Investigation into the role of bacteria biofilms in the initial attachment and colonization stages of filamentous green algae in lab bench-scale algal turf scrubbers

by

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Abstract

The design of reactor systems to cultivate filamentous green algae for remediation of nutrients from wastewater and for biomass production has long been pursued for various wastewater applications. The role of early colonization characteristics of algae on substrata in system performance has been poorly understood, however. In particular, the role of the overall background microbial community in contributing to attachment of filamentous green algae is not well known. Past research suggest that in mixed-community stream biofilms, bacteria, diatoms, and other microbes play an integral role in conditioning new surfaces through the exudation of extracellular polymeric substances (EPS), which forms an aggregate onto which algae cells settle and attach. This objective of this research was to investigate the role of a pre-existing bacteria biofilm in the colonization of polymer substrata in bench-scale floway systems by filamentous green algae (FGA). Specifically, it probes if the presence of microbial biofilms produced by communities sourced from FGA-favoring natural habitats results in faster and stronger attachment by the FGA *Rhizoclonium spp*, under varied flow, nutrient concentrations, bacteria community sources, and media types.

For the studies described, novel lab-bench scale systems were constructed and tested for operation and parameter setting. The effect of three bacteria biofilms, formed by communities sourced from the Tallapoosa River (Tallasee, Alabama, USA) and Town Creek (Auburn, Alabama, USA) and from the two mixed together, was tested using different strengths of the synthetic media (freshwater-modified) Proline F/2 (1/4 and 2x) and dilutions of tilapia aquaculture wastewater (1/4, ½, and full-strength) with different floway channel slopes (1%, 2%, and 3%). The impact of bacteria biofilms on attachment was assessed as speed and strength of

attachment, measured as amount of biomass and chlorophyll *a* over time, with the division of samples into two subsets for strength of attachment analysis.

It was found that the presence of the Tallapoosa River bacteria biofilm resulted in significantly faster attachment of *Rhizoclonium spp.* cells in the ¼ dilution of F/2 and with a channel slope of 3.00 ± 0.100%. The presence of the mixed bacteria biofilm also had a significant impact on attachment when parameters of a ¼-dilution of unfiltered tilapia effluent and a channel slope of 2.00 ± 0.100% were used, as indicated through *chl-a* measures. While statistical significance was not detected for other trial conditions, based on effect sizes and wide confidence intervals, and the fact that the graphical trend remained consistent and visible, it can be argued there is a need for greater power in the experimental design. There is reason to believe that given sufficient replication, biofilms formed by the tested microbial communities would demonstrate a statistically significant impact on speed of attachment under these other constraints.

From all results, it can be concluded that a microbial biofilm pioneer community can have impact on early colonization rates of FGA on virgin substrata. Also, it can be concluded that a lower nutrient concentration of the media results in a positive impact on speed of FGA attachment but altering the channel slope by 1% increments did not result in a noticeable effect. The research indicates that there is cause for further exploration of the microbial biofilm's role in initial FGA colonization of ATS systems.

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List of Abbreviations

ATS	Algal Turf Scrubber
EPS	Extracellular Polymeric Substances
DI	Deionized
FGA	Filamentous Green Algae
HPLC	High Performance Liquid Chromatography
LAB	Loosely Attached Biomass
SAB	Strongly Attached Biomass
SA index	Strength of Attachment Index

Chapter 1: Introduction

The world teeters precariously on the edge of a precipice, hemmed in by the snowballing threats of a rapidly expanding population, intensifying climate change, and the issues that stem from them. Among the dangers the spread of human influence has given birth to are the shrinking availability of already scarce resources (arable land, clean water, fossil fuels, etcetera) and the accumulation of harmful wastes, a large portion of which are arduous if not impossible to eliminate completely from the environment. Thus, humanity faces the challenges of sustaining itself and responsibly managing its byproducts at a level it and the world it inhabits are not currently prepared to handle and may lose the capacity to handle if measures are not enacted. Water quality is a concern as increased anthropogenic activity on land leads to greater contamination of natural waterbodies (negatively impacting them aesthetically, recreationally, environmentally, and economically) especially through diffuse waste loading, which continues to be a problem despite existing management techniques which may not be fully effective or widely applied (Sutherland & Craggs, 2017). Current agricultural methods are insufficient to keep up with the growing demand in a sustainable manner and produce considerable amounts of waste that if released untreated into the environment have the potential to have a negative impact on receiving waters and their ecosystems as well as posing a threat to human health. With the current state of the world, it is favorable to develop sustainable, efficient, and cost-effective methods of food production and waste treatment. Aquaponics is a field of agriculture that combines hydroponics and aquaculture, allowing the reuse of fish waste as a water and nutrient source for crop production. While the waste is put to good use, it still requires further polishing after passing through the crop production stage, in both decoupled and coupled systems.

The algal turf scrubber is a novel technology invented by Walter Adey in the 1980s that uses the natural processes of an artificial stream ecosystem populated primarily by attached filamentous green algae (but also diatoms, cyanobacteria, and bacteria) in channels of flowing water to capture and remove pollutants from contaminated waters (Adey & Bannon, 2008). By harnessing energy from the sun, this technology can treat natural waters and wastewater while also recycling otherwise detrimental constituents in the form of algal biomass that, once harvested, can be used in the production of valuable feedstock for animal feed, energy, fertilizer, and other vital products. The algae, besides uptaking nutrients like nitrogen and phosphorus from the water to fuel its growth, also removes carbon dioxide from the atmosphere, replacing it with oxygen produced during photosynthesis. In this way, ATS systems have the potential to contribute to the creation of a circular economy in an environmentally friendly manner, specifically incorporation as tertiary treatment in aquaponics processes.

However, while there have been multiple successful studies performed demonstrating ATS capabilities at pilot scale (W. Adey & Bannon, 2008; W. H. Adey et al., 2013; Kebede-Westhead et al., 2003) and some at smaller scale (Hariz et al., 2022; Salvi et al., 2021), further study is required for continued improvement and understanding of the system. One area that has not been extensively researched is the role of the bacteria biofilm in the initial attachment and colonization phase of the algal turf life cycle. Prior experiments provide evidence indicating that bacteria have the capacity to take on a beneficial role in algae-bacteria interactions (Hodoki, 2005; Kouzuma & Watanabe, 2015a; B. Zhang et al., 2020a). Existing literature on biofilms also suggests that certain bacteria play a vital role in the development of biofilms that periphytic algae cells flock to and integrate with and condition surfaces in a manner that aids attachment (Beleneva et al., 2017; Joint et al., 2007; Li et al., 2019a; Schnurr & Allen, 2015; Singh et al.,

2013). It therefore follows that the addition of a bacteria biofilm to ATS systems prior to the introduction of algae may promote faster or stronger algal attachment.

Section 1.1: Rationale and significance

The attachment of filamentous algae to substrata in channels is one of the primary advantages of ATS systems. The prohibitive harvesting costs associated with other algae-based wastewater treatment technologies, such as high-rate algae ponds and photobioreactors (Ahmed et al., 2022), are not present with ATS systems, whose biomass can be removed through scraping or vacuuming. From the base of algae left behind, healthy periphytic communities can regrow without reseeding. The establishment and development of a thriving algal turf takes time, and harvesting should not be performed until this phase is complete so the mat retains its recovery ability. Faster attachment can speed up this initial period. Stronger attachment allows the algae to resist shear forces from the flowing water and stay attached even in less-than-ideal conditions (Hariz et al., 2022). It also results in more "roots" remaining in the channel post-harvest, which may allow the turf to regrow faster. It is then important to research factors affecting attachment to better understand it and to potentially employ natural interactions to improve existing operations and setups.

Section 1.2: Purpose of Research

The primary intent of this research is to gain insight into the overall role of the bacterial biofilm in the initial attachment and colonization stages of filamentous green algae in lab-bench scale algal turf scrubber systems. In doing so, the research aims to add to the bases of knowledge regarding ATS systems, FGA colonization, and bacteria-algae interactions, contributing to the foundation on which future researchers may potentially build improvements to ATS design and operation. The elucidation of the relationship between bacteria and algae attachment as relates to

treatment of aquaculture waste may also help form a base for application-based study of bacteria biofilms in conjunction with algal turfs in aquaponics operations in the future.

Section 1.3: Objectives of Research

The overarching goal of this research is to determine the effect a bacterial biofilm community has on the initial attachment and colonization of filamentous green algae in algal turf scrubber systems. In these studies, this is quantified as the impact the presence the existing bacteria biofilm has on the speed and strength of attachment.

Previous studies involving similar experiments have demonstrated a correlation between bacterial presence and algal attachment (Gawne et al., 1998; Hodoki, 2005; Holmes, 1986), and bacteria are known to play a significant role in early biofilm development as initial pioneers, surface conditioners, and producers of extracellular polymeric substances (Brasell et al., 2015; Palmer et al., 2007; Schnurr & Allen, 2015). The overall hypothesis, then, was that the presence of a bacteria biofilm would increase the speed or strength of algal attachment. To test this hypothesis, the following objectives were formed:

1. Determine if biofilms formed from bacteria communities sourced from different filamentous algae-favoring waters stimulate attachment.
2. Determine if the results of objective one change if environmental parameters vary (flow regime altered through slope and nutrient concentration).
3. Determine if biofilms formed from bacteria communities sourced from filamentous algae-favoring waters stimulate attachment when the algae is grown in aquaculture wastewater.

Section 1.4: The potential of algae

Graham et al. (2016) refers to “algae” as a term that broadly encompasses a diverse grouping of eukaryotic photosynthetic organisms that range from unicellular to multicellular, reside in aquatic habitats, and possess the ability to perform oxygenic photosynthesis. The group shares traits with plants but is not itself classified as such due to lacking certain characteristics, including a vascular system. A number of species from multiple kingdoms and evolutionary lines fall under this vast umbrella. While algae are often viewed in the light of nuisance algae blooms (Vadeboncoeur et al., 2021), particularly in lakes, in recent times researchers have begun to focus on their potential as feedstock for bioproducts such as oil and fertilizer and their possible biotechnological applications. Algae may be broadly classed as macroscopic (usually seaweed falls into this group) or microscopic.

Microalgae possess an astounding array of valued molecules, including lipids, carbohydrates, complete proteins, and secondary metabolites (many difficult or impossible to synthesize through chemical methods), and are capable of producing others, namely pigments like carotenoids that are useful for bioproduction and biotechnological applications; they also excrete substances that enable them to act as biostimulants and antimicrobials, offering potential tools to fight pathogens (Sutherland et al., 2021). As a result, they constitute a valuable prospective resource with biophysical and chemical properties that can be exploited in many sectors, including pharmaceuticals, cosmetics, biofuels, agriculture, and wastewater treatment. Sutherland et al. (2021) highlights algae’s possible role in future solutions to global issues; it has the potential to help meet certain UN Sustainable Development Goals, including SDG 2 Zero Hunger, SDG 6 Clean Water and Sanitation, SDG 7 Affordable and Clean Energy, SDG 12 Responsible Consumption and Production, SDG 14 Life Below Water, and SDG 15 Life on Land.

The recognition of algae's varied advantages is not an entirely contemporary affair. Even as far back as the reign of the ancient Mayan civilization, different species of algae have served as a food source. Large scale microalgae cultivation, known as algaculture, has been around for decades (Trentacoste et al., 2015), but in recent times has seen an uptick in interest from many sectors, particularly energy, as the functionality of algae for many purposes became more well-known. Faster growth, less stringent cultivation requirements (less space requirement, the ability to use wastewater instead of freshwater for growth), and its incredible composition, has made integrating microalgae cultivation with current agricultural and aquacultural processes to promote sustainable practices and produce additional products to offset the costs of the addition an object of extensive studies. Algaculture straddles the agriculture and aquaculture worlds, though some consider it a new branch of agriculture in itself (Ullmann & Grimm, 2021). Currently, the majority of algal biomass production originates from the food and nutraceutical industries, but cultivation is not on the same scale as other foodstuffs due to bottlenecks making operations costly (Vieira et al., 2022).

Using algae for biofuel production, then, would not take away from global food availability on the same level as crop-based energy production, eliminating the moral argument often leveled against corn and other plant-stock fuels. Furthermore, microalgae are more oil- and lipid-rich than crops used in biofuel manufacturing, rendering the conversion of algae a more efficient and cost-effective method of energy production. For this reason, there has been and continues to be a great deal of focus on biofuel production from microalgae (Karimi et al., 2021), as well as other uses.

Despite the great potential of microalgae, commercialization and industrial scale applications face many challenges. Expense, mainly associated with harvesting, is a major

bottleneck. Currently, without optimizing growth and/or reducing costs, planktonic microalgae cultivation is not economically feasible on a large, industrial scale (Show et al., 2015). The use of filamentous green algae for coupled wastewater treatment and cultivation poses a possible solution to a portion of this problem due to the availability of less labor-intensive harvesting methods. Wastewater generally carries free nutrients (for instance, agricultural wastewater often has nitrogen and phosphorus from fertilizer use) that the filamentous green algae need to grow, which they are able to remove while also producing biomass for other purposes.

The concept of using microalgae to treat wastewater was conceived in the twentieth century and was built upon as researchers recognized its potential for recovering nutrients as feedstock for products while cleansing water for reuse (Paddock, 2019). Researchers have also invested time and energy into developing algae-based waste treatment technology, including high rate algal ponds, photobioreactors, and raceways (Sutherland & Craggs, 2017; Trentacoste et al., 2015). While much of the existing research and literature focuses on the cultivation and uses of planktonic microalgae due to their high lipid contents rendering them an appealing prospect for the production of biodiesel, there is less information about the applications and cultivation of filamentous green algae (Karimi et al., 2021). However, filamentous green benthic algae that attach to substrata have been shown to be effective in the removal of excess nutrients and pollutants from wastewater in many technologies, such as algal turf scrubber (ATS) systems.

Section 1.5: Algal turf scrubbers

As previously mentioned, ATS systems are a natural wastewater treatment technology invented by Walter Adey as a water quality measure and consist of a slightly inclined flow way wherein a turf or mat of filamentous green algae grows in a mini ecosystem. (W. Adey et al., 1993; W. Adey & Bannon, 2008; Craggs et al., 1996)

The shallowness and movement of the flowing water removes the need for aeration by promoting carbon dioxide and oxygen exchange across the air-water interface, while also bringing nutrients to the algal filaments and shifting them enough to help retard some of the self-shading seen in larger mats in the wild; contaminant removal is not limited to nitrogen and phosphorus, as the algae is also capable of removing metals and other pollutants (W. Adey & Bannon, 2008).

ATS systems have advantages over other treatment systems, including wetlands. Sutherland & Craggs (2017) state that periphyton nutrient removal systems, the wastewater treatment technology group to which ATSs belong, have half the land requirement but comparable nitrogen removal capacity to wetlands. The associated capital costs and establishment time are also lower, but it still takes weeks for the algal turf to establish itself. The presence of a bacteria biofilm formed by a community from natural waters may help the algae attach faster and more strongly to the substrata in systems used for treating agricultural waste.

Section 1.6: The colonization of surfaces by algae

Schnurr and Allen (2015) posits that biofilm development can be described in four stages, including an initial state where bacteria begin to colonize a substratum and exude extracellular polymeric substances to form a matrix. In later stages algae arrive and join this bacterial community, benefiting from the pre-established bacterial biofilm. While Schnurr and Allen's (2015) colonization and development theory ends with the mature biofilm, Karimi et al. (2021) proposes that filamentous algae growth does not end at the biofilm stage, but develops beyond it to form a three-dimensional canopy structure that behaves intrinsically differently from the biofilm. Figure 1 below shows the proposed stages of development. This research primarily focuses on the first stage and potential linkages to the later canopy stage.

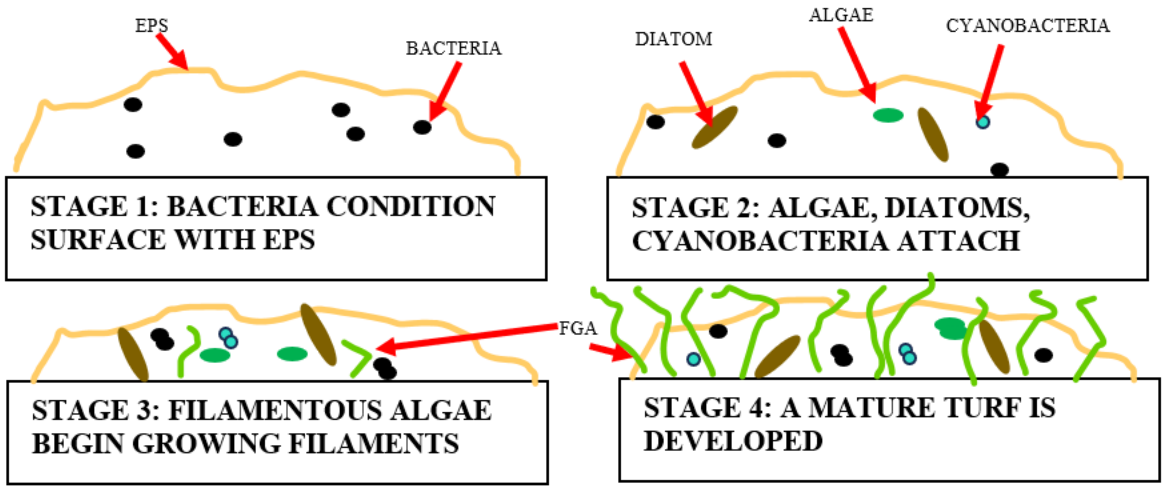


Figure 1: Algal turf development from biofilm, adapted from Schnurr & Allen (2015) Figure 1 (FGA: filamentous green algae).

Evidence strongly supports Schnurr & Allen's (2015) model, with the beginning stages of periphyton community development and succession, driven by bacteria; this is accomplished through their secretion of EPS, conditioning of surfaces for attachment, change of EPS matrix conditions, and feedback interactions between bacteria and algae (Brasell et al., 2015; Mieszkin et al., 2013). However, (Roeselers et al., 2007) found that the initial colonizer in phototrophic biofilms depends on light intensity, with algae taking on the role of pioneering organism at higher light intensities while bacteria did so at lower light intensities. This supports Mieszkin et al.'s (2013) warning that though the research supports bacteria's role as pioneering microorganisms in many situations, there are cases where it does not always fit into the role in the same way. In cases where bacteria are the pioneers, the EPS acts as a kind of adhesive that helps the algae cells stick. Upon settlement, algal cells first exude a mucilaginous sheath of their own that eventually hardens as attachment becomes irreversible. Certain species of FGA form a basal attachment structure from the hardened sheath (Fletcher & Callow, 1992; Tarakhovskaya, 2014). The initial secretion is an overproduction of cell wall products, including glycoproteins,

polysaccharides, and other components connected by covalent crosslinks; these molecules can form bonds with the proteins and polysaccharides in EPS, helping them attach to surfaces.

Section 1.7: Extracellular polymeric substances

EPS is the structural base and "glue" of the biofilm that algae eventually join and that serves to perform multiple functions that benefit them, so it is imperative to have a basic understanding of what it is. This section offers a very brief overview of EPS.

The secretion of EPS is one of the first steps in biofilm formation after a theoretical conditioning film of molecules and initial attachment of the bacteria to the substratum (Palmer et al., 2007). It holds the various organisms and molecules together within the biofilm and is responsible for cohesion and aggregation. EPS consists of multiple kinds of molecules, including polysaccharides, proteins, lipids, and extracellular DNA (H. C. Flemming, 2016; H. C. Flemming et al., 2007).

The EPS matrix is not rigid and unchanging. Instead, it adapts to circumstances, such as altered hydraulic regimes and nutrient availability (H. C. Flemming, 2016). It also offers protection against stressors like grazers, nutrient scarcity, and even antimicrobials. By holding water, conserving and assimilating nutrients, and acting as a digestive system through extracellular enzymes produced by bacteria the biofilm allows microbes to survive in otherwise harsh conditions (Flemming, 2016, Costa et al., 2018). While it is generally found that bacteria are largely responsible for conditioning substrata with EPS, microalgae, including green algae like *Chlorella vulgaris,* diatoms like *Amphora sp.*, and red algae like *Rhodella sp.*, can also produce EPS. Microalgae-produced EPS has been shown by studies to have great potential for the production of materials for medical, pharmaceutical, cosmetic, wastewater and other applications (Xiao & Zheng, 2016). Algae can also serve as substrata for the colonization of

bacteria (Besemer et al., 2007; Pohlon et al., 2010) so algal biomass can also promote bacterial density.

Section 1.8: Interactions between bacteria and algae in general

Algae and bacteria are often thought of as nuisances or as limiting factors in each others' growth. They may control each other through the release of allelochemicals and even antibiotics (Gubelit & Grossart, 2020). In certain situations, they also compete for available resources. However, both also have the potential to provide benefits to society, individually and in cooperation with each other. Bacteria have been found to be able to boost algae growth, promote beneficial actions, cooperate with algae to produce advantageous results, and aid in colonization and attachment (Hodoki, 2005; B. Zhang et al., 2020b).

Bacteria and algae have been shown to interact with each other in varying ways, both beneficial and limiting. Research has shown that bacteria secrete substances that promote algal growth or attachment while algae offer bacteria shelter. On the opposite end of the spectrum, species of each have been found to release chemicals/signals that result in the lysing of the other organism, and it has even been discovered that the way specific species of bacteria and algae interact can vary depending on cultivation conditions and can shift from mutualistic to hostile within the same environment based on changes in the surrounding conditions (B. Zhang et al., 2020b). Genomic evidence suggests that certain species of bacteria and algae co-evolved with each other and, within periphytic communities, bacteria play a critical role, and their absence can lead to slower growth. There is a theory that co-evolved bacteria and algae may have lost certain functions, meaning they must rely on each other to perform them (Gubelit & Grossart, 2020).

Kouzuma & Watanabe (2015b) state that bacteria-algae interactions fall under three basic concepts, gene transfer (bacteria and algae exchange genes), nutrient exchange (a cycle of

bacteria metabolism transforming nutrients into forms algae can uptake and algae producing organic matter), and signal transduction (where bacteria and algae communicate through the release of chemicals). Bacteria and algae also coexist in algal biofilms, with research showing that the role of bacterial biofilms in influencing algal attachment and colonization is significant at least in earlier stages, and that bacteria have the potential to promote algal growth and boost algal productivity and nutrient removal in wastewater removal systems. There is also evidence that bacteria-algae consortia in general perform better when treating wastewater than algae-only assemblages. Qian et al. (2023) found that a bacteria-microalgae consortia removed nutrients at a greater rate from soy sauce wastewater than communities comprised solely of bacteria or solely of algae, and the presence of proteins in EPS resulted in more algae attachment/greater biofilm development. While testing the efficacy of an indigenous microalgae-bacteria community sourced from food processing effluent, Amadu et al. (2023) found that the consortia performed better when polishing the effluent than when treating a synthetic media and theorized that, due to acclimation to the wastewater, it may perform better than an algae-only assemblage. Liu et al. (2017) attributed the greater effectiveness of attached algae-bacteria consortia to the ability to remove nutrients through multiple pathways and to utilize cooperative relationships. By working together, bacteria and algae can perform at a higher capacity than alone. This also applies when it comes to attachment.

Section 1.9: Bacteria and algae attachment

The literature indicates that in many cases there is a correlation between the presence of bacteria biofilms and algae attachment; this correlation can be positive or negative and is species-specific because bacteria-algae interactions are highly dependent on which bacteria and which algae are involved (Chan et al., 2022; Eigemann et al., 2013; Gawne et al., 1998; Gubelit

& Grossart, 2020). Examples of negative correlations include *Aquimarina salinaria* (Chen et al., 2012), which is algicidal, and *Pseudoalteromonas tunicata* (Egan et al., 2001), which is also algicidal, causing cell lysis of certain taxa of green and red algae.

Holmes (1986) demonstrated that the presence of a primary bacteria biofilm enhanced the attachment of algae that colonize and foul vinyl. Hodoki (2005) also showed that the presence of a bacteria biofilm prior to exposure to algae promotes the immigration and attachment of algae to substrata in a stream-like environment. His experiment provided evidence for a positive relationship between bacterial cell density and algae attachment. Roeselers et al. (2007) also found that polycarbonate substrata that had been preconditioned by heterotrophic bacteria saw faster algae growth.

Irving & Allen (2011) when studying attachment of *Scenedesmus obliquus* and *Chlorella vulgaris* on different substrata with sterile and non-sterile artificial media and effluent found that the interactions between other microbes and *S. obliquus* and *C. vulgaris* is very species-specific, but that for some species like *C. vulgaris*, the overall microbial community, including bacteria, is important for attachment and the development of an algae biofilm. In their study, when the nutrient source was non-sterile wastewater, *C. vulgaris* switched from 23.7% attachment (in sterile media) to 79.8% attachment. It also grew an approximately 7 times thicker biofilm in non-sterilized media versus sterilized. This behavior was not observed with *S. obliquus*, which developed biofilms of comparable thicknesses regardless of sterility or lack thereof. The authors attribute this phenomenon to the potential ability to produce EPS or other attachment promoting physical characteristics of the latter and the lack thereof of the former. The inability of either species to become dominant in biofilms formed under sterile conditions, however, also seems to

support the importance of the overall microbial community and bacteria in algae attachment and colonization.

Tong & Derek (2021) found attachment of three diatom species to polyvinylidene fluoride membrane to be strongly affected by EPS presence and composition due to molecules within the biofilm making adhesion easier. Singh et al. (2013) noted that *Ulva fasciata* spores settled on EPS-coated cover slips in greater quantities than on non-coated ones, and this settlement increased with incubation time, which can be speculated to be due to an increase in bacteria density over time. *Ulva lactuca* and *Undaria pinnatifida* also interact differently with different strains of bacteria, with some strains inhibiting attachment but promoting germination of their spores, some inhibiting attachment of *Ulva* but promoting attachment of *Undaria* and vice versa; these effects seemed to be the effect of metabolites released by the bacteria (Belenova et al., 2017).

Positive effects of bacteria on algae attachment may arise from more than one factor. In some cases, the bacterial biofilms adsorb algae and other materials in suspension in flowing waters. Essentially, they remove them from the stream and help them attach. Therefore, the presence of a bacteria biofilm promotes the colonization of rocks and other substrata by filamentous algae floating through streams and flow ways that have yet to set down roots.

One experiment found that bacterial biofilms, by virtue of their metabolism and ability to provide nutrients, can promote the settlement of algae on coral reefs. Li et al. (2019b) at the end of this study concluded that specific kinds of bacteria can be introduced to artificial reef systems to encourage settlement by algae. Amsler & Neushal (1989) found that certain kelps were aware of the presence of specific inorganic and organic nutrients and could respond to it; the presence of bacteria with metabolisms capable of transforming select nutrients could attract them. Zhang

et al. (2019) also found that the preference of the macrophyte *Potamogeton maackianus* can be correlated with the growth of epiphytic green filamentous algae, potentially at least partially due to the presence of certain bacterial taxa on the plant which might be needed for the growth and reproduction of the filamentous green algae. (Joint et al., 2000, 2002, 2007) found that regardless of whether the biofilms studied were axenic or not, in their studies there existed a common trend of biofilm bacteria density correlating positively to algae zoospore attachment, and this was likely due to the spores intercepting N -Acylhomoserine Lactone (AHL) signals used in bacteria quorum sensing, resulting in chemotaxis.

Overall, it can be concluded that initial bacteria biofilm development is very important to initial algae colonization. There is reason to believe that given the right bacteria community, the presence of a pre-existing bacteria biofilm can promote faster or stronger attachment through adhesive bonds, nutrient interactions, chemical signaling, or other means.

Chapter 2: Methods

Section 2.1. Development of a lab-bench scale ATS

The nature of the research required ATS systems at a lab-bench scale to maximize control. There were not openly available plans of lab-bench scale ATS systems suitable for the designed experiments at the time procedure and system development for this study began. Salvi et al. (2021) had designed a seeded lab-bench scale system for their research, but the arrangement did not fit the necessary criteria for the research described here, which required the development of systems small enough to fit multiple on a metal rack for efficient space utilization and replication. There was also a need for a built-in way to semi-isolate the environment within each channel to minimize contamination between systems, and the ones created by Salvi et al. (2021) were open.

Besides semi-isolation and compact size, other constraints taken into consideration were materials affordability and accessibility and easy disassembly for sterilization. Two generations of prototypes were built, with the finalized design using 2” PVC pipe and fittings, plastic storage bins, 3/8” hosing and fittings, LED strip lights, and 3D printed parts. Besides the 3D printed parts, all materials can be easily obtained from an in-person or online store.

The assembly of the second and final generation after testing of the first (conceptualization and construction performed by Research Engineer Bobby Bradford, Auburn University) did require a precision cutting tool that requires training to use and is not generally accessible. The main portion of the final system consists of a channel formed from half an approximately 23” length of PVC pipe glued to an endcap with three holes drilled in them for insertion of the inlet hose at different angles at the back end and a 90-degree elbow at the front end to which a drain pipe can be attached. The open portion of the channel (not covered by the

end cap and elbow) is approximately 20 ½" in length. A detachable lid constructed from the other half of PVC pipe approximately the same length as the open portion fits over it and helps reduce system to system splash contamination. Figure 2 shows a conceptualization of the final design.

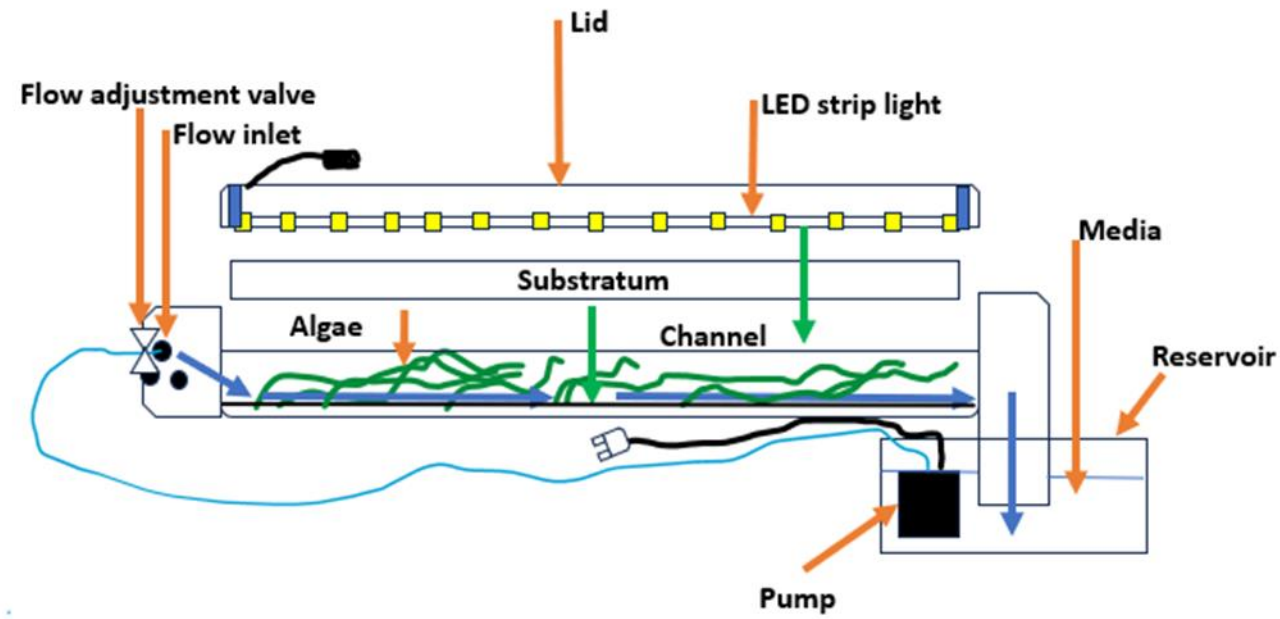


Figure 2: System design for a floway experimental system.

A strand of white LED strip lights is glued to each lid as a light source for the algae. Light intensity measurements were taken with an Apogee Instruments Model MQ-200 quantum meter; after measurements were taken, tape was placed over bulbs in certain lids to ensure the light intensity over the lid were within similar ranges for all the lids and measurements were retaken.

Figure 3 shows the light intensity from end to end of each lid measured in micromoles of photons per meter square per second. Lid 3 burnt out and was replaced with Lid 10 as of Trial 10, the trial testing the impact of the mixed bacteria community biofilm in fish waste diluted to half-strength. Certain bulbs on the LED strips for some lids also burnt out over the course of the trials. Figure 4 shows the final light intensity over the length of the lids as of the final four trials.

Each set of three lids/light strips were connected to a three-way splitter going to a 12 volt-2 amp adapter during the trial, and this same setup was used during the light measurements.

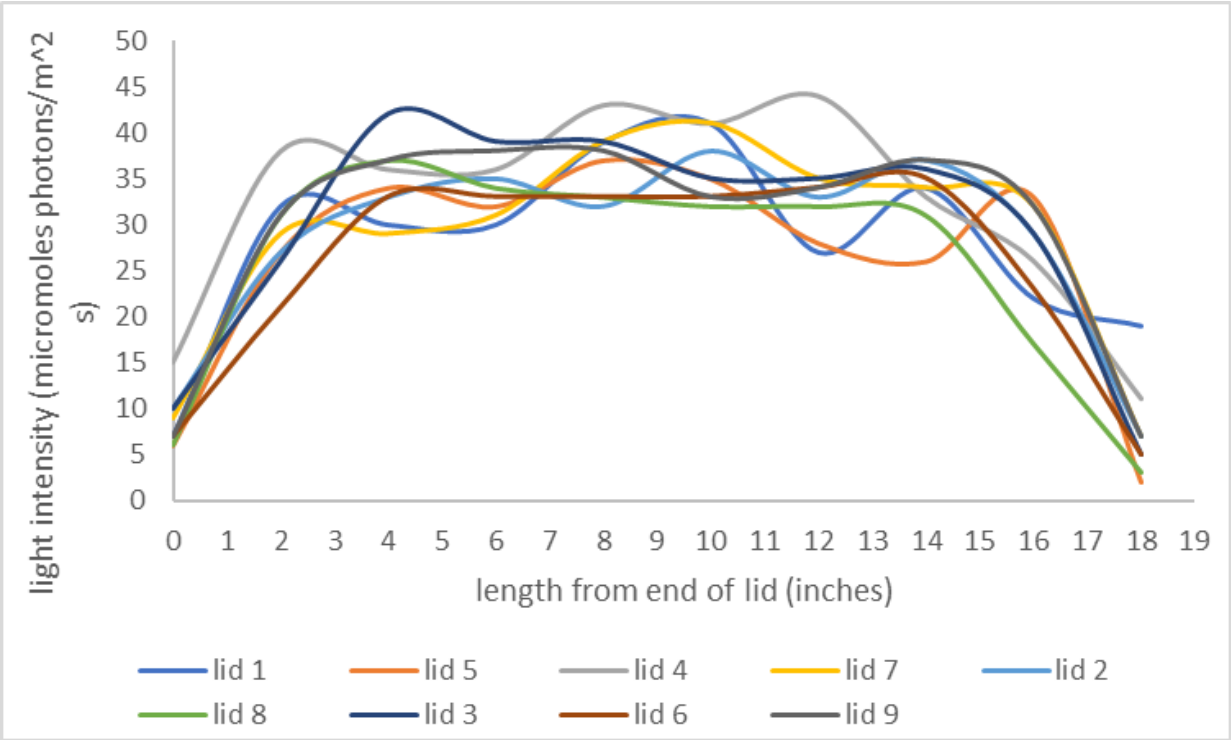


Figure 3: Light intensity across each lid's LED strip in micromoles photons per meter square per second when trials started.

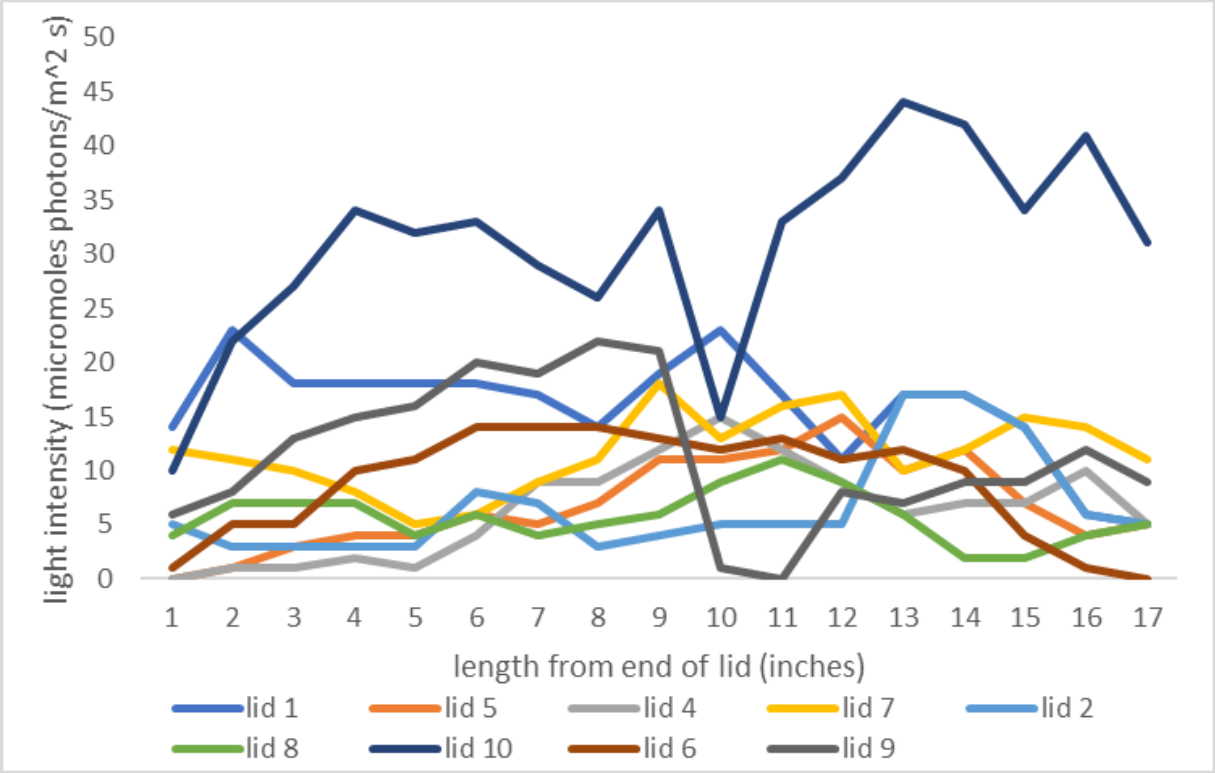


Figure 4: Light intensity across each lid's LED strip in micromoles photons per meter square per second when trials ended.

The average standard deviation between lids at the beginning and end respectively were 7.95 and 9.13 micromoles photons per meter squared per second (obtained by calculating the standard deviation between lids at each measured location then averaging the standard deviations in Microsoft Excel). The average standard deviation along the length of the lids (found by taking the average photon flux across all lids at each location then finding the standard deviation among these averages) went from 9.96 to 2.42 micromoles photons per meter squared per second from the beginning of the main experiments to the end. The average coefficient of variance (average standard deviation along length of lids/average of the mean measurement across all the lids at each location) went from 0.380 to 0.209. The mean difference of the mean of the average values at each measurement location was 14.6 micromoles photons per meter squared per second.

There was a large drop in the light intensity of the lids from the beginning of the trials to the end. This means light conditions were not the same across all trials; however, each trial is considered an isolated trial except for Trials 1-3, on which a mini meta-analysis was performed. However, it is unlikely that the light conditions changed significantly within the time span covered by the first three trials. Though there was some difference among lids, the average coefficient of variance remained medium and below both at the beginning and end of the trials. The plastic storage bins used as media reservoirs were Sterilite clear plastic storage boxes from Walmart and had a capacity of 5.7 liters.

A full picture with individual components labeled can be found in Appendix A.

Section 2.2. Procedures common to all main trials

The procedures listed below were used across all or most of the the main experiment trials, and some of them were also used in preliminary testing of the lab bench scale systems. Any exceptions are described in the description of conditions for each individual trial. A summary of certain beginning conditions of the main experiments is shown Tables 1 and 2. Note that the beginning nutrient concentrations are the means of five random samples taken at the beginning of each trial. The inlet flow rate is also the mean of the inlet flow rates of all systems.

Table 1: Beginning nutrient concentrations for all experimental conditions.

BACTERIA SOURCE	MEDIA	DILUTION	BEGINNING NITRATE (PPM)	BEGINNING PHOSPHATE (PPM)	BEGINNING NITRITE (PPM)	BEGINNING AMMONIA (PPM)
TALLAPOOSA RIVER	PROLINE F/2	¼ X	2.77	1.92	NA	NA

TALLAPOOSA RIVER	PROLINE F/2	¼ X	NA (estimate: 3.27)	2.65	NA	NA
TALLAPOOSA RIVER	PROLINE F/2	¼ X	3.76	3.67	NA	NA
TALLAPOOSA RIVER	PROLINE F/2	2 X	NA (estimate: 55.6)	NA (estimate: 9.70)	NA	NA
TOWN CREEK PARK	PROLINE F/2	¼ X	2.65	2.06	NA	NA
TOWN CREEK PARK	PROLINE F/2	2 X	55.6	9.70	NA	NA
TOWN CREEK PARK	FISH WASTE (FILTERED)	½ X	223.3	19.9	0.750	0.700
MIXED	FISH WASTE	¼ X	168.1	12.8	0.227	0.763
MIXED	FISH WASTE	FULL STRENGTH	5.80×10^2	46.7	1.75	1.08
MIXED	FISH WASTE	½X	225	22.9	0.349	0.508
MIXED	FISH WASTE	1/4X	136	10.5	0.0728	0.357
MIXED	FISH WASTE	1/4X	133	9.52	0.0879	0.339

Table 2:Beginning trial conditions for all trials.

BACTERIA SOURCE	MEDIA	DILUTION	DURATION (DAYS)	SLOPE (%), AVERAGE FULL INLET FLOW RATE (ML/S)	BACTERIA INOCULATi-ON TIME (HRS)	ESTIMATED ALGAE INOCULUM DENSITY (MG/ML)	ESTIMATED BEGINNING BIOFILM BIOMASS (G/CM^2)
TALLAPOOSA RIVER	PROLINE F/2	¼ X	8	3, 18.6	69.7	0.500	NA
TALLAPOOSA RIVER	PROLINE F/2	¼ X	8	3, 19.4	72.0	0.300	NA
TALLAPOOSA RIVER	PROLINE F/2	¼ X	8	3, 18.7	44.8	0.340	2.96×10^{-5}

TALLAPOOSA RIVER	PROLINE F/2	2 X	5	3, 18.5	72.0	2.58	1.94 x 10-5
TOWN CREEK PARK	PROLINE F/2	¼ X	8	3, 16.8	72.0	1.18	2.84 x 10-5
TOWN CREEK PARK	PROLINE F/2	2 X	6	3, 16.4	72.0	N/A	2.13 x 10-5
TOWN CREEK PARK	FISH WASTE (FILTERED)	½ X	7	3, 14.5	65.6	N/A	N/A
MIXED	FISH WASTE (UNFILTERED)	¼ X	7	2, 15.2	48.0	5.64	1.36×10^{-5}
MIXED	FISH WASTE (UNFILTERED)	FULL STRENGTH	4	2, 16.9	45.4	5.64	1.13 x 10-5

MIXED	FISH WASTE	½X	12	2, N/A	N/A	5.51	1.53×10^{-5}
MIXED	FISH WASTE	1/4X	4	1, N/A	48.7	5.45	2.75×10^{-5}
MIXED	FISH WASTE	1/4X	7	3, 16.3	72.0	5.45	3.26×10^{-5}

2.2.1. Algae and algae inoculation

A variety of algae was used in preliminary experiments. However, the main experiments utilized *Rhizoclonium spp.* due to its easy accessibility (collected from plant nursery in Biological Engineering Research Laboratory at Auburn University, Auburn, Al, where it grew naturally) and possession of desirable attachment characteristics. *Rhizoclonium* is a uniserial, usually unbranched filamentous green algae from the family Cladophoraceae (Zhao et al., 2018). Due to the nature (long, crystalline fibers) and high content of its cellulose, it has potential to be used as part of the wood pulp needed for paper manufacture (Hwang et al., 2022). Its lipid content also makes it possibly suitable for biodiesel production feedstock (Satpati et al., 2015). Images of *Rhizoclonium spp.* viewed under a light microscope at different magnifications can be seen in Figure 5.

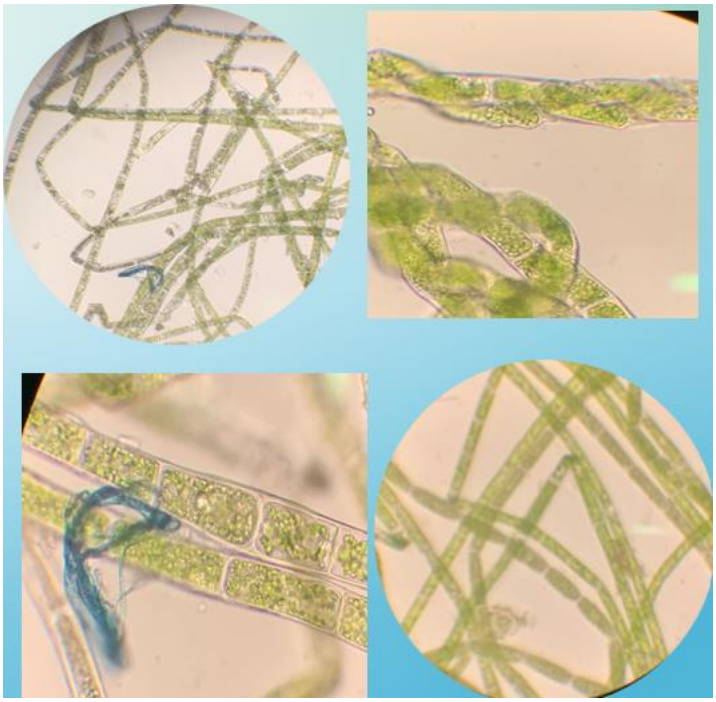


Figure 5: Images of Rhizoclonium spp. under the microscope at 40x (top left), 100x (bottom right), and 400x magnification (top right and bottom left).

To prepare the algae inoculum for each trial, clumps of the algae were placed in a blender with deionized water and blended for between 15 and 30 seconds to create a homogenized inoculum. Two methods were used to gain an approximate quantification of the biomass per milliliter of inoculum. In the first, 1 milliliter of inoculum each was pipetted into five pre-weighed aluminum pans, after which the pans were dried at 45 – 105°C for 3-24 hours. After drying, the pans were cooled in a desiccator for at least 30 minutes, then weighed. The original pan weights before the addition of the biomass were subtracted from these new weights to find the dry weight of the biomass. The five biomass weights were averaged to approximate the grams of algae biomass per milliliter of inoculum. The second method, which was transitioned into at the start of and used for all of the aquaculture waste trials, involved weighing a set amount of wet algae biomass (3.00 ± 0.100 grams wet weight) prior to inserting it in 550 milliliters of DI water and blending

it. At the start of each trial, 40-50 milliliters of the blended inoculum were added to each reservoir, with exceptions noted in discussion of each trial's specifics (note that the amount varied from trial to trial when method 1 was used but was approximately the same for each system within a trial unless an incident occurred).

2.2.2. Bacteria and bacteria inoculation

Bacteria was sourced from two locations, the Tallapoosa River boat launch in Tallassee, Alabama (32°30'32.6"N, 85°53'28.7"W) and Town Creek Park, Auburn, Alabama (32°34'54.7"N, 85°28'36.0"W, images of which can be viewed in Figure 6.

Figure 6: From left to right, Tallapoosa River Boat Launch, Tallassee, Alabama and Town Creek Park, Auburn, Alabama.

Water was collected from these sites and used to culture bacteria communities in sterilized nutrient broth made by mixing nutrient broth powder from VWR International with tap water treated with sodium thiosulfate crystals. At the beginning of each trial, samples of the algae inoculum, media, and bacteria inoculum (in random amounts) were taken and poured into plastic centrifuge tubes, then stored in a freezer. The first two represent the background bacteria. The water sourced from the environment was poured into or had bacteria transferred by loop to nutrient broth and incubated at 37°C for 24 hours to create the initial cultures used. These cultures were poured into glass mason jars, each containing one rolled-up substratum, and the jars were incubated for 24-72 hours (depending on the trial) at 37°C as shown in Figure 7. The jars were removed from the incubator and manually shaken in a horizontal circular motion at random intervals. One of these new jars was saved to use as the new inoculum source. This was repeated for both bacteria communities used, the one sourced from Town Creek Park and the one sourced from the Tallapoosa River, as well as the mixed community formed from mixing bacteria cultures from both locations.

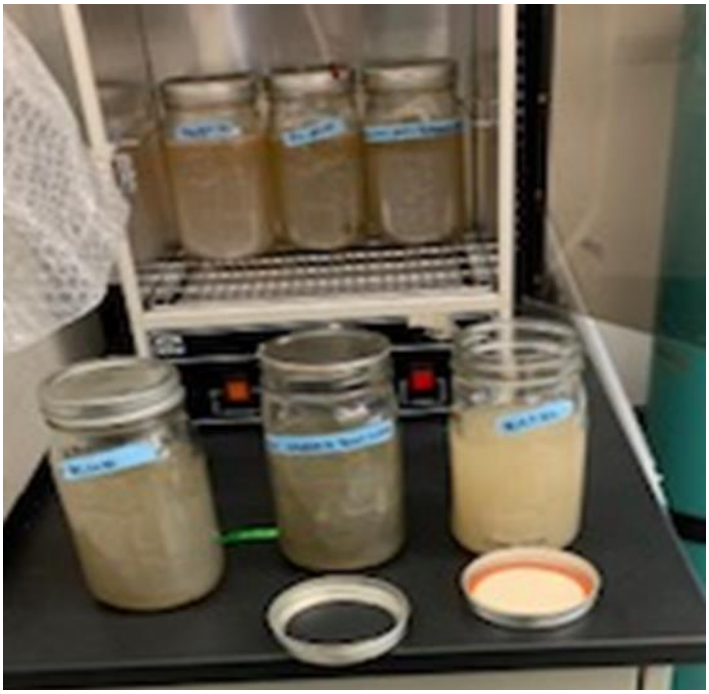


Figure 7: Substrata inoculation jars in and out of incubator.

At the end of the experiments, select samples were sent off to Molecular Research DNA lab (MR. DNA) for 16S RNA sequencing to identify the major bacteria present within the systems during the trials. The samples were as follows:

- A sample to represent the Tallapoosa River bacteria community;
- A sample to represent the Town Creek Park bacteria community;
- Two samples to represent the mixed bacteria community;
- A sample to represent the background bacteria from synthetic media;
- A sample to represent the background bacteria from the algae inoculum;
- A sample to represent the background bacteria from the aquaculture wastewater.

Even though samples were not taken from each trial, it is assumed that community structure for trials using the same community source would not shift significantly between trials, so these

samples can be taken as representative of the structures for trials from which samples were not processed.

2.2.3. Media preparation

Two types of media were used in this study, a synthetic media, freshwater modified ProLine F/2 from Pentair, which is modeled after Guillard's F/2 media, and aquaculture waste. The later was obtained from barrels gathered at least a year prior to the start of the experiments and stored at 4°C since, resulting in the elimination or minimalization of the effluent's native microbial community. The artificial media was prepared by pipetting the required ProLine F/2 nutrient solutions A and B into two five-gallon buckets and adding tap water, then dechlorinating the resulting media with sodium thiosulfate crystals. To ensure an even mixture of both buckets in case of pipetting or measurement error, a measuring cup was used to scoop 2 liters from each bucket into each reservoir for a total volume of 4 liters each. The fish waste was similarly diluted when applicable with conditioned tap water in two five-gallon buckets or placed into one bucket while the other bucket held treated tap water and distributed evenly from both buckets to the reservoirs. In the trial where the fish waste was filtered, Fisher Brand P8 20.5-centimeter diameter filters with 20 to 25 micrometer particle retention were used.

