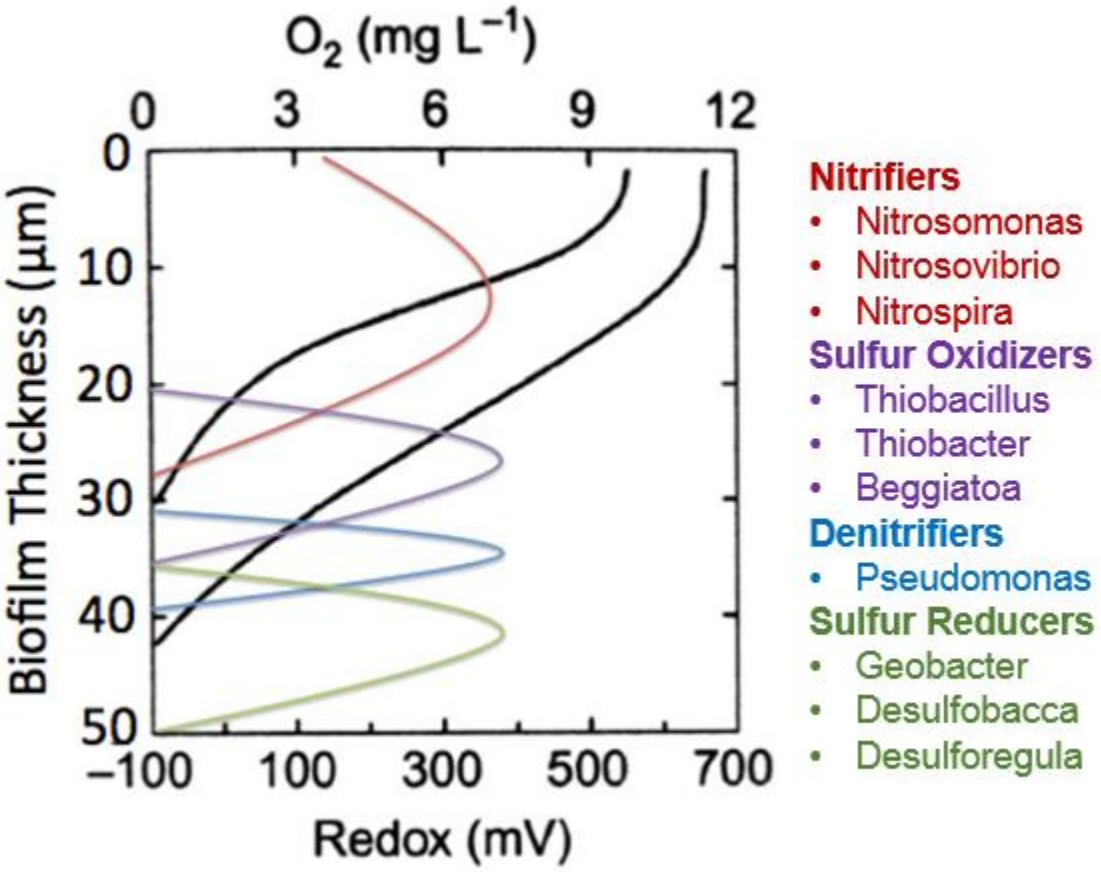


Figure 20. A modification from a Dodds & Whiles (2010) figure on nutrient cycling. The figure displays a redox gradient in relation to oxygen concentration and functional groups. The functional groups listed in the figure are those that were characterized in this study.