2.2.4. Trial set-up

At the beginning of each trial, after filling the reservoirs, the drain pipes were attached to the systems' front ends, and the systems were placed on the rack with the front end with drain pipe going down into the reservoir. A Corollata brand DC107 automatic digital angle gauge (manufacturer: AOSYCO) was used to adjust the slope by removing and adding various items used as wedges close to the inlet end where the systems sat on wooden blocks.

The substrata were then removed from the jars and secured at the bottom of each channel with binder clips. The control substrata, which had no biofilm, were also placed in the channels at this time, after which the lids were slid into place. The inlet hoses were inserted into the inlet holes in the back end of the system and secured with tape where necessary. The lids/LED strips were plugged in and the algae inoculum was added. The power strips where the pumps and lights were plugged in were then turned on to start the trial.

2.2.5. Initial bacteria biomass enumeration

At the beginning of the substrata inoculation period, 3 coupons of roughened polypropylene film (3.02 x 7.62 cm^2 in dimension) were also placed into each jar. These were removed at the same time as the substrata. The coupons were placed in aluminum pans as shown in Figure 8 and then dried in a muffler furnace at 55 – 105°C for 24-48 hours.

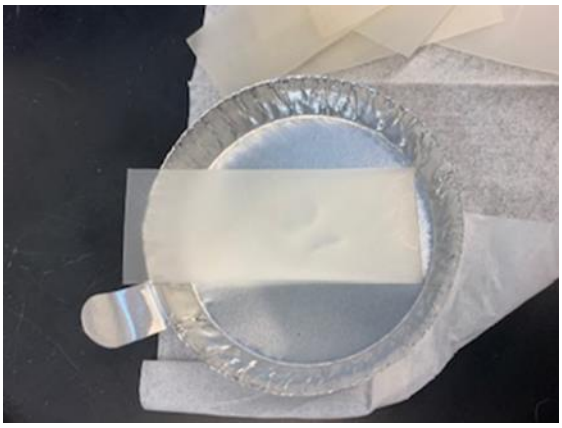


Figure 8: 3.02 cm x 7.62 cm roughened polypropylene bacteria enumeration strip in aluminum pan.

After removal from the muffler furnace, the pans with the coupons were placed in a desiccator for a minimum of 30 minutes and allowed to cool to ambient air temperature, after which the pans with the coupons were weighed. The coupons were removed from the pans, and the

roughened sides were scraped with a piece of plastic or a pair of tweezers. After replacing the coupons in the pans, the pans and coupons together were weighed again and this weight subtracted from the initial to get an estimate of the biofilm mass covering the coupons. The biofilm mass per area was then estimated by dividing the biomass by the coupon surface area. The biofilm masses per area were averaged for each substratum, then the averages were averaged together to get the average estimated starting biofilm mass per area for each trial.

2.2.6. Harvest and analyses

At the end of each trial, the biomass was harvested from the entire surface area of each substratum, leaving nothing visible behind for regrowth since each trial was a separate trial requiring sterilization before and after. The substratum was first removed from the channel and placed on the lab bench. A known amount of deionized water in a squeeze bottle was used to rinse biomass off the substratum going in an up-and down zigzag motion from left to right, repeated down the length of the material. The biomass rinsed off into a beaker in this step was classified as loosely attached biomass (LAB). Half of the LAB (measured with a graduated cylinder) was filtered through a dried and pre-weighed 47 mm diameter, 0.7-micron pore size Whatman GF/F filter using a vacuum filtration system, and the filter was placed in an aluminum pan.

The pan and filter were placed in a muffle furnace and dried at 45-105 °C for 24-48 hours, cooled in a desiccator for a minimum of thirty minutes, and weighed. The original filter weight was then subtracted from the weight of the filter + LAB to obtain the dry weight. The other half of the LAB was filtered in the same manner on a separate filter, but this filter was placed in a 5-milliliter centrifuge tube wrapped in aluminum foil to block the light. Four milliliters of a 90% acetone and saturated magnesium carbonate solution was then pipetted in,

and chlorophyll *a* was extracted in the dark at 4°C for 3-24 hours. Absorbances were then measured on the spectrophotometer and chlorophyll *a* was calculated as per Chapter 17 of *Methods in Stream Ecology, Second Edition* (Hauer & Lamberti, 2007).

In addition, for all but the first two trials, the absorbance of the extract at 665 nanometers before acidification was obtained and the total chlorophyll pigments calculated as a check on the chlorophyll *a* measurements (see Calculations and Analysis section for total chlorophyll pigments equation). A small cylindrical tube brush was used to detach the remaining biomass from the substratum, classified as strongly attached biomass (SAB), and the brush and substratum were then rinsed with a known amount of deionized water in a squeeze bottle. The rest of the steps were the same as for the LAB. This procedure was repeated for each system and is visualized in the diagram in Figure 9. The ratio of SAB to LAB is the strongly attached index, or SA index, and is used as the measure of strength of attachment for this research.

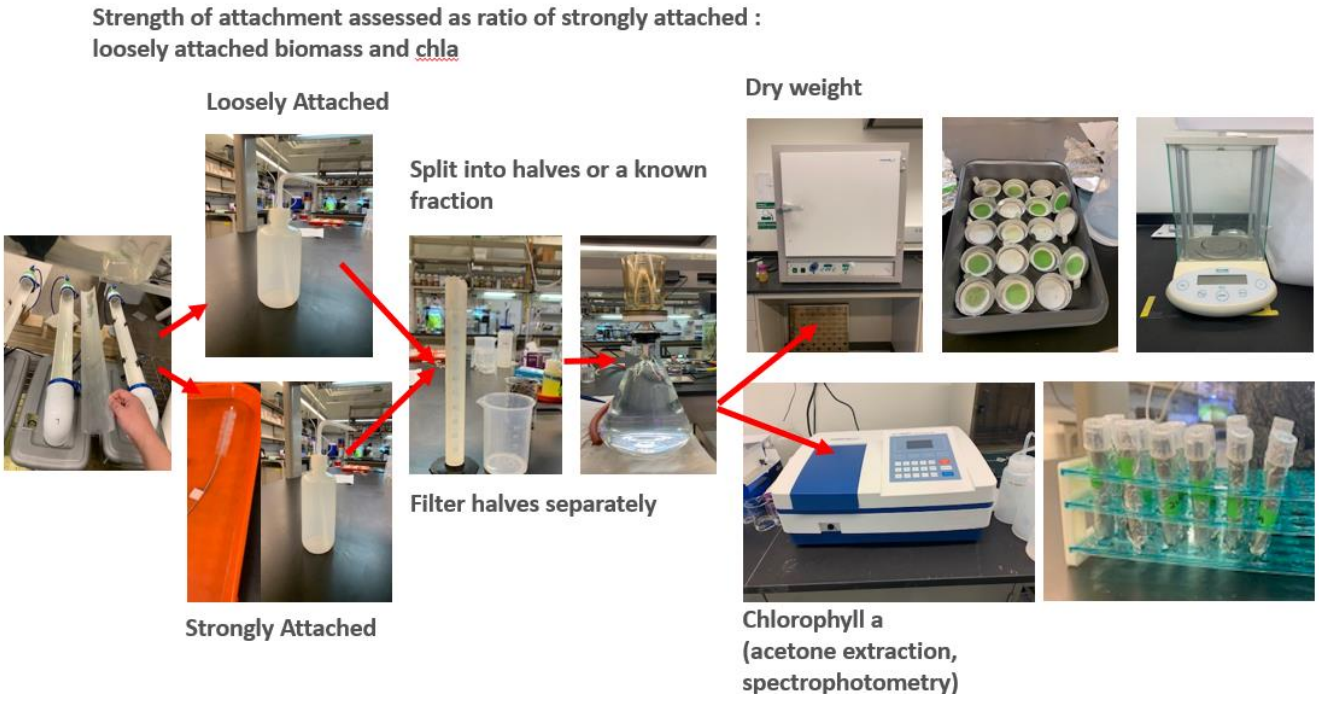


Figure 9:Diagram of harvesting process and workflow.

2.2.7. Flow measurements

Two flow measurements were taken at the end of each trial, inlet flow in milliliters per 2 seconds and approximate channel travel time. To obtain the former, the amount of fluid that filled a centrifuge tube at the inlet in 2 seconds was measured five times for each system, then averaged. The averages of all the averages for each system were then averaged together and divided by 2 to get an overall average inlet flow rate in milliliters/second for the trial. To measure channel travel time, a drop of food coloring was dropped at the beginning of the open portion of the channel, and its travel was recorded using an iPhone. The time it took the tip of the dye trail to travel from the beginning to the end of the open portion of the channel in seconds was then obtained from the video. This was only performed once for each channel for each trial.

2.2.8. Nutrient analysis

At the beginning of each trial, five water samples were taken from personally selected reservoirs (different for each trial) for nutrient quality analysis. At the end of each trial, each reservoir was filled back to the 4-liter mark with deionized water to account for evaporation, then a water sample was taken from each one for nutrient analysis. The end water samples were filtered through 47 mm diameter, 0.70-micron pore size Whatman GF/F glass fiber filters using a vacuum filtration setup to eliminate interference from organic particulate matter. Nitrate and phosphate measurements were taken for each trial from the water samples using Chemetrics Vacu-vials (kits K-6903, which uses the cadmium reduction method, and K-8503, which uses the Vanadomolybdophosphoric Acid method for orthophosphate, respectively). In addition, nitrite nitrogen and ammonia nitrogen measurements were taken for the fish waste trials, also using Chemetrics Vacu-vials (kits K-7003, which uses the Azo dye test method, and K-1513, which uses the direct Nesslerization method, respectively) and converted to ppm of nitrite and

ammonia. The samples were diluted with DI water in the provided sample cups as needed to get them within the range of the Vacu-vials (for nitrate, dilution was performed either by diluting in a separate cup or adjusting the amount of sample added using the syringe).

2.2.9. Environmental measurements

A Hanna HI98129 combination electrical conductivity/temperature/pH probe was used to take measurements of pH, temperature in degrees Celsius, total dissolved solids in parts per million, and electrical conductivity in microSiemens a minimum of three times during each trial, at the beginning, once during the trial, and at the end of the trial, directly from the reservoir through a slot cut in the lid as shown in Figure 12. At the end of each trial, measurements were taken of the final condition of the media, but an additional measurement was taken after the reservoirs were filled back up to the 4-liter mark with deionized water to account for evaporation losses. Temperature and pH were not controlled, and the reservoir levels were replenished only at the end of each trial before taking water samples.

Figure 10: Hanna combination probe

2.2.10. Sterilization

Between every trial, the systems were disassembled and sterilized with alcohol and bleach separately. The lids were removed and wiped down with 70% isopropyl alcohol. The channels were removed, and the drain pipes were detached; the outsides were wiped down with 70% isopropyl alcohol, and more alcohol was sprayed into the channels and allowed to sit for at least 60 seconds. The insides of the channels were then rinsed with tap water and scrubbed with a bleach and detergent solution. The hoses and drain pipe were placed back in the still full reservoirs, and bleach was added to the reservoir; the reservoir and components placed in it were allowed to soak for at least 3 hours. The reservoir was then scrubbed with a bleach and liquinox solution, and it, the hoses, and the drain pipe were rinsed with tap water.

Section 2.3. Preliminary trials

2.3.1. Biomass trials

A series of growth trials were performed to determine if the prototypes and the final systems could grow the kind of algae (filamentous green algae) desired for the planned experiments. These trials were primarily qualitative. Generation 1 prototypes and Generation 2 prototypes (the first 3) were inoculated with liquid *Stigeoclonium spp.* (see Figure 11a) cultures and clumps of *Rhizoclonium spp.* (see Figure 11b) in several separate trials.

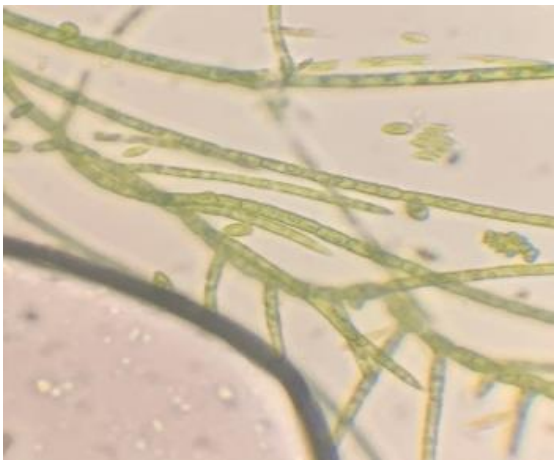
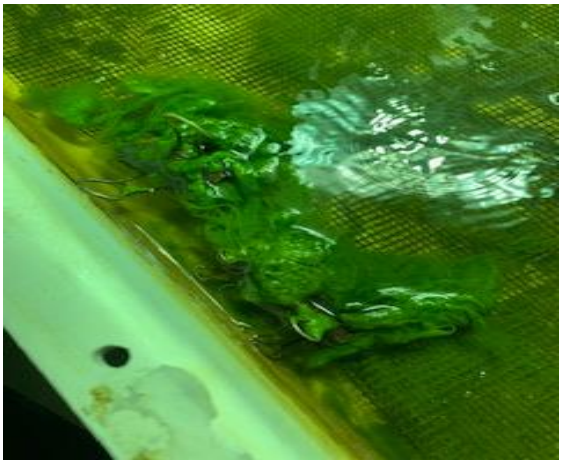
a.  b. 

Figure 11: (a) Stigeoclonium spp., (b) source of Rhizoclonium spp. Clumps.

The biomass was harvested by flask vacuuming (using a vacuum flask where the opening has a plug that is sealed except for an opening through which a small piece of a glass pipette with a hose attached is inserted and the side is hooked up to a vacuum line for suction) at regular intervals and air- or oven-dried, and the turf was allowed to regrow. These trials were solely to see if the desired attached filamentous green algae would grow at the small scale of the lab-bench ATS systems to determine if the systems were suitable for the planned experiments. As a result, quantitative data is not available, but an image timeline of growth can be viewed in Appendix B.1.

2.3.2. Length, concentration, sterility experiment trials

It was determined that a set of preliminary experiments testing combinations of trial length, media nutrient concentration, and sterilization was necessary to define parameters for the main experiment. The purposes of the experiment, called the Length, Concentration, Sterility Experiment (LCSE), were to see if the trial periods could be shortened from a week to save time and reduce the amount of time the algae had to grow filaments from attached cells, (which would distort gathered data), by raising the nutrient concentration, determine the minimum trial

duration needed, and see the effect of sterilization of media on general attachment. These were basic biomass growth trials with dry weight as the measure of attachment. Dry weight was obtained in the same way as discussed in the common methods section, but for these preliminary trials the biomass was harvested by flask vacuuming and taken all at once, with no distinguishing between loosely and strongly attached biomass. Different combinations of F/2 concentration and trial duration were tested.

However, while carrying out these trials, it was observed that the algae seemingly growing "attached" to the black substratum (a black polyethylene netting traditionally used by the lab group by Industrial Netting, Maple Grove, Minnesota, USA) laid in the bottom of the channels were actually not attached in a way detectable by unenhanced vision. Instead, the algae biomass was trapped beneath the water of the net. The observation raised concerns that the algae was not actually attaching to the substratum and would not attach fast enough for the planned experimentation, so LCSE was abandoned part way through. Results before experiment shutdown can be viewed in Appendix B.2, but the values shown represent biomass present in the channel at the end of each trial rather than attached biomass.

2.3.3. Substratum trials

The substrata originally used in preliminary testing was black polypropylene netting (bat exclusion netting with 1/4-inch mesh from Industrial netting) because it has traditionally been found to be a surface that filamentous green algae and other periphytic species find favorable for attachment and is known to be chemically inert, reducing noise. It has also been used successfully in different studies and has a history of use within the lab group. Gross et al. (2016) also found polypropylene and nylon to be the substrate materials best suited for algae attachment in an attached revolving biofilm system. However, throughout the preliminary trials, it became

clear that the attachment was not taking place at a rate suitable for the range and nature of the experiments planned, as described above. Because the stages being studied were initial attachment and colonization, it was preferable to have attachment take place within three to seven days. Even though attachment may have been occurring on a microscopic scale, it was difficult to verify this, so the attachment referred to here is visible attachment. Visible attachment to the black netting did not occur within this duration; what appeared to be attachment and was harvested through vacuuming was algae trapped in the water beneath the netting, which could not be differentiated from actual attached biomass because of lack of visibility.

Four substratum materials were tested to determine if a different substratum would be more suitable for the envisioned trials: white polypropylene film, white polypropylene film roughened with sandpaper, white polypropylene felt, and the previously used black polypropylene netting as shown below in Figure 12. The film (ASTM D4101, FDA Compliant 21 CFR 177.1520, UL 94 HB) and felt (unrated) were manufactured by McMaster-Carr. The roughened film was roughened by sanding the surface with an x-pattern going down the length of the substratum twice, beginning with a left to right stroke the first time and a right to left stroke the second time. The sandpaper used was 220 grit sandpaper.

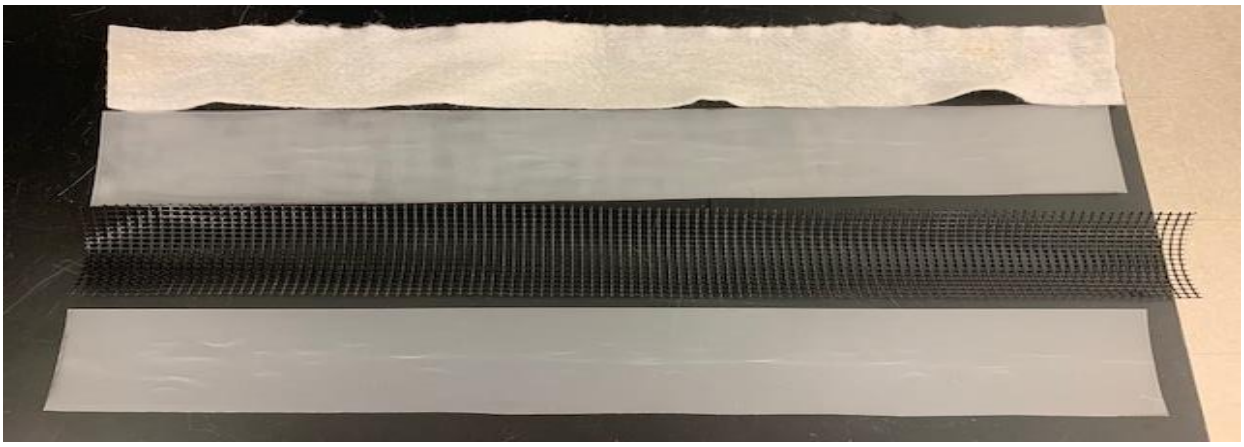


Figure 12: From top to bottom: polypropylene felt, roughened polypropylene film, polypropylene netting, and unroughened polypropylene film.

Three 8-day growth trials were run with double strength, freshwater-modified F/2 as the media and *Klebsormidium spp.* (see Figure 13) for the first and second trials and *Stigeoclonium spp.* for the third trial as the algae.

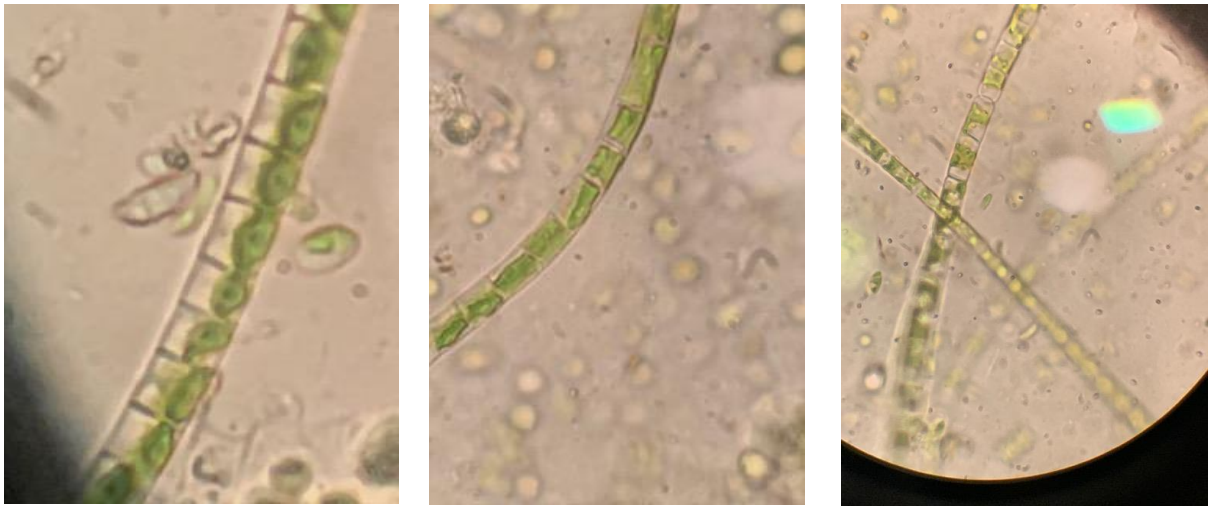


Figure 13: Klebsormidium spp. at 400x magnification.

Other operating parameters were a slope of 0% (set using a level) and full inlet flow rate. For the first trial, there were three replicates of systems with each kind of substratum, film, felt, and netting, for a total of nine systems run. The second trial was a repeat of the first, but based

on the qualitative observation that there was no visible attachment on the netting, the netting systems were not harvested. The final substrata trial tested the film, felt, and roughened film.

These were simple biomass attachment trials, with dry weight being the sole measure of biomass since there was no need to differentiate between a bacteria biofilm and the algae. Harvest was achieved through flask vacuuming, with no distinction between loosely and strongly attached biomass, but dry weight was obtained using the same method as described in the common methods section. A one-way ANOVA and a Tukey's test were performed for the final substrata trial, using the ANOVA function from data analyzer in Microsoft Excel and Tukey's HSD test calculations in Microsoft Excel.

2.3.4. Variability trials

Two trials were run to determine the variability among the nine final systems. These were simple 4-day biomass growth trials with all systems run under the same conditions (0% slope, full inlet flow, 20 mL liquid *Klebsormidium spp.* inoculum cultured in a flask, roughened polypropylene film as substrata). These trials were run like main experiment trials, using the same common methods, but there were no treated systems and no chlorophyll *a* analysis, only dry weight analysis.

Section 2.4. Main experiments

The following sections describe conditions for the various trials and any deviations from the standard methods related previously. Some of the information related in Tables 1 and 2 is reiterated here for the purpose of creating separate concise images of each particular investigation. The experiments are numbered as trials in order to reference them later in the document without having to reiterate the conditions tested repeatedly.

2.4.1. Trials 1-3: The effect of Tallapoosa River water bacteria biofilm on attachment in low nutrient synthetic media

The impact of bacteria biofilms formed by a community cultured from Tallapoosa River water on attachment in a ¼ dilution of synthetic media was tested in three separate trials. While these tested the same condition, 0.25x Proline F/2 (low nutrient synthetic media made with freshwater) and the Tallapoosa River microbial community, they were not exact replicates of each other and were analyzed completely separately. They are referred to as Trial 1, Trial 2, and Trial 3 in the order they were conducted. The trial duration for each was 8 days because in the first trial the algae took longer to visibly attach than had been observed previously and the following two were run for same duration to keep them similar. The substrata incubation times and average algae inoculum biomass density were 69.7, 72.0, and 44.8 hours and 0.500, 0.300, and 0.340 mg/mL for Trials 1, 2, and 3 respectively. Beginning bacteria biomass estimations were not estimated for the first two runs, but for the third one, it was estimated that the substrata began with $2.96 \times 10^{-5} \pm 7.79 \times 10^{-6}$ g/cm^2 biofilm biomass. The average beginning nitrate concentrations for Trials 1 and 3 were 2.77 ± 0.274 and 3.76 ± 1.70 ppm respectively. When measured, the average beginning nitrate concentration for Trial 2 was measured as 10.9 ± 2.27 ppm, but because the value is so high and not consistent with the other two, this is believed to be an inaccurate value caused by an error in the measurement process. The average beginning phosphate concentrations for the three trials in order were 1.92 ± 0.669, 2.65 ± 0.733, and 3.67 ± 1.55 ppm respectively. Though the values are different, they are still in range of each other and the expected phosphate concentration, so they are considered accurate for their respective trials, with a mistake during media preparation considered the cause behind the variation. Slope was set at 3.00 ± 0.100% with full inlet flow; the average inlet flow rates were 18.6 ± 1.75, 19.4 ± 1.19, and 18.7 ± 1.08

mL/s for Trials 1, 2, and 3 respectively. No final pH, temperature, total dissolved solids, and electrical conductivity data were taken on harvest day for Trial 2.

2.4.2. Trial 4: The effect of Tallapoosa River water bacteria biofilm on attachment in high nutrient synthetic media

The impact of the Tallapoosa River water bacteria biofilm on attachment in high nutrient synthetic media was tested using double strength freshwater-modified F/2 over a five-day period using substrata incubated for 72 hours and algal inoculum with an average algae biomass density of 2.58 mg/mL. The estimated average beginning biofilm biomass was $1.94 \times 10^{-5} \pm 3.00 \times 10^{-6}$ g/cm^2. The beginning nutrient concentration data was lost but based on other nutrient measurements taken from 2x strength F/2, the beginning nitrate and phosphate concentrations should have been around 55.6 and 9.70 ppm respectively, and these values were used when calculating the change in nutrient concentrations over the course of the trial. The slope was set to 3.00 ± 0.100% with full inlet flow and an average inlet flow rate of 18.5 ± 2.23 mL/s.

2.4.3. Trial 5: The effect of Town Creek Park Water bacteria biofilm on attachment in low nutrient synthetic media

The impact of a biofilm formed from a bacteria community sourced from Town Creek Park's stream on attachment in low nutrient synthetic media was tested using quarter strength freshwater-modified F/2 over an eight-day period using substrata incubated for 72 hours and algal inoculum with an average algae biomass density of 1.18 mg/mL. The estimated average beginning biofilm biomass was $2.84 \times 10^{-5} \pm 6.02 \times 10^{-6}$ g/cm^2. The beginning nitrate and phosphate concentrations were 2.65 ± 0.195 and 2.06 ± 0.0898 ppm respectively, and these values were used when calculating the change in nutrient concentrations over the course of the

trial. The slope was set to 3.00 ± 0.100% with full inlet flow and an average inlet flow rate of 16.8 ± 1.56 mL/s. Deviations in methodology were that systems 7, 8, and 9 were harvested the day after the remainder, but the water flow and lights for these systems were turned off at the same time to inhibit growth. The biomass for dry weight was dried at 55°C for 48 hours.

2.4.4. Trial 6: The effect of Town Creek Park water bacteria biofilm on attachment in high nutrient synthetic media

Trial 6 tested the effect of the Town Creek Park water bacteria biofilm on attachment when double-strength freshwater-modified F/2 was used as the growth media over a six-day period using substrata incubated for 72 hours. The numbers to find the average biomass density of the algae inoculum were lost; due to a spillage, five milliliters of the inoculum plus an algae clump of unknown mass were added to each system. The estimated average beginning biofilm biomass was $2.13 \times 10^{-5} \pm 9.24 \times 10^{-6}$ g/cm^2. The beginning nitrate and phosphate concentrations were 55.6 ± 3.47 and 9.72 ± 0.113 ppm respectively. The slope was set to 3.00 ± 0.100% with full inlet flow and an average inlet flow rate of 16.4 ± 1.85 mL/s. The dry weight filters were dried for 47 hours at 105 °C.

2.4.5. Trial 7: The effect of Town Creek Park water bacteria biofilm in half-diluted, filtered aquaculture waste

The impact of the Town Creek Park water bacteria biofilm on attachment was tested in aquaculture wastewater that was diluted to half-strength with tap water treated with sodium thiosulfate. The waste had been preserved at 4°C for over a year and was filtered with a Fischer brand P8 20.5 cm diameter filter with 20-25 micron particle size retention capacity prior to dilution. The trial took place over a 7-day period and used substrata incubated for 65.6 hours

with an unknown beginning bacteria biofilm mass and algal inoculum with an unknown biomass density (both unknowns arose from data loss due to a scale error). The beginning nitrate, phosphate, nitrite, and ammonia concentrations were 267 ± 21.6 ppm, 19.5 ± 1.55 ppm, 0.227 ± 0.00 ppm, and 1.02 ± 0.421 ppm respectively. The slope was set at 3.00 ± 0.100 % with full inlet flow and an average inlet flow rate of 14.5 ± 3.29 mL/s. There were four major changes in methodology in this trial. The first is that this trial was harvested with tap water instead of DI water, and the reservoirs were refilled with tap water before water samples were taken for final nutrient analysis. It is assumed for the purposes of this trial that any nutrients in the tap water are present in a negligible amount compared to the amount in the fish waste (which had a considerable amount of nutrients). The second change is that for this trial instead of splitting the harvested biomass in half (half for dry weight measurement and half for chlorophyll *a* measurement) like in previous trials, the biomass was split (with a graduated cylinder) so that 3/8 was used for dry weight measurement and 5/8 for total chlorophyll *a* measurement to try and reduce some of the error caused by having such a small amount of chlorophyll *a* available for analysis. The third change was that the biomass was blended for approximately 15s before splitting and filtration (this was only carried out in this trial and Trial 8). The final change was that the filter and pan were weighed together instead of only weighing the filter, and this change was adopted for all trials after (the other three were not). As of this trial, lid 3 was replaced with lid 10 because the former burnt out.

2.4.6. Trial 8: The effect of a mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste

In this trial, the impact of a mixed bacteria biofilm (the inoculation jars were inoculated with bacteria from both the Tallapoosa River and the Town Creek Park waters) on attachment when

unfiltered aquaculture waste diluted to quarter strength with sodium thiosulfate-treated tap water was used as the media was tested over 7 days, using substrata inoculated for 48 hours with a beginning estimated bacteria biofilm biomass of $1.36 \times 10^{-5} \pm 5.38 \times 10^{-6}$ g/cm^2, and algae inoculum with a biomass density of 5.64 mg/mL. The waste used was the same waste used in Trial 7, and all trials after also used this waste. The beginning average nitrate, phosphate, nitrite, and ammonia concentrations were 168 ± 31.0 ppm, 12.8 ± 1.43 ppm, 0.227 ± 0.00 ppm, and 0.763 ± 0.158 ppm respectively. The slope was set to 2.00 ± 0.100% with full inlet flow and an average inlet flow rate of 15.2 ± 3.43 mL/s. (The reduction of the slope from 3 to 2% was in the hopes that any true effect of the biofilm, if present, would be more apparent through any outside noise in unfavorable conditions; increasing the slope increases the channel velocity, and higher channel velocity favors attachment up to a point because of the transfer of nutrients to the algae via movement. Reducing the slope should theoretically have made the environment less favorable for attachment.)

For this trial, the bacteria biofilm mass estimation strips were air dried for 3 days instead of being oven dried. Starting with this trial and continuing in trials after, algae inoculum was made by pre-weighing a select amount of algae biomass to 3.00 ± 0.100 g wet weight and adding it to a known amount of DI water (5.50×10^2 mL).

2.4.7. Trial 9: The effect of a mixed biofilm bacteria biofilm in undiluted, unfiltered aquaculture waste

The effect of the mixed bacteria biofilm (Tallapoosa River and Town Creek Park water bacteria) on attachment when the media is undiluted, unfiltered fish waste was tested over a 4-day period. The substrata were incubated for 45.4 hours and the average beginning bacteria biofilm biomass was $1.13 \times 10^{-5} \pm 6.59 \times 10^{-6}$ g/cm^2. The algae inoculum had an average biomass density of 5.64

mg/mL. The average beginning nitrate, phosphate, nitrite, and ammonia concentrations were 601 ± 32.6 ppm, 46.7 ± 4.79 ppm, 0.581 ± 0.0439 ppm, and 22.4 ± 0.806 ppm respectively. (Only 4 nitrate measurements, 4 ammonia measurements, and 3 nitrite measurements were averaged to obtain the average beginning nitrate, ammonia, and nitrite concentrations.) Slope was set at 2.00 ± 0.100% with full inlet flow rate and an average inlet flow rate of 16.9 ± 2.28 mL/s. On day 3, the lights and flow to the systems were cut off for a few hours.

2.4.8. Trial 10: The effect of mixed bacteria biofilm in medium diluted, unfiltered aquaculture waste

The mixed bacteria (Tallapoosa River and Town Creek Park water bacteria) biofilm's impact on attachment in unfiltered aquaculture waste diluted to half-strength with tap water treated with sodium thiosulfate was tested over 12 days. The longer trial duration was due to an unavoidable scheduling conflict; though it led to some extension of filament, which may have slightly distorted the amount of attachment versus growth, the amount of outgrowth is assumed to be negligible here. The substrata were incubated for an unrecorded period and began with an estimated average biofilm mass density of $1.53 \times 10^{-5} \pm g/cm^2$. The algae inoculum biomass density was 5.51 mg/mL. The average beginning nitrate, phosphate, nitrite, and ammonia concentrations were 225 ± 24.2 ppm, 22.9 ± 0.419 ppm, 0.349 ± 0.0416 ppm, and 0.508 ± 0.111 ppm respectively. The slope was set to 2.00 ± 0.100% slope with full inlet flow. There is no average inlet flow rate data available for this trial. The dry weight samples were air dried. There is no intention to compare across different trials so as long as the samples dry the same amount relevant to each other, there is no need to ensure all water is removed for the purposes of the experiments described in this manuscript. In addition, though DI water was used to refill the

reservoirs at the end of the trial before taking final water samples for nutrient analysis, the actual harvest was performed with tap water.

2.4.9. Trial 11: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with reduced slope

The effect of the mixed (Tallapoosa River plus Town Creek Park water) bacteria biofilm was tested using a quarter-dilution of unfiltered fish waste (diluted with tap water) and with a considerably reduced slope of 1.00 ± 0.100% slope with full inlet flow (the average flow inlet flow rate was not obtained for this trial). The trial duration was 4 days, and the substrata were incubated for 48.7 hours, resulting in an average beginning biofilm biomass density of $2.75 \times 10^{-5} \pm 1.14 \times 10^{-5}$ g/cm^2. The algae inoculum had a biomass density of 5.45 mg/mL. The beginning nitrate, phosphate, nitrite, and ammonia concentrations were 136.2 ± 11.0, 10.5 ± 0.718, 0.0728 ± 0.00680, and 0.357 ± 0.0313 ppm respectively. The lights on systems 1, 4, and 9 were flashing on day 3; the amount of time they had been flashing is unknown but was under 24 hours. The adapter for those three systems were replaced with a 12 V, 1.5 A adapter for the rest of the trial. This trial was meant to test the effect of changing slope (dropping from the 2% that had been used for all aquaculture waste trials with the mixed bacteria biofilm 1%) in tandem with biofilm presence; the quarter dilution of fish waste was used because statistical significance was observed in the trial where the dilution was used as the media.

2.4.10. Trial 12: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with increased slope

Trial 12 tested the impact of the mixed bacteria biofilm (Tallapoosa River plus Town Creek Park water bacteria) when quarter strength fish waste was used and the slope was set to 3.00 ± 0.100%

slope with full inlet flow and an average inlet flow rate of 16.3 ± 2.54 mL/s. In this trial, because there was more than a minimal difference (on the scale of 5 mL/s) between the inlet flow rates of some of the individual systems, a t-test was also run in R to see if there was a difference in total dry weight or total chlorophyll *a* because of the flow rate difference. To do this, a label of high was assigned to those systems with inlet flow rates of greater than 15 mL/s and a label of low to those with an inlet flow rate less than that. No statistically significant difference was found, meaning any effect the difference in inlet flow rates had on attachment can be ignored. The channel depth was also measured in this trial only (by dipping the end of a strip of paper into the center of the channel and measuring the length of the wet portion, repeated five times for each channel) and found to be a maximum of 0.159 cm across all systems. The substrata were incubated for 72 hours, resulting in an average beginning biofilm biomass density of $3.26 \times 10^{-5} \pm 1.28 \times 10^{-5}$ g/cm^2, and the algae inoculum had a biomass density of 5.45 mg/mL. The systems were not harvested until day 8, but they were turned off (lights and flow) on day 7 to prevent further attachment, making this a 7-day trial. The beginning nitrate, phosphate, nitrite, and ammonia concentrations were 133 ± 10.6 ppm, 9.52 ± 0.236 ppm, 0.0879 ± 0.0197 ppm, and 0.224 ± 0.0188 ppm respectively. This trial was meant to test the effect of changing slope in tandem with biofilm presence (trials with quarter dilution of aquaculture waste and mixed bacteria biofilm had been tested only at 1 and 2% slopes prior to this trial); the quarter dilution of fish waste was used because statistical significance was observed in the trial where the dilution was used as the media.

Section 2.5. Calculations and Analysis

The data were originally gathered in paper form, then transferred to Microsoft Excel where total dry weight, chlorophyll *a*, total chlorophyll pigments, and SA indices (SAB:LAB, see previous section on harvesting) were calculated.

Chlorophyll *a* was calculated using the equations from *Methods in Stream Ecology* (Hauer & Lamberti, 2006), listed as Equations 1-3 below, with the exception that it was not normalized over the surface area as in the text:

$$\text{Chlorophyll } a \text{ } (\mu g) = 26.7 \, (E_{664b} - E_{665a}) \times V_{ext}/L \qquad (1),$$

where,

E_{664b} = [{Absorbance of sample at 664 nm−Absorbance of blank at 664 nm} − {Absorbance of sample at 750 nm−Absorbance of blank at 750 nm before Acidification}] (2),

E_{665a}= [{Absorbance of sample at 665 nm−Absorbance of blank at 665 nm} − {Absorbance of sample at 750 nm−Absorbance of blank at 750 nm after Acidification}] (3),

V_{ext} = Volume of 90% acetone used in the extraction (mL)

L = length of path light through cuvette (cm)

26.7 = absorbance correction (derived from absorbance coefficient for chlorophyll a at 664 nm [11.0] ×correction for acidification [2.43])

1.7 = maximum ratio of E_{664b}:E_{665a} in the absence of pheopigments.

Total chlorophyll pigments were calculated using Equation 4, which was adapted from Goltermann's (1969) *Methods for chemical analysis of fresh waters IBP Handbook No 8*, using Parsons and Strickland's (1963) extinction coefficients for chlorophyll *a* in aqueous acetone as

listed in Table 7.1 in the same book and to use absorbance read at 665 nm instead of 663 nm (see Appendix E.1 for derivation).

$$P_{chl} = ({}^{U}E_{665} - {}^{U}E_{750} - {}^{A}E_{665} + {}^{A}E_{750})(\text{Vol. ext./L}) \quad (4)$$

where,

P_{chl} = total chlorophyll pigments in $\mu g/cm^2$,

${}^{U}E_{665}$ = absorbance of unacidified extract at 665 nm,

${}^{U}E_{750}$ = absorbance of unacidified extract at 750 nm,

${}^{A}E_{665}$ = absorbance of acidified extract at 665 nm,

${}^{A}E_{750}$ = absorbance of acidified extract at 750 nm,

Vol. ext. = extract volume in mL, and

L = light path through cuvette in cm.

Generally, chlorophyll *a* and total chlorophyll pigments are expressed in terms of total area or volume. However, the substrata used differed slightly in surface area, ranging from 360.10 to 445.16 cm^2 due to slight cutting errors. As the algae could not attach to the entire substratum due to the nature of its placement in the channel, these slight differences were not sufficient to cause serious deviation in results, but it was also deemed more accurate to relay findings in a non-normalized format.

Excel's F.TEST function was used to determine if the control and treated groups had equal variances for each variable calculated; the F-test results can be viewed in Appendix E.3. The data was then analyzed using t.test() for equal or unequal variance (based on the F-test results) in R, with an alpha of 0.05. The 95% confidence intervals were also obtained from the t.test() function. These show the range of potential true mean differences such that, if these trials were repeated multiple times, in 95% of the replicate trials, the true population mean difference

between the treated and control groups would be in the intervals. The width of a confidence interval is also an indicator of precision.

Cohen's d effect size was calculated in Excel by dividing the mean difference between the control and treated groups by the pooled standard deviation, where pooled standard deviation is:

$$\mathrm{SQRT}[\{(N_1\text{-}1) \times SD_1^2 + (N_2\text{-}1) \times SD_2^2)\}/(N_1 + N_2 - 2)] \quad (5),$$

where,

N_1 = sample size of group 1,

N_2 = sample size of group 2,

SD_1 = standard deviation of group 1,

and,

SD_2 = standard deviation of group 2.

Equivalence testing was also performed, to determine if a meaningful effect was present, using the tsum_TOST() function from the TOSTER library in R since Cohen's d by itself is a point value susceptible to influence by large standard errors. The equivalence bounds for the equivalence testing were set at Cohen's d effect sizes of -0.5 to 0.5, because it is the threshold for medium size effects and in this case an assumption is made that a medium effect size is the minimum effect needed to be meaningful. This benchmark method of determining bounds was chosen in the absence of other reasonable methods. Lakens (2017) mentions using benchmarks as a method of last resorts, but other means they mention could not be used here due to a lack of needed information. Similarly, the means of setting bounds described by Limentani et al. (2005) require prior knowledge and replicates not present in this study. The mean differences input into the TOST_tsum function were calculated by multiplying the pooled deviation by this chosen

minimum effect size of interest. Though equivalence testing has historically been used primarily in clinical settings to date, it is applicable in other fields of research (Limentani et al., 2005). Graphical visualization was performed in both Excel and R. For Trials 1-3 only a mini meta-analysis was performed following Goh et al.'s (2016) instructions (the Cohen's d was calculated as stated above, but equations 6-10 below are as they specify) described below (this was the only study which had more than one replicate trial) for total dry weight and total chlorophyll *a*. The Cohen's d for each trial was converted to Pearson's correlation coefficient using Equation 5 (Goh et al., 2016).

$$r = \sqrt{\frac{d^2}{d^2 + \frac{1}{P*Q}}} \quad (6),$$

where,

r = Pearson's correlation coefficient,

d = Cohen's d

P = proportion of sample in group 1 and,

Q = proportion of sample in group 2.

Fisher's *z* transformation to r_z was then performed on the *r*-values using Excel's fisher(x) function. The weighted mean effect size was then calculated with Equation 7.

$$r_{zwm} = \frac{\sum(N-3)r_z}{\sum(N-3)} \quad (7)$$

where,

r_{zwm} = weighted mean effect size,

N = sample size for given effect size, and

r_z = Fisher-transformed *r*.

The weighted mean effect size was then converted back into r, then into a Cohen's d-value using Equations 8 and 9 below.

$$r = \frac{e^{2r_z} - 1}{e^{2r_z}} \quad (8)$$

$$d = \frac{2r}{\sqrt{1-r^2}} \quad (9).$$

Planetcalc's online calculator (Timur : planetcalc member, n.d.) was used to obtain the Z-score for t-value for each trial and Equation 10 was used to find a combined summary Z-score, which was then converted back into a summary p-value for the three trials combined using the soscistatistics.com calculator ("Quick P Value From Z Score Calculator," n.d.).

$$Z_{combined} = \frac{\sum Z}{\sqrt{k}} \quad (10)$$

where,

$Z_{combined}$ = summary Z-score ,and

k = number of Zs combined.

Chapter 3: Results

Section 3.1. Preliminary Trials

3.1.1. Variability trials

The results of the variability trials are shown graphically combined in a bar plot in Figure 14. Only four points were able to be used from the first trial due to a drying error giving negative biomass values. Graphically, there appears to be a large difference between the systems. However, the coefficient of variances were 0.386 and 0.394 for Trials 1 and 2 respectively. These are less than 1.00, the threshold for high variability, but higher than 0.300, the threshold for low variability. Overall, there is some unidentified factor or noise resulting in innate medium variability among the systems. Relative to the mean, standard deviation is almost 40% of the mean value. It was expected that the values within each trial would be close to each other if not exactly the same since the conditions were the same across all systems in both trials. System 2 is an outlier; this may be due to some unknown factore inhibiting its growth. For instance, the inoculum introduced to that system may have had more dead cells. The inclusion of system 2 may also have raised the coefficient of variation beyond what it actually is.

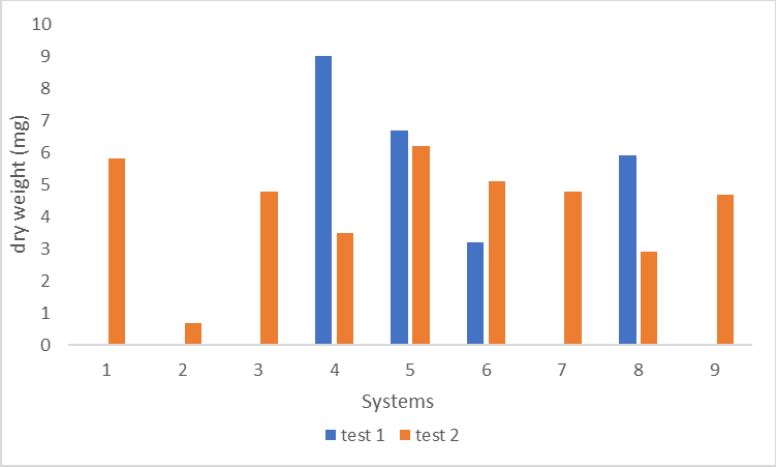


Figure 14: Variability trials combined results showing Dry Weight generated per system.

3.1.2. Substrata experiment

Figure 15 below show the results of trials 1 and 2 of the substrata trials. Figure 16 shows the mean grams of biomass per square centimeter harvested in trial 3 of the substrata trials with standard deviation bars. Netting was eliminated from consideration after the first and second trials. It is visually apparent from the graphs that felt had the most attachment on average at the end of each 8-day trial. From the trial 3 graphs, roughened film appears to have the second-most attachment.

Based on a one-way ANOVA performed in Microsoft Excel, there was a significant difference (p-value = 4.94×10^{-4}) between roughened film, unroughened film, and felt in trial 3. A Tukey's test in Microsoft Excel showed a significant difference between the amount of attachment on felt versus the other two substrata, but no significant difference between attachment on unroughened and roughened film. However, based on the graphical difference, white polypropylene film roughened with P120 Power Sanding paper (in a crisscross motion down the length of the substrata, twice) was chosen as the substratum.

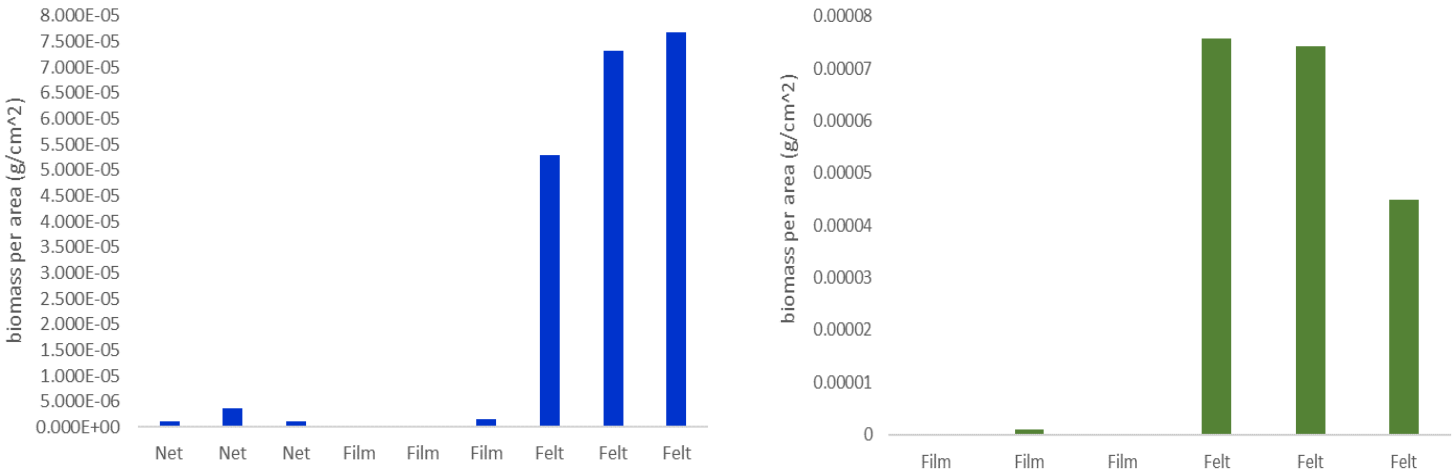


Figure 15: Substrata trials 1 and 2 results.

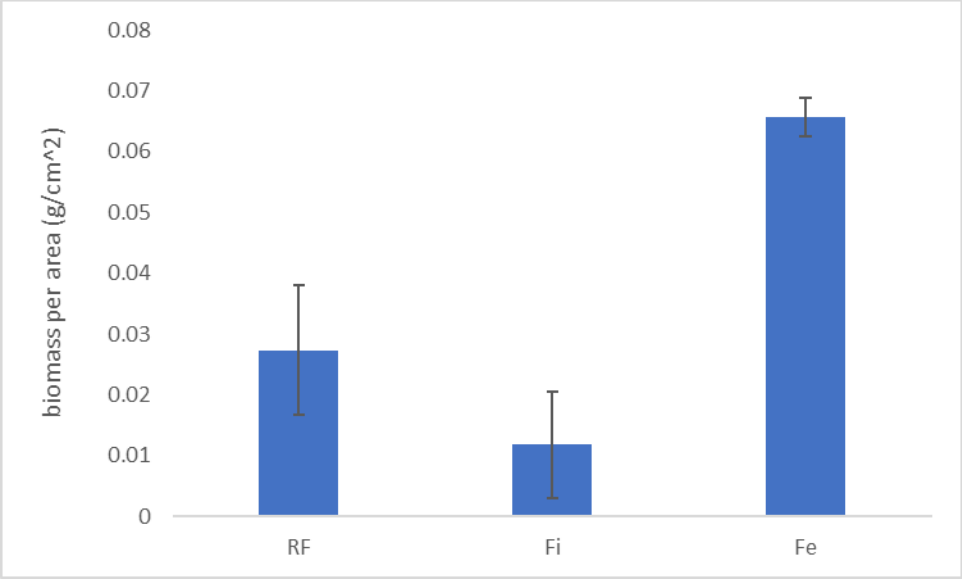


Figure 16: Substrata experiment trial 3 mean with standard deviation bars (RF = roughened film, Fi = unroughened film, Fe = felt).

Section 3.2. Artificial media trials

3.2.1. Trials 1-3: The effect of Tallapoosa River water bacteria biofilm on attachment in low nutrient concentration synthetic media

Out of the three trials (referred to here as Trial 1, Trial 2, and Trial 3 in the sequence they were carried out) run using a bacteria community sourced from the Tallapoosa River and 1/4-strength freshwater modified ProLine F/2, two had results that agreed with each other, but the final one did not agree with either of them. Trials 1 and 2 both came out significant (p-value = 0.0339 with CI[-0.00232, -0.000123] and p-value = 0.0285 with CI[-0.00292, -0.000232]) for total dry weight, but not for total chlorophyll *a* (p-value = 0.284 with CI[-11.9, 4.07] and p-value = 0.207 with CI[-35.4, 9.88]). The final trial came out significant for chlorophyll *a* (p-value = 0.0290 with CI[-75.4, -6.20] but not for dry weight (p-value = 0.219 with CI[-0.00717, 0.00202]). Figure 21 and Figure 22 below show the mean total dry weight and chlorophyll *a*

values for the three trials with sample standard deviation bars (calculated separately for each trial, not the combined values). Even though there is no statistical significance to support it, the graph shows the same trend throughout all 3 trials; the mean values of both total dry weight and total chlorophyll *a* are consistently larger for the treated systems. There is also a trend of large standard deviation from the mean. This potentially indicates that unspecified noise is distorting the signal of the impact of the biofilm on the speed of attachment and that the sample size is not large enough for any effect to show through the distortion on a significant level.

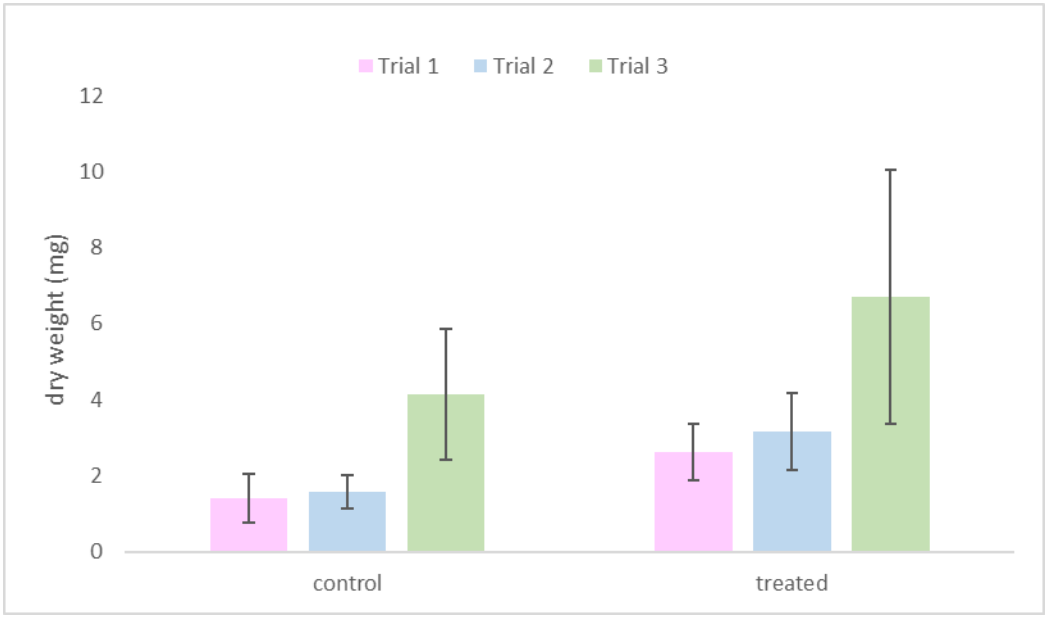


Figure 17: Mean total dry weight for Trials 1-3 of the Tallapoosa River biofilm in low nutrient concentration synthetic media experiment with standard deviation bars (separate for each trial).

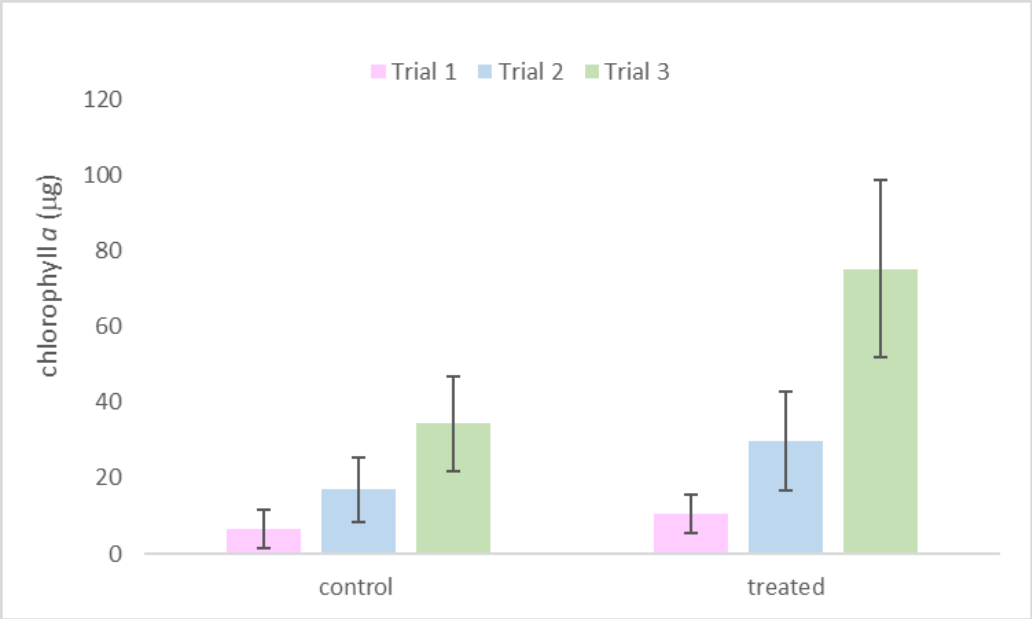


Figure 18: Mean total chlorophyll a for Trials 1-3 of the Tallapoosa River biofilm in low nutrient concentration synthetic media experiment with standard deviation bars (separate for each trial).

The Cohen's d effect sizes for Trial 1, Trial 2, and Trial 3 were 1.69, 4.97, and 0.970 for total dry weight and 0.778, 1.21, and 2.32 for total chlorophyll *a* respectively. These are all greater than 0.500, making them large effect sizes. While they may be somewhat affected by the large standard deviation, they also potentially support the theory that the trials are indeed underpowered and needed more replication to demonstrate the significance of the biofilm's impact. The results of equivalence tests with equivalence bounds of d = -0.500 to d = 0.500 were non-significant for all three trials, indicating that the means between the treated groups and the control groups are not equivalent regardless of non-significance and that we cannot reject the idea that there is a meaningful effect, supporting the legitimacy of the Cohen's d values. The TOST results for the equivalence tests, including p-values, can be viewed in full in Appendix E.4.1. For Trial 1, the lower TOST for total dry weight did come out significant, meaning that an

effect more extreme than -0.500 (effects more extreme than medium-sized effects in the direction of the control) can be rejected. Similarly, based on p-values < 0.5, effects more extreme than the lower bounds could be rejected for both total dry weight and total chlorophyll *a* for Trial 2, and for total chlorophyll *a* for Trial 3.

The large width of the non-significant results' confidence intervals is also indicative of the large error among the results and that a true effect cannot be ruled out. This is not unexpected as the variability trials indicated that there exists an innate medium amount of variability among the systems.

For Trial 2, system 9 leaked out and the chlorophyll *a* data for system 1 was lost, so only 8 systems were included in the analysis of dry weight and 7 for the analysis of chlorophyll *a*. During the harvesting process in Trial 3, the dry weight filter for system 3 was dropped, resulting in its removal from analysis and only 8 systems being included in the dry weight statistical testing. Systems 8 and 4 were also excluded from the chlorophyll *a* analysis as a result of spilling the harvest suspension and extract respectively, leading to a 7-system chlorophyll *a* analysis. These losses in sample size further reduced the power of the studies. While the trials test the same condition, they are not exact replicas, with certain factors being different, which may also be partially responsible for the variation in results.

Because the chlorophyll *a* results do not agree with the dry weight results and the three trials do not agree with each other, it cannot be concluded from each individual trial that in low nutrient concentration artificial media (specifically F/2) the Tallapoosa River bacteria biofilm had an effect on the speed of attachment. The dry weight values cannot be taken as evidence of an effect on their own without the chlorophyll *a* confirmation as there is no measure of the bacteria and biofilm fraction of the harvest. The chlorophyll *a* values from Trial 3 could be taken

as evidence of an effect on their own, but the lack of agreement between the trials prevents it. Given the graphical trend, the large effect sizes, and the equivalence test results, the presence of an effect cannot be rejected either. Looking at each individual trial separately, the results are inconclusive, with strong evidence that there is an effect present that the studies are simply too underpowered to detect.

However, the fixed effect mini meta-analysis of the trials yielded highly significant results; for total dry weight and total chlorophyll *a* respectively, the combined summary p-values were 6.90×10^{-4} and 9.21×10^{-3}. This confirms the likely presence of an effect on *Rhizoclonium spp.* attachment in the low nutrient synthetic media when substrata are pre-inoculated with a Tallapoosa River bacteria biofilm.

The average amounts of both measurements increase in general from Trial 1 to Trial 3, which may be attributed to the difference in beginning average nutrient concentration, with higher nutrient concentrations resulting in more biomass in general.

The SA ratio was also not significant for either total dry weight or total chlorophyll *a* for all three trials (in order for dry weight SA index: p-value = 0.464 with CI[-8.10, 15.5] and d = -1.37, p-value = 0.630 with CI[-4.59, 3.01] and d = 0.360, and p-value = 0.428 with CI[-1.34, 0.636] and d =0.300 ; in order for total chlorophyll *a* SA index: p-value = 0.686 with CI[-15.1, 10.6] and d = 0.310, p-value = 0.581 with CI[-29.3, 18.4] and d = 0.451, and p-value = 0.876 with CI[-0.563, 0.640] and d = -0.126). There was also high standard deviation for all measures of the SA index, which can be seen by the large confidence intervals. The equivalence tests came out non-significant, indicating we cannot reject the presence of a meaningful effect, but all the effect sizes shown here are small, while the equivalence bounds were set to exclude any effects less than medium-sized. In addition, the Cohen's d-values are different signs for different trials

and measures, indicating contrasting effect directions. The high standard error seems to be obscuring the true signal, and unlike with the total dry weight and total chlorophyll *a,* measurements, there is not enough consistency to tease out a signal. Nothing about the impact of the Tallapoosa River bacteria biofilm on the attachment strength of *Rhizoclonium spp.* in low nutrient concentration synthetic media can be concluded from these trials. Additional graphs for Trials 1-3, including SA index mean with standard deviation bars charts can be viewed in Appendix C.1.1, including total chlorophyll pigments graphs for Trial 3 (it can be noted that the total chlorophyll pigment results mirrored the total chlorophyll *a* results for this trial, also coming out significant with p-value = 0.0302 and CI[-2.71×10^3, -0.208×10^3]).

Observations of note are that in Trial 3, the filtrate was pink as opposed to the normal clear and a considerable fraction of the attached biomass was brown, indicating potentially dead algae which could lead to an underestimation of chlorophyll *a,* as seen in Figure 19 below. The cause of the pink color could not be identified.

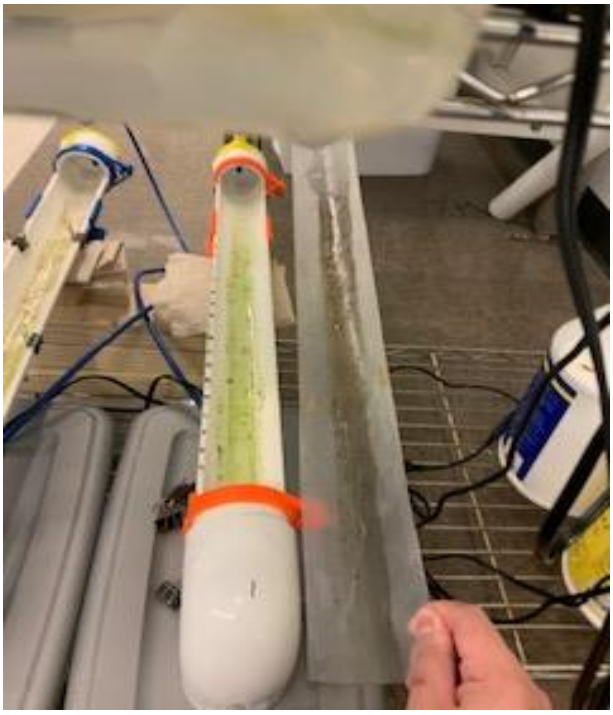


Figure 19: From left to right: Pink filtrate and substratum with mix of green and brown attachment from Trial 3 of experiment testing Tallapoosa River bacteria biofilm in low nutrient synthetic media.

3.2.2. Trial 4: The effect of the Tallapoosa River water bacteria biofilm on attachment in high nutrient synthetic media

Trial 4 tested the impact of the Tallapoosa River water bacteria biofilm on attachment in double-strength freshwater modified F/2 media. Both the measures of attachment, total dry weight and total chlorophyll *a*, were statistically non-significant (p-value = 0.648 with CI[-0.00262, 0.00174] and p-value = 0.468 with CI[-11.2, 5.71] respectively). However, the confidence intervals are wide, indicating a large standard error and a lack of precision. Figure 20 below shows the mean total dry weight and mean total chlorophyll *a*. The graphs show a similar trend to trials 1-3, with the mean of the treated group being larger than the mean of the control group for both measurements and the standard deviation being high.

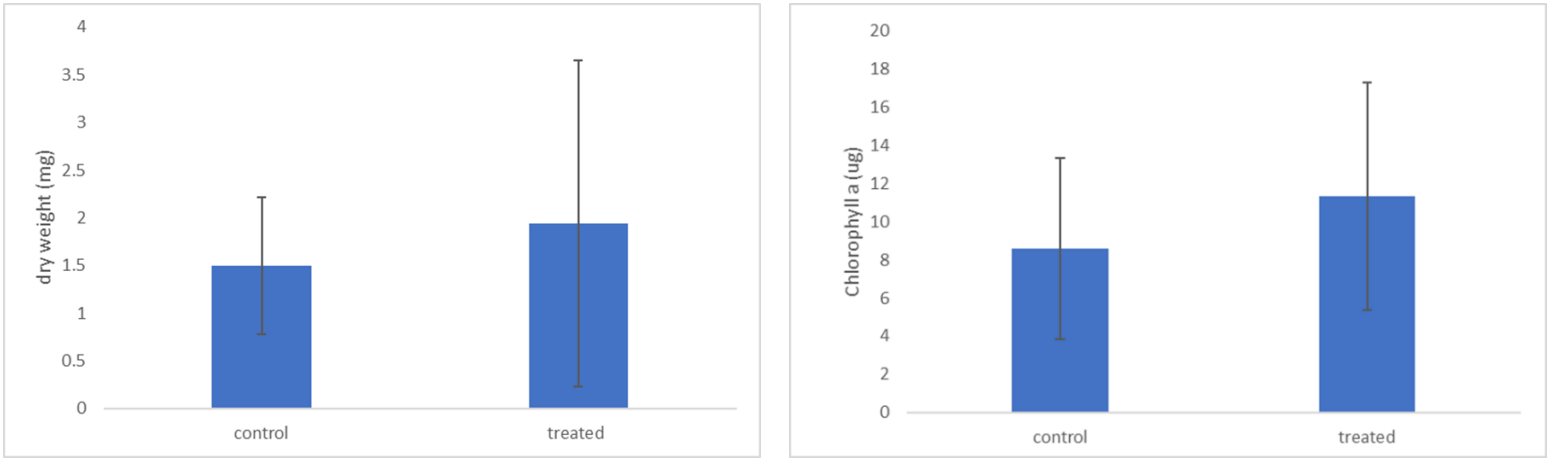


Figure 20: Mean total dry weight (left) and mean total chlorophyll a with standard deviation bars for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media.

The total chlorophyll pigments analysis showed the same result as the total chlorophyll *a*, with their being no significance (p-value >> 0.05). The Cohen's d effect sizes were 0.320 and 0.501 for total dry weight and chlorophyll *a* respectively, which are considered indicative of small and medium effects, but based on equivalence tests with bounds of -0.500 to 0.500, we cannot reject the possibility of a more extreme effect for both (see Appendix E.4.2 for TOST results). Based on these results, the trial was non-conclusive. It cannot be concluded that the biofilm had an effect in the double strength F/2 or not, but the effect sizes and equivalence test results combined with the graphical trend suggest that there is an effect present that cannot be distinguished on a statistical level because of a large standard error from some unidentified noise because the study is underpowered.

The analysis on strength of attachment also came out non-significant for both dry weight and chlorophyll *a* (as well as total chlorophyll pigments, which confirms the chlorophyll *a* result), with p-values being 0.639 with CI[-19.9, 13.1] and 0.817 with CI[-1.87, 2.29] and d = -1.47 (large effect size) and d = -0.161 (small effect size) respectively (the total chlorophyll pigments SA index also had a small negative effect size and were non-significant). The sign of the effect sizes indicates an effect in the direction of the control group, as does the graphical trend (see Appendices C.2c and C.2f), which shows the control group having a higher mean for SA index, but extremely large error bars. The equivalence tests also came out non-significant, meaning the possibility of significant effects as defined for this study cannot be rejected. Thus, Trial 4 was also inconclusive with regards to effect on strength of attachment, with some indication that the study lacks enough replication to distinguish the true effect. Overall, more trials with larger replication need to be carried out to distinguish any effect of the Tallapoosa

River bacteria biofilm on attachment strength or speed in high nutrient synthetic media. Additional figures and charts associated with this trial can be viewed in Appendix C.2.

3.2.3. Trial 5: The effect of Town Creek Park Water bacteria biofilm on attachment in low nutrient synthetic media

The impact of a biofilm formed from bacteria sourced from a Town Creek Park stream on attachment in low nutrient concentration (quarter-strength freshwater modified F/2) synthetic media was tested. Both measures of attachment, total dry weight (p-value = 0.201 with CI[-0.0130, 0.00353]) and total chlorophyll *a* (p-value = 0.180 with CI[-109.5, 26.9]) , were non-significant. In addition, the total chlorophyll pigment measurements were also non-significant (p-value = 0.188 with CI[-4.00×10^3, 1.02×10^3]), confirming the results of the total chlorophyll *a* analysis. The corresponding Cohen's d effect sizes are, respectively, 1.23 and 1.19 (1.17 for total chlorophyll pigments), which are large effect sizes; furthermore, equivalence tests with bounds of -0.500 to 0.500 and alpha = 0.500 were non-significant, meaning that the presence of extreme effects cannot be rejected and the two groups' means are not equivalent. The results seem to indicate the study needs more replication to tease out the signal of effect. The widths of the confidence intervals also seems to support this. Figure 21 below shows the mean total dry weight and total chlorophyll *a* values; the large standard deviation bars indicate that the some environmental static may be causing the effect to be unclear.

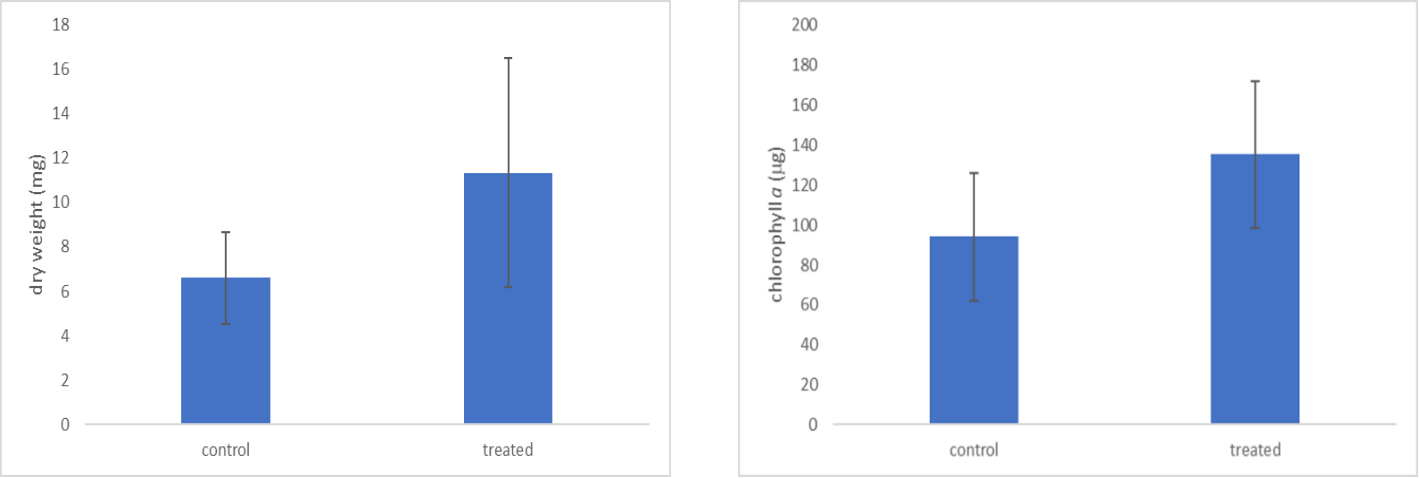


Figure 21: Mean total dry weight (left) and mean total chlorophyll a (right) with standard deviation bars for Trial 5: The effect of Town Creek Park water bacteria biofilm on attachment in low nutrient synthetic media.

The analyses on strength of attachment also resulted in non-significant results (p-value = 0.379 with CI[-90.3, 46.0] for dry weight SA index and p-value = 0.385 with CI[-12.2, 5.61] for chlorophyll *a* SA index, and p-value = 0.445 with CI[-4.19, 6.63] for total chlorophyll pigments. The equivalence tests (see Appendix E.4.3 for TOST results) also came out non-significant, indicating lack of equivalence, but the Cohen's d effect sizes, though in the medium to large range, contradict each other in terms of direction: they were 0.665, 0.727, and -0.846 for dry weight SA index, chlorophyll *a* SA index, and total chlorophyll pigments SA index respectively. This may also be due to the large standard error that is present, which is apparent from looking at Figures 22 below, which shows the mean with standard deviation charts for the SA indices.

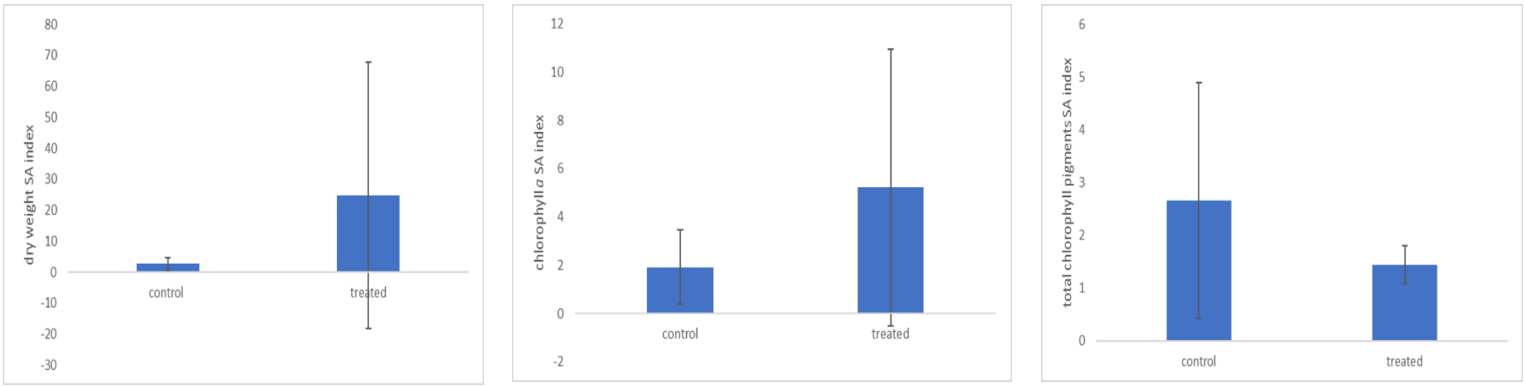


Figure 22: Mean dry weight SA (left), chlorophyll a (center), and total chlorophyll pigments (right) indices with standard deviation bars for Trial 5: The effect of Town Creek Park water bacteria biofilm on attachment in low nutrient synthetic media.

Trial 5 can be concluded to be inconclusive for both speed and strength of attachment, though the results seem to indicate the possibility of an effect on speed of attachment that the study is underpowered to detect on a statistically significant level given the high error. The strength of attachment data showed such a high standard deviation and contrasting results, so that there is no hint of a potential effect in either direction. Other figures and tables for this trial can be viewed in Appendix C.3.

Other observations of note are that system 2 leaked out and system 8 was removed from the analysis because an unknown amount of harvest solution was spilled. This reduced the sample size from 9 to 7 (3 control and 4 treatment systems), which may also have contributed to the inability to fully distinguish any effects potentially present. During the harvest process, blue filtrate was also produced by some of the treated systems; the constitution of this is unknown.

3.2.4. Trial 6: The effect of Town Creek Park water bacteria biofilm on attachment in high nutrient synthetic media

Trial 6 tested the impact of the Town Creek Park water bacteria biofilm when double-strength freshwater modified F/2 was used as a media. Due to a drying error, no dry weight data is

available for this trial, so the only analyses performed were on chlorophyll *a* and total chlorophyll pigments; system 1 was excluded from the analysis due to spillage of the strongly attached biomass extract, reducing the sample size to 8, 4 treated and 4 control systems. The results came out non-significant for both measurements (p-value = 0.242 with CI[-0.0592, 0.0182] for total chlorophyll *a* and p-value = 0.236 with CI[-2.18, 0.656] for total chlorophyll pigments). However, the Cohen's d effect sizes, 0.918 and 0.931 for total chlorophyll *a* and total chlorophyll pigments respectively, are large ones and the equivalence tests came out non-significant (see Appendix E.4.4 for TOST results), indicating the means of the control and treated groups are not equivalent. These along with the large width of the confidence intervals seems to indicate that the study is underpowered. Figure 23 below shows the mean total chlorophyll *a* and total chlorophyll pigments values for each group with standard deviation bars. Both show large error bars, which supports the idea that there is not enough replication for any effect to show through the noise. The mean of the treated group is also larger than that of the control group, which coincides with the sign of the effect sizes (positive indicating an effect towards the treated group).

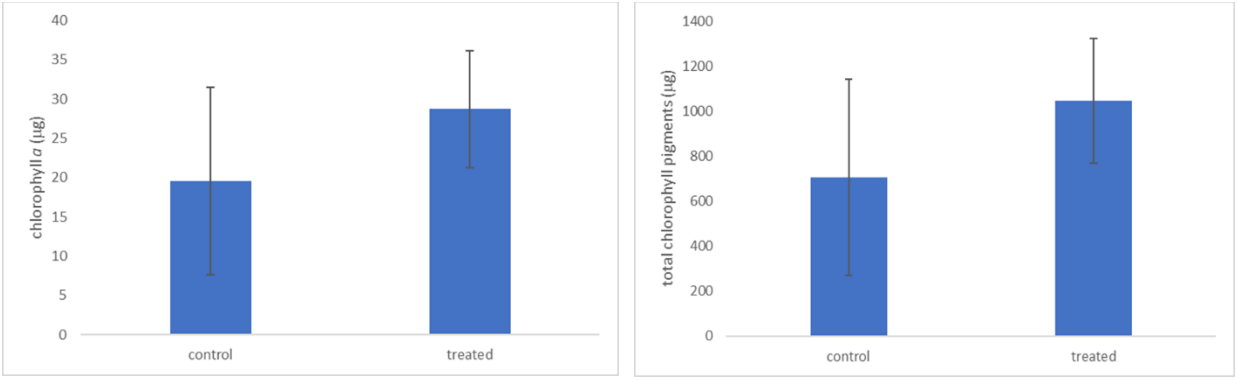


Figure 23: Mean total chlorophyll a (left) and mean total chlorophyll pigments (right) with standard deviation bars for Trial 6: The effect of Town Creek Park water bacteria biofilm on attachment in high nutrient synthetic media.

The trial was inconclusive for speed of attachment, but there is evidence to suggest that the high standard error and lack of power are contributing to masking a true effect.

The strength of attachment analysis similarly came out non-significant for both measures, with p-value = 0.762 and CI[-25.3,19.5] for chlorophyll *a* SA index and p-value = 0.578 with CI[-24.6, 40.3] for total chlorophyll pigments. However, the direction of the effect sizes contradict each other (d = 0.224 and -0.416 for total chlorophyll *a* SA index and total chlorophyll pigments SA index respectively) as do the charts of the means shown in Figure 24 below. Thus, even though the equivalence tests came out non-significant for the SA indices as well, it is not possible to discern any potential effect in either direction or to say that increasing replication might reveal an effect.

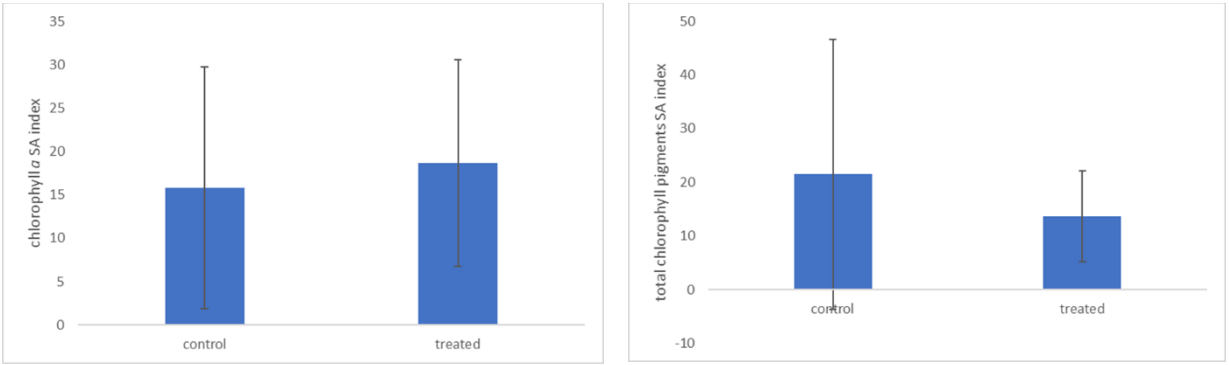


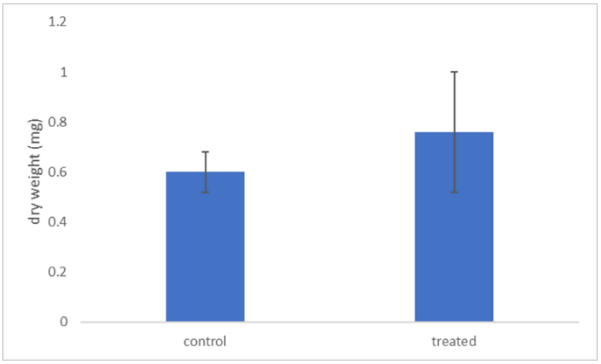
Figure 24: Mean total chlorophyll a (left) and mean total chlorophyll pigments (right) SA indices for Trial 6: The effect of Town Creek Park water bacteria biofilm on attachment in high nutrient synthetic media.

Additional graphs associated with Trial 6 can be viewed in Appendix C.4.

Section 3.3. Aquaculture waste trials

3.3.1. Trial 7: The effect of Town Creek Park stream bacteria biofilm in half-diluted, filtered aquaculture wastewater

Trial 7 tested the effect the Town Creek Park water bacteria biofilm has on attachment when filtered fish wastewater (assumed to possess no or minimal native bacteria as a result of prolonged storage at 4℃) diluted to half-strength is used as the media. Both the total dry weight (p-value = 0.249 with CI[-0.000461, 0.000141]) and total chlorophyll *a* (p-value = 0.597 with CI[-4.11, 2.81] results came out non-significant, but with high and low effect sizes of d = 0.843 and d = 0.651 respectively. Similarly to total chlorophyll *a*, the analysis of total chlorophyll pigments revealed no significance (p-value = 0.710 with CI[-131, 101]), but it also showed a large effect size of 1.38. The equivalence test results came out non-significant (see Appendix E.4.5 for TOST results), indicating that the control and treated groups' means are not equivalent. For speed of attachment, Trial 7 is inconclusive because the study is underpowered. The sign of the effect sizes and the means of the groups for both measures, as seen in Figure 25 below indicate that if an effect does exist it exists in the anticipated direction, with the treatment resulting in higher attachment/a higher mean. The high error (especially for total chlorophyll *a*) as indicated by the large standard deviation bars may be obscuring the true effect.



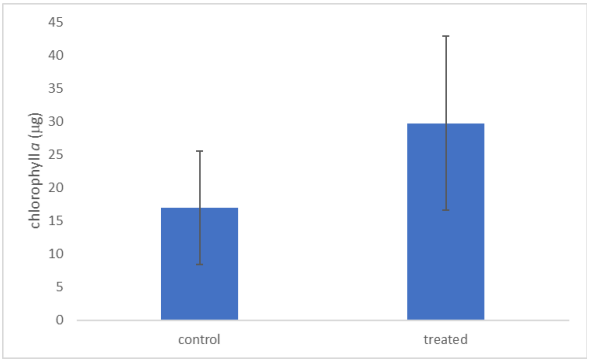


Figure 25: Mean total dry weight (left) and mean total chlorophyll a (right) with standard deviation bars for Trial 7: The effect of Town Creek Park water bacteria biofilm on attachment in medium dilution of filtered aquaculture wastewater.

The SA indices also came out non-significant (p-value > 0.5 for all measures) and with non-significant equivalence tests indicating non-equivalence, but they had conflicting Cohen's d effect sizes and extremely large errors. The Cohen's d effect sizes were -1.15, a large effect size indicating an effect on strength of attachment in the direction of the control group, while the total chlorophyll *a* and total chlorophyll pigments had d = 0.173 and d = 0.305 respectively, indicating a small effect but in the opposite direction. Large standard deviation bars can be viewed in Figure 26 below, which shows the mean values of the SA indices.

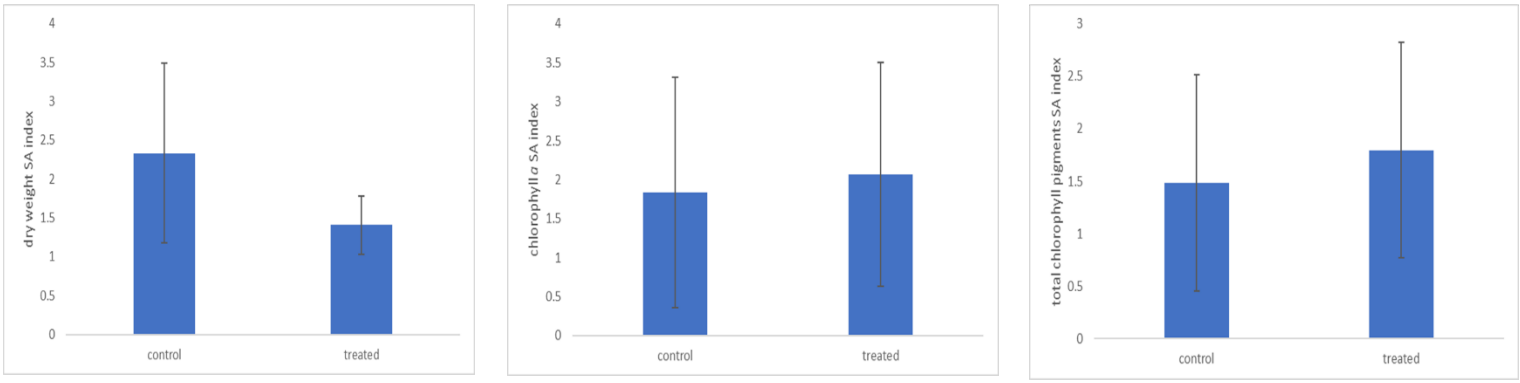


Figure 26: Mean dry weight (left), chlorophyll a (center), and total chlorophyll pigments (right)o SA indices with standard deviation bars for Trial 7: The effect of Town Creek Park water bacteria biofilm on attachment in medium dilution of filtered aquaculture wastewater.

Trial 7 is also inconclusive for strength of attachment, but the large error and contrasting results render it impossible to determine what direction a potential effect may be in given more replication. Additional figures and tables for this trial can be viewed in Appendix C.5.

3.3.2. Trial 8: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture wastewater with reduced slope

This trial tested the effect of the mixed bacteria (Town Creek Park and Tallapoosa River) biofilm on attachment when the media was unfiltered aquaculture wastewater diluted to quarter-strength

with dechlorinated tap water and the slope was decreased to 2%. No dry weight data is available for this trial due to a drying error. However, the total chlorophyll *a* data were significant (p-value = 0.0417 with CI[-3.74, -0.0952]) and had a large effect size (d = 1.67) and a non-significant equivalence test result, indicating that the significance is practical as well as statistical. The total chlorophyll pigments data was not significant (p-value = 0.0586 with CI[-131, 3.05]) ; however, the effect size is high (d = 1.51) and the equivalence test (see Appendix E.4.6 for TOST results) came out non-significant, indicating non-equivalence of the means of the treated and control groups. It can also be seen from Figure 27 below, which shows the mean total chlorophyll pigment values for each group with standard deviation bars, that there is a high error.

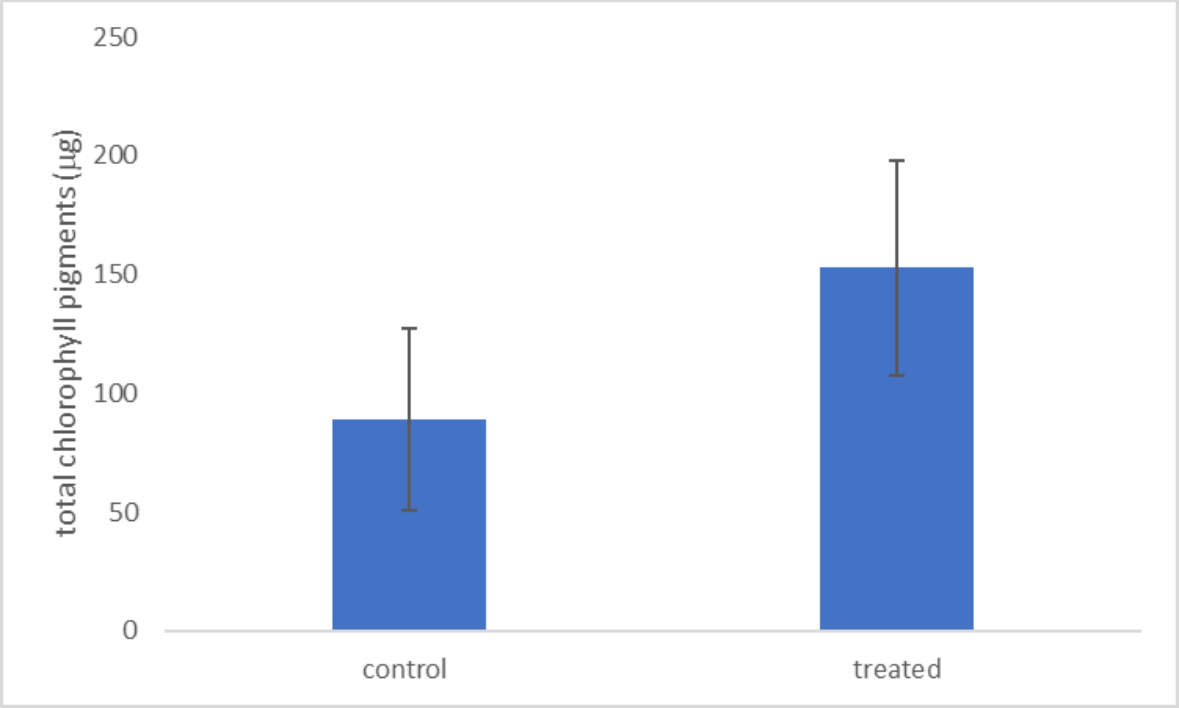


Figure 27: Mean total chlorophyll pigments for Trial 8: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture wastewater with reduced slope.

The mean of the treated group is still higher than the mean of the control group, and the confidence interval is large. It can then be said that the difference is caused by standard error and

the study is too underpowered to discern the effect through the total chlorophyll pigments measurement. It can also be concluded that the mixed biofilm had a positive effect on speed of attachment based on the total chlorophyll *a* analysis.

Both the chlorophyll *a* SA index and the total chlorophyll pigments SA index came out non-significant (p-values = 0.739 and 0.389 respectively) and had very broad confidence intervals ([-7.41, 5.51] and [-13.9, 6.68] respectively), indicating large error but also a potentially large effect, though the effect sizes themselves were alternately small (0.233) and medium (0.571). Equivalence tests for both also came out non-significant, suggesting a meaningful effect cannot be rejected. Overall, the strength of attachment analyses were inconclusive due to lack of power. Additional charts and figures for Trial 8 can be viewed in Appendix C.6).

3.3.3. Trial 9: The effect of mixed bacteria biofilm in undiluted, unfiltered aquaculture wastewater with reduced slope

Trial 9 tested the mixed bacteria biofilm's effect on attachment in undiluted, unfiltered fish wastewater when the slope was set at 2%. For this trial, three treatment systems leaked out, and one control system was removed from the trial to even out the numbers, reducing the sample size to 6. System 4 was not included in the total chlorophyll *a* analysis because the extract was lost. No chlorophyll *a* and chlorophyll pigments SA index analyses were performed due to not having enough usable data. Both measurements had non-significant results, with p-value = 0.0656 with CI[-0.0210, 0.00103] for total dry weight and p-value = 0.205 with CI[-80.3, 23.6] for total chlorophyll *a*. However, they had high and medium Cohen's d effect sizes, 2.06 and 0.713 respectively (total chlorophyll pigments had an effect size of 4.17, though it was also non-significant, with p-value = 0.254 and CI[-103, 39.8]), and the equivalence tests (see Appendix E.4.7 for TOST results) came out non-significant, indicating that we cannot dismiss these effect

sizes as mere inflation from high standard error. The standard error is high, however, as seen in Figure 28 (total dry weight and total chlorophyll *a* means for each group) below, which may be preventing any true effect's signal from showing on a statistical level.

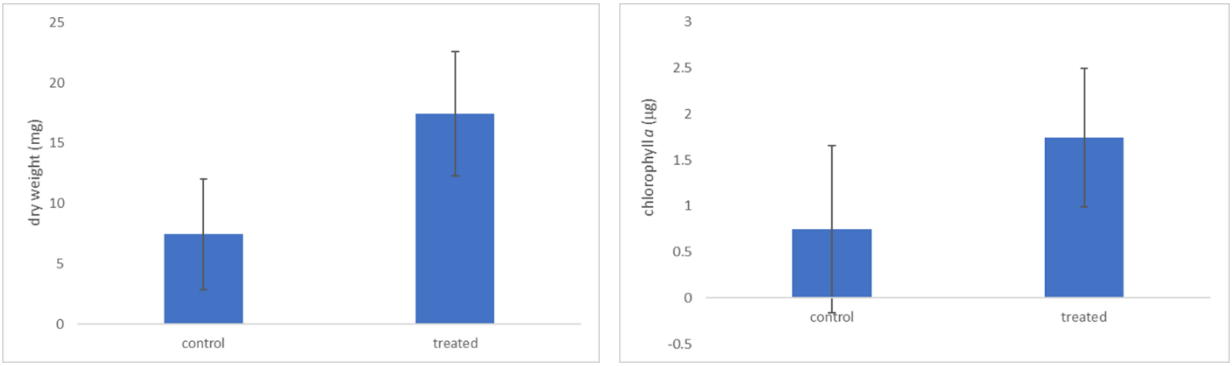


Figure 28: Mean total dry weight (left) and mean total chlorophyll a (right) with standard deviation bars for Trial 9: The effect of mixed bacteria biofilm on undiluted, unfiltered aquaculture wastewater with reduced slope.

It can also be seen though that in both cases the means of the treated groups are higher than the means of the control groups, matching the direction the signs of the effect sizes indicate. The width of the confidence intervals also hints at the possibility of a large effect.

The dry weight SA index came out non-significant (p-value = 0.210 and CI[-2.13, 7.09]). It's Cohen's d effect size, -1.22, is large and indicates an effect in the direction of the control group and matching the skew and broad range of the confidence interval. Equivalence testing on the dry weight SA index also came back non-significant, indicating non-equivalence. Overall, Trial 9 was inconclusive for both speed and strength of attachment, but based on the equivalence test results, large confidence intervals, and effect sizes, it can be inferred that the study is underpowered to discern the signal of any effect through the large error present. Other charts and figures from this trial can be viewed in Appendix D.7.

3.3.4. Trial 10: The effect of mixed bacteria biofilm in medium diluted, unfiltered aquaculture wastewater with reduced slope

Trial 10 tested the mixed bacteria biofilm's impact on attachment when unfiltered aquaculture wastewater diluted to half-strength with sodium thiosulfate-treated tap water was used as the media and the slope was set at 2%. Systems 5 and 8 leaked out and were not included in the trial, reducing the sample size to 7. System 8 was also excluded from the SA index analyses. Both the total dry weight and total chlorophyll *a* results were non-significant (the total chlorophyll pigments results were also non-significant, p-value >> 0.05), with p-values of 0.784 with CI[-0.0321, 0.0256] and 0.513 with CI[-147, 83.6] respectively, and Cohen's d effect sizes of 0.221 (small) and 0.537 (medium). The difference in effect sizes for the measures seems to indicate heavy influence by standard error, especially since the confidence interval for total chlorophyll *a* is wider than that for total dry weight. However, the equivalence tests for both measures were non-significant, indicating that meaningful effects cannot be rejected (see Appendix E.5.8 for TOST results). Figure 29 below shows the means of the two measures for the two groups, including very large standard deviation bars.

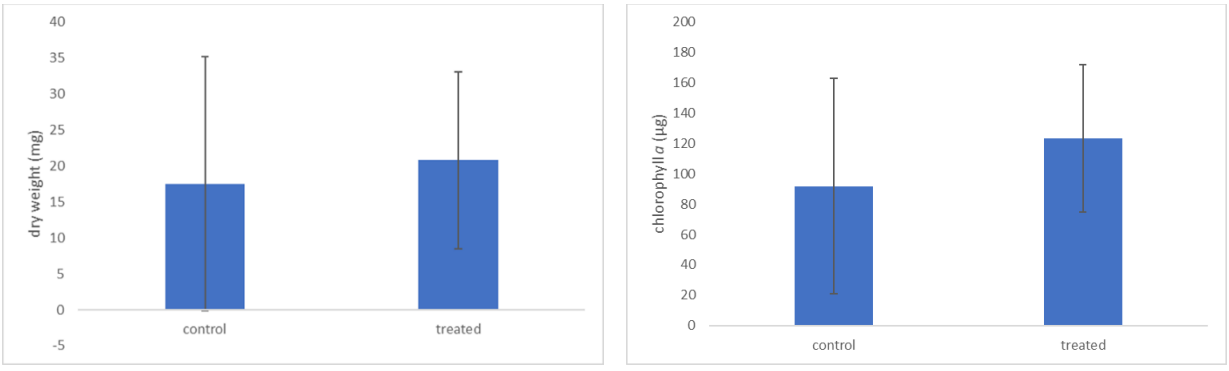


Figure 29: Mean total dry weight (left) and mean total chlorophyll a (right) with standard deviation bars for Trial 10: The effect of mixed bacteria biofilm in medium diluted, unfiltered aquaculture wastewater with reduced slope.

However, the means of the control group is less than that of the treated group in both figures. It can thus be said that for speed of attachment, Trial 10 was inconclusive due to not enough replication.

The SA indices for all measures also came out non-significant for standard null hypothesis testing (p-value >> 0.05) but with non-significant equivalence tests and with wide confidence intervals ([-108, 70.0] for dry weight SA index and [-44.4, 78.0] for chlorophyll *a* SA index]). The width of the confidence intervals indicates both large error and potential for large effects. The Cohen's d effect sizes contradict each other: 0.479 for dry weight SA index and -1.07 for chlorophyll *a* SA index. The former suggests a small effect in the direction of the treated systems, while the latter hints at a large effect in the direction of the control systems. The study is inconclusive for strength of attachment as well, and there is no way to discern if an actual effect might be present or in what direction given more power. Additional figures and charts are in Appendix C.8.

3.3.5. Trial 11: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture wastewater with heavily reduced slope

Trial 11 tested the effect of the mixed bacteria biofilm on attachment in a quarter dilution of the fish wastewater when the slope was reduced to 1%. Both the total dry weight and total chlorophyll *a* results showed no statistical significance; the total chlorophyll pigments analysis also resulted in no significance, confirming the total chlorophyll *a* result. The p-values were 0.724 with CI[-0.00304, 0.00224], 0.628 with CI [-20.1, 14.1], and 0.620 with CI[-725, 506] for total dry weight, total chlorophyll *a*, and total chlorophyll pigments respectively. However, the confidence intervals are large, indicating both large error and the potential for large effects, which is given further credence by the fact that the equivalence tests for the measures returned a

result of no significance (TOST results in Appendix E.5.9), indicating that the possibility of large effects cannot be rejected, though the Cohen's d effect sizes are small (0.262, 0.378, and 0.388 for total dry weight, total chlorophyll *a*, and total chlorophyll pigments respectively). It can also be seen from Figure 30, which shows the mean total dry weight and total chlorophyll *a* values (total chlorophyll pigment charts can be viewed in Appendix C.9 along with other charts and figures for this trial) that the mean of the treated group is higher than the mean of the control group for both measures. The standard deviation bars are large, which may be contributing to masking any true effect that may be present.

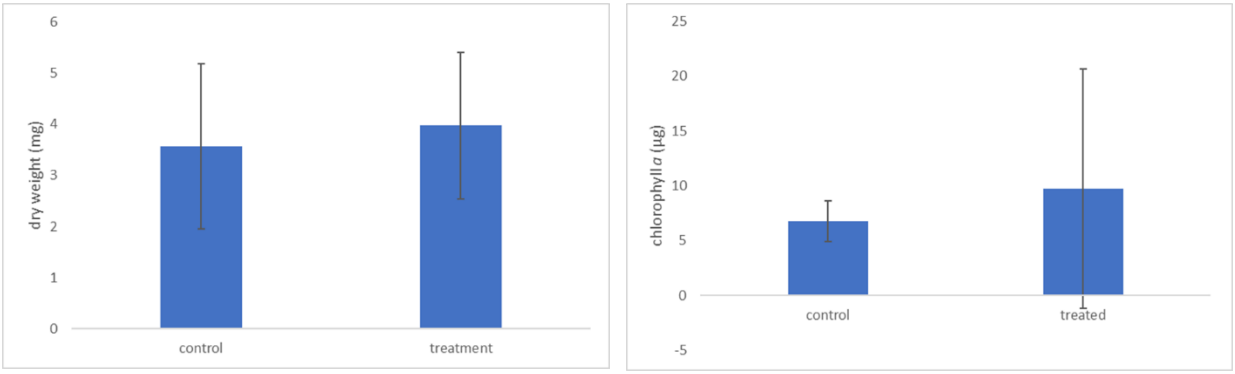


Figure 30: Mean total dry weight (left) and mean total chlorophyll a (right) with standard deviation bars for Trial 11: The effect of mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture wastewater with heavily reduced slope.

The SA indices results were also non-significant (p-value = 0.159 with CI[-3.72, 0.950], 0.0522 with CI[-3.28, 0.028], and 0.0712 with CI[-3.35, 0.248] for dry weight SA index, chlorophyll *a* SA index, and total chlorophyll pigments SA index respectively. The effect sizes were large (1.30, 2.18, and 1.93 respectively) which, along with non-significant equivalence tests, indicates that the study is underpowered to detect any effect that may be present. However, seeing the mean values of the dry weight and chlorophyll *a* indices in Figure 31 below, it is apparent that the mean of the treated group is higher than the mean of the control group for both measures, though there is high error.

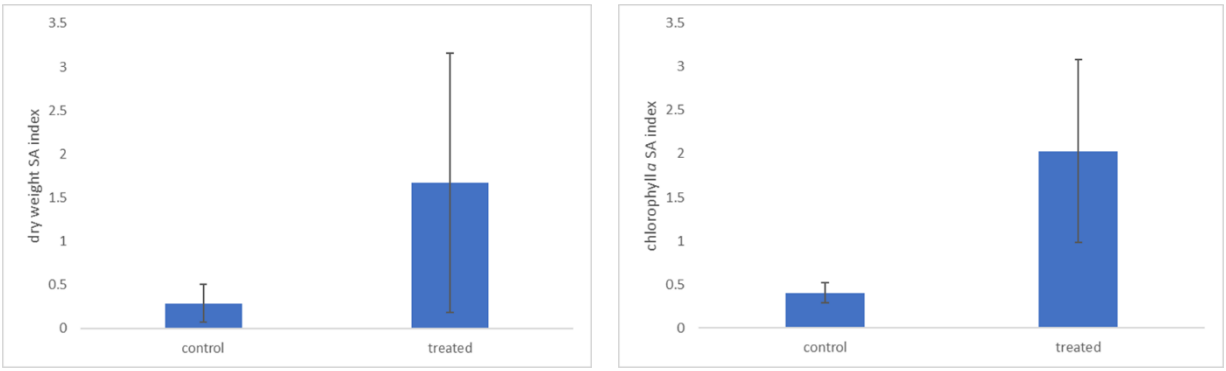


Figure 31: Mean dry weight (left) and chlorophyll a (right) SA indices with standard deviation bars for Trial 11: The effect of mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture wastewater with heavily reduced slope.

Trial 11 was inconclusive for both speed and strength of attachment. However, there is evidence that indicates that there may be an effect present obscured by high standard error that the trial was too underpowered to detect on a statistically significant level.

3.3.6. Trial 12: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture wastewater

Trial 12 tested the mixed bacteria biofilm's impact on attachment when a quarter-strength dilution of unfiltered aquaculture wastewater and a 3 % slope were used. There is no dry weight SA index analysis for this trial. In addition, certain negative total dry weight values were converted to 0 for this trial under two assumptions: one, given the lack of visible biomass on the filters, it is believable that the value is 0 and that the negative value comes about as a result of a balance error, and two, that these negatives were in the fourth decimal place, which represents the highest precision the balance used possesses, and were close to 0.

The total dry weight values came out significant, with p-value = 0.0174 and CI[-0.00278, -0.000372], and the Cohen's d effect size was 2.08, indicating that the effect was practically as well as statistically significant. The equivalence test for total dry weight (see Appendix E.5.10

for Trial 12 TOST results) also came out non-significant, indicating that the effect size is large simply because of the standard error.

Contrary to this, the total chlorophyll *a* measurements (and the total chlorophyll pigments measurements, p-value = 0.147 with CI[-0.970, 0.184]) came out non-significant, with a p-value of 0.143 and CI[-0.0274, 0.00504]. However, the equivalence test for this measure came out non-significant, and the effect size, 1.19, was large, indicating that the study may simply be underpowered to detect the effect. (The total chlorophyll pigments data was similarly non-significant, but with a large effect size and non-significant equivalence test.) Figure 32 shows the mean total chlorophyll *a* values for each group; it can be seen that the mean of the treated group is higher than the mean of the control group and that there is high standard deviation, which supports this theory.

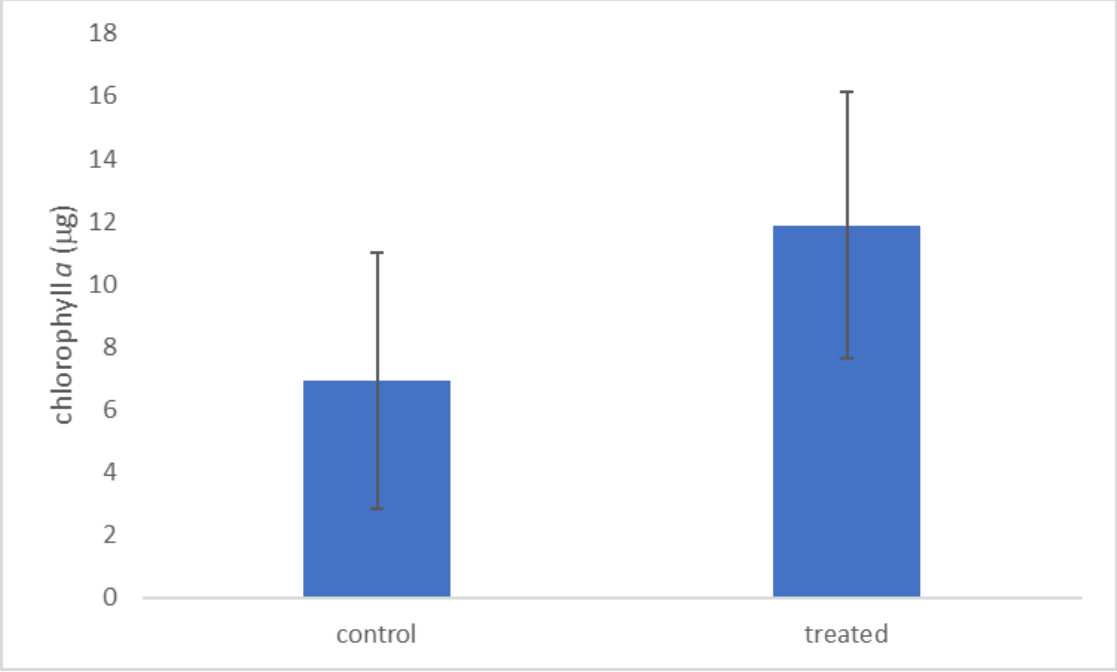


Figure 32: Mean total chlorophyll a for Trial 12: The effect of mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture wastewater.

Since dry weight measurements cannot be taken as proof of an effect on their own given the bacterial fraction is unknown, Trial 12 is inconclusive for speed of attachment, but there is reason to believe that further testing with more replicates is warranted.

The SA indices also came out non-significant (p-values = 0.526 with CI[-52.6, 30.5] for chlorophyll *a* and 0.867 with CI[-65.5, 57.0] for total chlorophyll pigments), but with effect sizes that are medium (d = 0.520 for chlorophyll *a*) and on the high end of small (d = 0.437 for total chlorophyll pigments) and non-significant equivalence tests. It can be seen in Figure 33, which shows the mean values of the SA indices, that the mean of the treated groups is higher than the mean of the control groups, but there is a very large error.

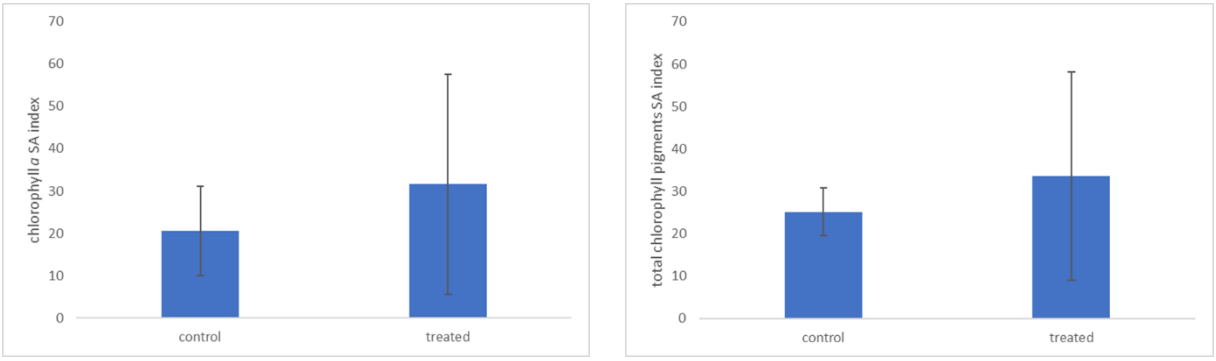


Figure 33: Mean chlorophyll a (left) and total chlorophyll pigments SA indices for Trial 12: The effect of mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture wastewater.

Trial 12 can be concluded to be inconclusive for strength of attachment as well due to being underpowered. Other figures and tables associated with this trial can be viewed in Appendix C.10.

Environmental measurements, including full nutrient measurements, for all trials can be viewed in Appendix D. The schedule on which the main experiments took place can be viewed in Appendix E.2.

Section 3.4. Nutrient analyses

Due to the small scale of the systems and the short trial durations, large shifts in nutrient concentration over the course of the trials were not expected. Significant differences in nutrient removal between the control and treated systems were also not anticipated. The results were as expected for phosphate and ammonia with a few outliers. However, nitrate increased from the beginning to the end of the trial for all synthetic media trials. Nitrite also increased for all aquaculture waste trials. The increase may be due to the presence of nitrifying and denitrifying bacteria and organic matter from decomposing portions of the algal inoculum.

Section 3.5. Bacteria community analysis

The most common phyla, classes, and genera identified in the representative microbial samples sent off are summarized in Figures 53-55 below, with the y-axis in each figure representing the percentage of the total community each phylum (Figure 52), class (Figure 53), and genus (Figure 54) represents.

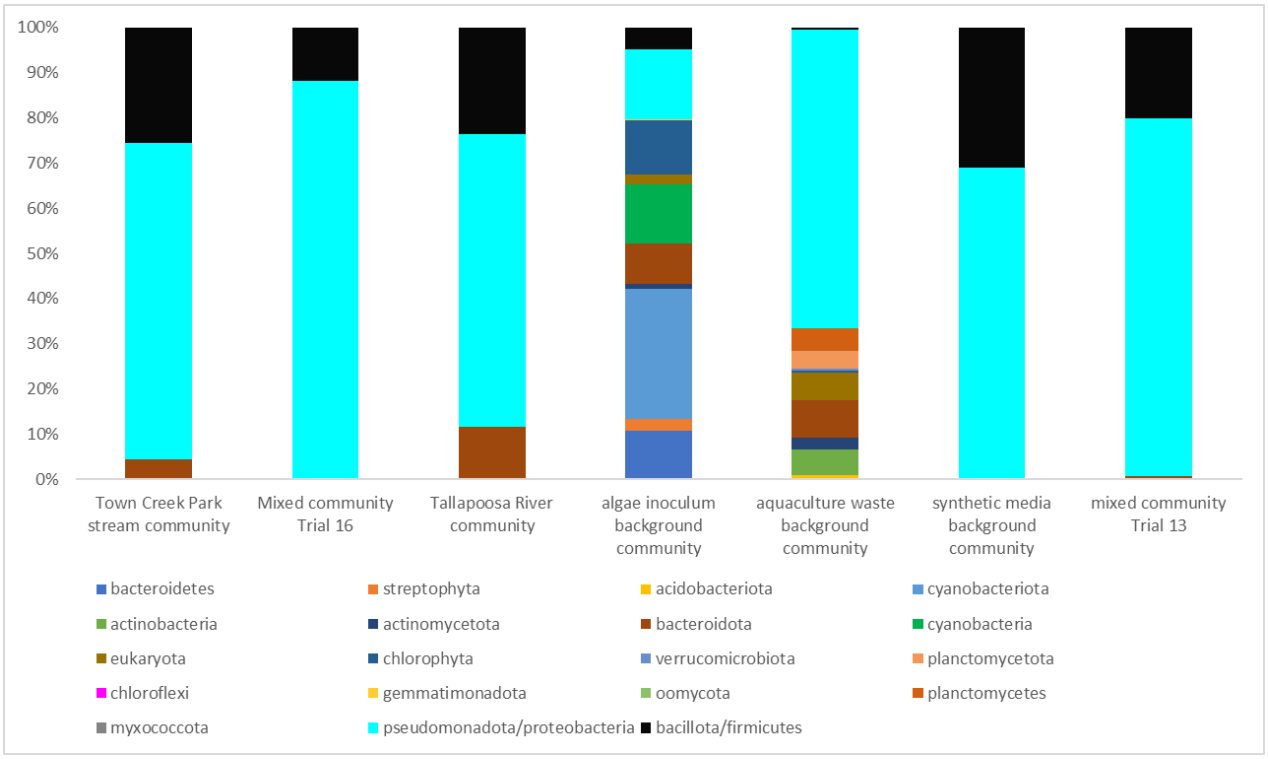


Figure 34: Phyla identified as belonging to each microbial community tested.

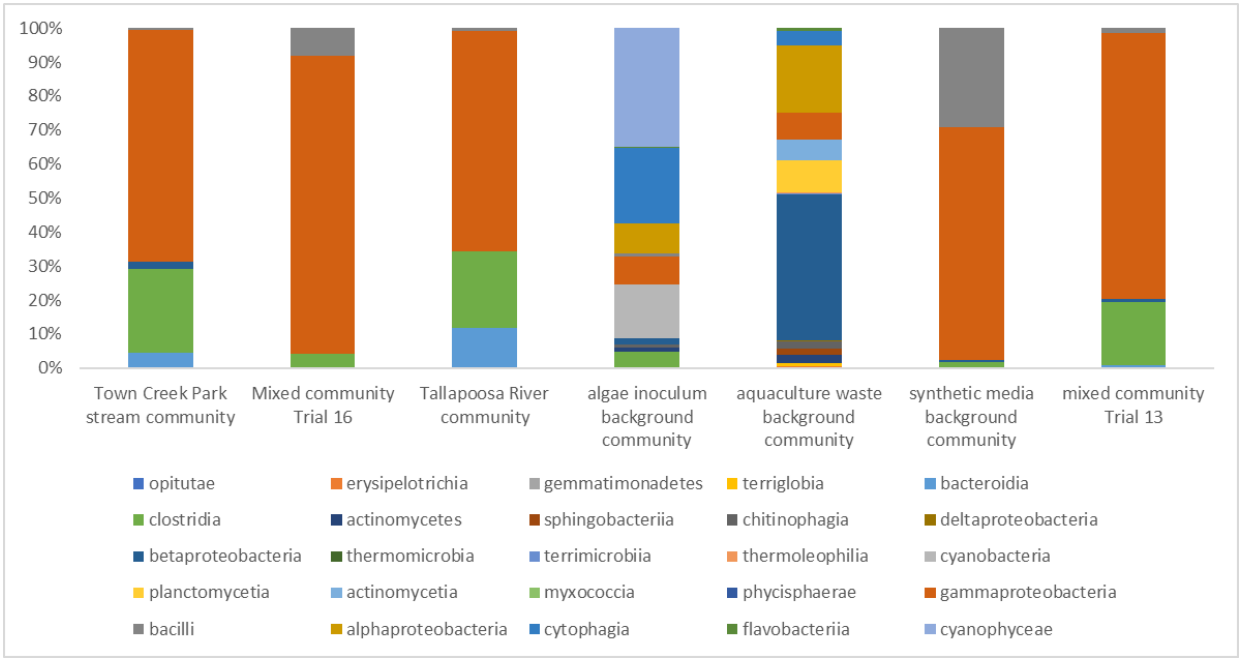


Figure 35: Classes identified as belonging to each microbial community tested.

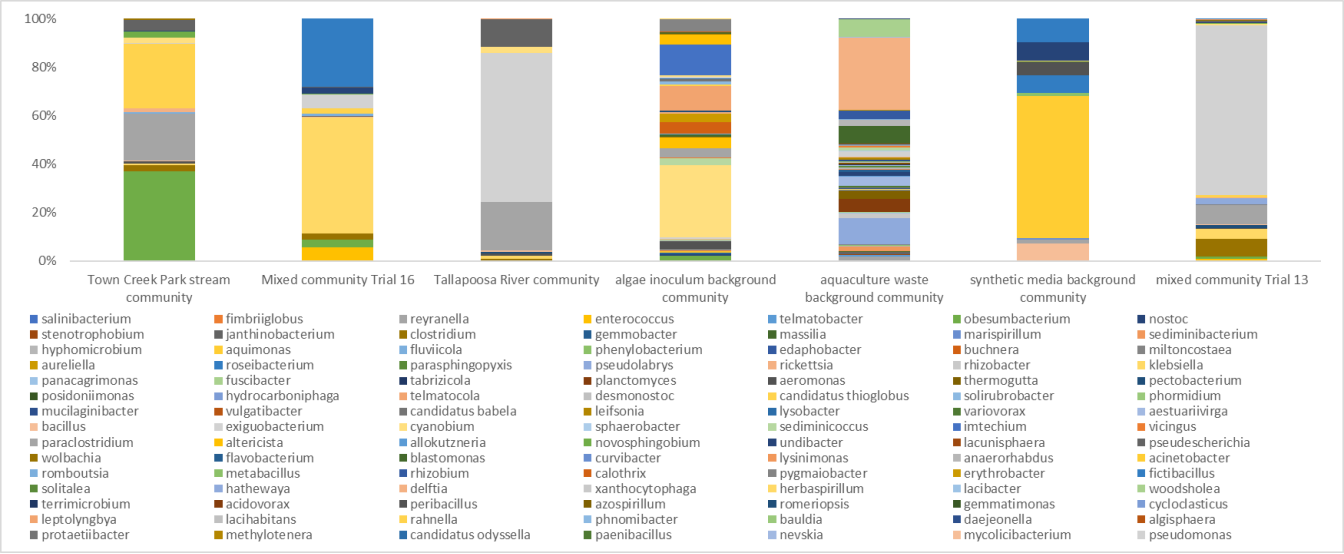


Figure 36: Genera identified as belonging to each microbial community tested.

Pseudomonadata, also known as Proteobacteria, comprised large component of the microbial communities analyzed. In fact, members of it made up the largest portion of all the communities, except for the algae inoculum background community (most dominant was Cyanobacteria), in which it was the second most dominant phylum. Members of the phylum proteobacteria have been found to be capable of facilitating microbial attachment prior to biofilm formation (Mhedbi-Hajri et al., 2011; Qian et al., 2023). Battin et al. (2016) also identified it as one of the two dominant phyla commonly found in stream biofilms. The phylum Bacillota/Firmicutes was the second most dominant among all the communities except the background aquaculture wastewater and algae inoculum communities, which had Bacteroidota and Proteobacteria/Pseudomonodata as the second most dominant phyla respectively. The portions comprising Bacillota and Firmicutes can thus be considered one portion.

Battin et al. (2016) also identified Firmicutes as a phylum common to stream biofilms, while Qian et al. (2023) found both Firmicutes and Proteobacteria to be two of the three most dominant phyla present in the raw soy sauce wastewater bacterial community used in his

communities prior to algal biofilm formation, though Proteobacteria completely dominated the biofilm by the end of their experiments. Their research found that the bacteria promoted algae attachment. Members of the phylum Bacteroidota were not present in as large amounts in the mixed bacteria communities as they were in the other communities.

Common classes identified were gamma- and betaproteobacteria, which are also common proponents of stream microbial assemblages. *Pseudomonas spp*., which are known biofilm producers, were also noted to be present in the Tallapoosa River and mixed bacteria communities. *Bacilli* is also among the top three common genera identified for the mixed bacteria community, and several species of the genus are known to be able to produce biofilms (Vlamakis et al., 2013). The members of the microbial communities used, and even the background bacteria, seem to lend themselves to the formation of biofilms such as the ones common in stream environments. The communities' presence should have resulted in a positive effect on algae attachment. The three most common families, classes, and genera for each community are summarized in Table 3, with 1 indicating the most common.

Table 3: Three most dominant families, classes, and genera for main and background microbial communities.

	Tallapoosa River Bacteria	Town Creek Park stream bacteria	Mixed community bacteria	Algae inoculum background bacteria	Fish waste background bacteria	Synthetic media background bacteria
Class 1	Gammaproteobacteria	Gammaproteobacteria	Gammaproteobacteria	Cyanophyceae	Betaproteobacteria	Gammaproteobacteria

Family 1	Pseudomonadaceae	Hafniaceae	Pseudomonadaceae and Enterobacteriaceae	Prochlorococcaceae	Methylophilaceae	Moraxellaceae
Genus 1	Pseudomonas	Obesumbacterium	Klebsiella and Pseudomonas	Cyanobium	Methylophilus	Stenotrophomonas
Class 2	Clostridia	Clostridia	Bacilli and Clostridia	Cytophagia	Alphaproteobacteria	Bacilli
Family 2	Peptostreptococcaceae	Yersiniaceae	Clostridiaceae and Xanthomonadaceae	Amoebophilaceae	Xanthobacteraceae	Bacillaceae
Genus 2	Paraclostridium	Rahnella	Stenotrophomonas and Paraclostridium	Candidatus amoebophilus	Pseudolabrys	Lysinibacillus

Class 3	Bacteroidia	Bacteroidia	Clostridia and Bacilli	Alphaproteobacteria	Planctomycetia	Clostridia
Family 3	Dysgonomonadaceae	Peptostreptococcaceae	Peptostreptococcaceae and Pseudomonadaceae	Scenedesmaceae	Comamonadaceae	Xanthomonadaceae
Genus 3	Dysgonomonas	Paraclostridium	Pseudomonas and Clostridium	Leptolyngbya	Mycobacterium	Bacillus

Stenotrophomonas was the most common genus found in the background synthetic media community. The species in this genus are known to be very versatile, playing important roles in the nitrogen and sulfur cycles and shielding plants and encouraging their development (Ryan et al., 2009). Their inclusion in a biofilm could offer similar protection to algae cells as well as transforming nutrients into uptakeable forms. *Methylophilus* was the most common genus in the background aquaculture waste community. Members of this genus can process methanol and use it as an energy source (Jenkins et al., 1987). Though it is assumed that the living bacteria community in the fish waste is dead or minimal, its presence is still interesting.

There are two entries for some of the classes and genera for the mixed bacteria community because the two samples sent off did not agree. Even though the genera for Trials 16 and 13 differed, indicating that there were changes in the mixed bacteria communities in trials,

the dominant phyla and classes are similar, indicating the community overturn was not significant. Note that though bacteria were identified as being present in the fish waste, these results do not indicate that living cells were present, as identification procedures can be performed on dead cells.

Pie charts representing the classes present in each of the tested samples can be viewed in Appendix E.5.

Chapter 4: Discussion and Conclusions

The literature indicates that the presence of beneficial bacteria biofilms should result in a positive effect on algae attachment (Gawne et al., 1998; Hodoki, 2005). Only two trials out of twelve, agreed with it on a statistical level. These trials were the weakest dilutions (1/4) of synthetic media and aquaculture waste. The effect on attachment may have been stronger in these trials, and thus more likely to show through the noise, because the algae and bacteria, both collected from natural environments that typically do not have high nutrient concentrations, are more acclimated to lower nutrient concentrations. The higher concentrations may have led to a shock-like effect. Graphical trends, equivalence test results, and Cohen's d effect sizes seem to indicate though that in the other ten trials, an effect is present, but muffled by the noise, though nothing can be concluded from these non-significant result trials. However, statistics also indicate that an effect on the speed of attachment may be present in the ten inconclusive trials (see Table 4 below for summary of trial results) but muffled by noise causing large standard deviation.

Table 4: Summary of trial results.

BACTERIA SOURCE	MEDIA	DILUTION	SLOPE (%)	TESTED PARAMETER	STATISTICALLY SIGNIFICANT	RESULT
TALLAPOOSA RIVER	PROLINE F/2	¼ X	3	LOW NUTRIENT SYNTHETIC	YES (COMBINED, NOT INDIVIDUAL)	POSITIVE EFFECT ON SPEED OF ATTACHMENT

TALLAPOOSA RIVER	PROLINE F/2	2 X	3	HIGH NUTRIENT SYNTHETIC	NO	INCONCLUSIVE
TOWN CREEK PARK	PROLINE F/2	¼ X	3	LOW NUTRIENT SYNTHETIC	NO	INCONCLUSIVE
TOWN CREEK PARK	PROLINE F/2	2 X	3	HIGH NUTRIENT SYNTHETIC	NO	INCONCLUSIVE
TOWN CREEK PARK	FISH WASTE (FILTERED)	½ X	3	MEDIUM DILUTION FISH WASTE	NO	INCONCLUSIVE
MIXED	FISH WASTE	¼ X	2	HIGH DILUTION FISH WASTE	YES	POSITIVE EFFECT ON SPEED OF ATTACHMENT
MIXED	FISH WASTE	NONE	2	UNDILUTED FISH WASTE	NO	INCONCLUSIVE
MIXED	FISH WASTE	½X	2	MEDIUM DILUTION FISH WASTE	NO	INCONCLUSIVE

MIXED	FISH WASTE	1/4X	1	REDUCED CHANNEL SLOPE	NO	INCONCLUSIVE
MIXED	FISH WASTE	1/4X	3	INCREASED CHANNEL SLOPE	NO	INCONCLUSIVE

A statistically significant effect on speed of attachment appeared in the trial testing 2% slope in combination with the mixed bacteria biofilm and a quarter dilution of aquaculture waste, but not in the trials also testing the mixed bacteria biofilm and a quarter dilution of aquaculture waste, but with 1% and 3% slope. However, the graphical indicators of the treated group having a higher mean than the control group but high standard deviation were still present, and the equivalence tests based on bounds of Cohen's d effect sizes of -0.500 to 0.500 were still non-significant, indicating an inability to reject the chance of a statistical effect given more power. If an effect was truly not present in those cases, it may be due to the change in flow regime. Decreasing the slope increases the wetted perimeter and reduces the hydraulic retention time, and channel velocity; while this may lessen the likelihood of shear stress removing cells before they are permanently attached, it also lowers the amount of cells and nutrients passing through the channel in a given time. Increasing the slope has the opposite effects on wetted perimeter, hydraulic retention time, and channel velocity. It also increases the shear force applied to the attached cells but raises the speed at which nutrients are brought to them. Liu et al. (2016), when studying *Stigeoclonium spp.* and *Klebsormidium spp.,* found that higher flow rates resulted in

improved biomass growth and nutrient removal, but they also reduced FGA dominance in outdoor ATS systems, likely due to the shear stress breaking the algal filaments.

In the case that the effect was not present in the other trials where results were not statistically significant, possible reasons include competition between the microbes for nutrients, production of algicidal products, or other unknown species-specific interactions. The bacteria communities tested, though similar in terms of some phyla and classes, still had differences, and strains from the same genera have been known to have different effects on different algae strains (Belenova et al., 2017).

Section 4.1. Conclusions

Five findings can be reported. There is evidence to believe that the presence of a Tallapoosa River biofilm, dominated by genera *Pseudomonas*, *Paraclostridium*, and *Dysgonomonas*, can lead to faster *Rhizoclonium spp*. attachment when a quarter dilution of F/2 and an ATS channel slope of 3% are used. There is also evidence to believe that the mixed bacteria biofilm, dominated by the genera *Pseudomonas, Clostridium*, *Stenotrophomonas*, and *Paraclostridium*, has a positive effect on attachment speed when a quarter dilution of fish waste and slope of 2% are the parameters. This study was generally underpowered and requires further testing. In future, when researching this topic, it may be of benefit to perform a power analysis prior to starting. However, this may be difficult, as it requires estimating the expected population effect size, which can most easily be done by looking at similar studies, of which there are only a few at the scale used in this research. Lowering the nutrient concentration had a positive effect on speed of attachment, while changing slope by 1% increments had no effect.

Section 4.2. Limitations of the research

One major limitation of the research was the noise associated with the procedures and systems used. Standard deviation was high for every trial, particularly for the chlorophyll *a* data. This noise may originate in the methods. More precise methods, such as sonication for harvest of strongly attached biomass to ensure all the EPS has been removed and high performance liquid chromatography (HPLC) for chlorophyll *a* analysis (Santos, 2003; Wright et al., 1991) may remove some of the noise. More replication and trial iterations could also have helped reduce the impact of outside interference had time not been a constraint since the small, uneven samples sizes themselves could have introduced some error. Based on the variability trials, it can also be said that there is inherently moderate variability among the set of systems in the set-up used, along with the inherent variability that comes with working with biological entities (though the variability trials also need to be repeated for a more accurate variability measure). There are many possible sources of error that could have been identified and removed given more time for testing of the systems.

Another potential source of noise may have been contamination. While the lids on the systems themselves and the lids of the reservoirs were intended to reduce splashing-related contamination between systems, elimination of air travel of bacteria between the channels as spores could not be confirmed. In hindsight, separation from the treated systems and/or complete isolation of the control systems may reduce the possibility of contamination. Another option would be to control bacterial invasion of the control systems through an antibiotic treatment regime. As to the potential effects of this possible contamination on the experiments described in this work, while it is not impossible for a bacteria biofilm to have developed over the course of

the trial, the effect of the treatment should still have been evident in some fashion. Any bacteria biofilm developed in the control systems would have been lesser in density.

The literature (Hodoki, 2005) suggests that a thicker biofilm and greater bacteria density are correlated with faster attachment. If the bacteria biofilm interacts with the algae in an attachment-stimulating manner, then the increased density of the biofilm in the treated systems should have resulted in greater attachment than in contaminated control systems. However, contamination could have reduced the mean difference between the control and treated systems, causing the effect to not appear on a statistical level.

It was also not verified that the entire roughened surface of the substrata were evenly inoculated by the biofilm, which could lessen the area of interaction between the bacteria and algae. The estimation of the biofilm mass at the beginning of the trials was a rough estimate that comes with three important notes. Firstly, particles (small bioflocs and nutrient broth powder that stayed after the liquid portion dried) from the nutrient broth that were not attached could still cling to the subsample strips, distorting the final measurements. Secondly, when the water first hit the actual substrata in the systems, loosely attached EPS may have been removed by the sudden flow. Finally, the biofilm amount is variable throughout the trial; it grows and sloughs off, so the final biofilm mass would be different than the initial estimated amount. The total fraction of biomass contributed solely by the bacteria could not be verified completely accurately. Though measures were taken to roughen the substrata in a similar manner, it was not confirmed that roughness was equal for each substratum. Measuring the roughness of each one could help verify that there was no significant difference in roughness adding to the noise.

Chapter 5: Potential Future Work

There is potential to improve and expand upon this research. Because bacteria-algae interactions are so species-specific, there is room to study the effects of bacteria communities sourced from other locations. Alternatively, future research could be narrowed to study the impact of specific strains known from prior investigation to produce biofilms. Many environmental variables have significant impact on algae development. This research only explored the effect of two, flow regime and nutrient concentration, in a limited range. Future research could expand on this by testing the impact of different flow and nutrient conditions, as well as other parameters, such as light cycle and intensity, temperature, and pH in conjunction when a bacteria biofilm is present. There is also a need to study how a pre-existing bacteria biofilm interacts with the microbial community inherent to the fish waste, which was not present in the effluent used in these experiments. In the trials performed, no attempt was made to eliminate the introduction of the background laboratory bacteria under the assumption that since outdoor systems are also exposed to outside bacteria that may interact with an introduced biofilm, it was important to include the potential for similar interference here. However, a study excluding it may elucidate more on the role of the bacteria biofilm by itself. The experiments performed here could be repeated for the sake of performing mini meta-analyses on the individual trials. There is also room to perform upscaled experiments. Overall, there is considerable basis for further study of this topic.

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Appendix A: System Design

Appendix A.1: List of components

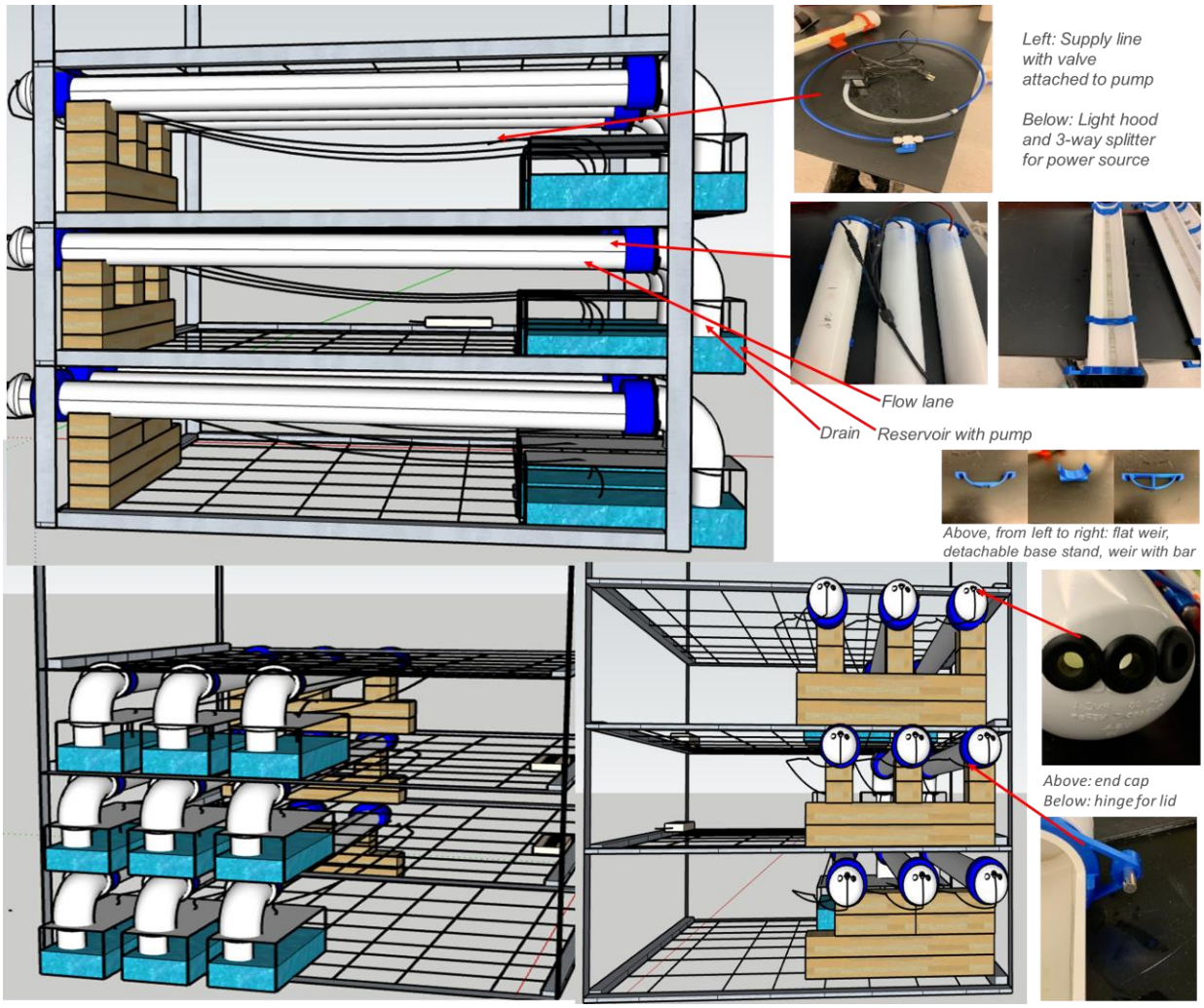
Component	Material	Size	Notes
Reservoir	Plastic (clear with gray lid)	6 quarts (5.7 liters); 14”L x 8” W x 4 7/8” H (35.6 cm x 20.3 cm x 12.4 cm)	Filled only to 4 liters for all experiments; holes cut in lid for drain, pump cord, hosing, and measurements
Hosing	Plastic (transparent hosing zip-tied to plastic hosing	Transparent hosing: 3/8” OD, length: approximately 14” Blue hosing: ¼” OD, length of part entering valve: approximately 47”, length of part exiting valve (entering inlet hole of system): approximately 2 1/16”	Detachable

		Total hosing when zip-tied together (excluding part entering inlet hole of system and valve): approximately 59 ½”	
Valve	Plastic	¼”	
Pump	Plastic	95 gph	Pulaco pump PL-128X2
Base (excluding drain and end cap)	PVC	Approximately 21 3/16” length (including drain and end cap, approximately 27 ¼” length)	
Lid	PVC	Approximately 19 15/16” length	Detachable
Lights	White LED strip lights	Approximately 16 5/8” length	Average light range of approximately 21 (at ends) to 67 photons/m^2/s
Adapter	NA	NA	Input: AC100-240 V, 50/60 Hz, 1.2 A

			Output: 12 V, 2 A Each one is used with a 3-way splitter, powering the lights for 3 systems at once (see pictures on poster)
End cap	PVC	2”	3 inlet holes to alter flow rate
Drain (elbow + length of pipe)	PVC	Elbow: 2” Length of pipe: 2”, approximately 4” length	Elbow and length of pipe detachable from each other and main system
Weirs	PLA	NA	Two kinds; for substrata testing, flat one placed at approximately: 6 ½” down the channel from the opening; Weir with bar placed at approximately: 16 ¼” down the channel from the opening;

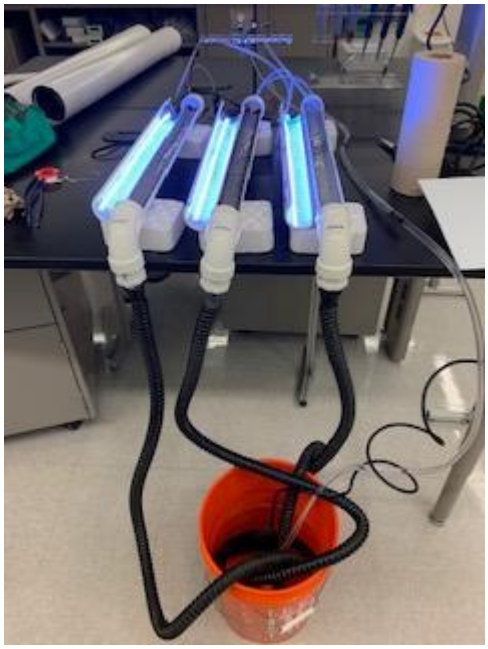
			replaced with binder clips for main experiments
Binder clips	NA	NA	NA
Other parts	PLA	NA	Hinges, attachment pieces, detachable base stand (see poster for picture), hold-in-place pieces

Appendix A.2: System setup

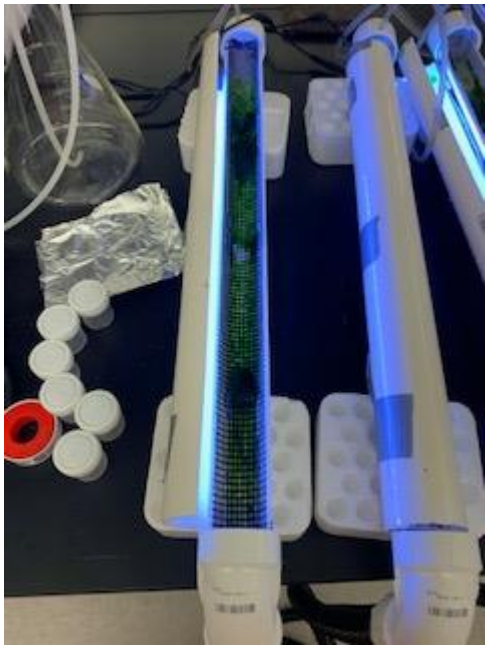


Appendix B: System Testing

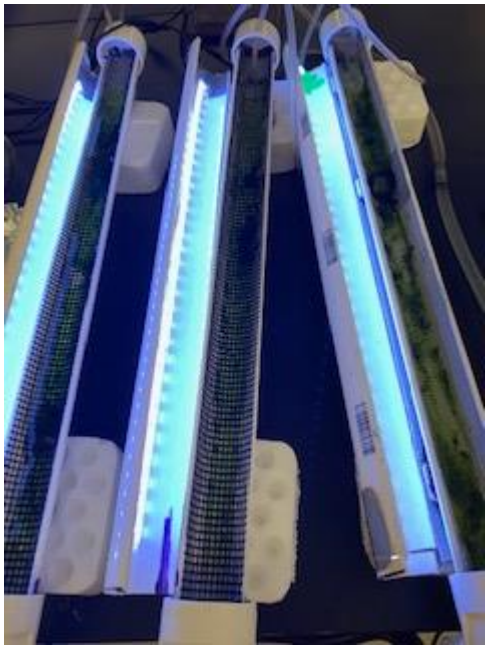
Appendix B.1: Image timeline of preliminary qualitative biomass testing



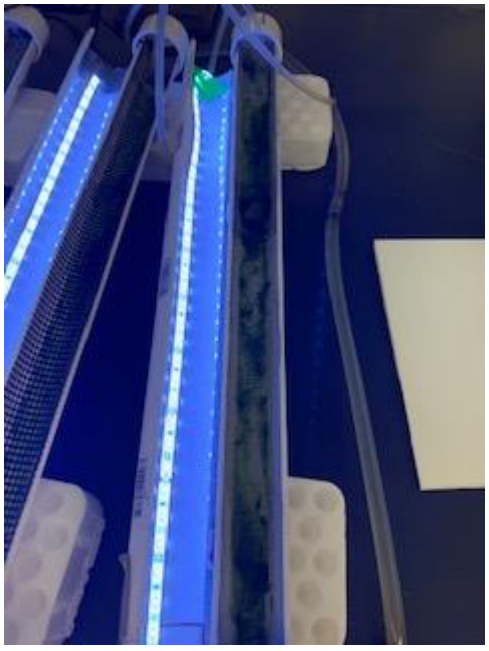
May 6, 2022, systems seeded



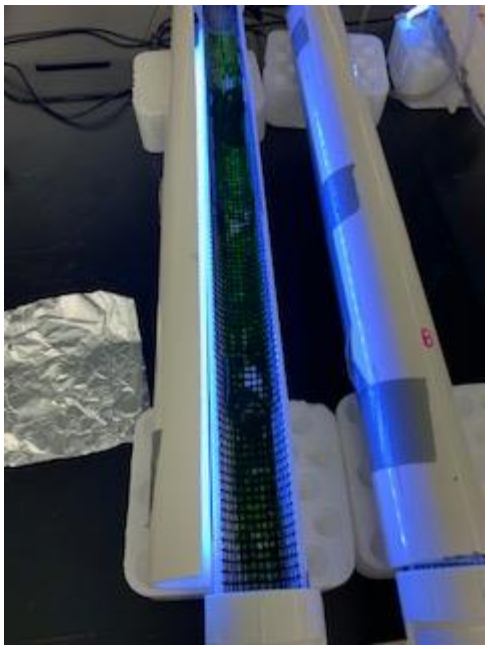
May 12, 2022, Day 6 growth in system A



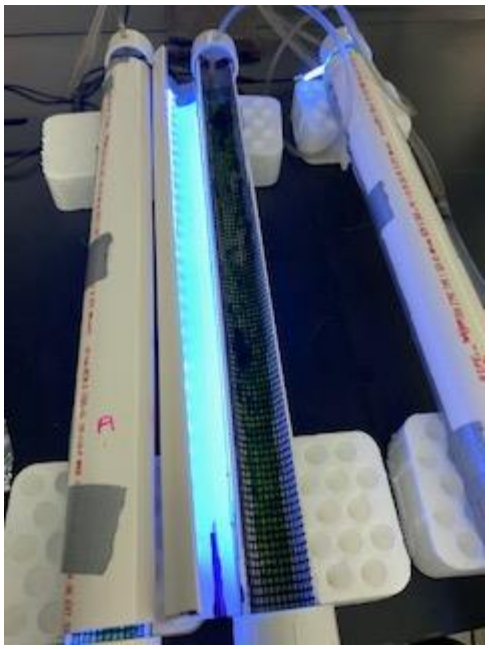
May 12, 2022, Day 6 growth in system B



May 12, 2022, Day 6 growth in System C



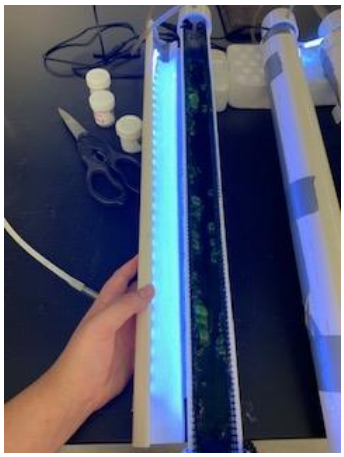
May 13, 2022, Day 7 growth in system A before harvest 1



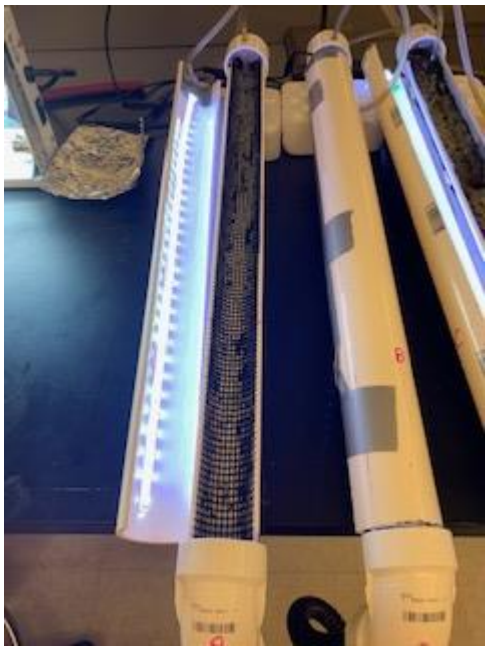
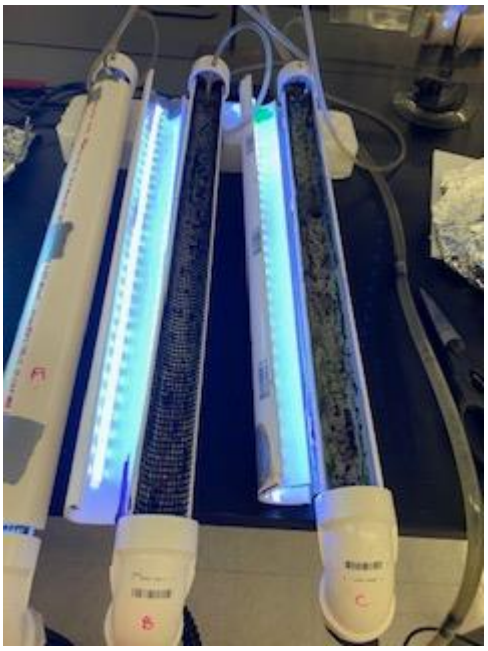
May 13, 2022, Day 7 growth in system B before harvest 1

Appendix B.1.1: Preliminary test 1 growth period 1 (from right to left, systems are A, B, C – 7-day biomass was 0.0831 g and 0.0978 g for A and B respectively, data for C lost) for generation 1 prototypes

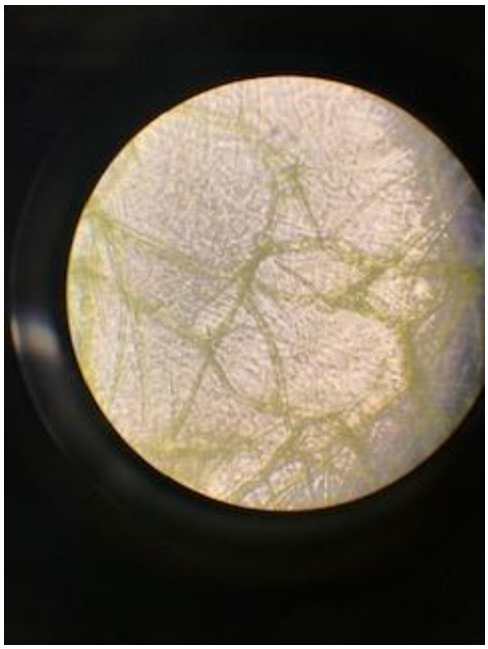
May 16, 2022, Day 4 regrowth after harvest 1



May 20, 2024, Day 7 regrowth after harvest 1, before harvest 2

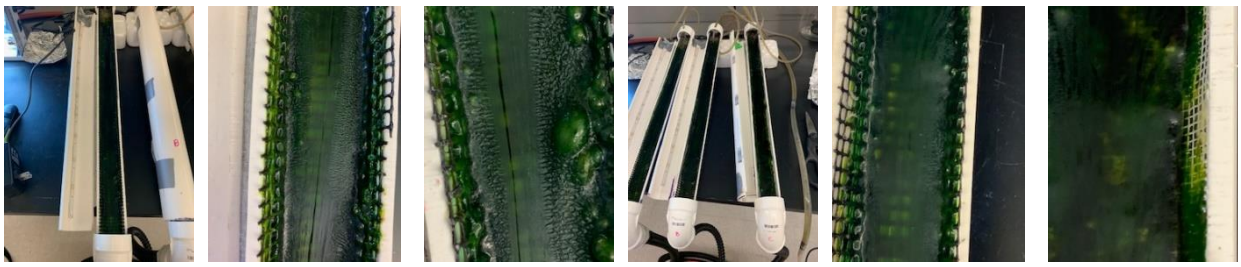



May 20, 2022, after harvest 2



May 16, 2022 algae sample from system A under microscope

Appendix B.1.2: Preliminary test 1 growth period 2 for generation 1 prototypes (total biomass for all 3 systems was

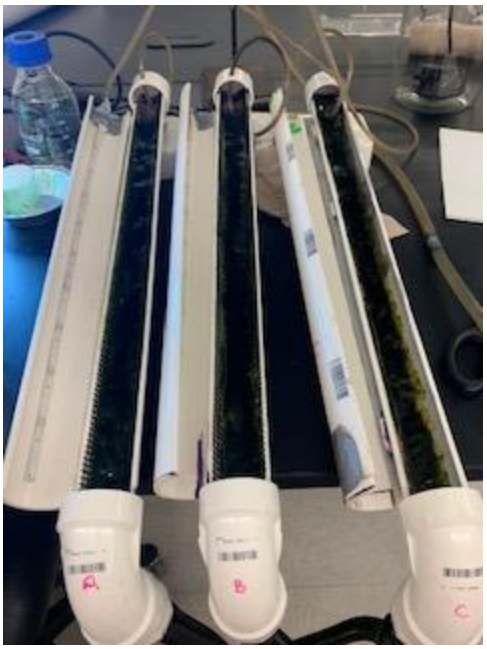


May 24, 2022, Day 4 of regrowth after harvest 2

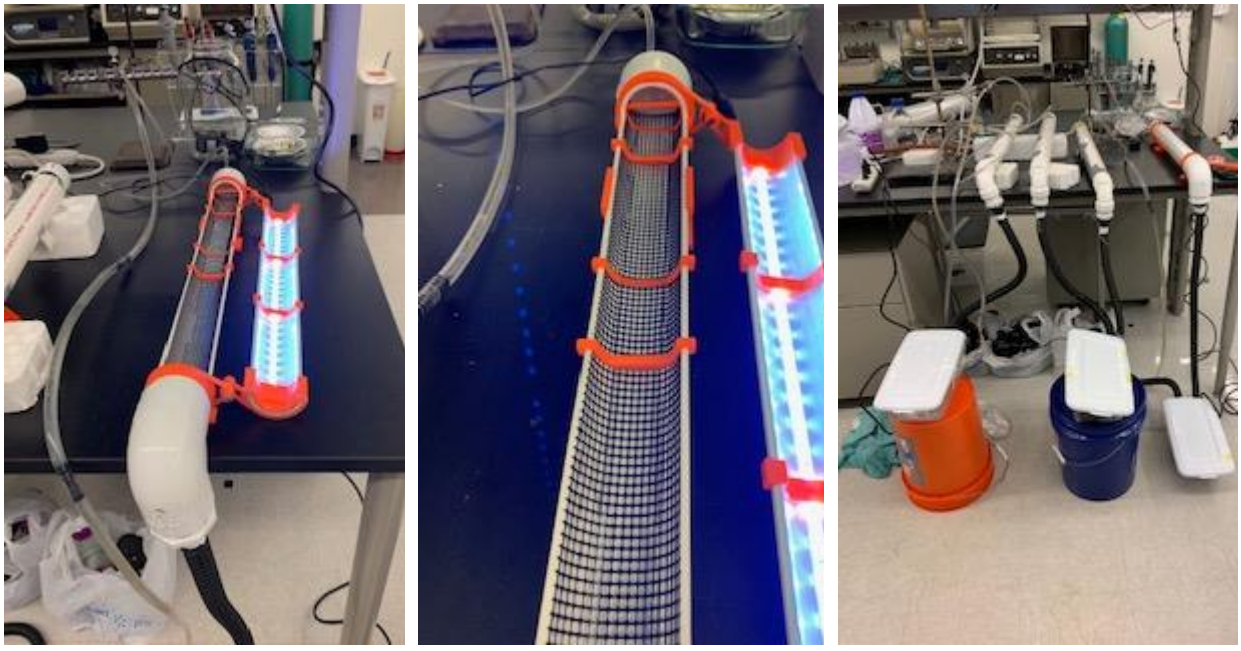
May 27, Day 7 regrowth after harvest 2, before harvest 3

May 24 sample from system B under microscope

Appendix B.1.3: Preliminary test 1 growth period 3 for generation 1 prototypes

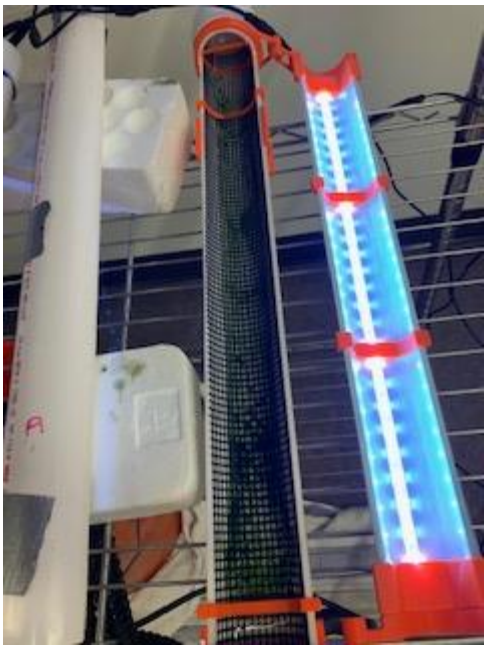


Appendix B.1.4: Preliminary test 1 growth period 4, day 7 (6/03/2022) regrowth, before harvest 4 (harvest 4 7-day total biomass for 3 systems combined = 1.88 g) for generation 1 prototypes

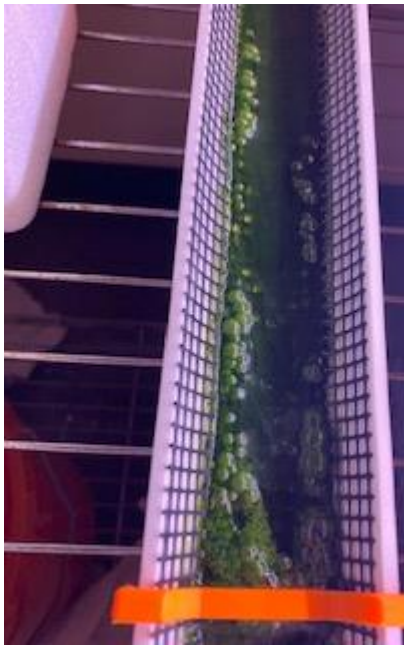


June 30, 2022, start of growth trial with systems A, B, C, and generation 1, prototype 1

July 7, systems moved to rack



July 18

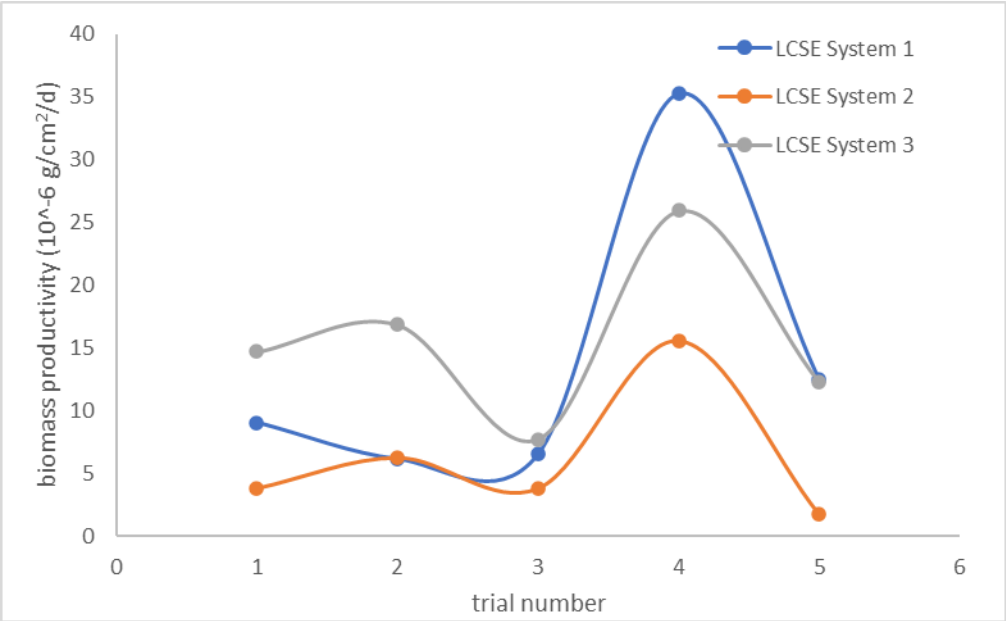


July 27

Appendix B.1.5: Preliminary test 2 for generation 2, system 1

Appendix B.1.6: Preliminary testing biomass for generation 2, systems 1, 2, and 3

Appendix B.2: Results from aborted LCSE trials



Appendix C: Additional Figures and Tables

Appendix C.1 Additional figures and tables for Trials 1-3: The effect of the Tallapoosa River water bacteria biofilm on attachment in low nutrient synthetic media

(Return to: 3.2.1)

Appendix C.1.1: Trial 1 additional figures and tables

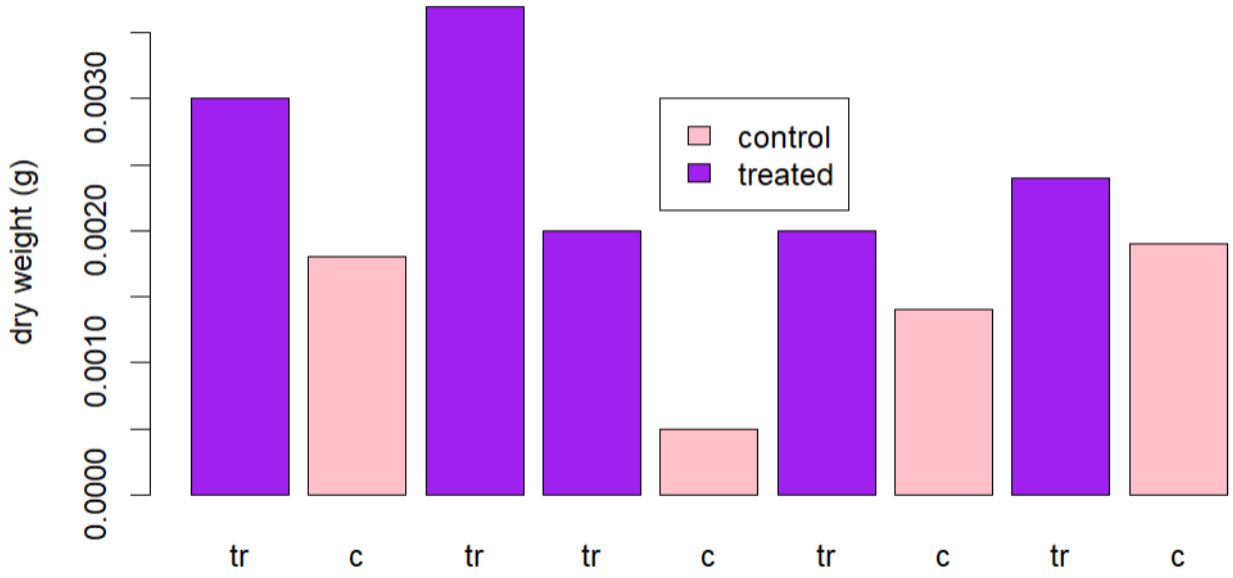


Figure C.1.1.1: Total dry weight for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

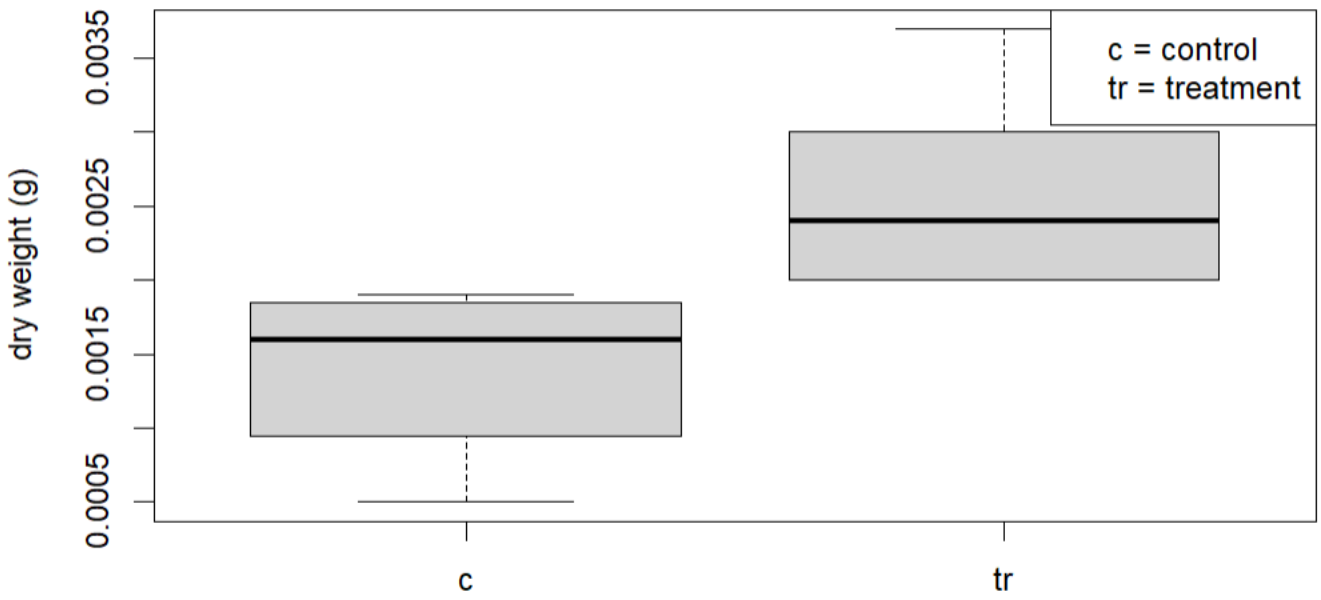


Figure C.1.1.2: Total dry weight boxplots for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

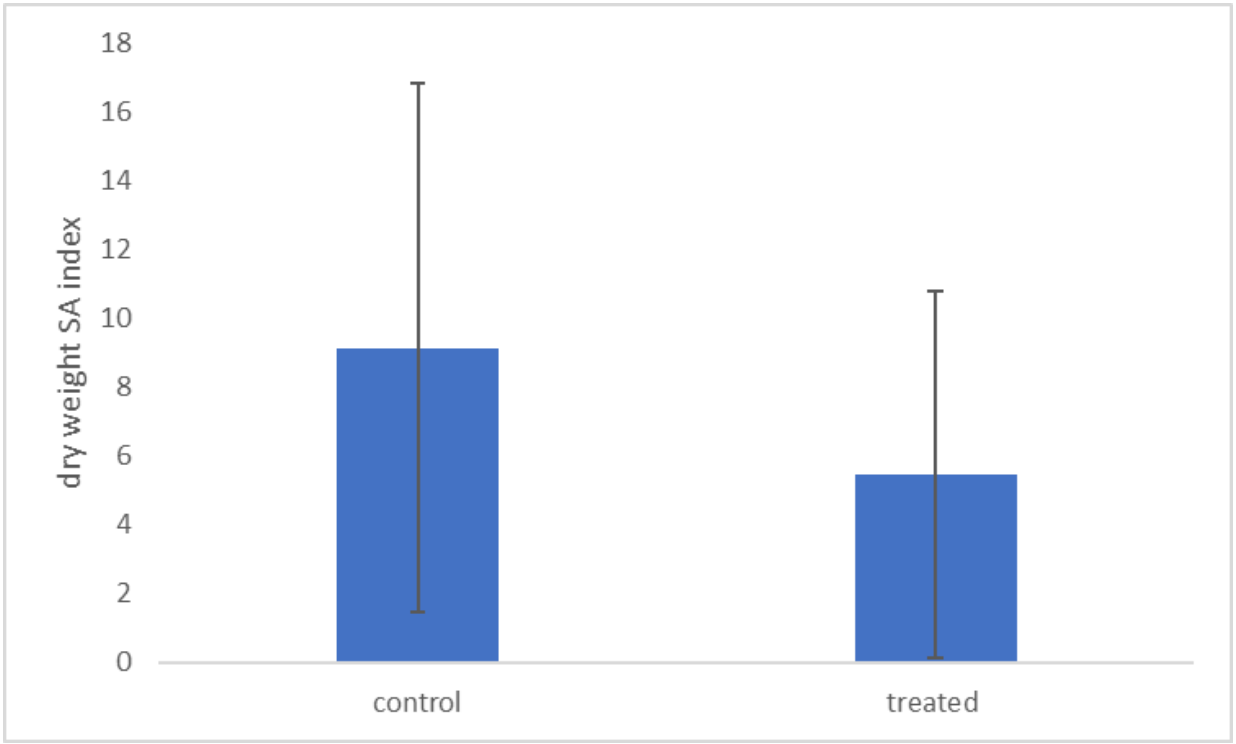


Figure C.1.1.3: Mean dry weight SA index with standard deviation bars for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

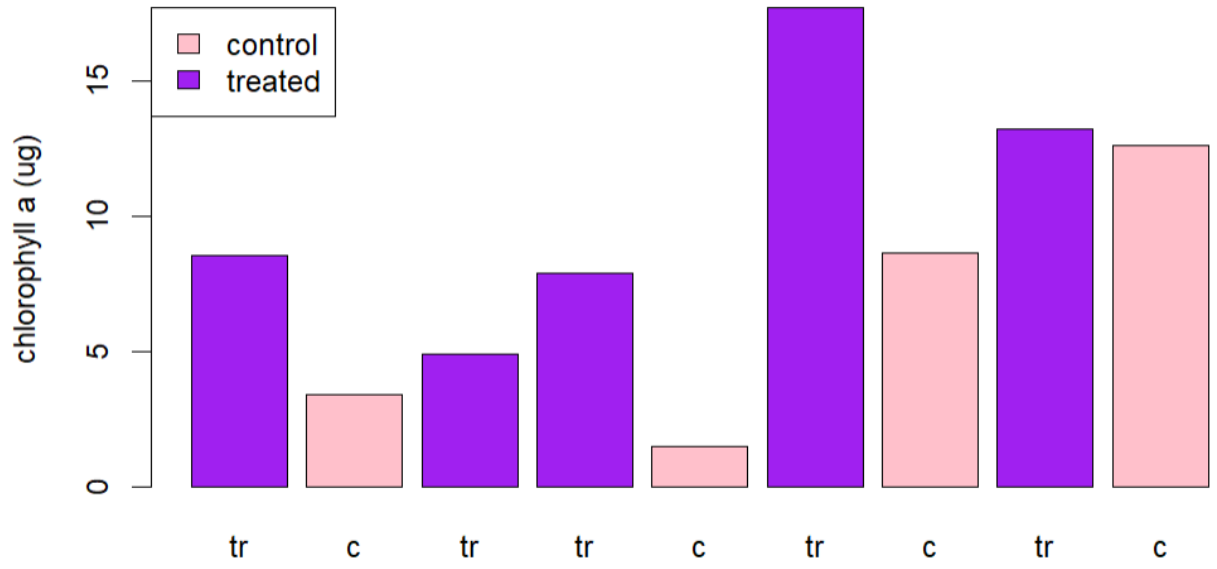


Figure C.1.1.4: Total chlorophyll a for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

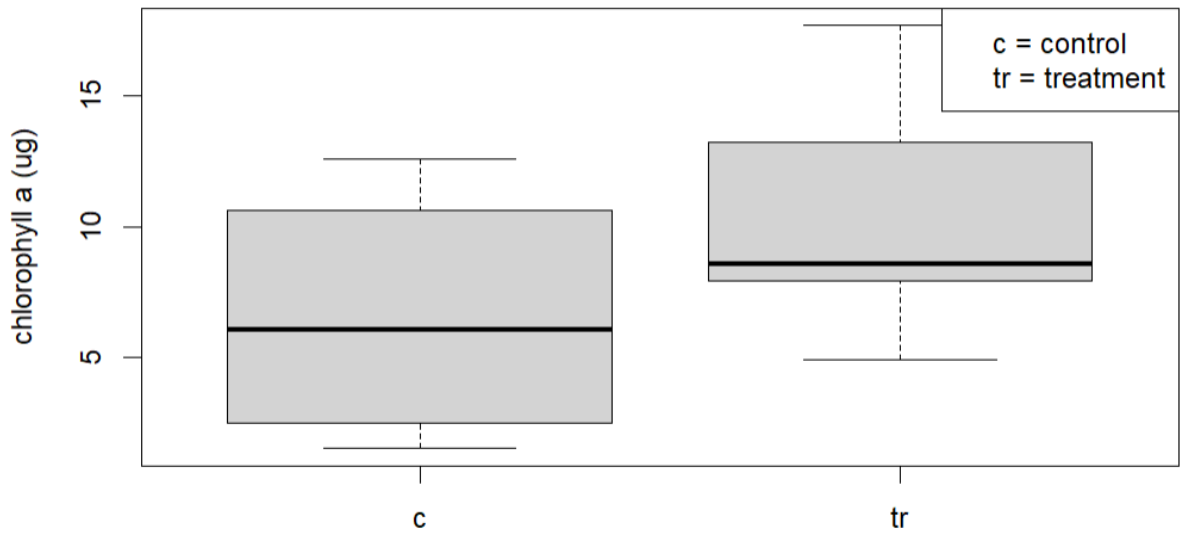


Figure C.1.1.5: Total chlorophyll a boxplots for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

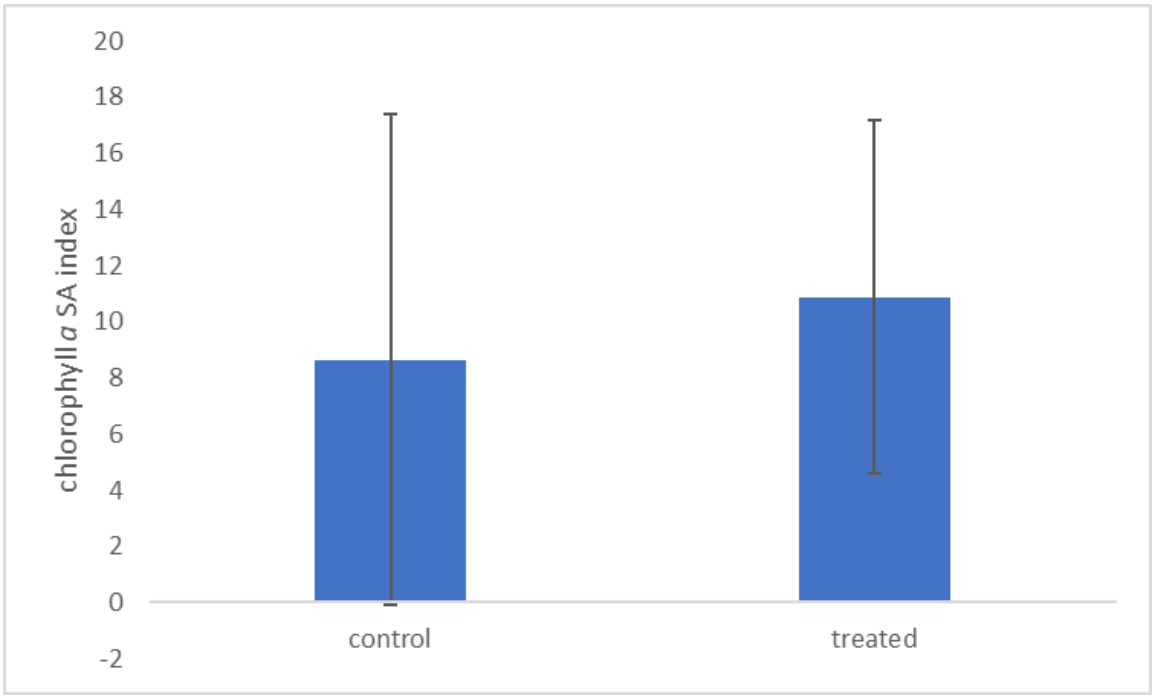


Figure C.1.1.6: Mean chlorophyll a SA index with standard deviation bars for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

*Table C.1.1.1: Placement set-up for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
9	8	4	9 (c)	5 (c)	7 (c)	2	1	5
5	1	3	6 (tr)	3 (tr)	2 (c)	7	3	4
2	6	7	1 (tr)	4 (tr)	8 (tr)	9	8	6

Appendix C.1.2: Trial 2 additional figures and tables

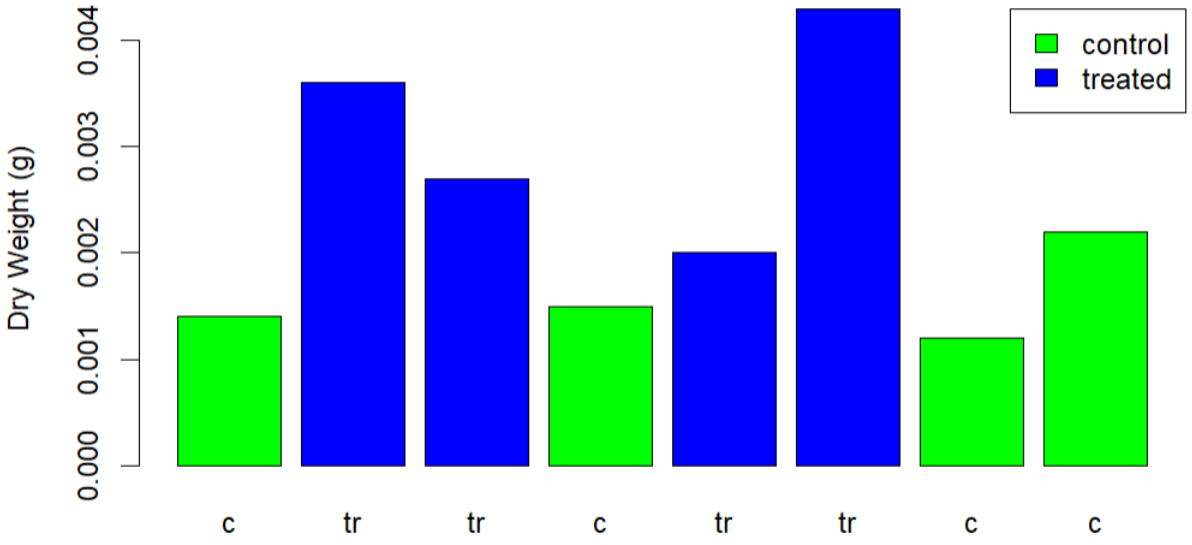


Figure C.1.2.1: Total dry weight for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

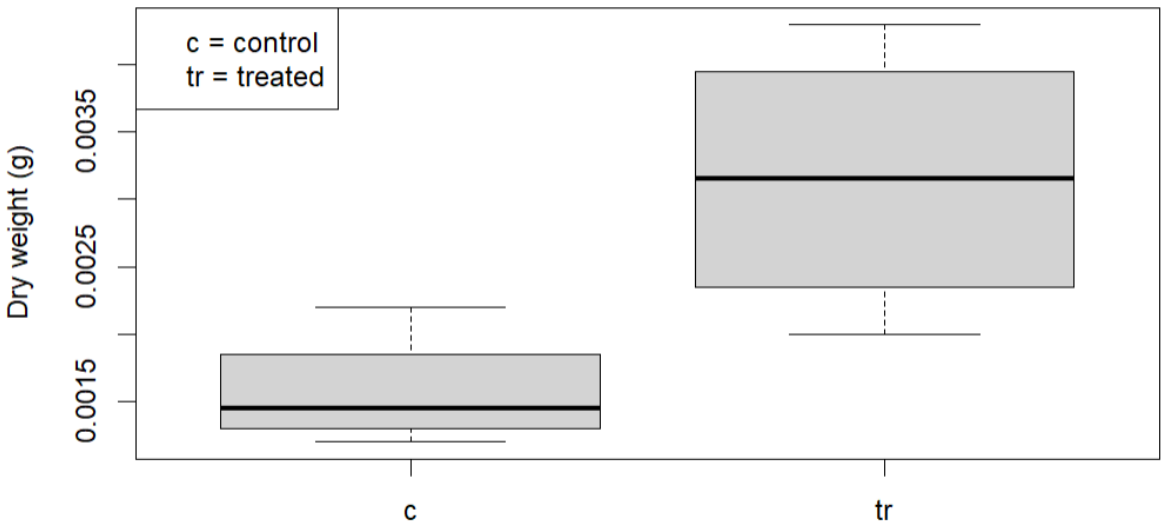


Figure C.1.2.2: Total dry weight boxplots for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

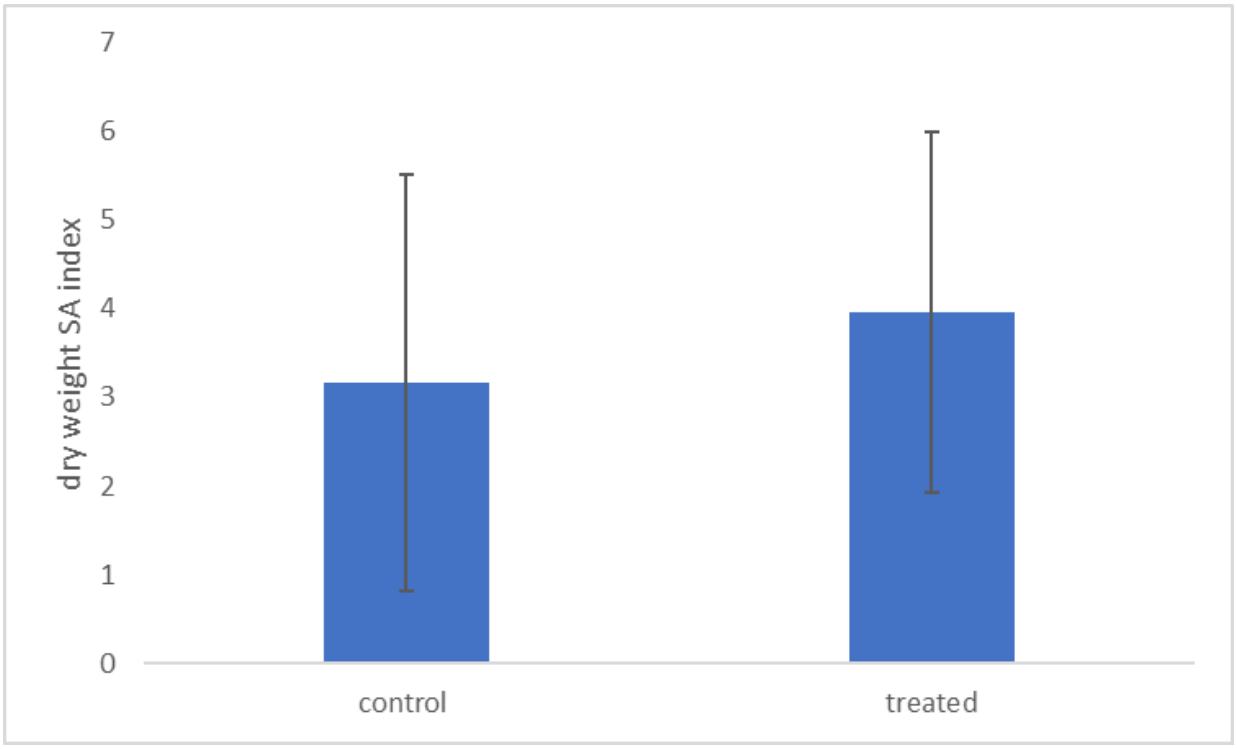

7
6
5
4
3
2
1
0
dry weight SA index
control
treated

Figure C.1.2.3: Mean dry weight SA index with standard deviation bars for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

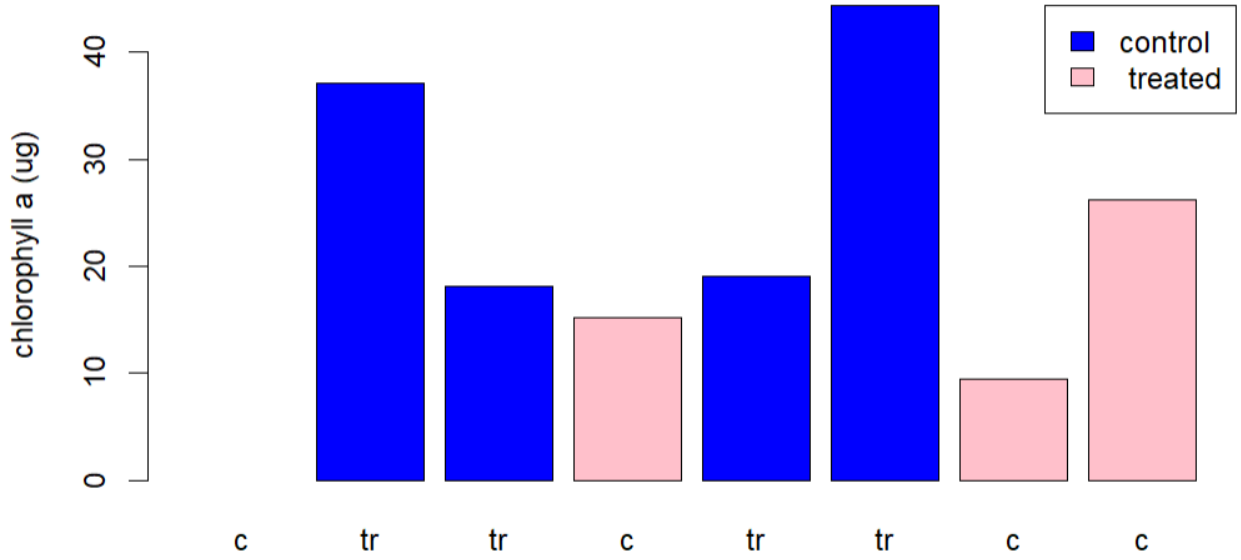


Figure C.1.2.4: Total chlorophyll a for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

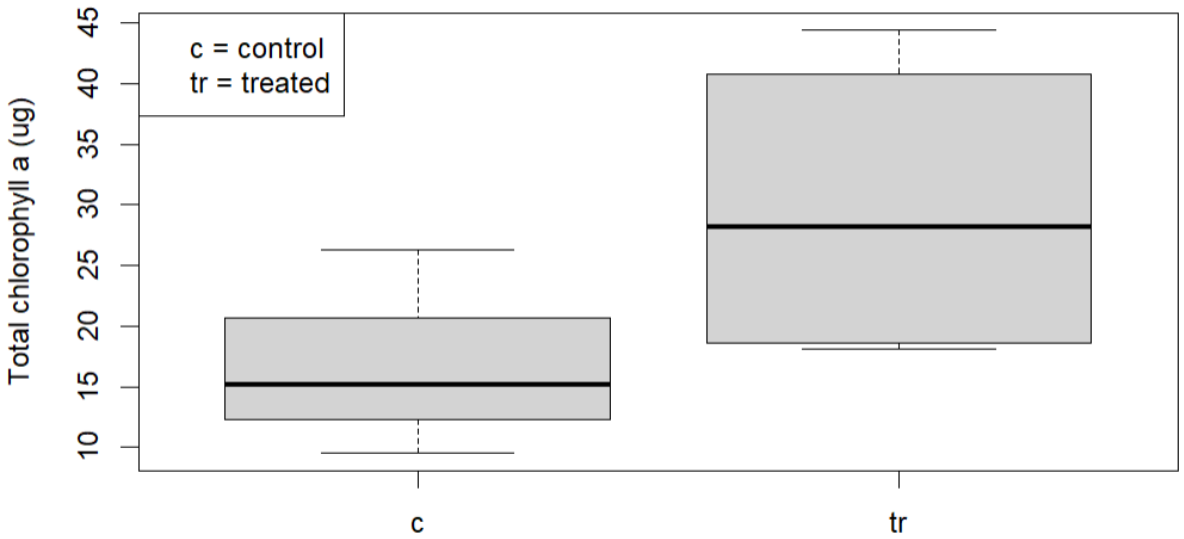


Figure C.1.2.5: Total chlorophyll a boxplots for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

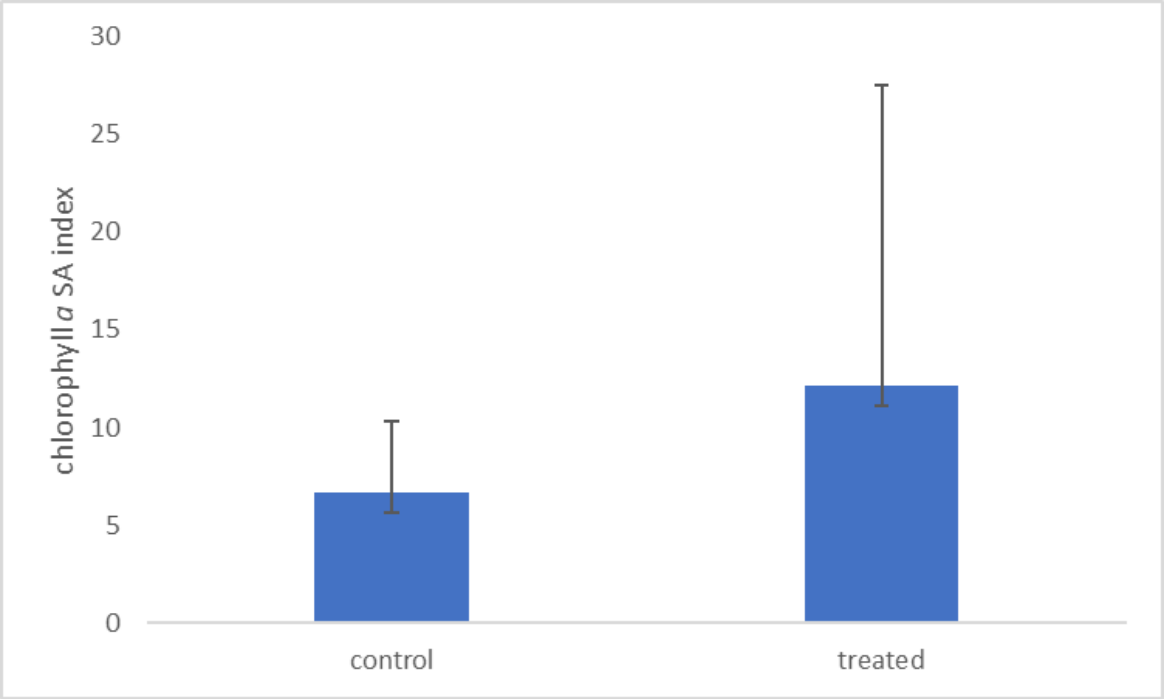


Figure C.1.2.6: Mean chlorophyll a SA index with standard deviation bars for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

*Table C.1.2.1: Placement set-up for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids	Systems	Substrata

5	8	4	9 (tr)*	5 (tr)	4 (c)	6	9	3
6	3	7	8 (c)	7 (c)	2 (tr)	2	1	8
2	9	1	3 (tr)	6 (tr)	1 (c)*	7	4	5

Appendix C.1.3: Trial 3 additional figures and tables

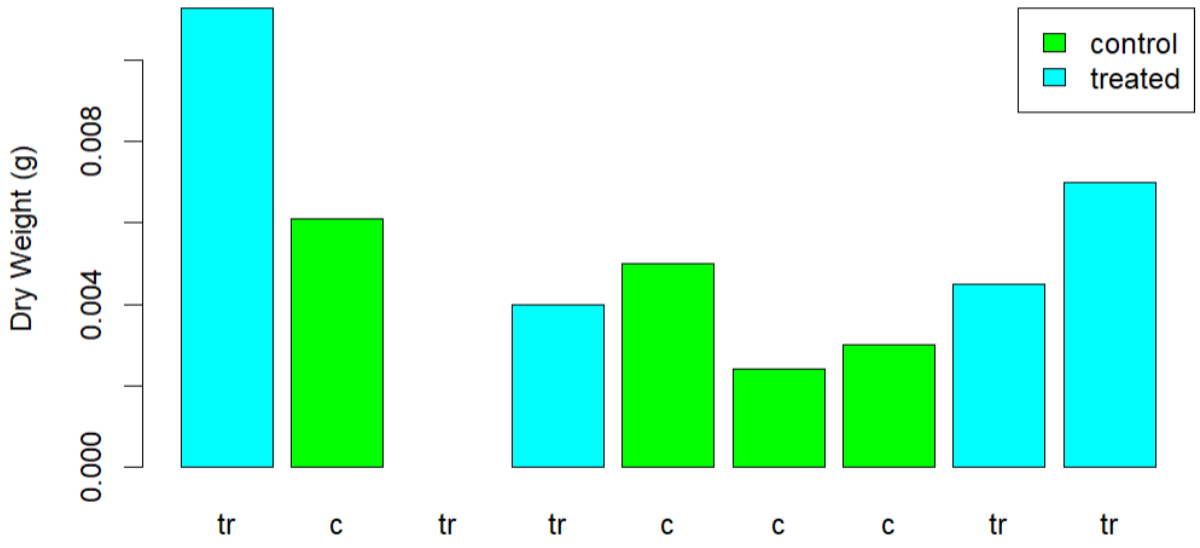


Figure C.1.3.1: Total dry weight for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

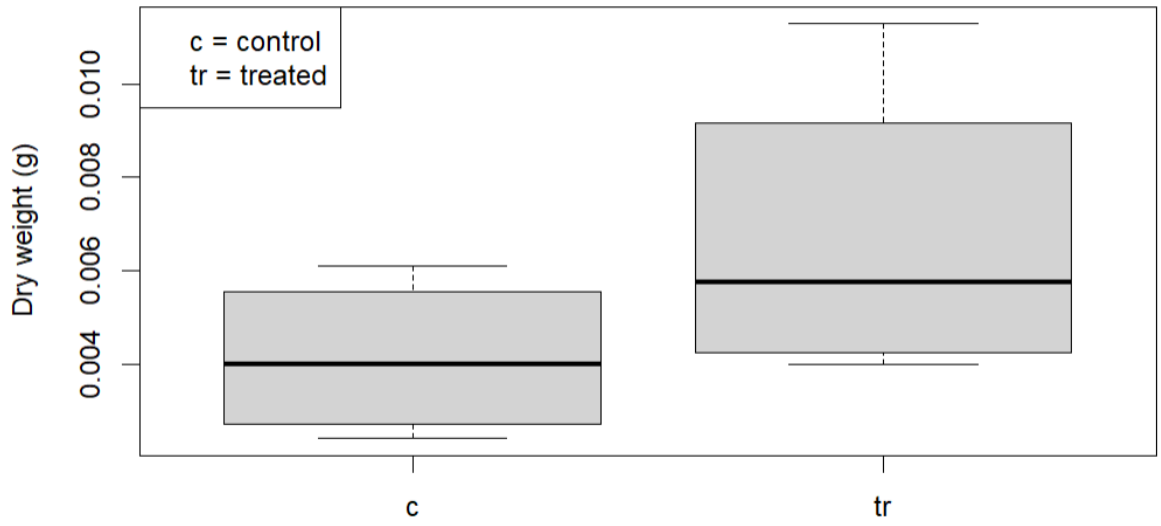


Figure C.1.3.2: Total dry weight boxplots for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

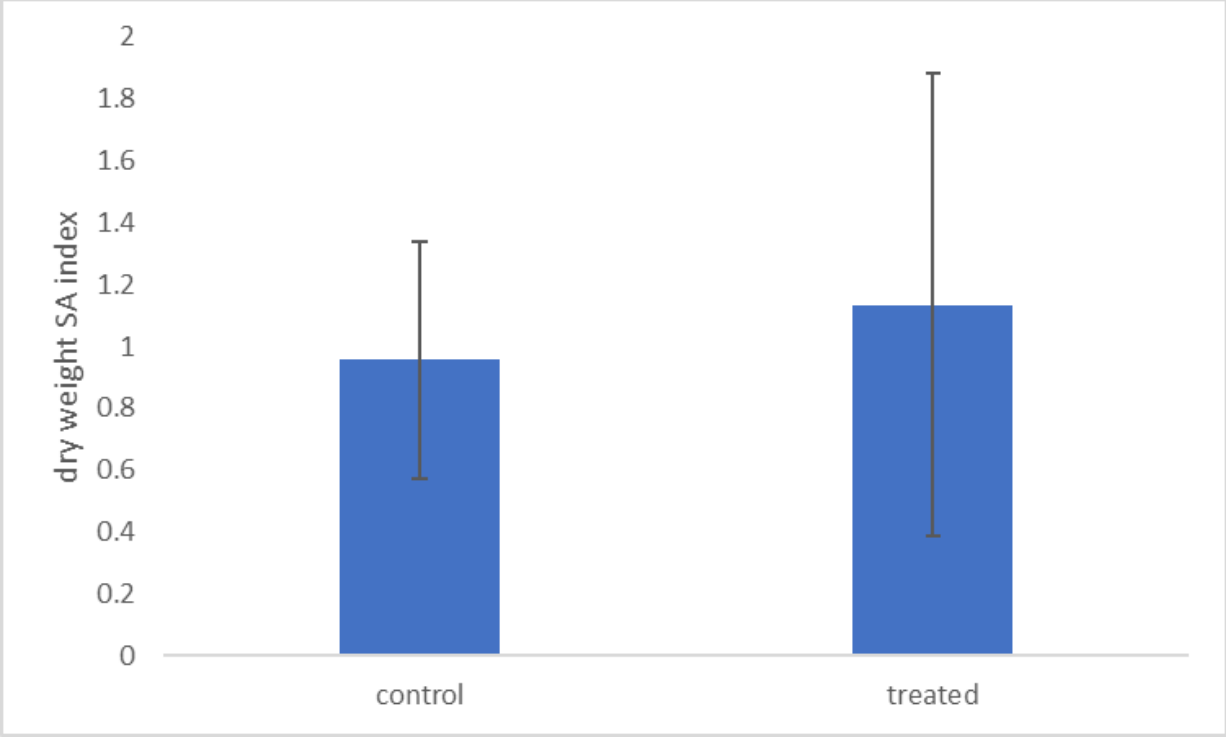


Figure C.1.3.3: Mean dry weight SA index with standard deviation bars for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

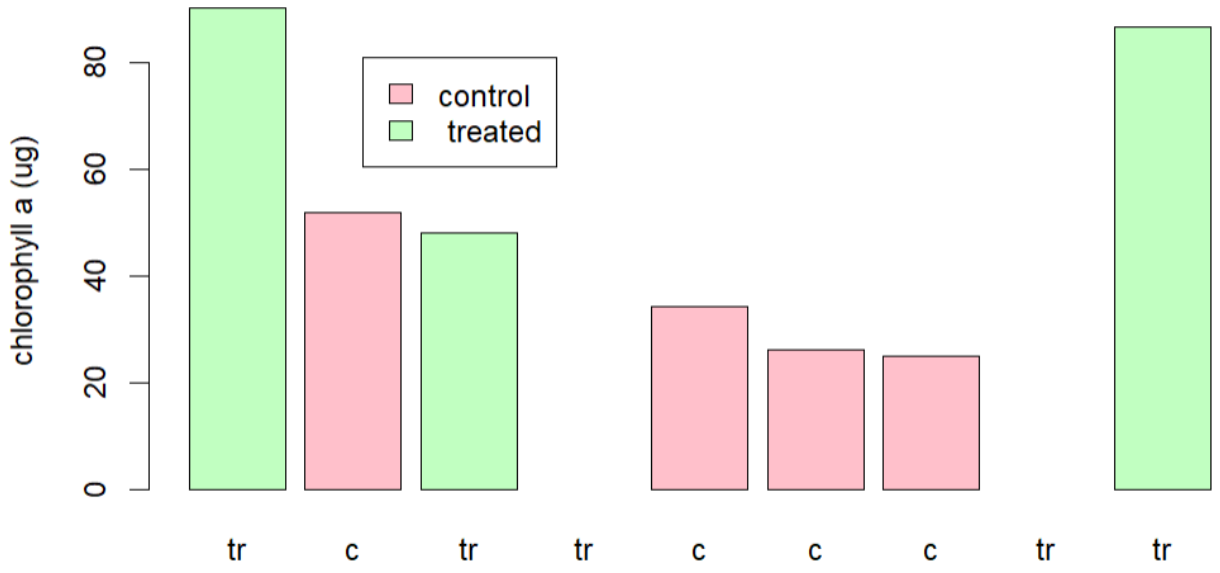


Figure C.1.3.4: Total chlorophyll a for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

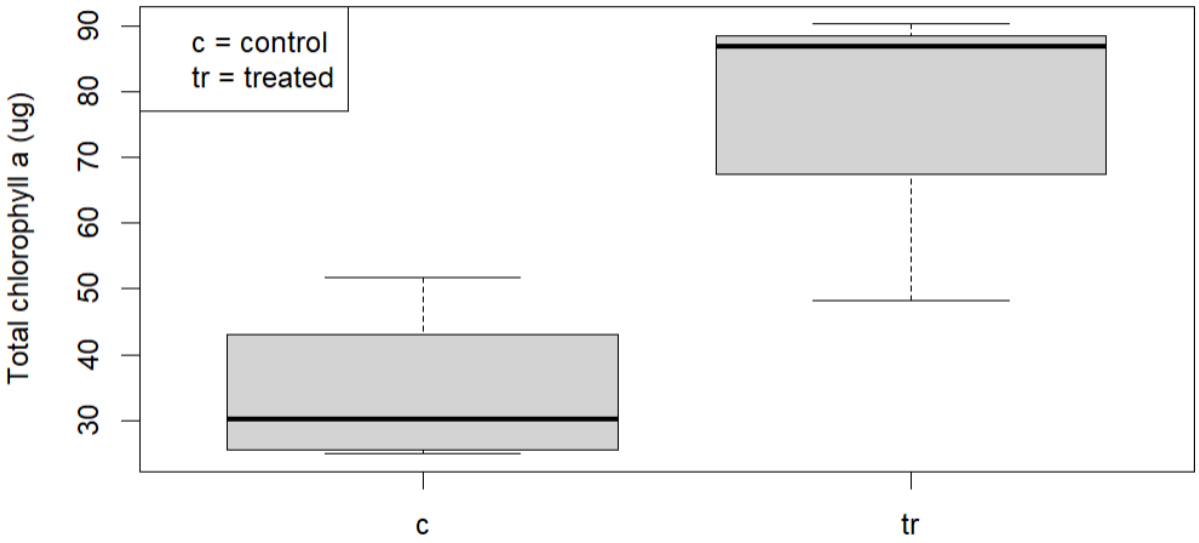


Figure C.1.3.5: Total chlorophyll a boxplots for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

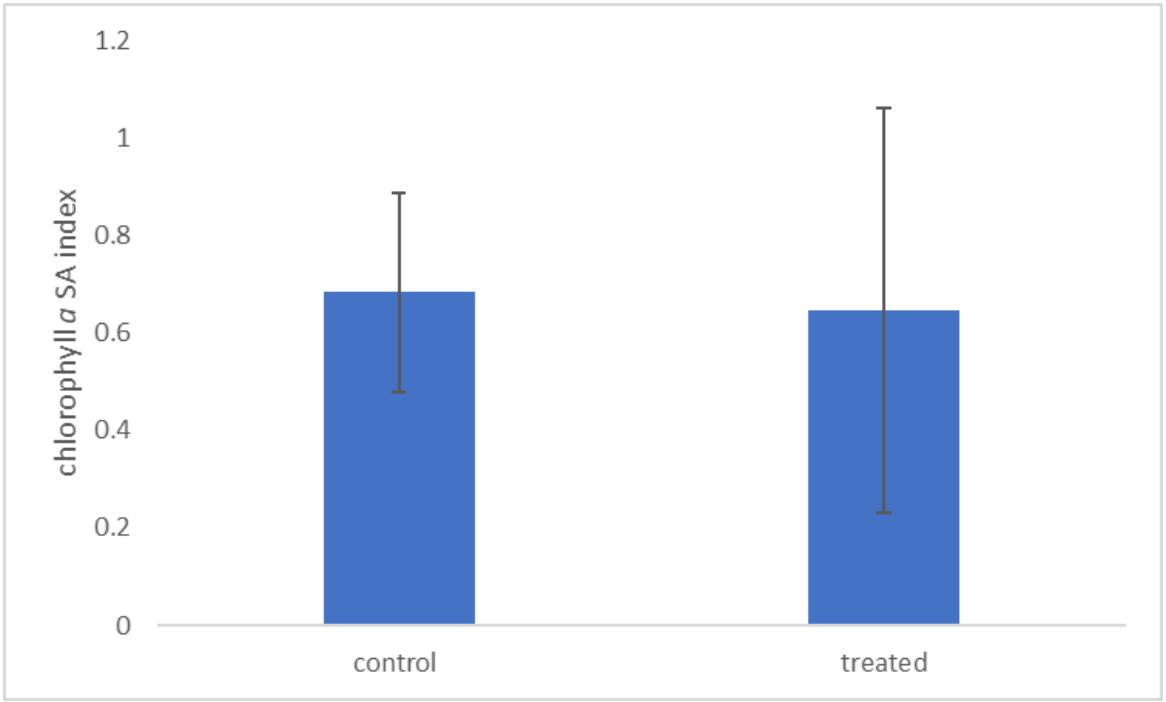


Figure C.1.3.6: Mean chlorophyll a SA index with standard deviation bars for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

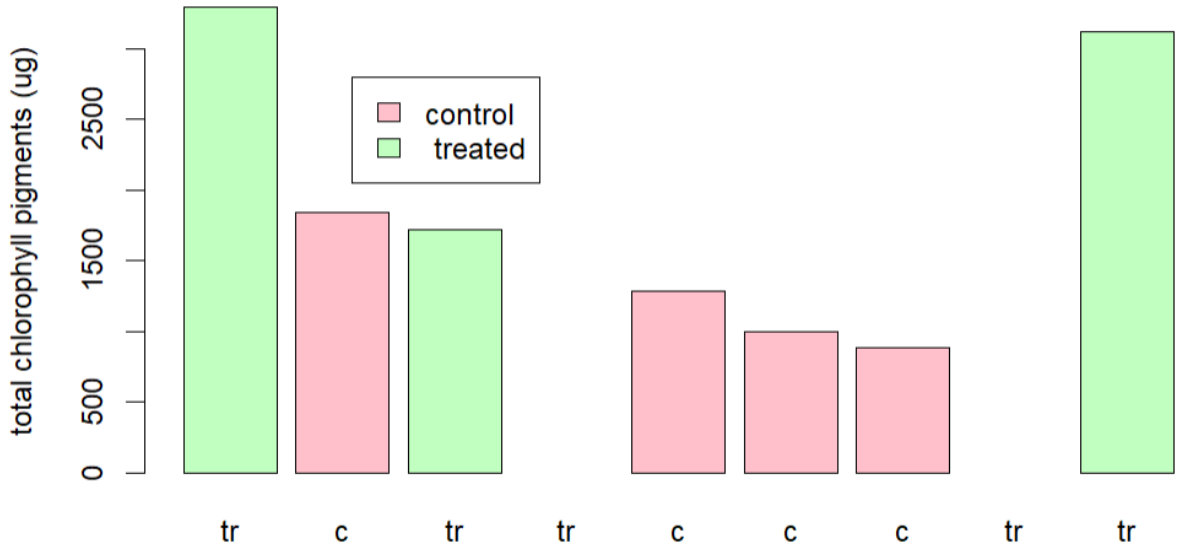


Figure C.1.3.7: Total chlorophyll pigments for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

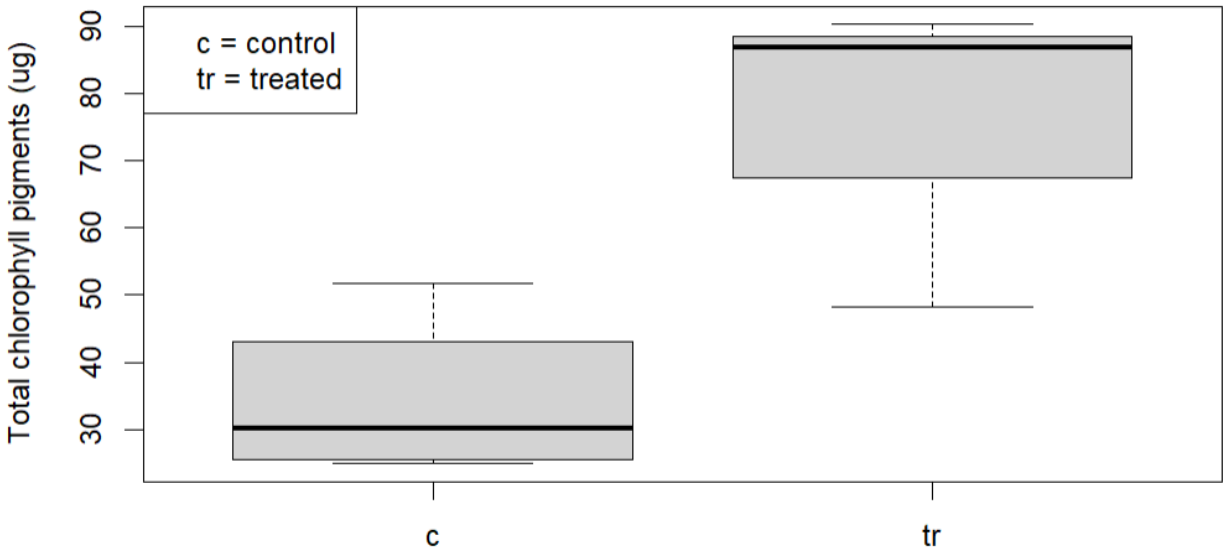


Figure C.1.3.8: Total chlorophyll pigments boxplot for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

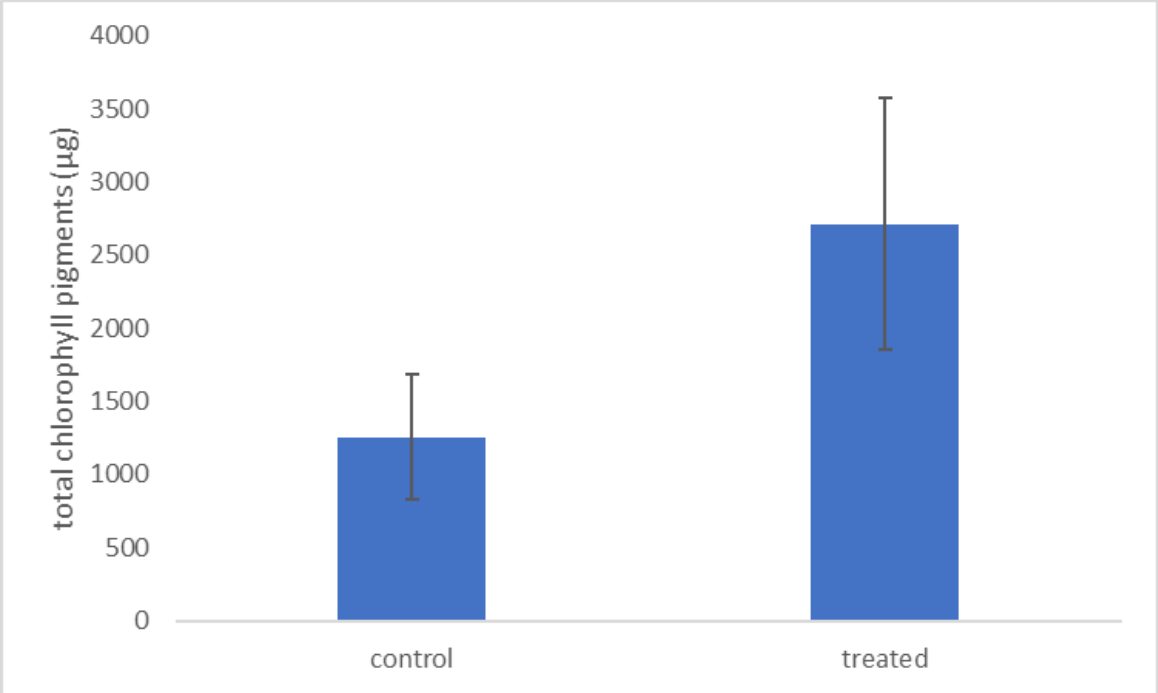


Figure C.1.3.9: Mean total chlorophyll pigments with standard deviation bars for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

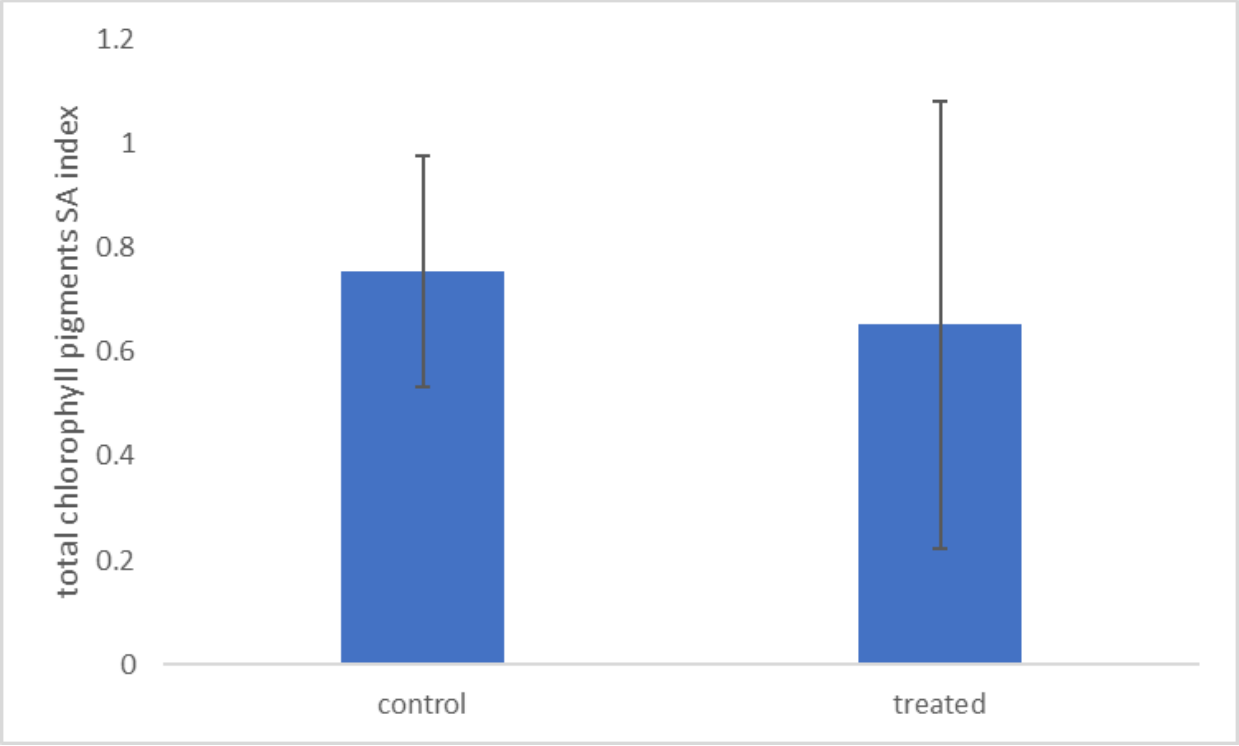


Figure C.1.3.10: Mean total chlorophyll pigments SA index with standard deviation bars for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

Table C.1.3.1: Placement set-up for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a

**indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
8	2	1	7 (c)	4 (tr)*	2 (c)	5	4	7
4	7	6	3 (tr)*	5 (c)	9 (tr)	9	8	2
5	9	3	6 (c)	8 (tr)*	1 (tr)	3	1	6

Appendix C.2 Additional figures and tables for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

(Return to: 3.2.2)

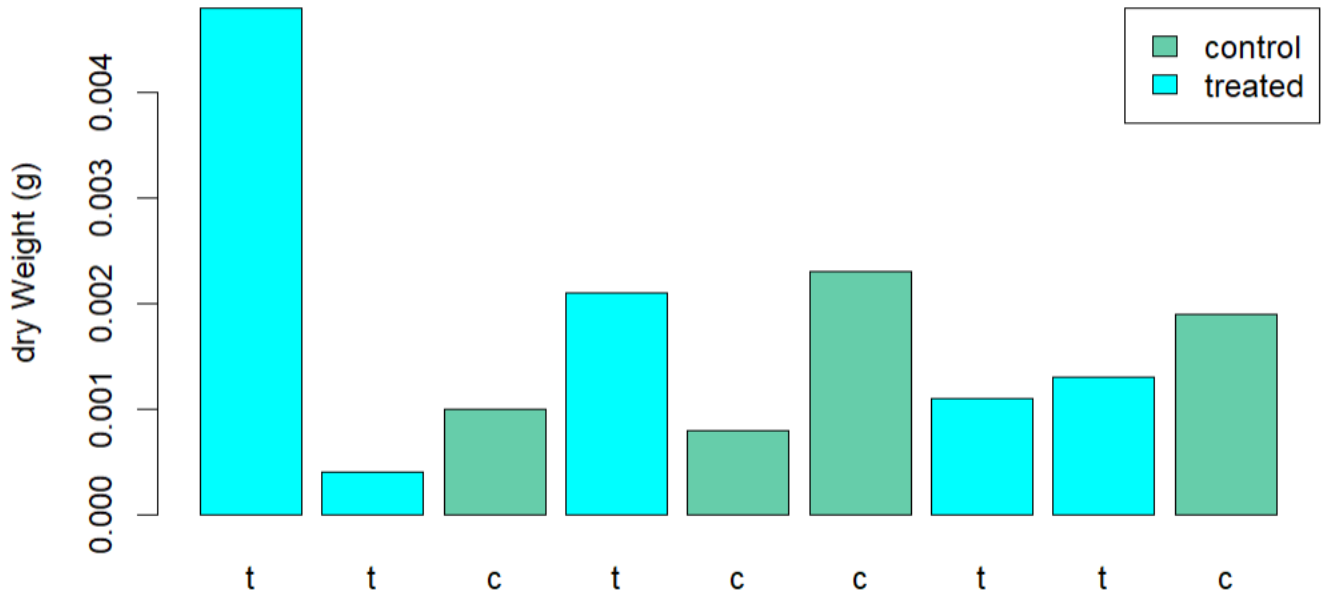


Figure C.2a Total dry weight for Trial 4: the effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

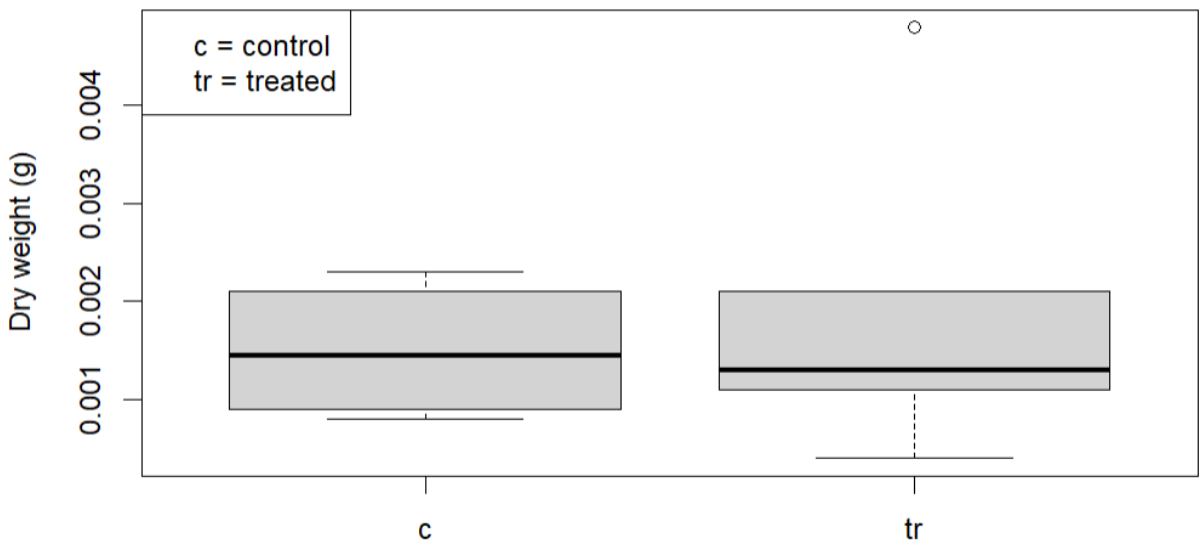


Figure C.2b Total dry weight boxplots for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

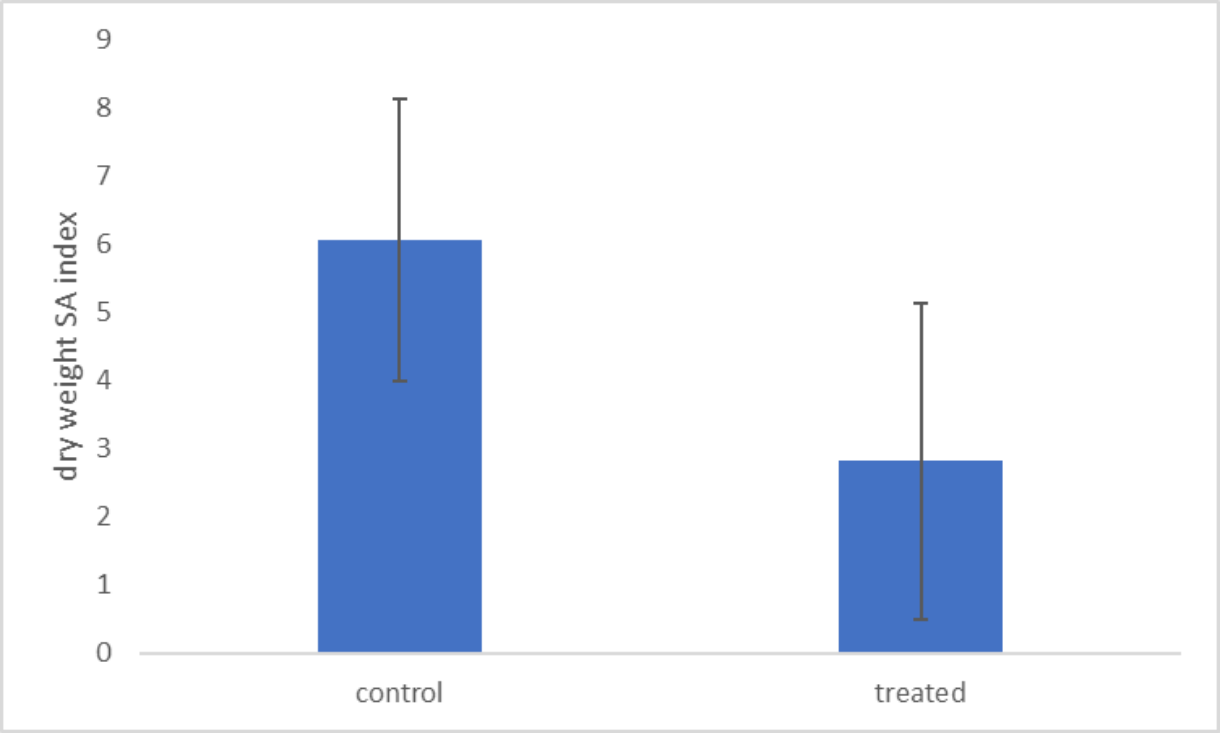


Figure C.2c: Mean dry weight SA index with standard deviation bars for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

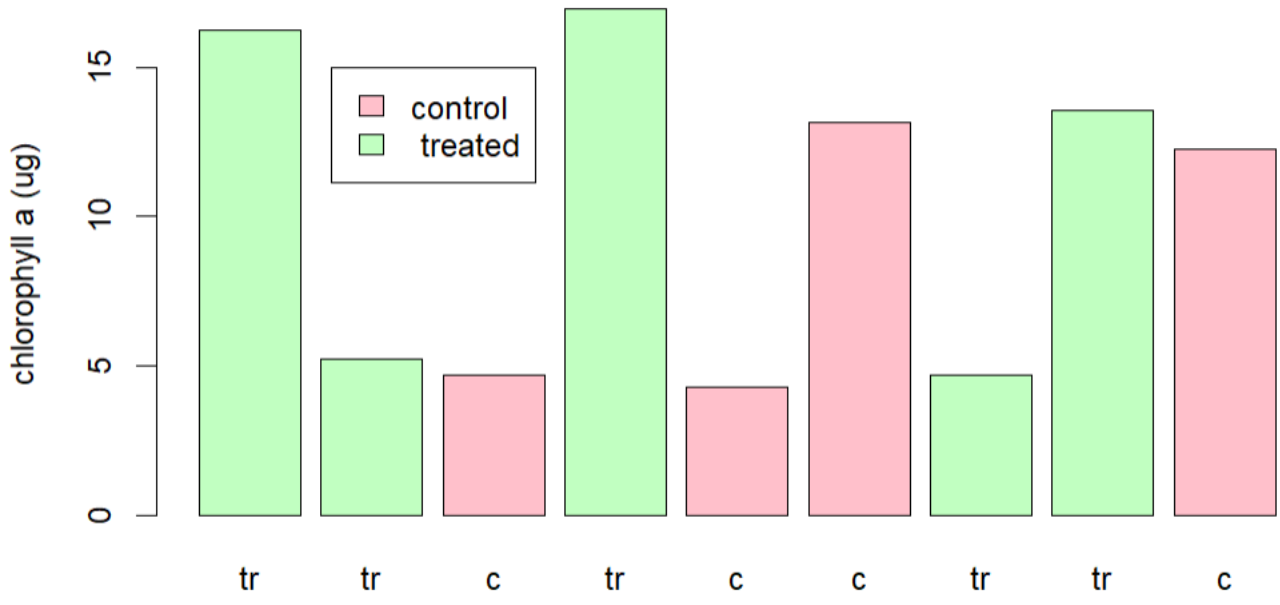


Figure C.2d Total chlorophyll a for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

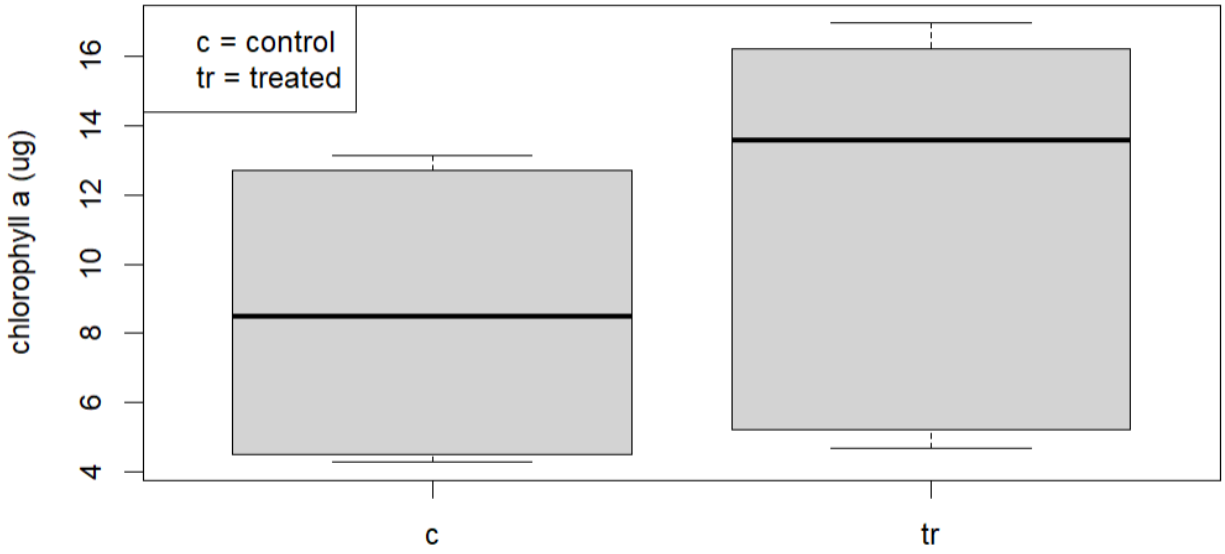


Figure C.2e Total chlorophyll a boxplots for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

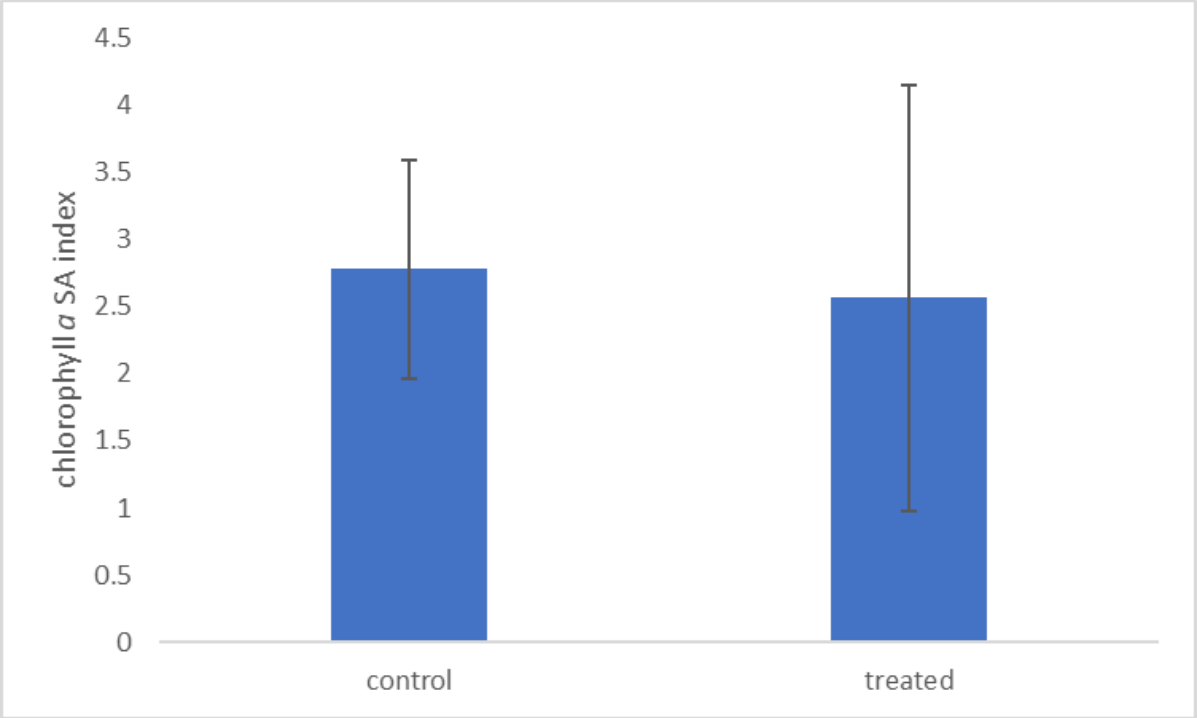


Figure C.2f Mean chlorophyll a SA index with standard deviation bars for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

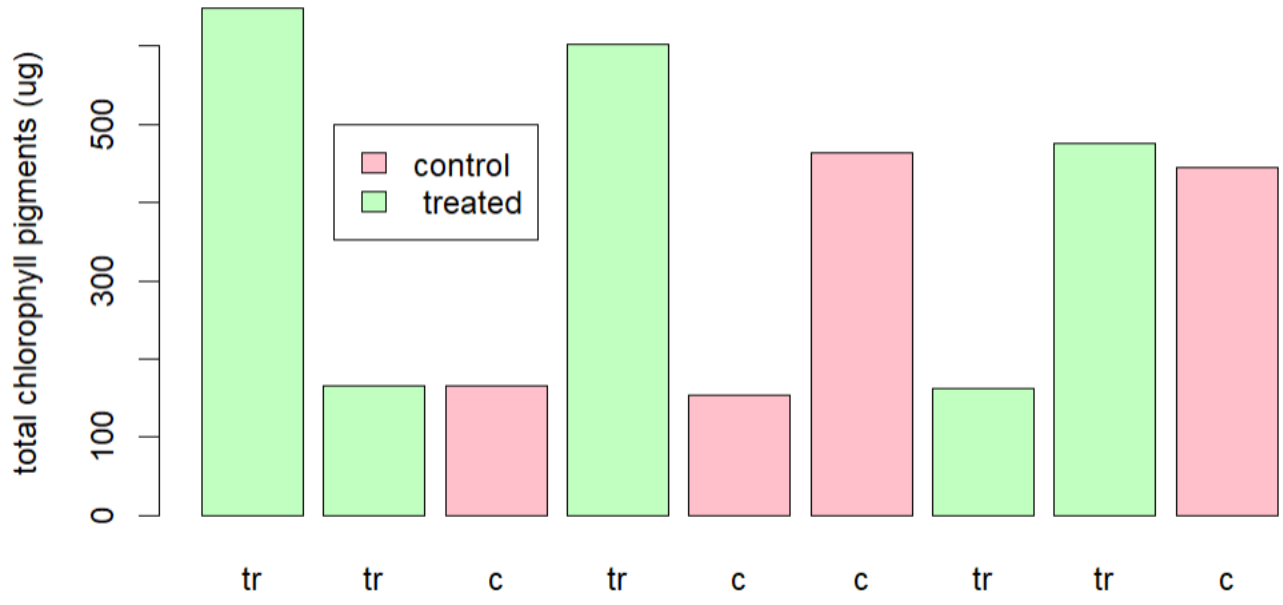


Figure C.2g: Total chlorophyll pigments for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

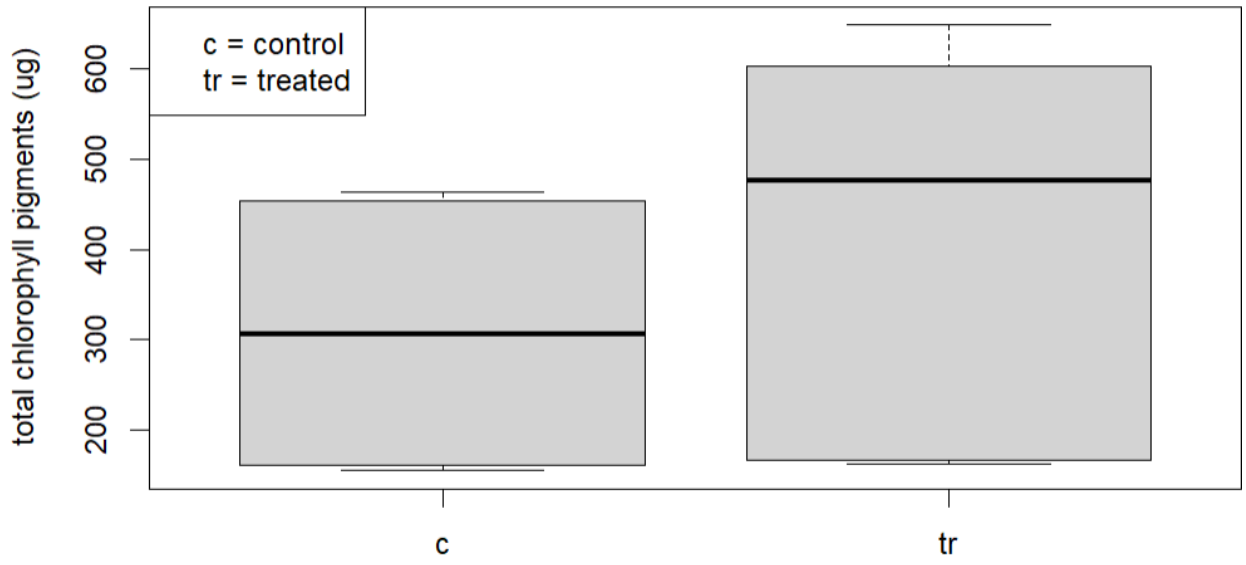


Figure C.2h: Total chlorophyll pigments boxplot for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

Figure C.2i: Mean total chlorophyll pigments with standard deviation bars for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

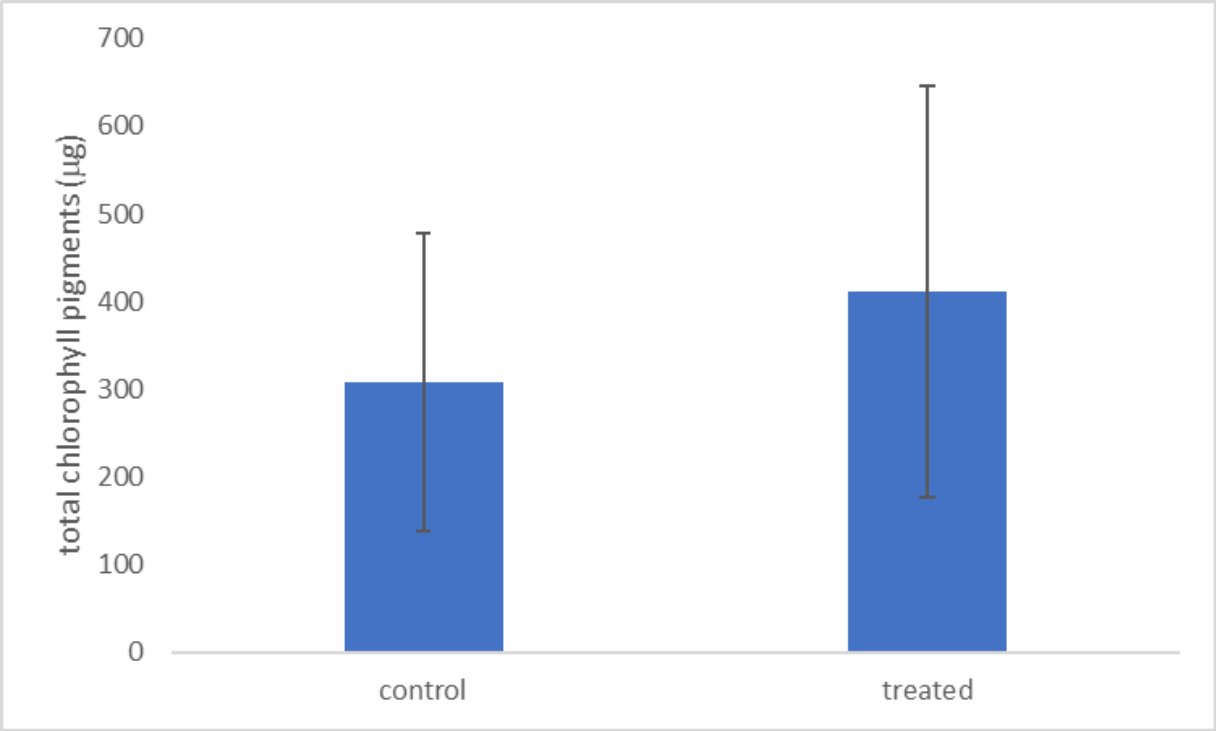


Figure C.2j: Mean total chlorophyll pigments SA index with standard deviation bars for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

*Table C.2a: Placement set-up for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of*

some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)

Lids			Systems			Substrata		
NA	NA	NA	6 (c)	1 (tr)	8 (tr)	9	8	4
NA	NA	NA	2 (tr)	7 (tr)	9 (c)	2	7	6
NA	NA	NA	4 (tr)	5 (c)	3 (c)	5	1	3

Appendix C.3 Additional figures and tables for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient water

(Return to: 3.2.3)

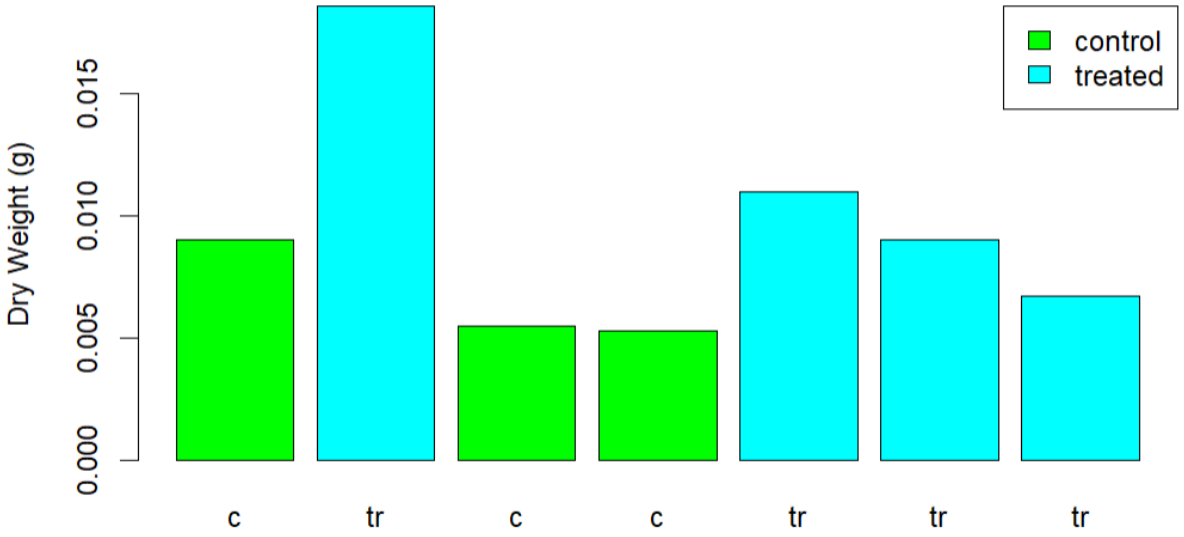


Figure C.3a: Total dry weight for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

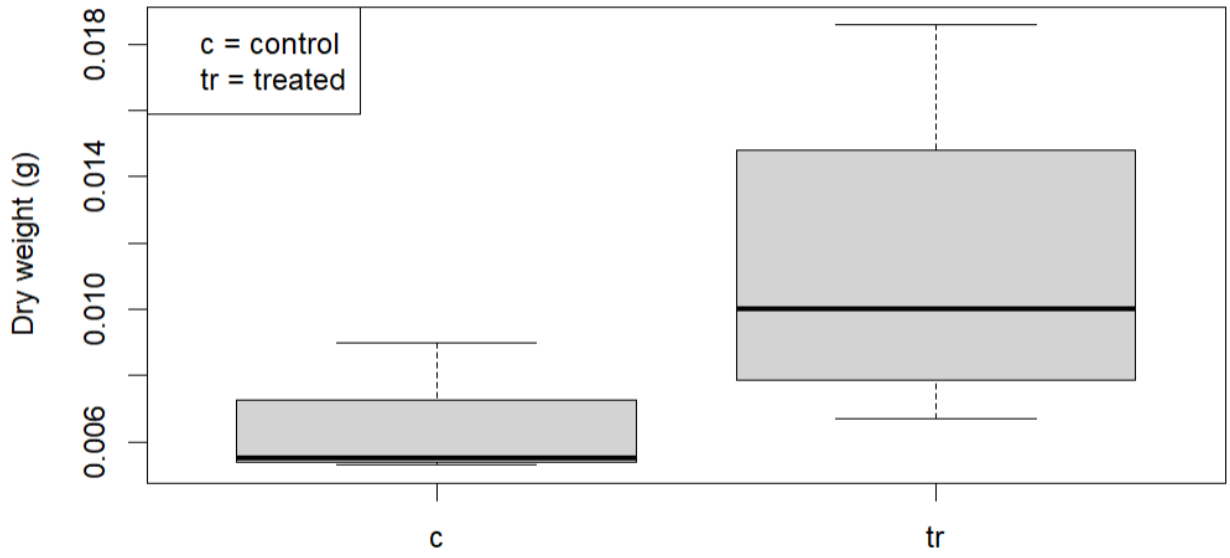


Figure C.3b: Total dry weight boxplots for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

Figure C.3c: Total chlorophyll a for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

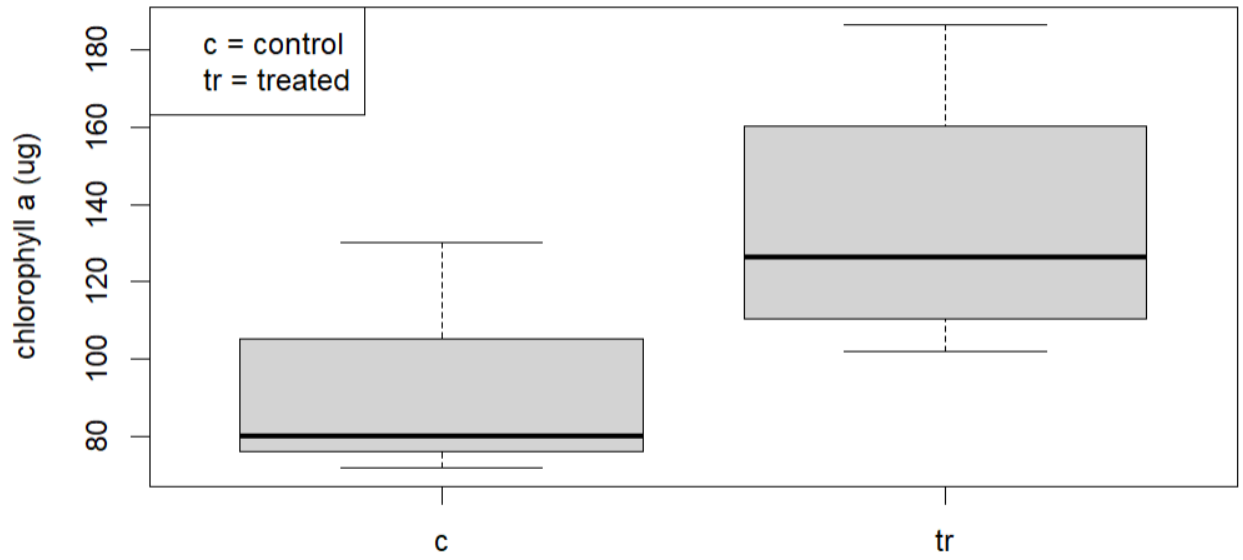


Figure C.3d: Total chlorophyll a boxplots for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

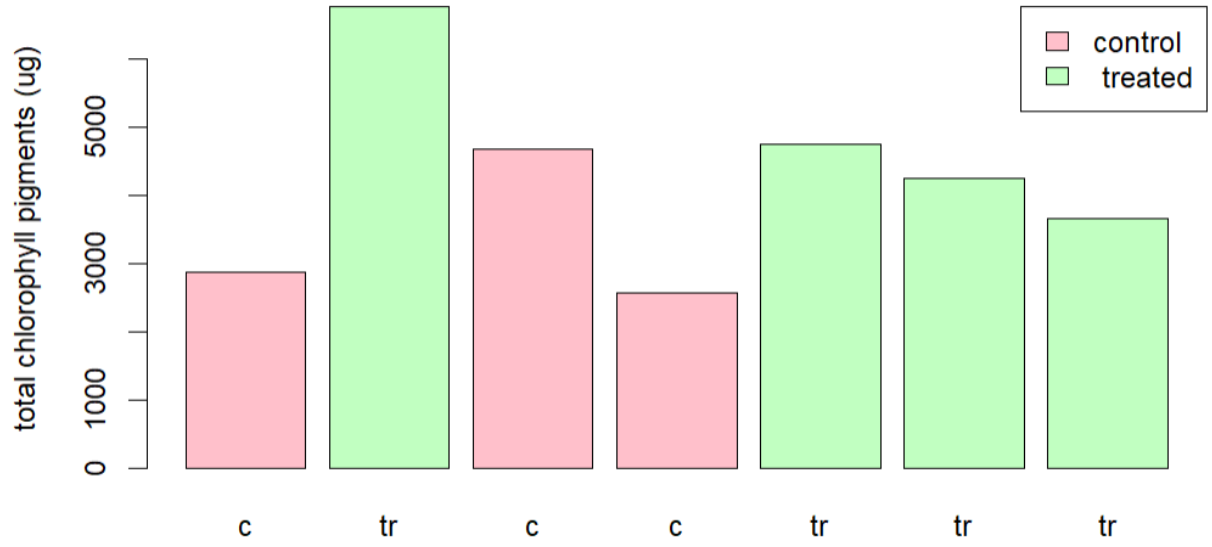


Figure C.3e: Total chlorophyll pigments for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

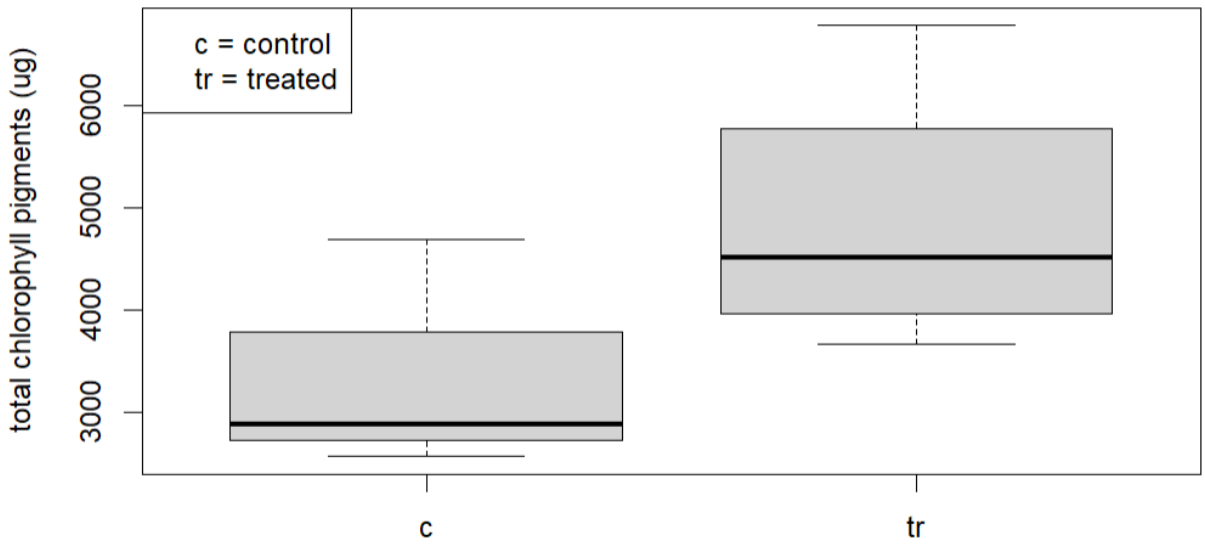


Figure C.3f: Total chlorophyll pigments boxplot for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

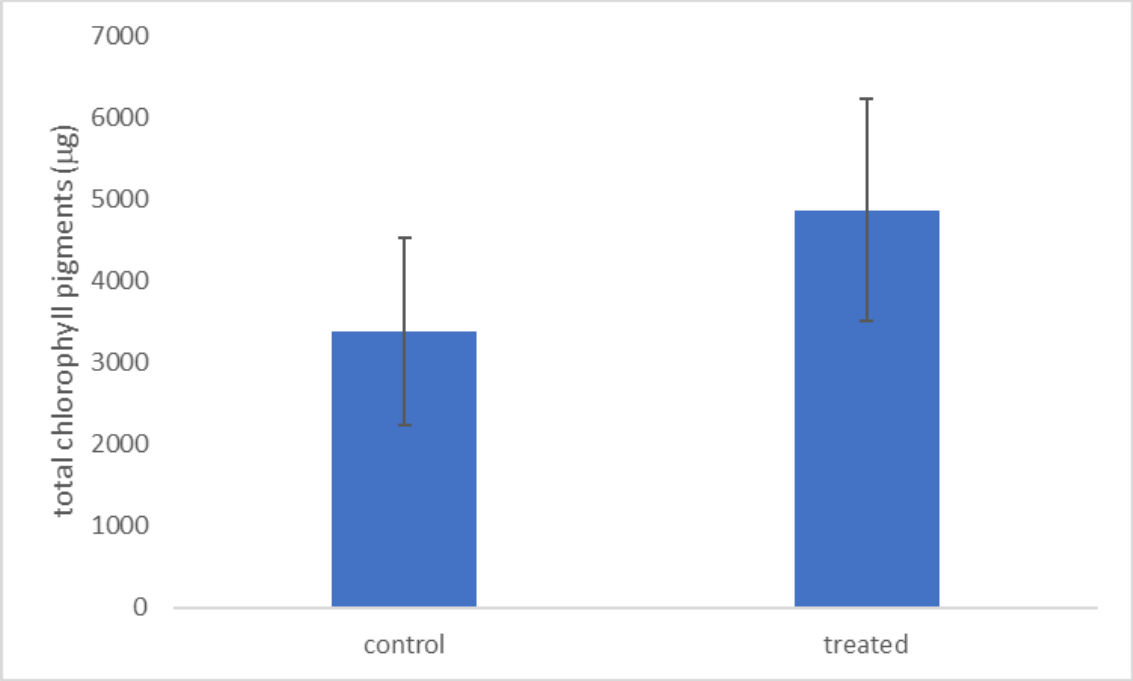


Figure C.3g: Mean total chlorophyll pigments with standard deviation bars for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

*Table C.3a: Placement set-up for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
1	5	9	3 (tr)	4 (c)	5 (c)	9	3	7
8	3	6	9 (tr)	6 (tr)	7 (tr)	5	2	4
7	2	4	1 (c)	2 (tr)	8 (c)	8	6	1

Appendix C.4 Additional figures and tables for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

(Return to: 3.2.4)

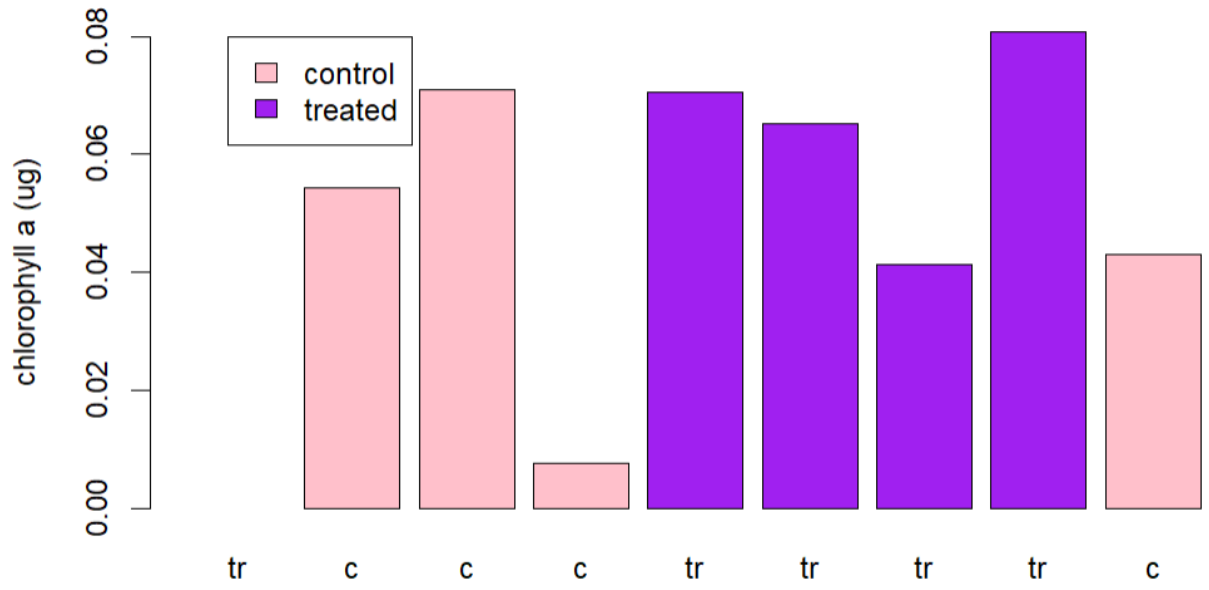


Figure C.4a: Total chlorophyll a for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

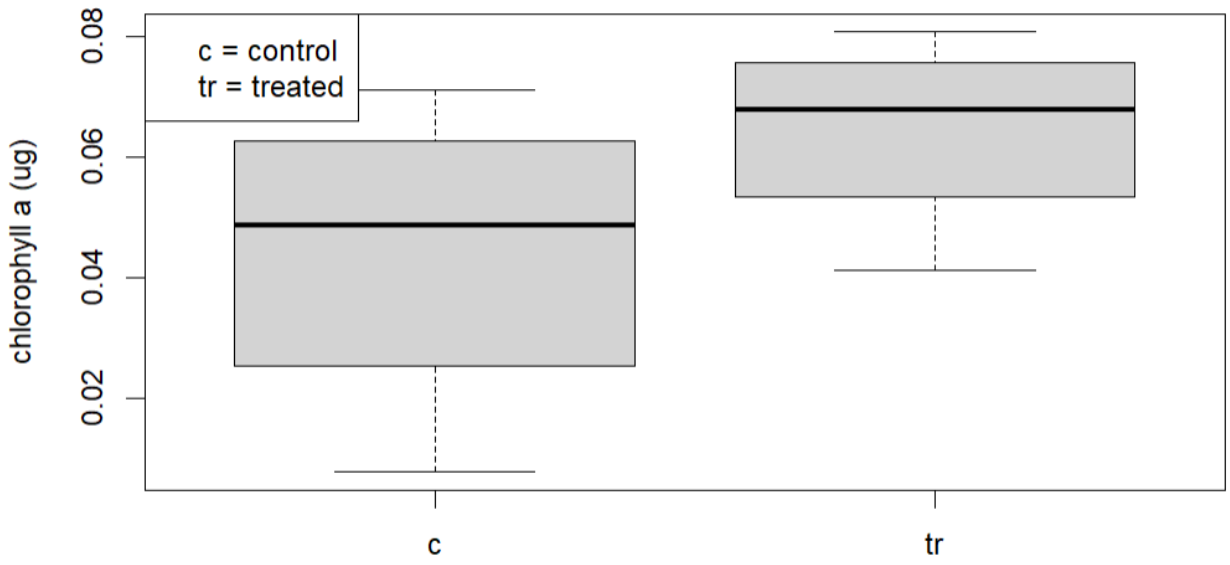


Figure C.4b: Total chlorophyll a boxplots for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

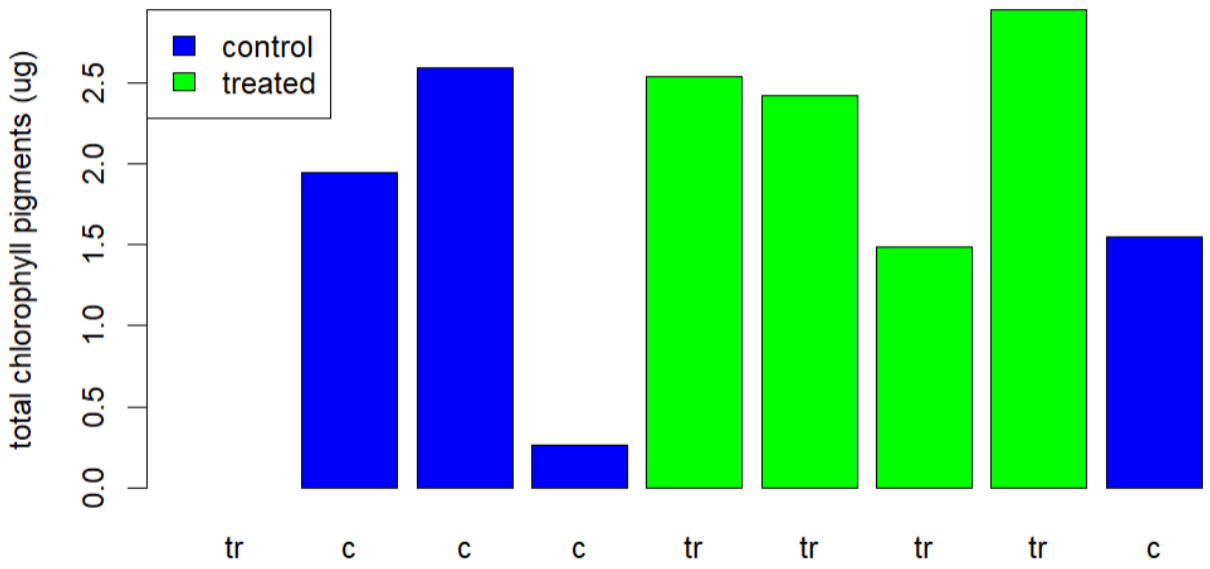


Figure C.4c: Total chlorophyll pigments for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

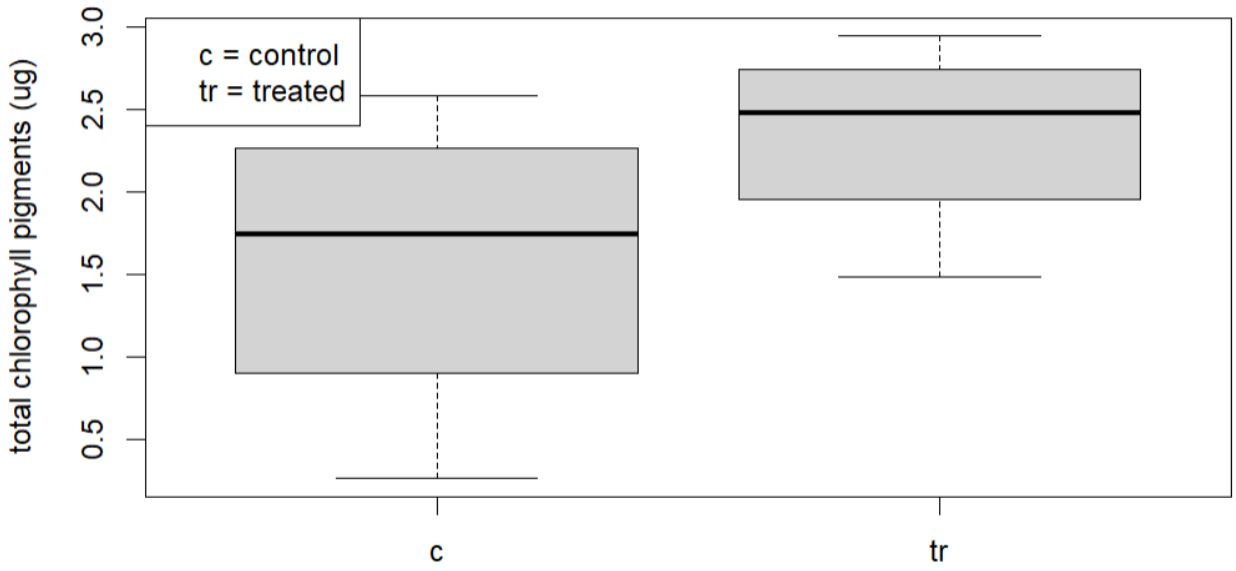


Figure C.4d: Total chlorophyll pigments boxplot for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

*Table C.4a: Placement set-up for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
2	9	3	8 (tr)	1 (tr)	2 (c)	5	9	6
8	5	1	5 (tr)	9 (c)	3 (c)	3	1	8
6	4	7	6 (tr)	7 (tr)	4 (c)	2	4	7

Appendix C.5 Additional figures and tables for Trial 7: The effect of the Town Creek Park stream bacteria biofilm in half-diluted, filtered aquaculture waste

(Return to: 3.2.5)

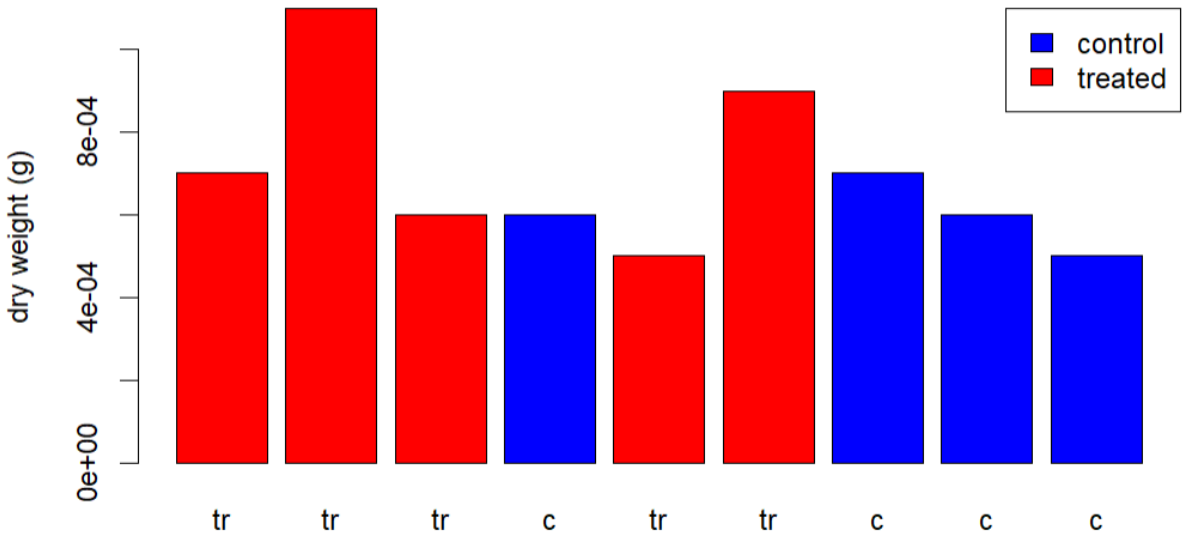


Figure C.5a: Total dry weight for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in half-diluted, filtered aquaculture waste

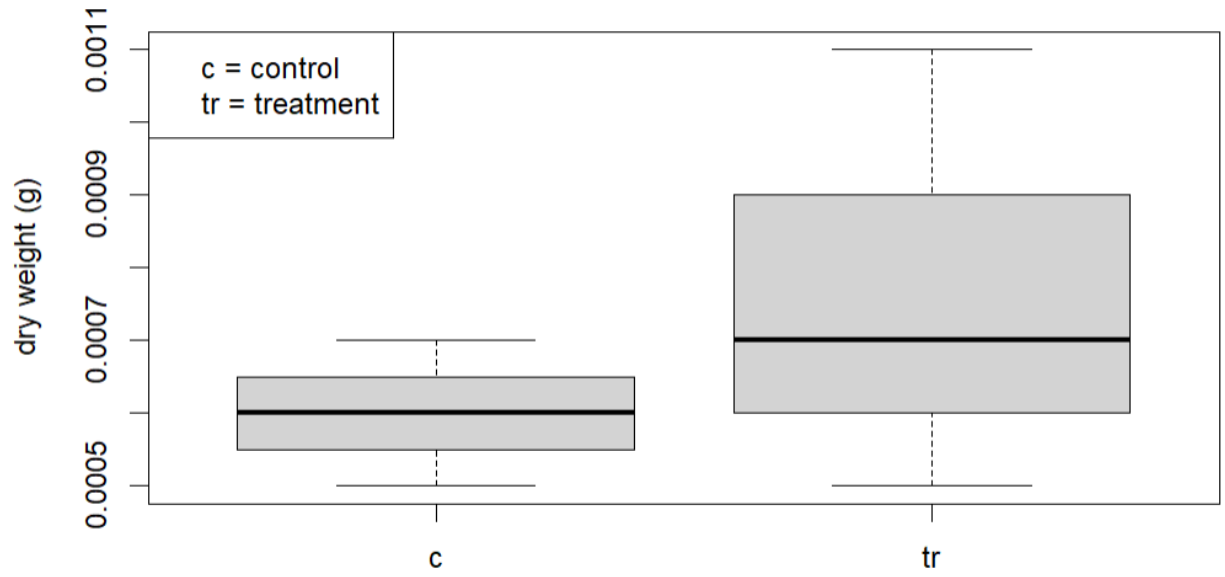


Figure C.5b: Total dry weight boxplots for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in half-diluted, filtered aquaculture waste

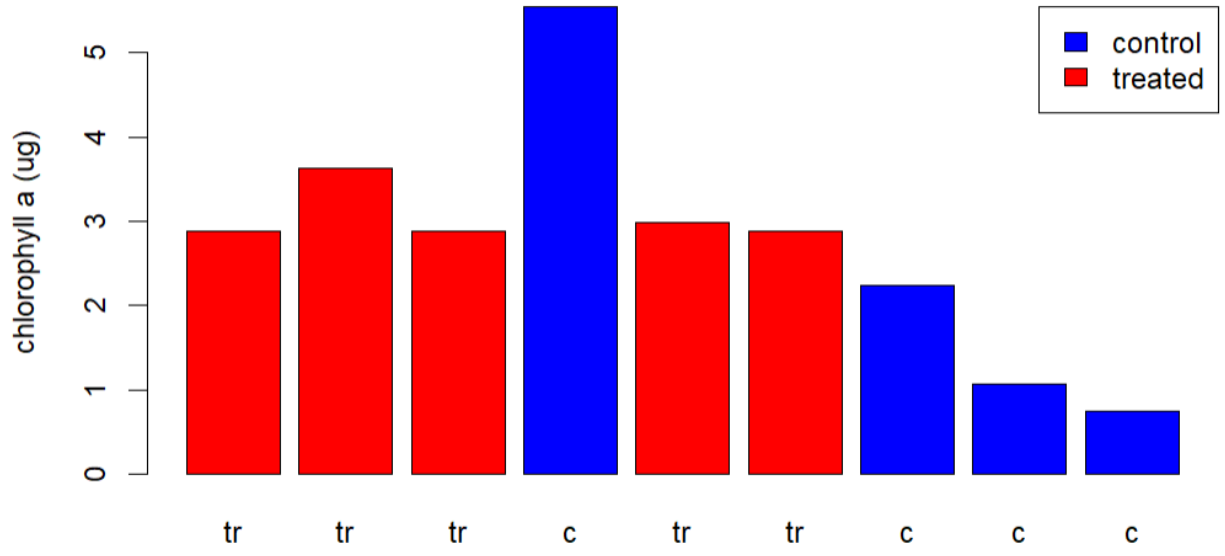


Figure C.5c: Total chlorophyll a for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in half-diluted, filtered aquaculture waste

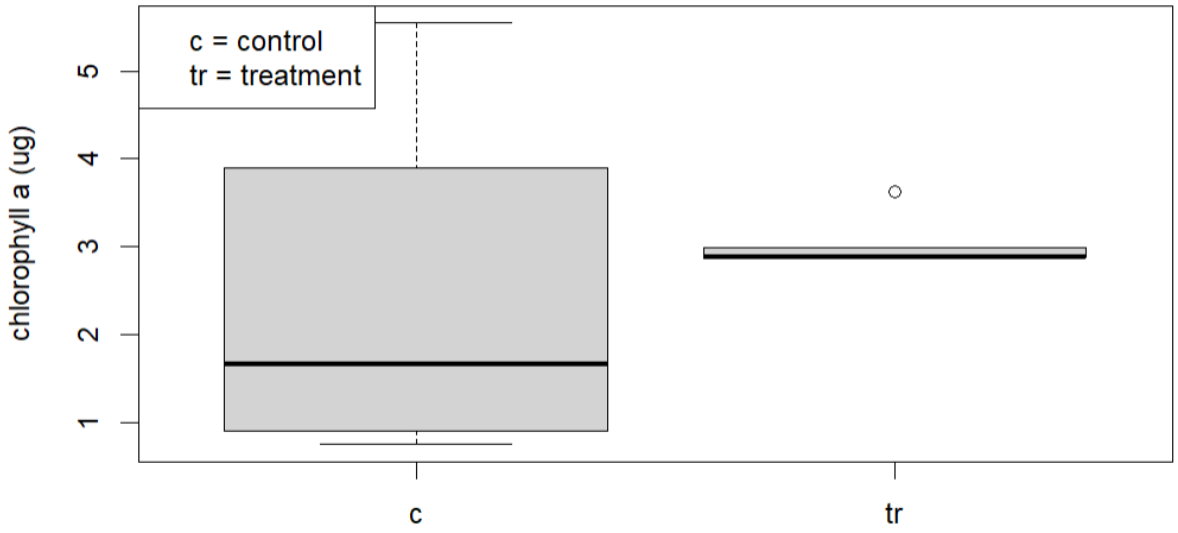


Figure C.5d: Total chlorophyll a boxplots for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in half-diluted, filtered aquaculture waste

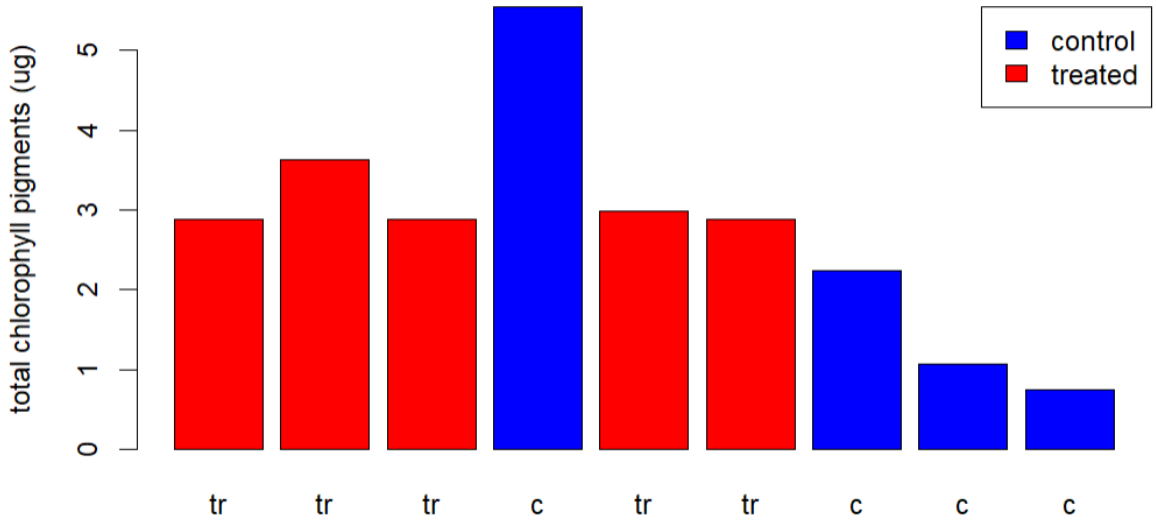


Figure C.5e: Total chlorophyll pigments for Trial 7: The effect of the Tallapoosa River bacteria biofilm on attachment in half-diluted, filtered aquaculture waste

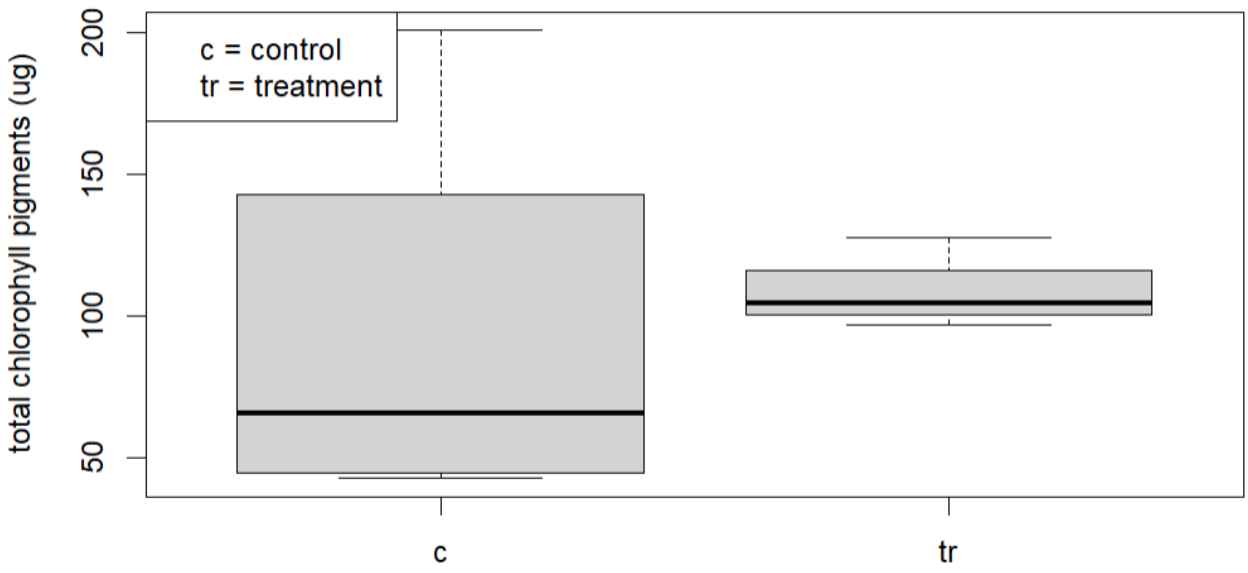


Figure C.5d: Total chlorophyll pigments boxplot for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in half-diluted, filtered aquaculture waste

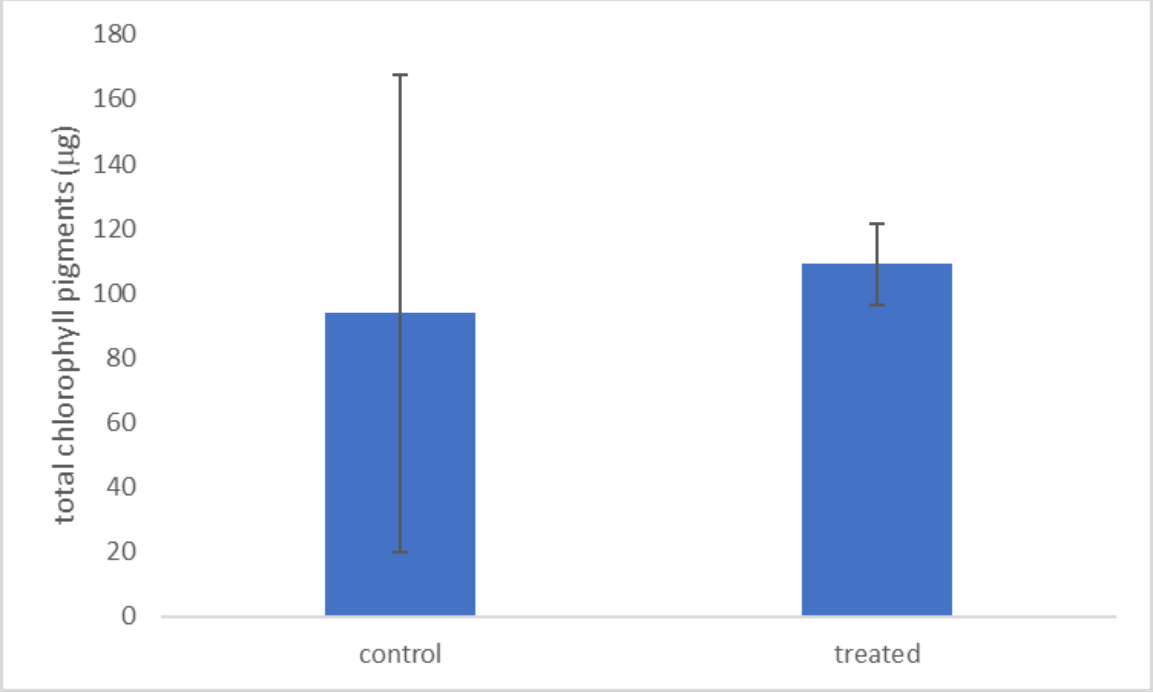


Figure C.5e: Mean total chlorophyll pigments with standard deviation bars for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste

*Table C.5a: Placement set-up for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
1	7	10	8 (c)	3 (tr)	7 (c)	3	2	6
8	2	9	2 (tr)	1 (tr)	4 (c)	1	7	5
4	6	5	5 (tr)	6 (tr)	9 (c)	8	4	9

Appendix C.6 Additional figures and tables for Trial 8: The effect of the mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with reduced slope

(Return to: 3.2.6)

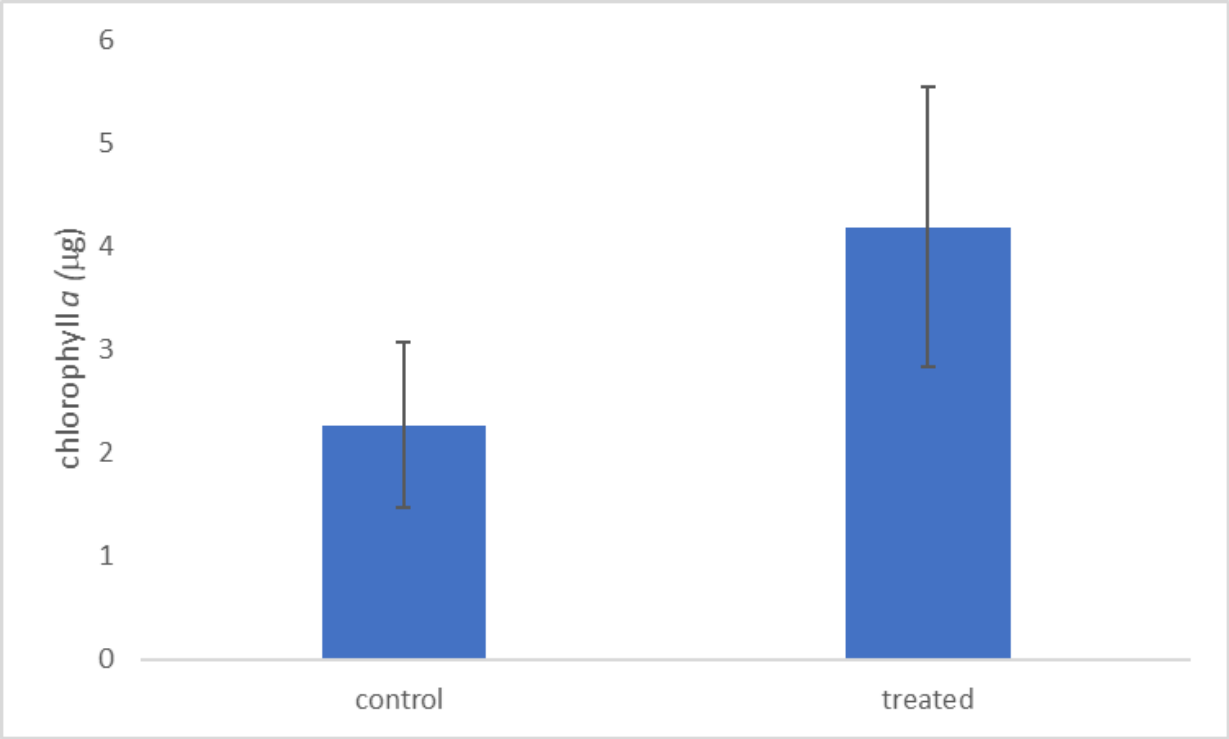

6
5
4
3
2
1
0
chlorophyll a (µg)
control
treated

Figure C.6a: Mean total chlorophyll a with standard deviation bars for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

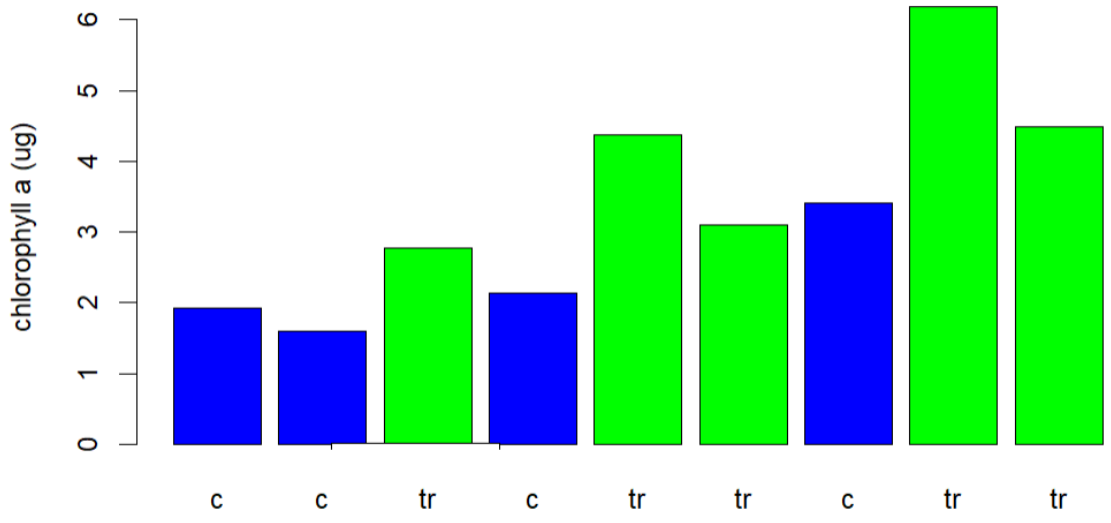


Figure C.6b: Total chlorophyll a for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

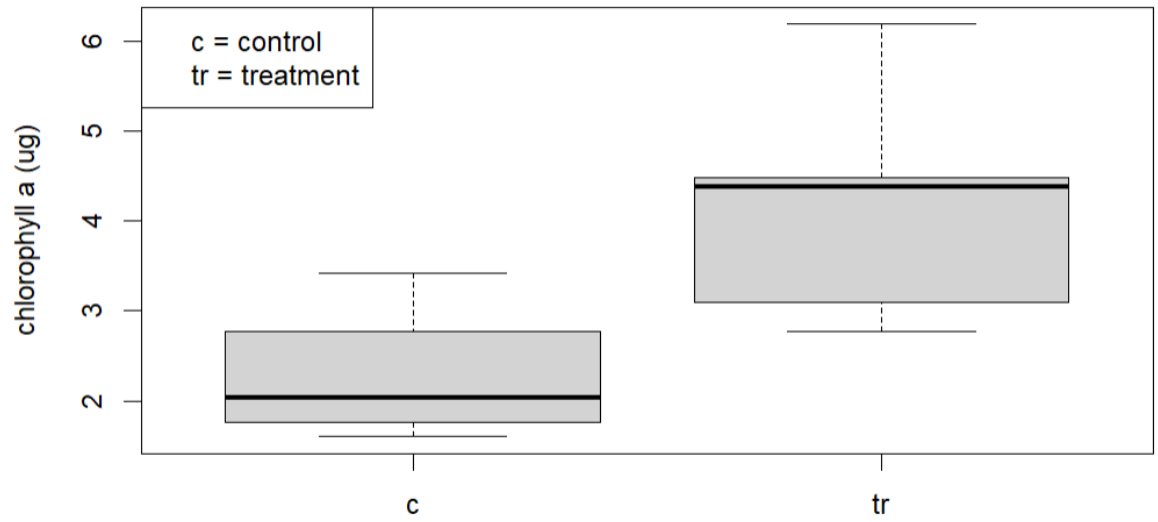


Figure C.6c: Total chlorophyll a boxplots for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

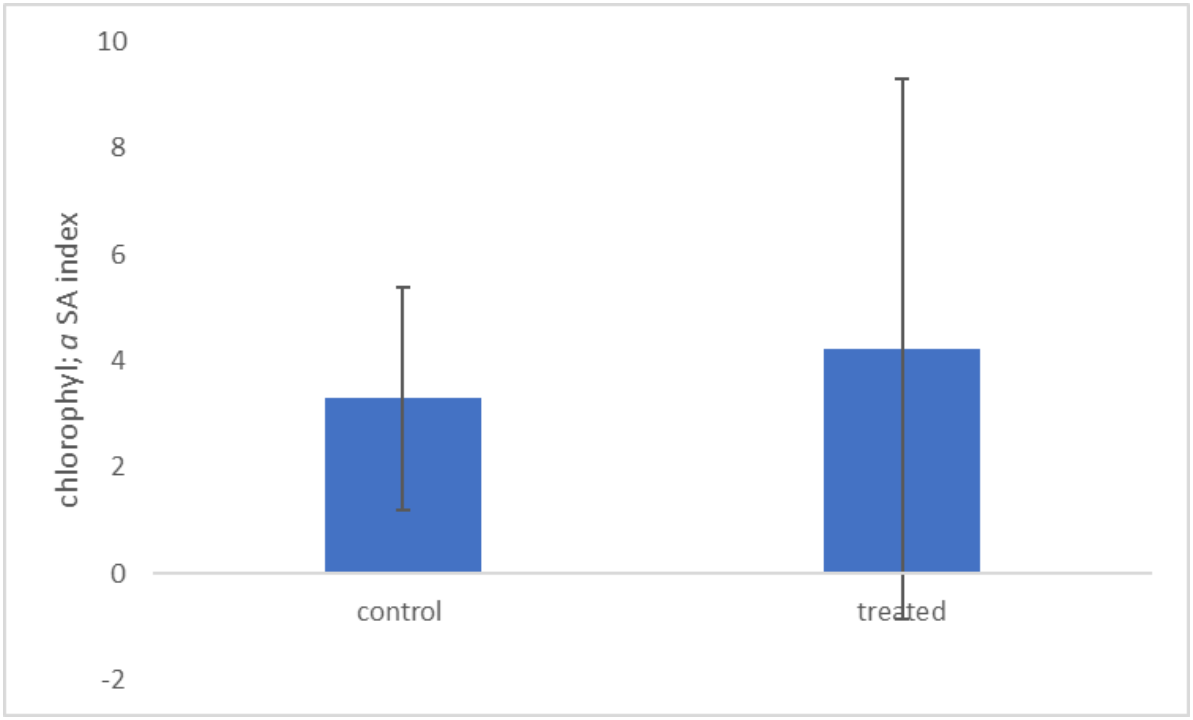


Figure C.6d: Mean chlorophyll a SA index with standard deviation bars for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

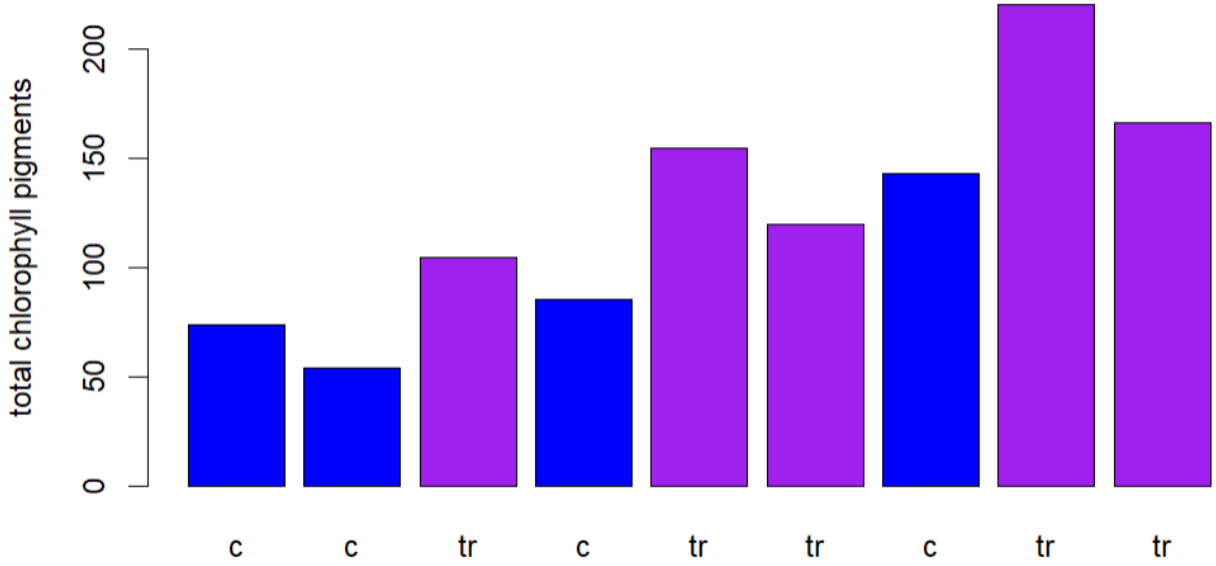


Figure C.6e: Total chlorophyll pigments for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

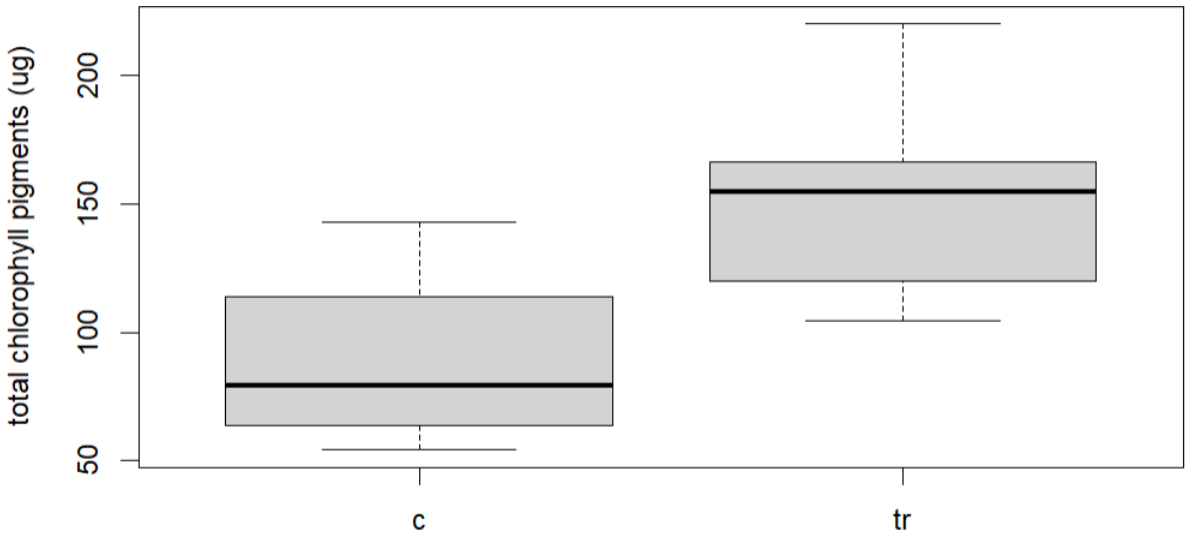


Figure C.6f: Total chlorophyll pigments boxplot for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

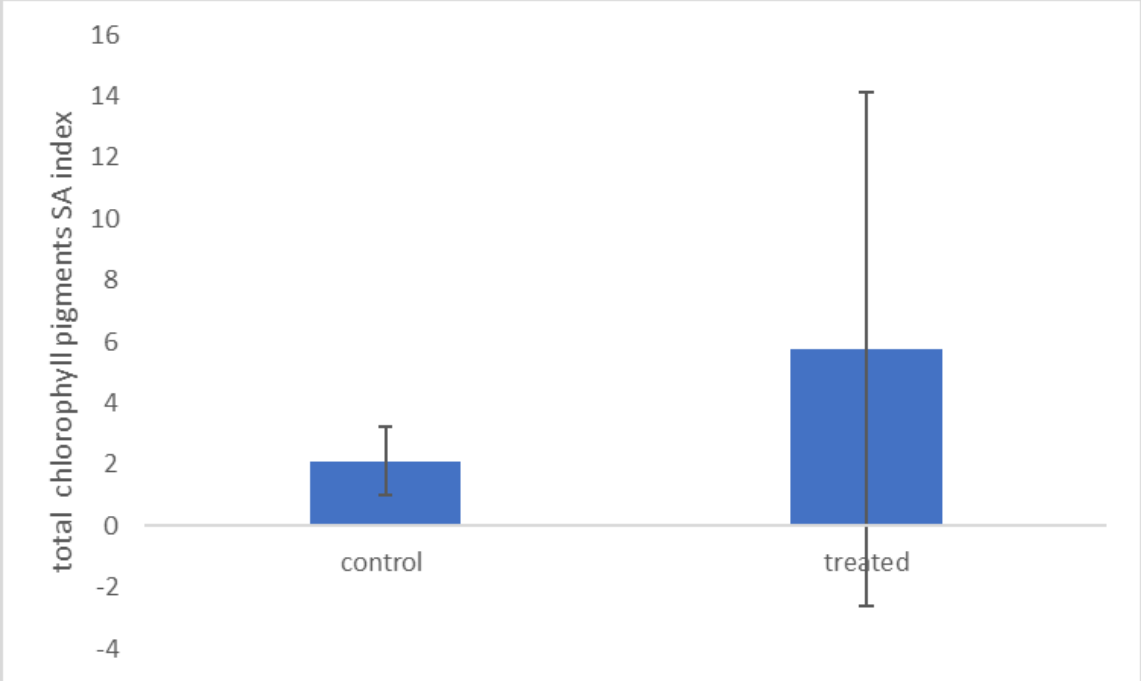


Figure C.6g: Mean total chlorophyll pigments SA index with standard deviation bars for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

*Table C.6a: Placement set-up for experiment testing Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
8	10	2	4 (c)	8 (tr)	2 (c)	7	2	1
7	4	5	7 (c)	3 (tr)	1 (c)	5	8	9
1	6	9	9 (tr)	6 (tr)	5 (tr)	3	4	6

Appendix C.7 Additional figures and tables for Trial 9: The effect of mixed bacteria biofilm in undiluted, unfiltered aquaculture waste with reduced slope

(Return to: 3.2.7)

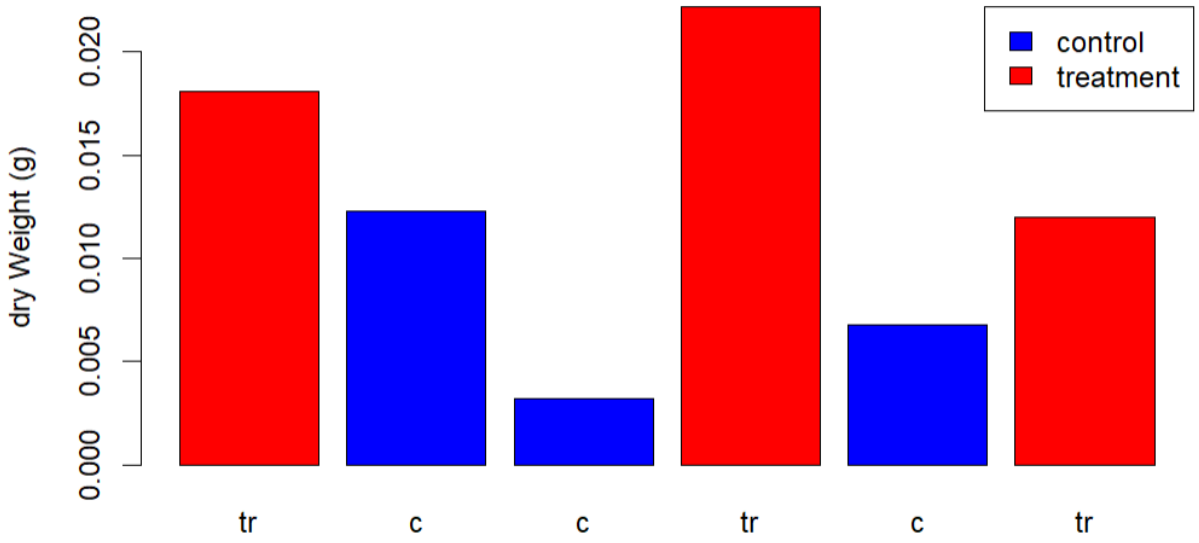


Figure C.7a: Total dry weight for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

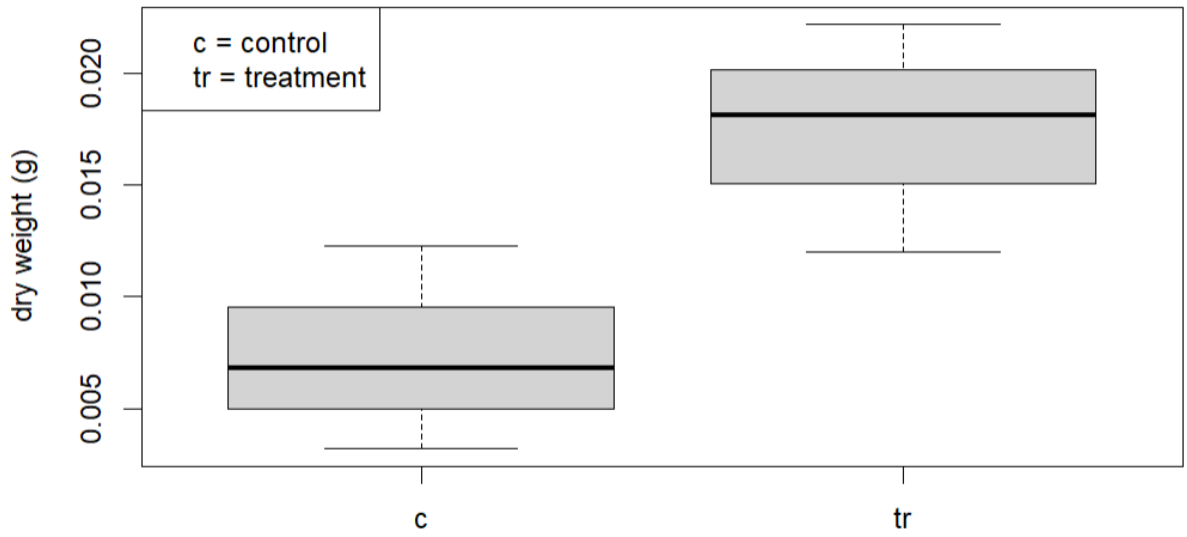


Figure C.7b: Total dry weight boxplots for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

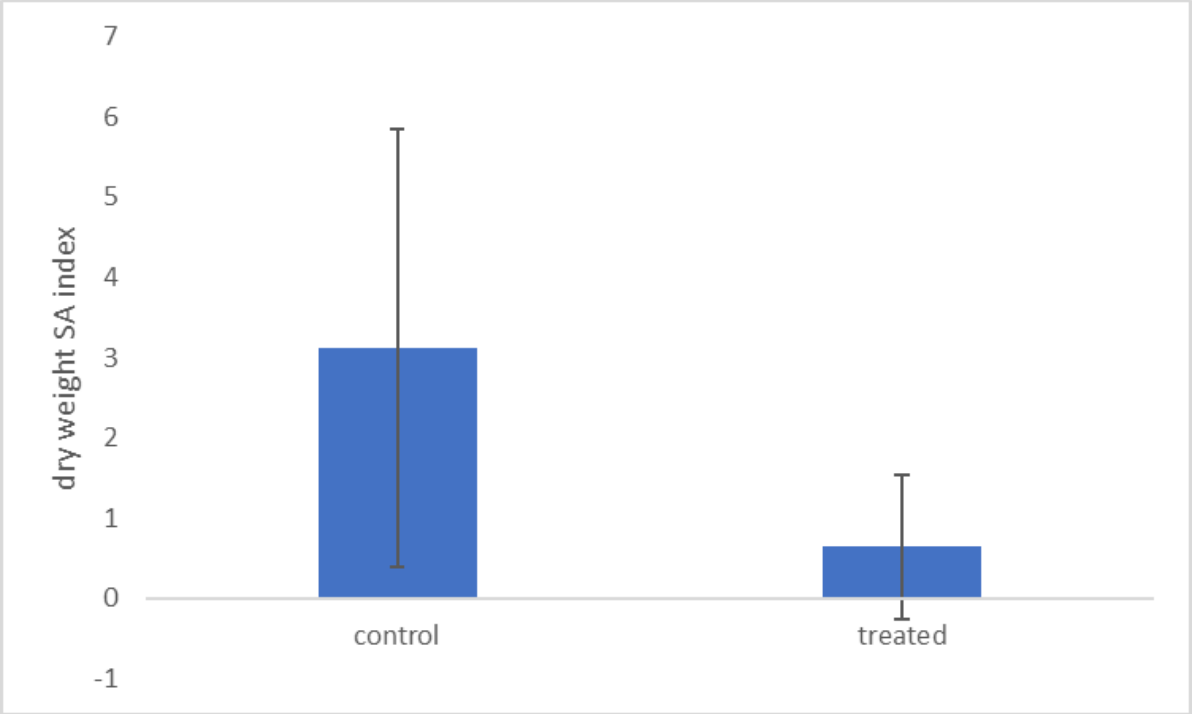


Figure C.7c: Mean dry weight SA index with standard deviation bars for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

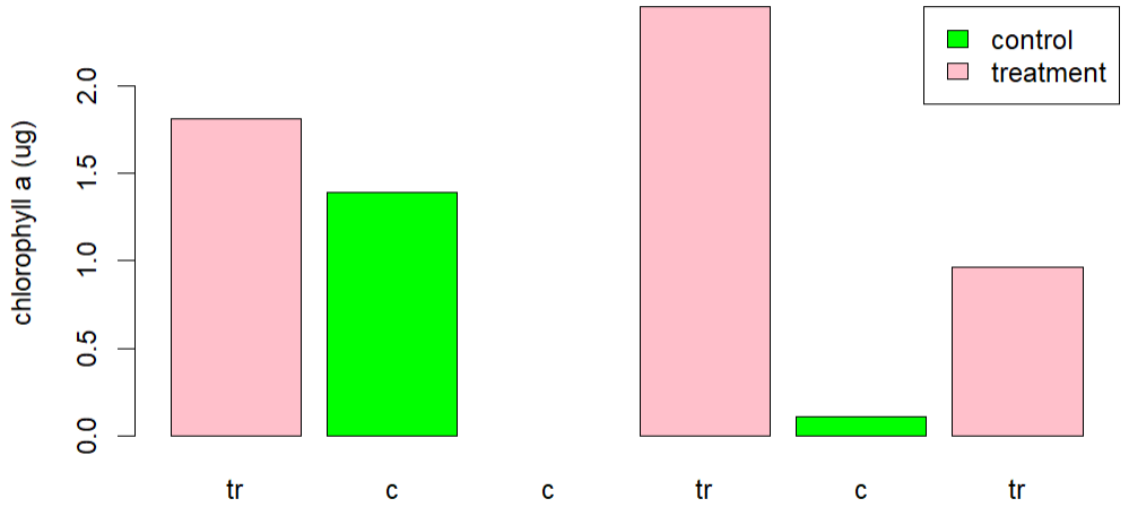


Figure C.7d: Total chlorophyll a for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

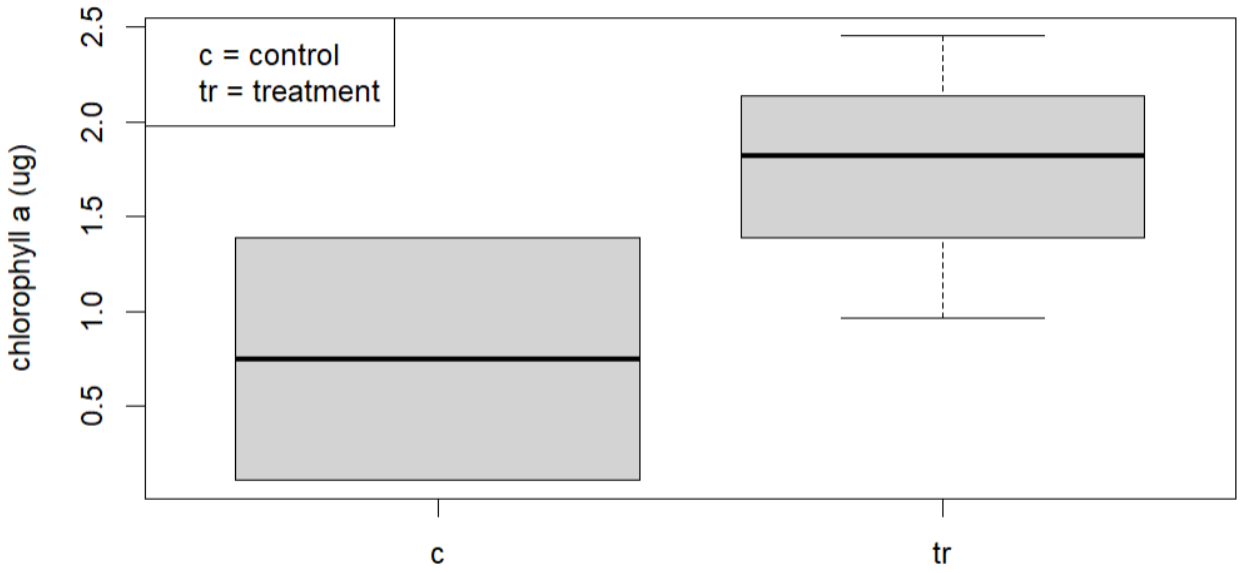


Figure C.7e: Total chlorophyll a boxplots for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

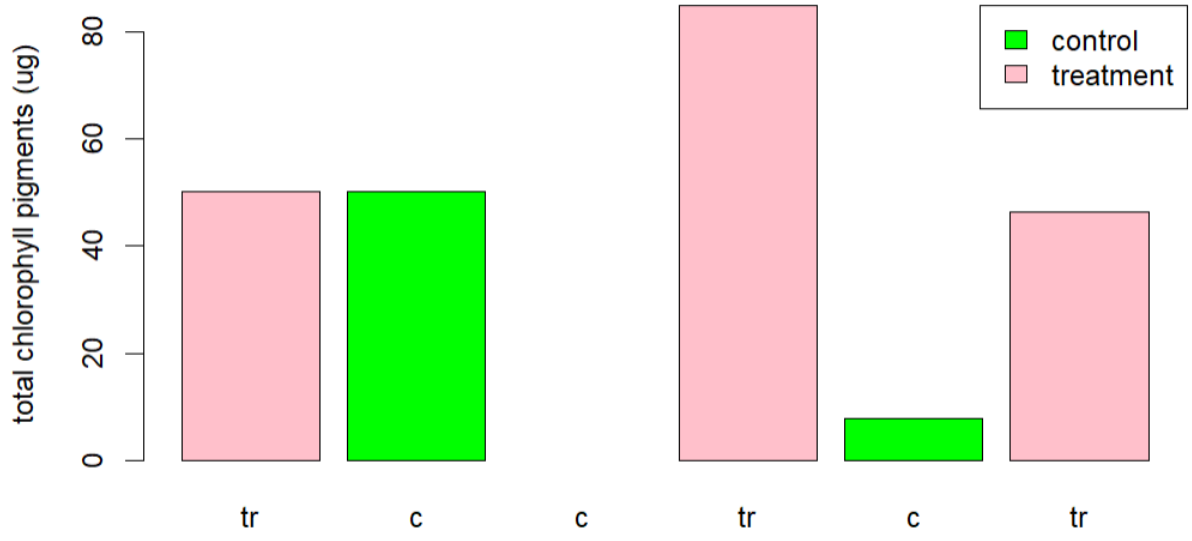


Figure C.7f: Total chlorophyll pigments for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

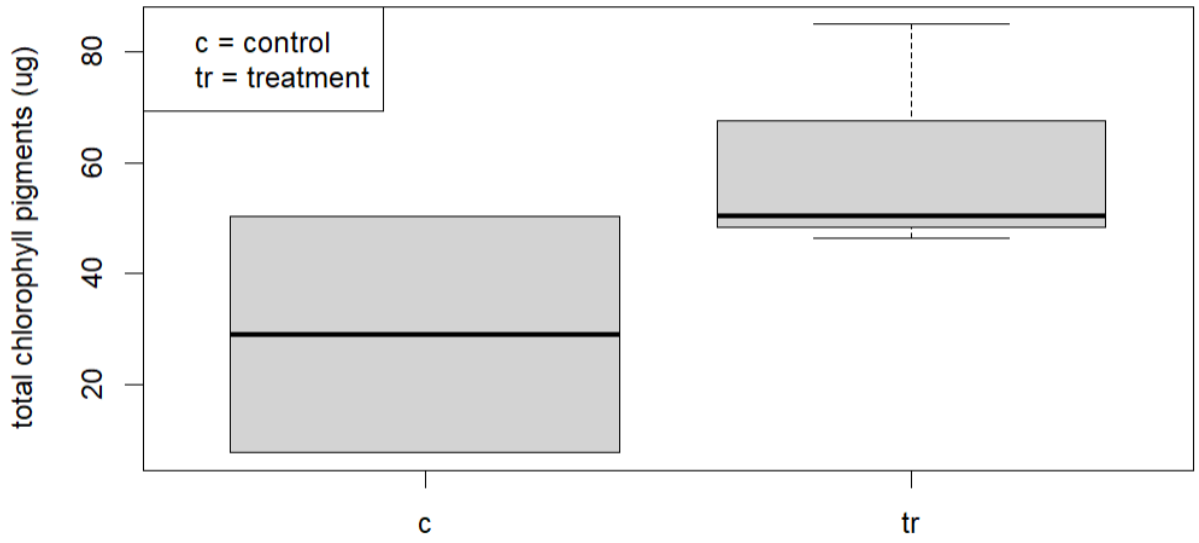


Figure C.7g: Total chlorophyll pigments boxplot for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

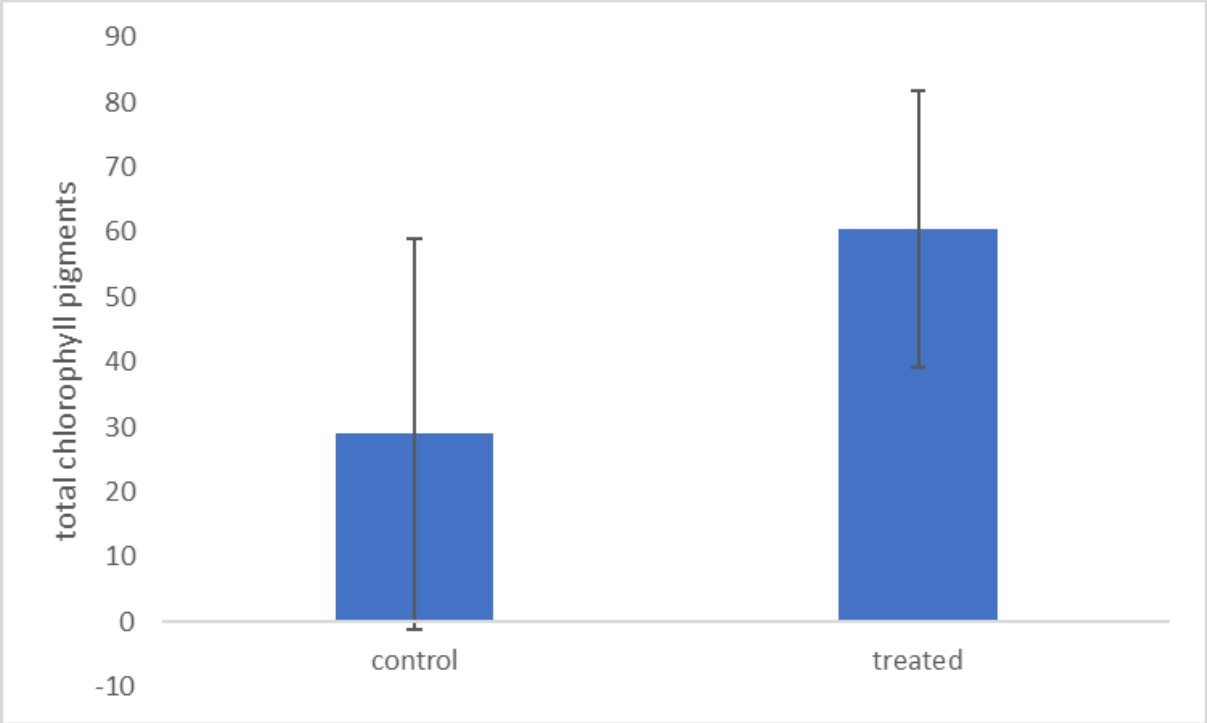


Figure C.7i: Mean total chlorophyll pigments with standard deviation bars for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

*Table C.7a: Placement set-up for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
9	7	6	7 (c)	3 (c)	6 (tr)	5	6	8
4	10	1	9 (tr)	8 (tr)	2 (c)	2	1	3
5	2	8	1 (tr)	4 (c)	5 (tr)	9	4	7

Appendix C.8 Additional figures and tables for Trial 10: The effect of mixed bacteria biofilm in half-diluted, unfiltered aquaculture waste with reduced slope

(Return to: 3.2.8)

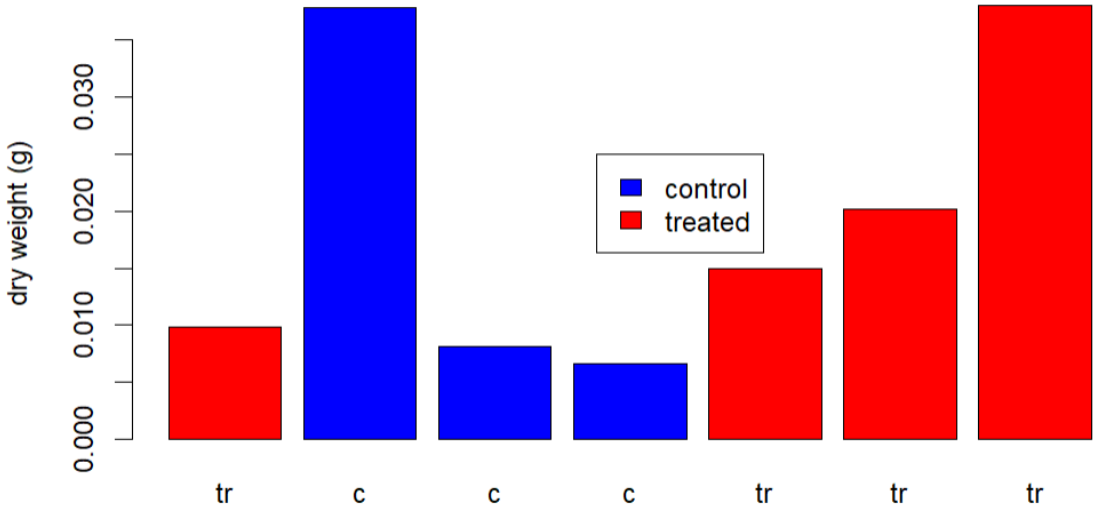


Figure C.8a: Total dry weight for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

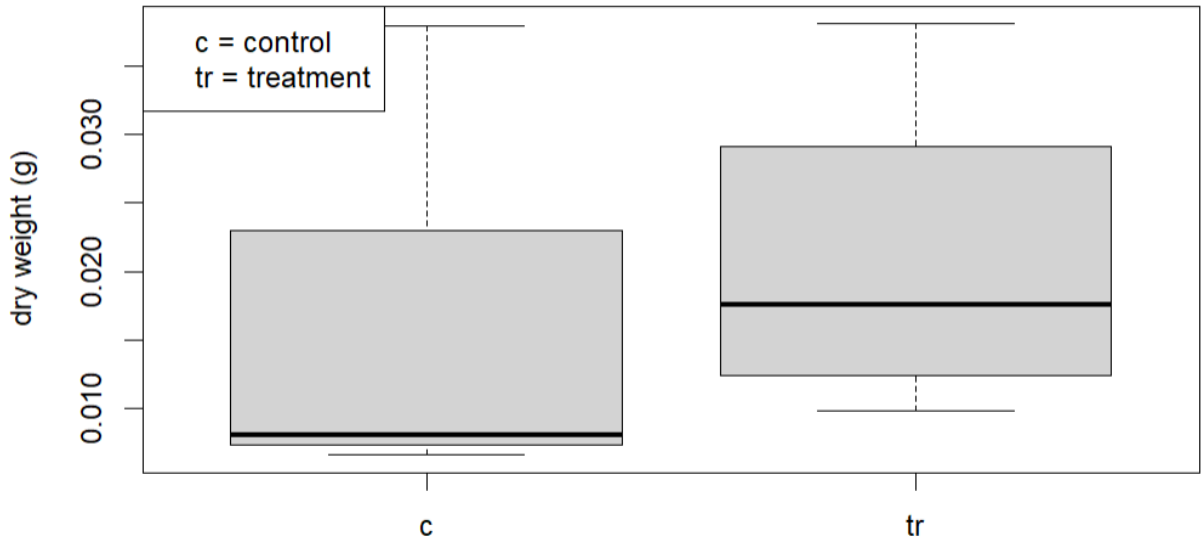


Figure C.8b: Total dry weight boxplots for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

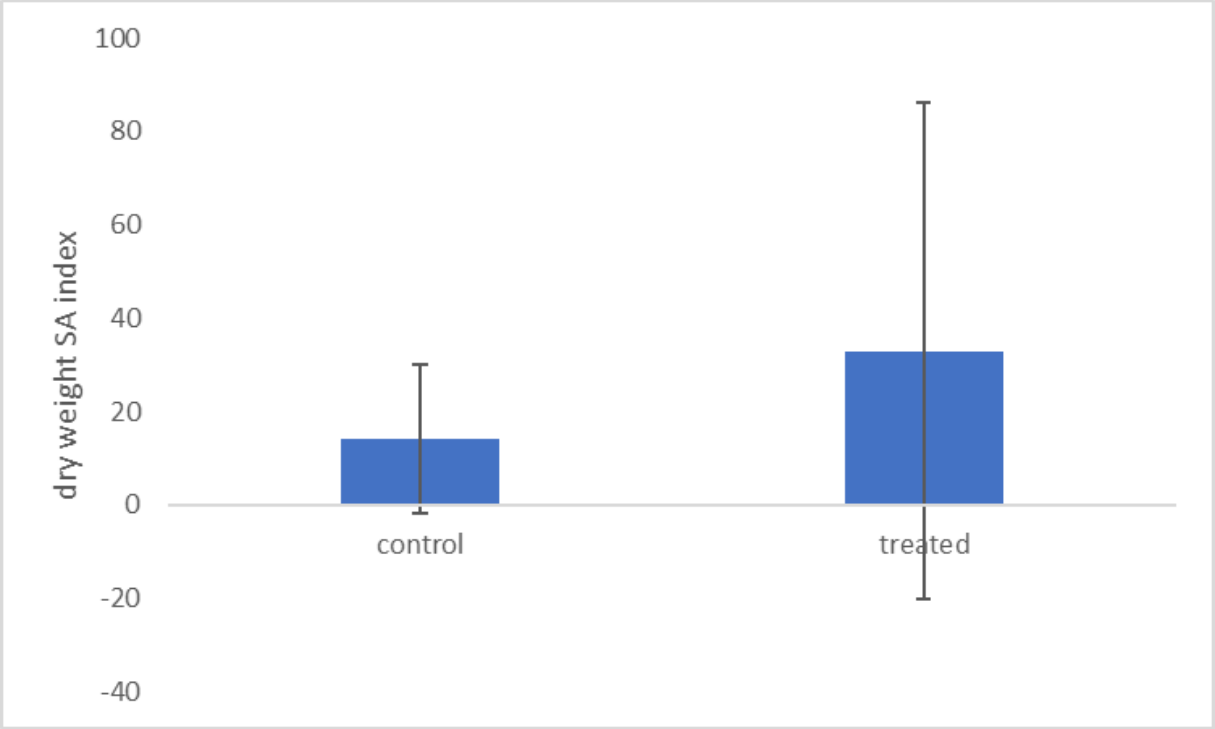


Figure C.8c: Mean dry weight SA index with standard deviation bars for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

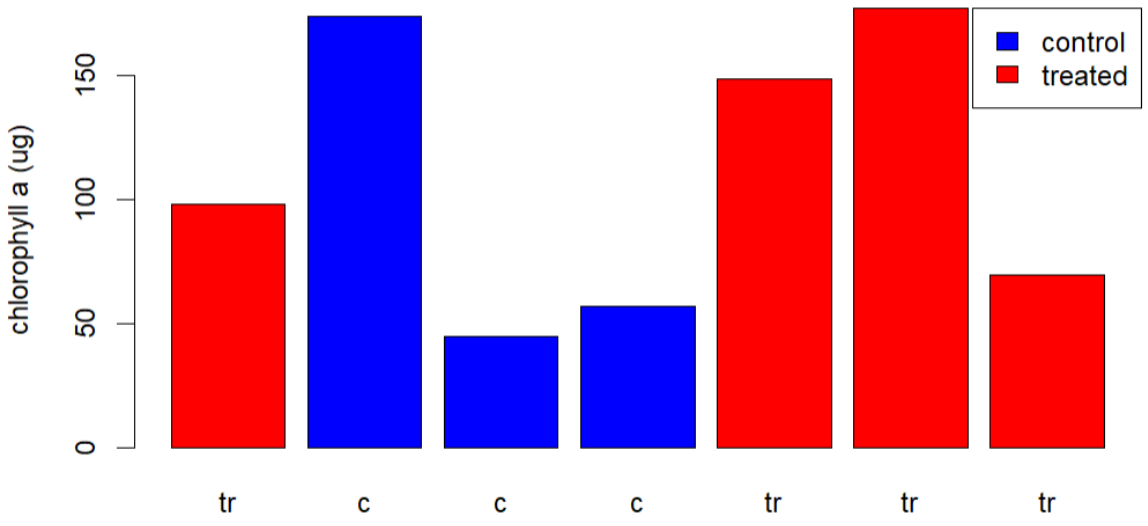


Figure C.8d: Total chlorophyll a for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

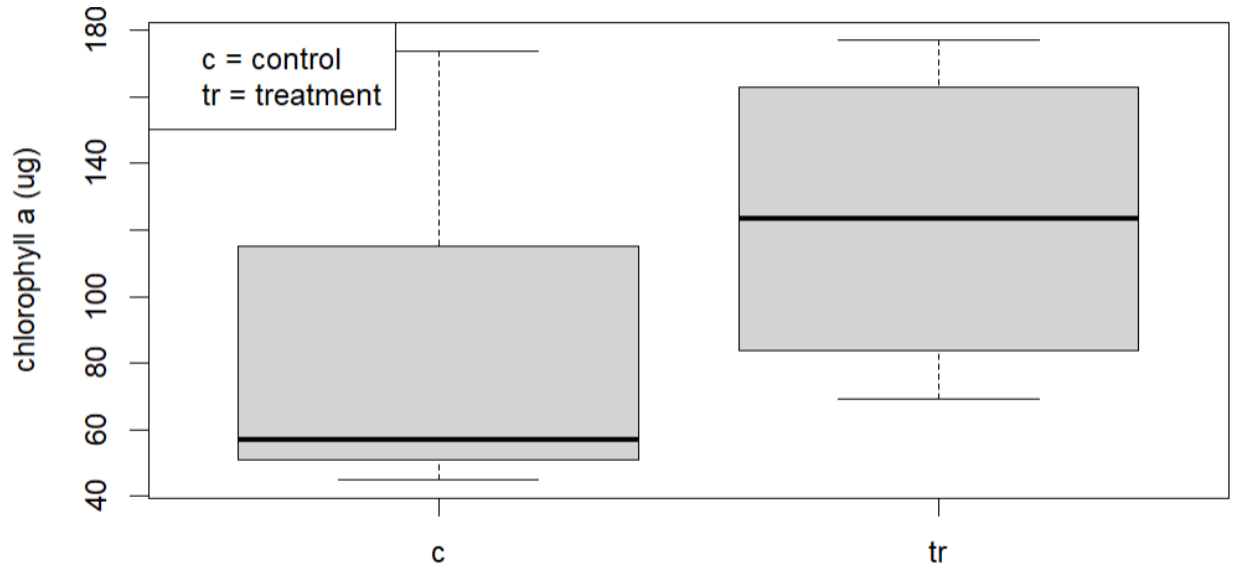


Figure C.8e: Total chlorophyll a boxplots for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

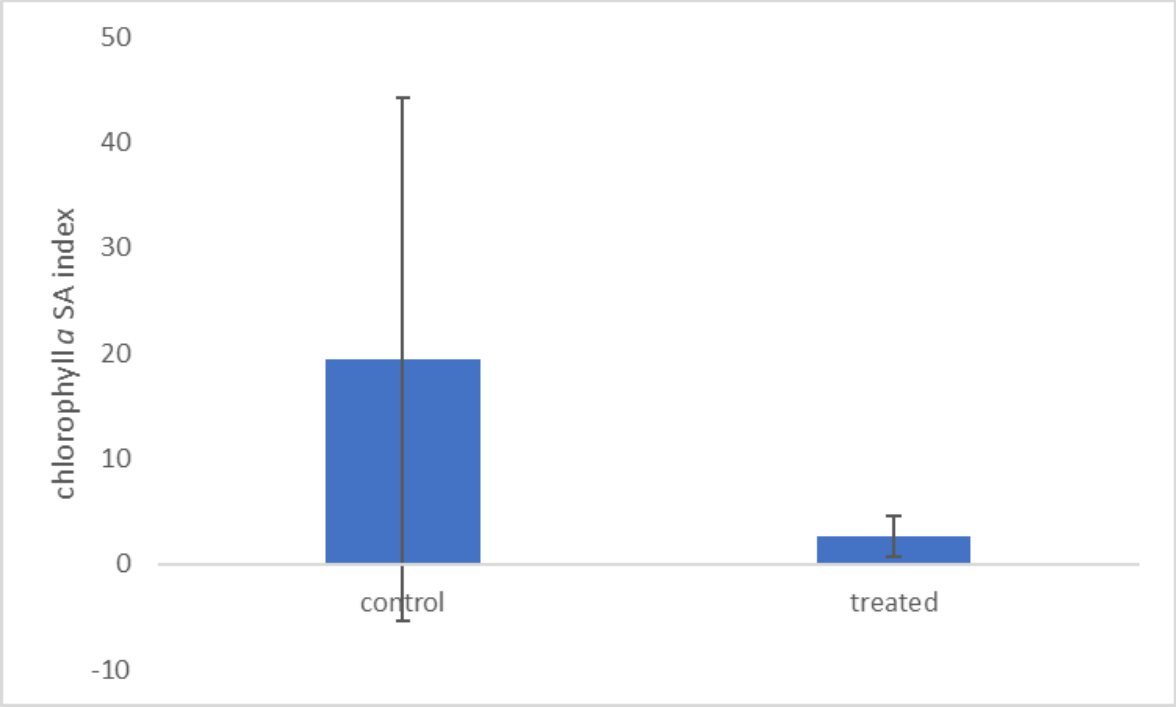


Figure C.8f: Mean chlorophyll a SA index with standard deviation bars for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

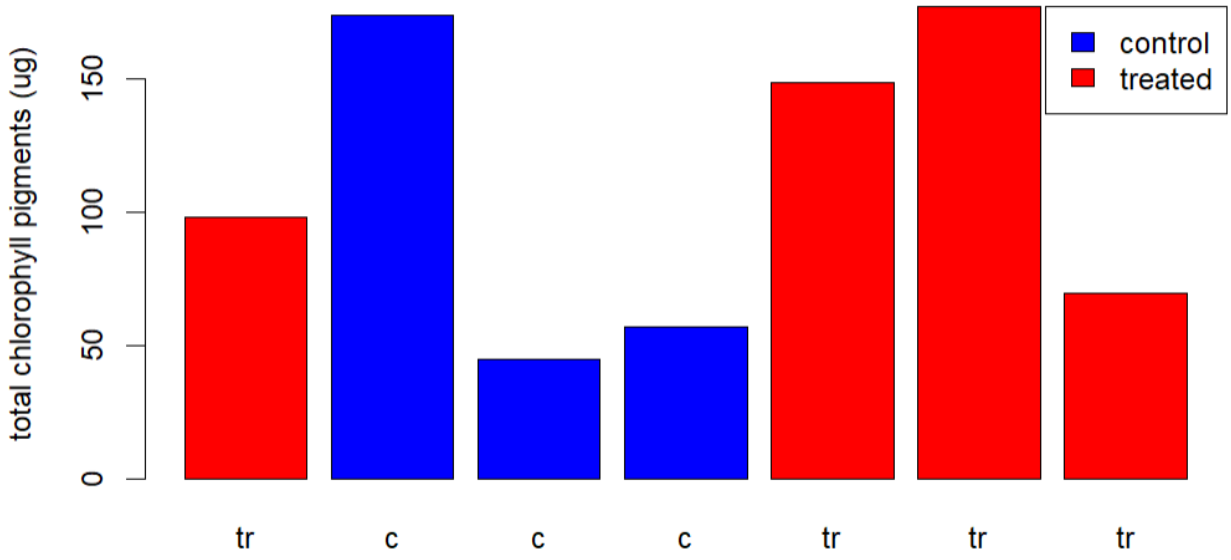


Figure C.8g: Total chlorophyll pigments for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

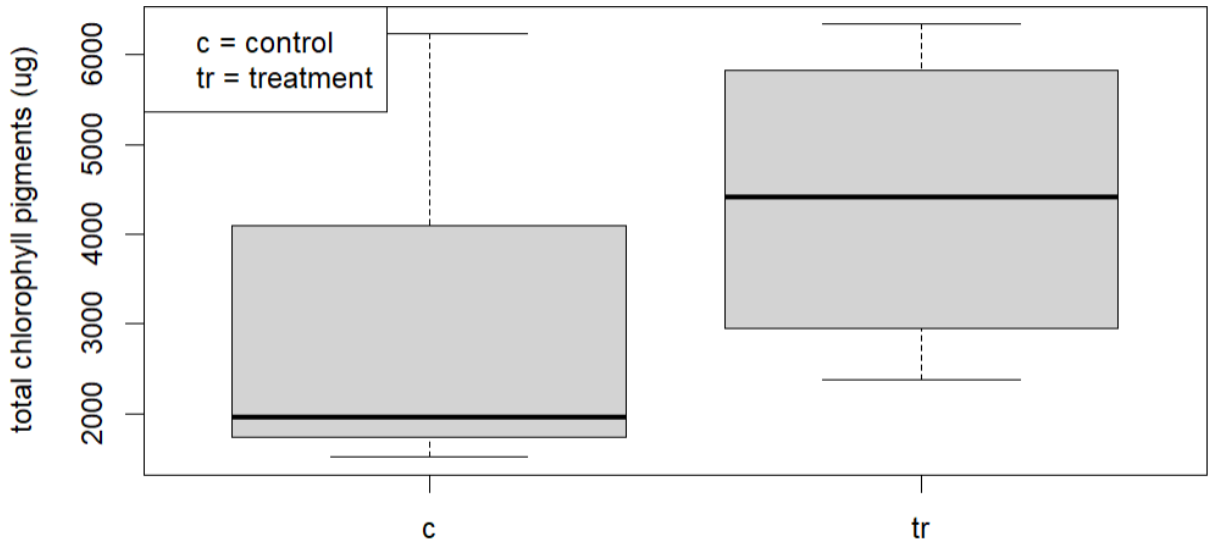


Figure C.8h: Total chlorophyll pigments boxplot for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

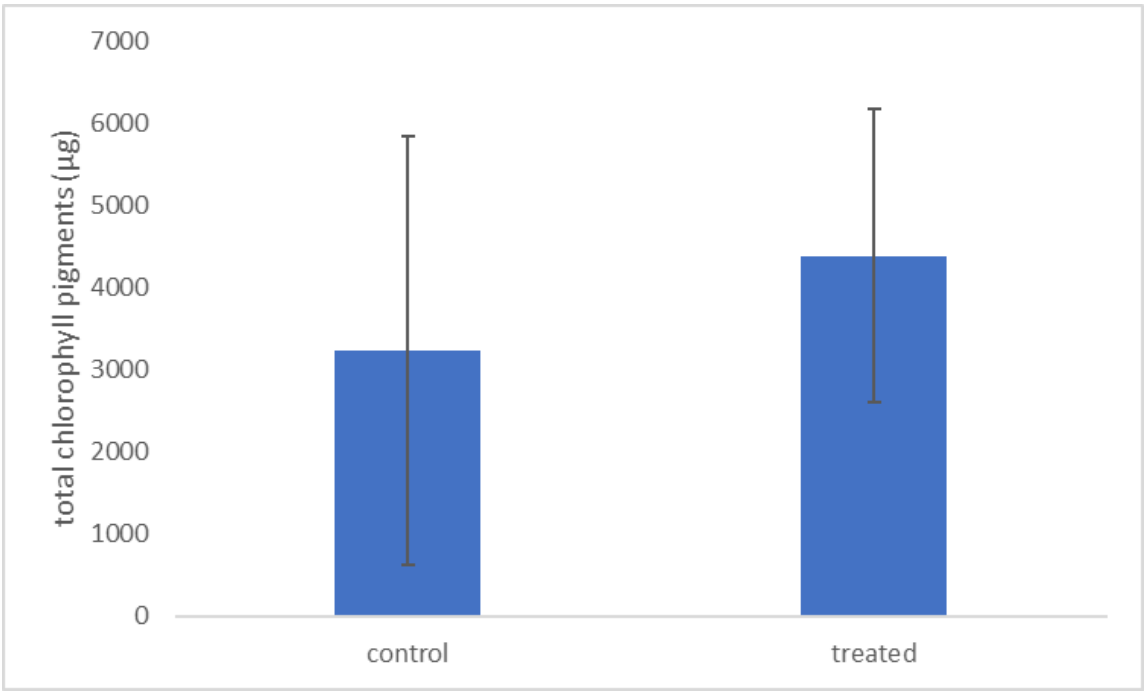


Figure C.8i: Mean total chlorophyll pigments with standard deviation bars for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

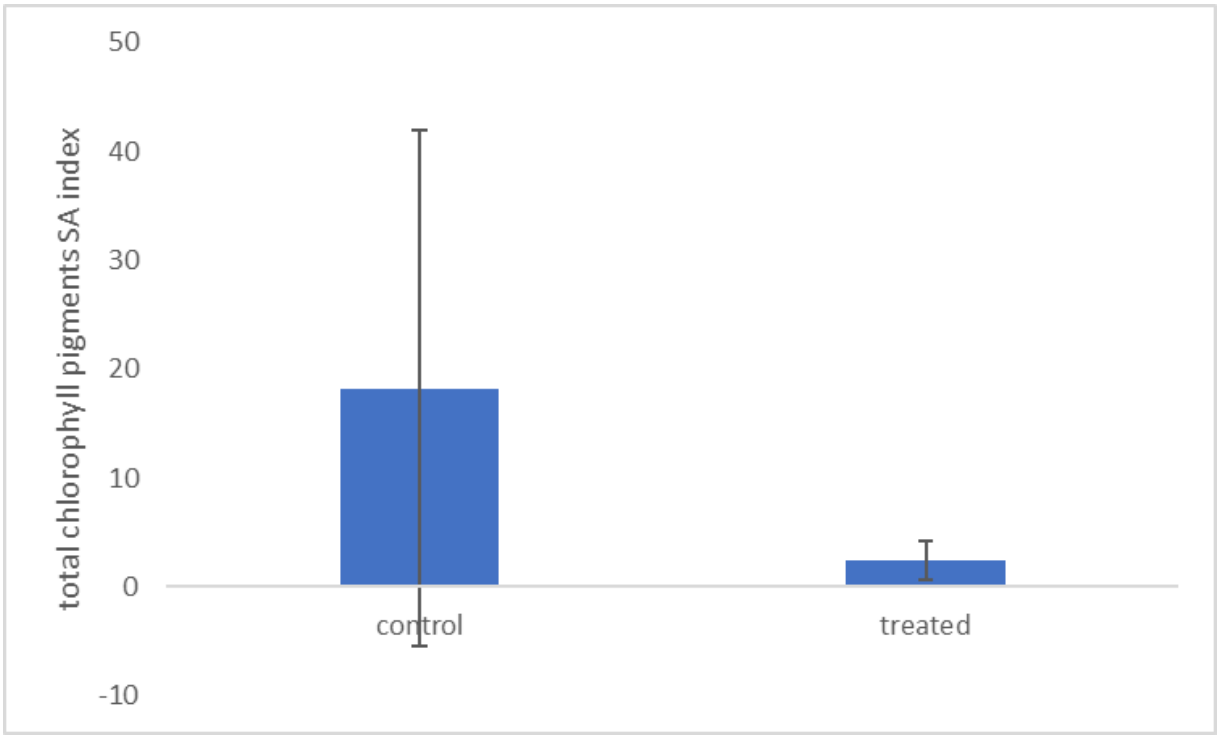


Figure C.8j: Mean total chlorophyll pigments SA index with standard deviation bars for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

Table C.8a: Placement set-up for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a

**indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
8	5	2	5 (c)	4 (c)	1 (tr)	7	3	5
6	9	1	9 (tr)	8 (tr)	2 (c)	4	6	1
10	7	4	7 (tr)	3 (c)	6 (tr)	9	2	8

Appendix C.9 Additional figures and tables for Trial 11: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

(Return to: 3.2.9)

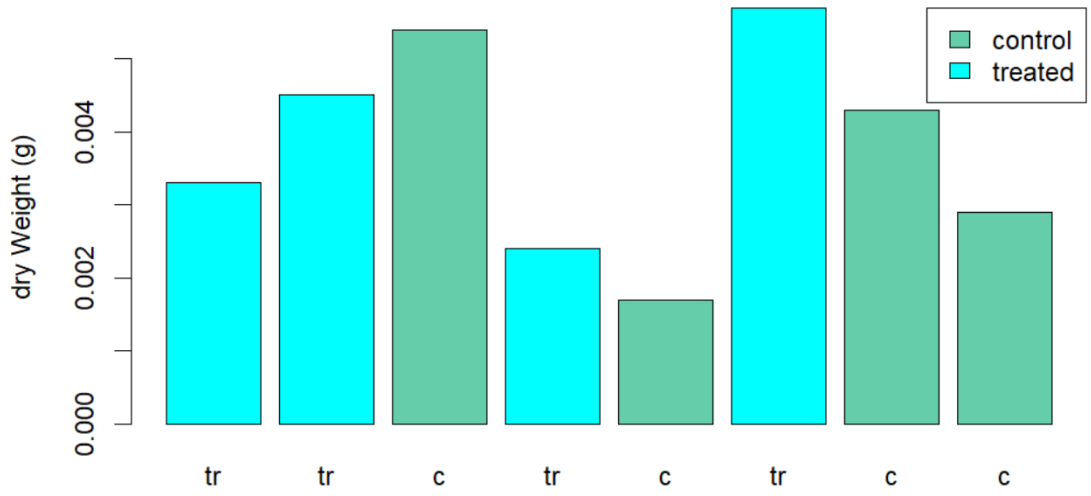


Figure C.9a: Total dry weight for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

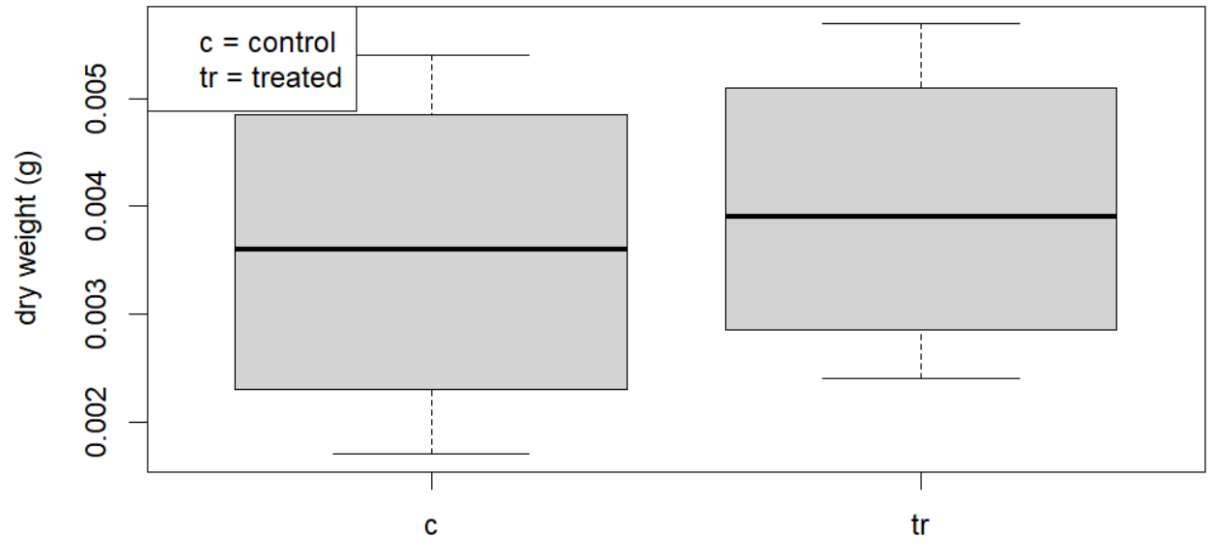


Figure C.9b: Total dry weight boxplots for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

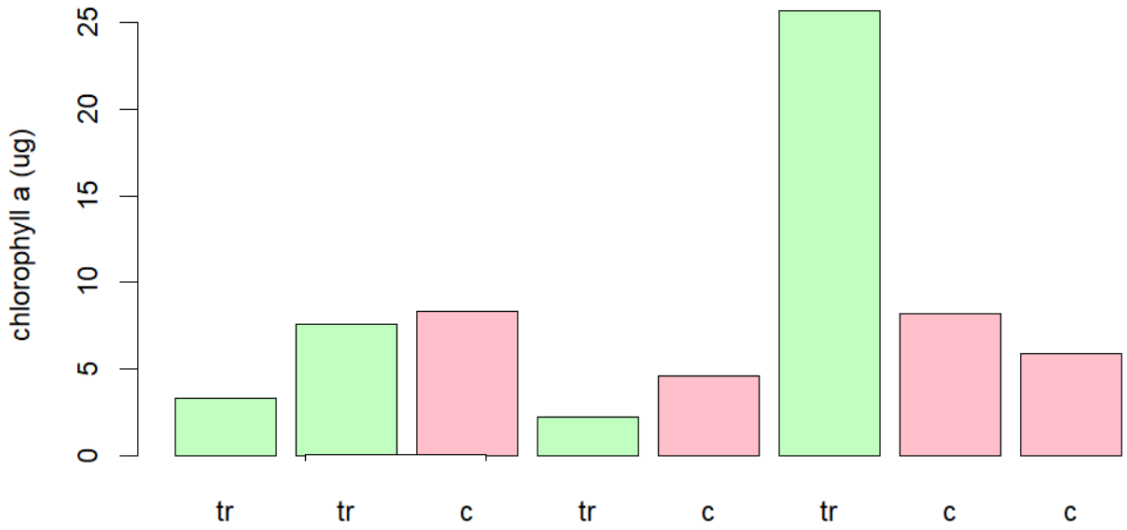


Figure C.9c: Total chlorophyll a for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

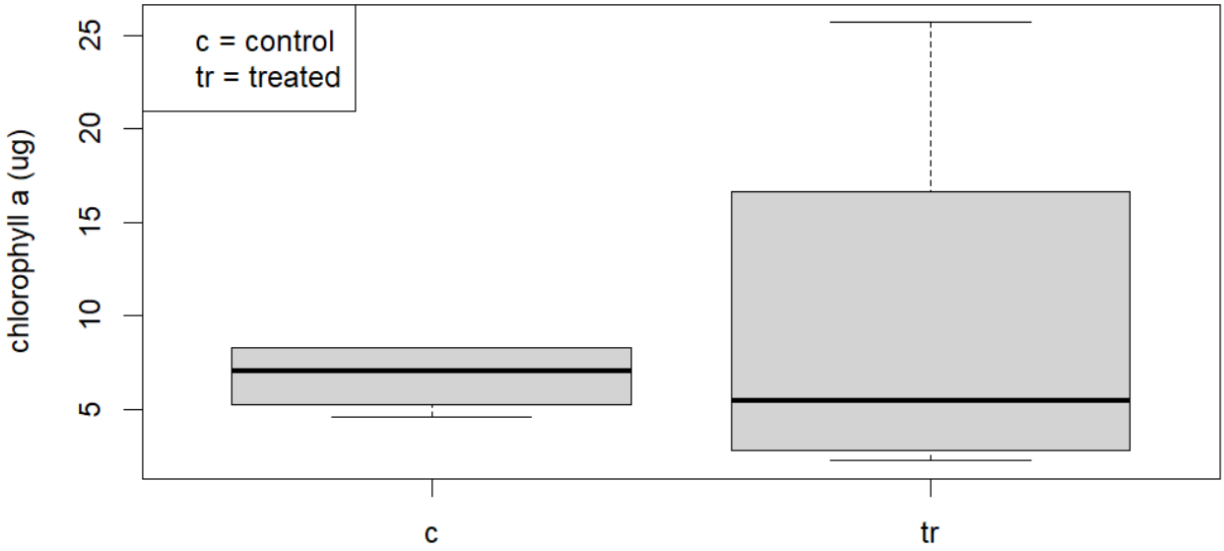


Figure C.9d: Total chlorophyll a boxplots for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

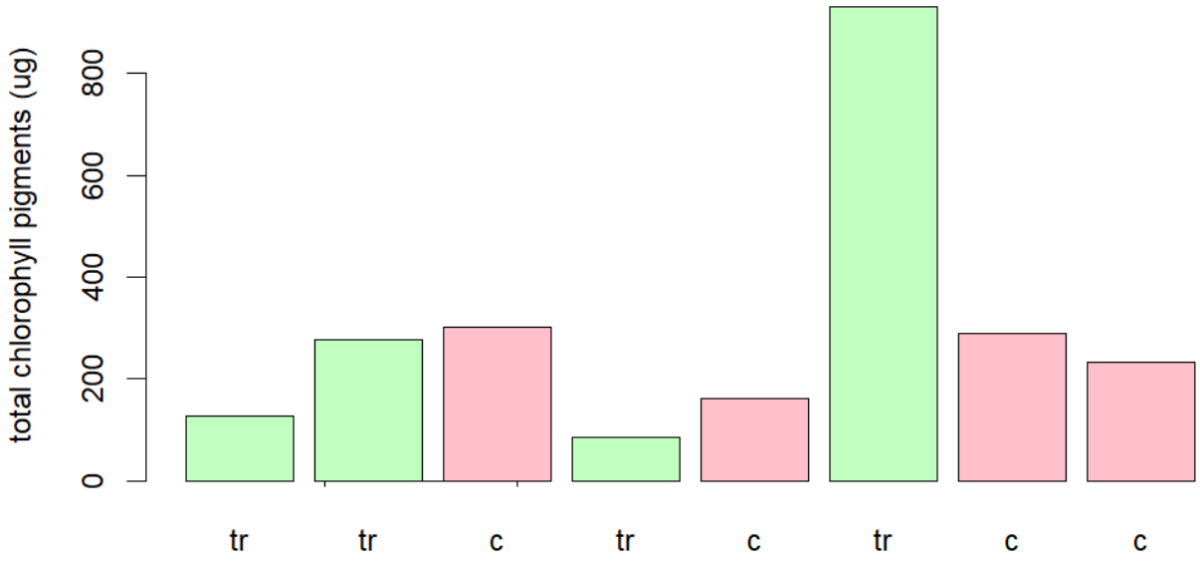


Figure C.9e: Total chlorophyll pigments for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

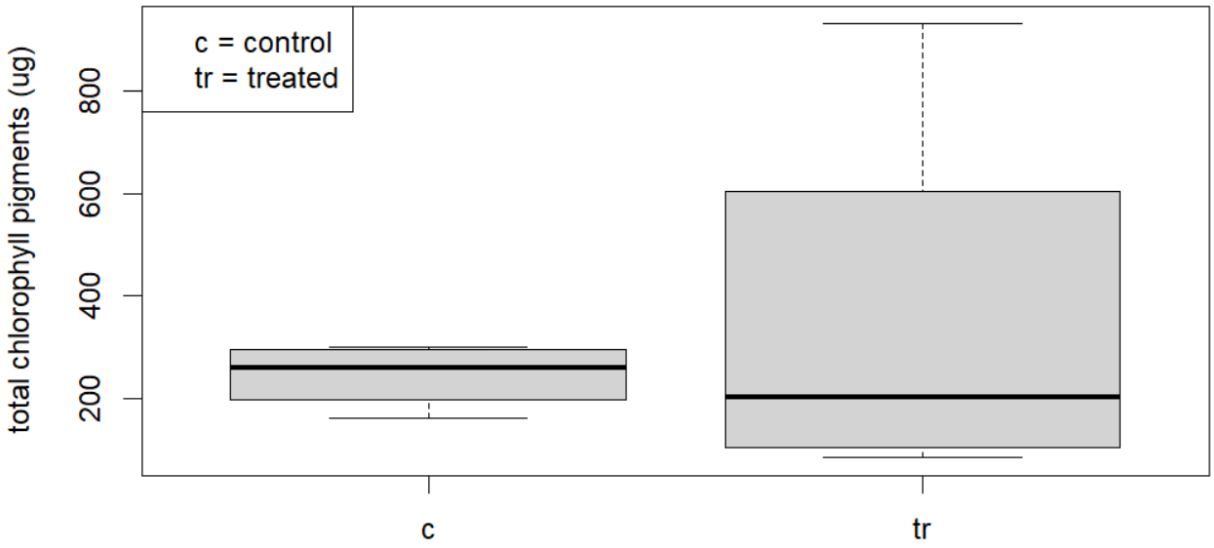


Figure C.9f: Total chlorophyll pigments boxplot for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

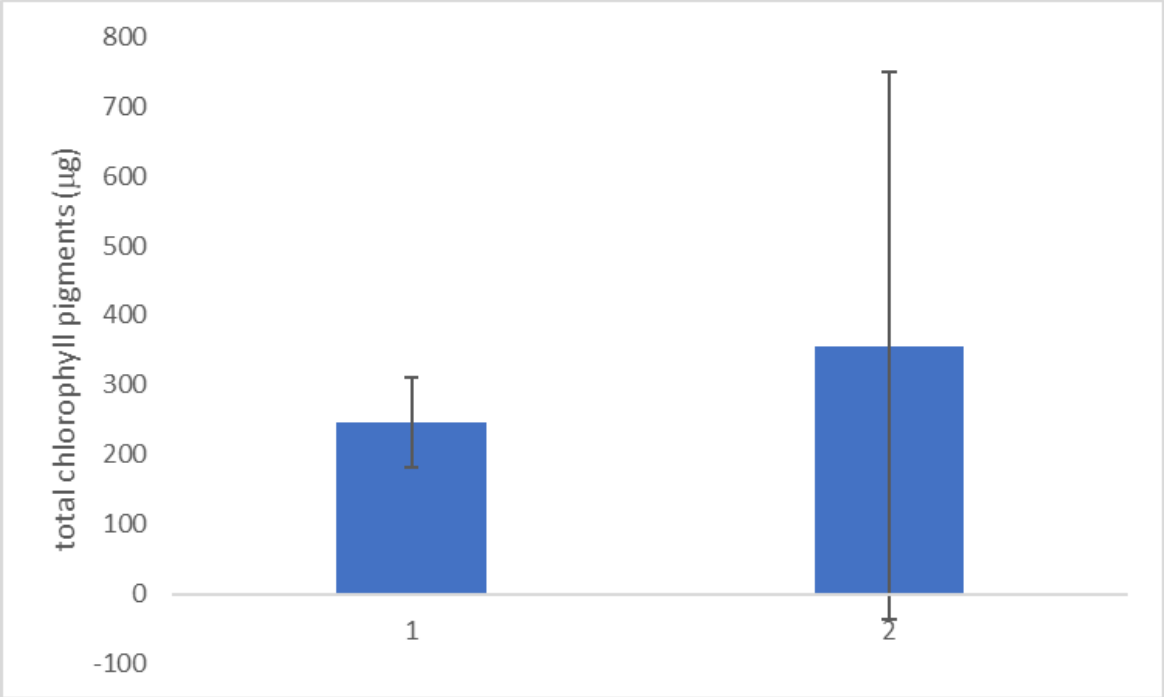


Figure C.9g: Mean total chlorophyll pigments with standard deviation bars for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

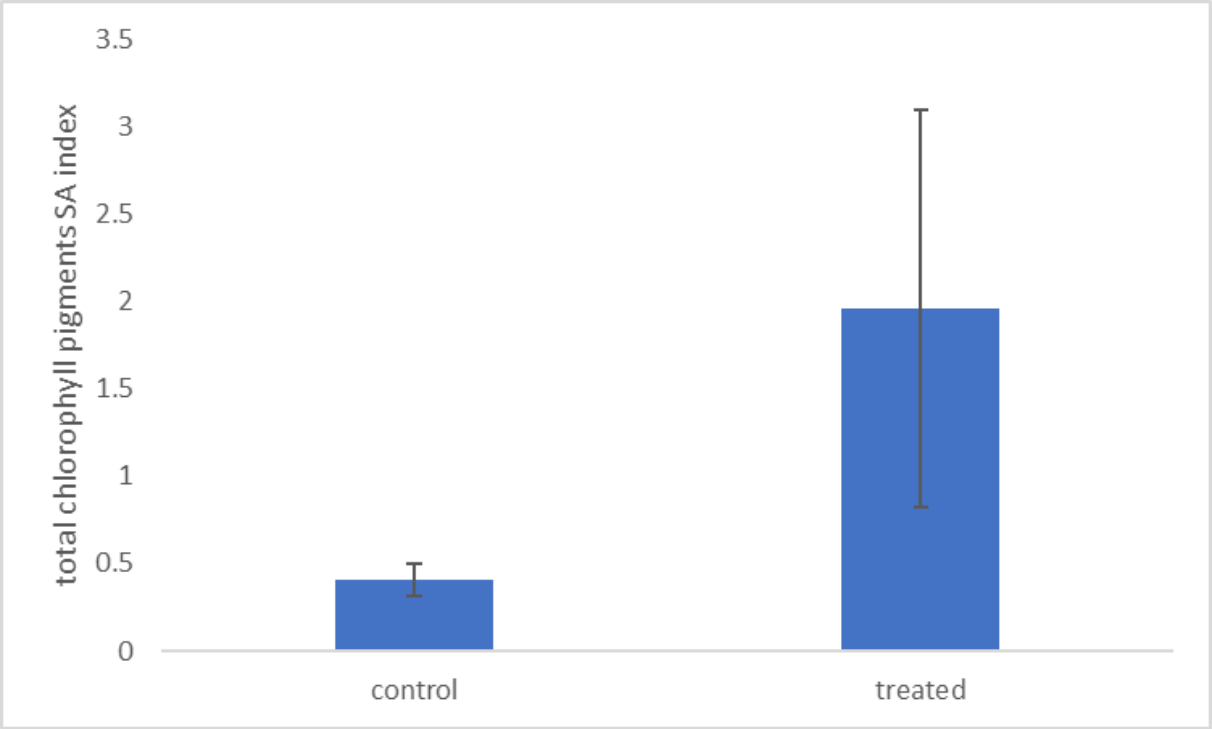


Figure C.9h: Mean total chlorophyll pigments SA index with standard deviation bars for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope

Table C.9a: Placement set-up for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with heavily reduced slope with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to

*bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and "c" indicates a control system)*

Lids			Systems			Substrata		
7	4	10	3 (c)	2 (c)	6 (c)	3	7	8
2	6	5	9 (c)	1 (tr)	3 (tr)	5	6	2
9	1	8	5 (tr)	8 (tr)	7 (tr)	9	4	1

Appendix C.10 Additional figures and tables for Trial 12: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste

(Return to: 3.2.10)

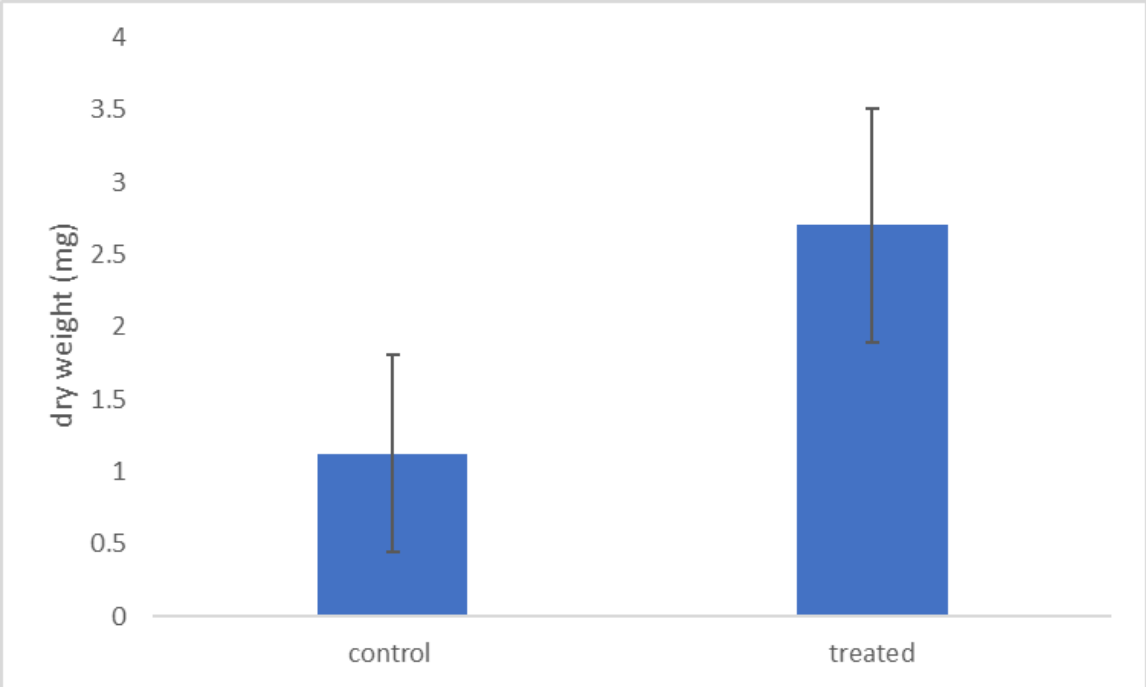


Figure C.10a: Mean dry weight with standard deviation bars for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

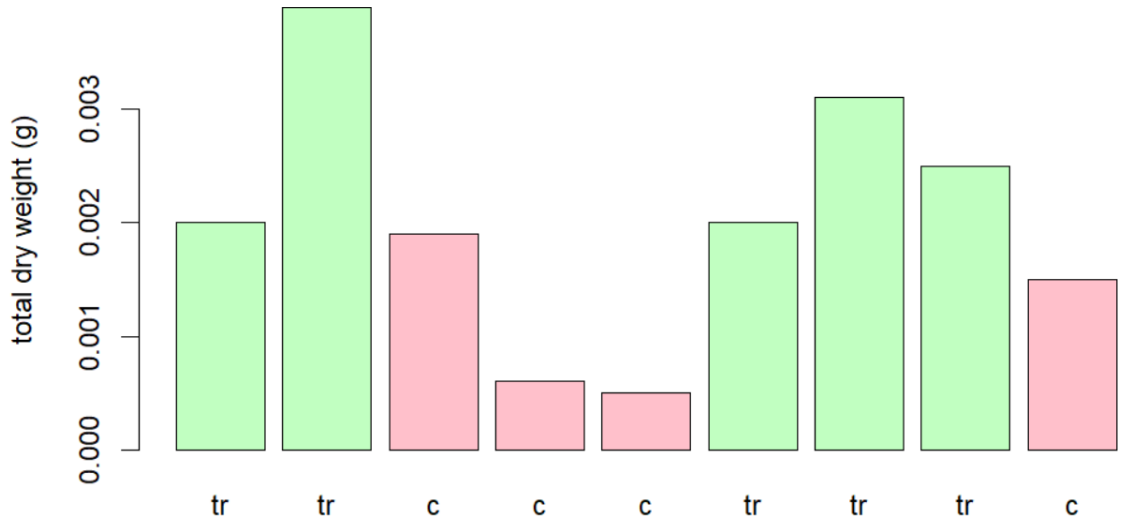


Figure C.10b: Total dry weight for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

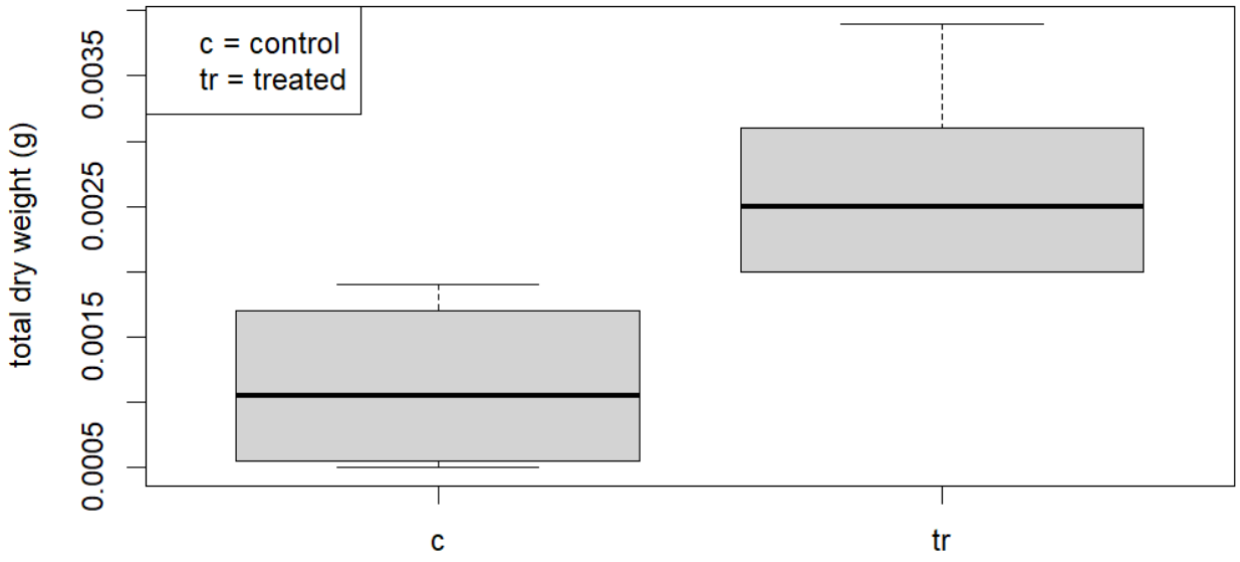


Figure C.10c: Total dry weight boxplots for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

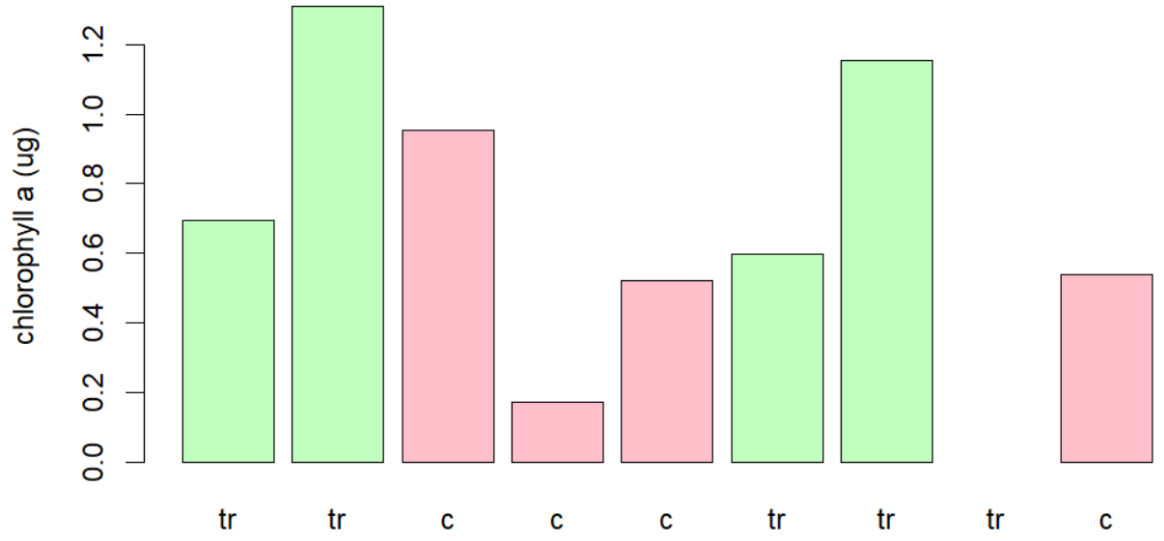


Figure C.10d: Total chlorophyll a for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

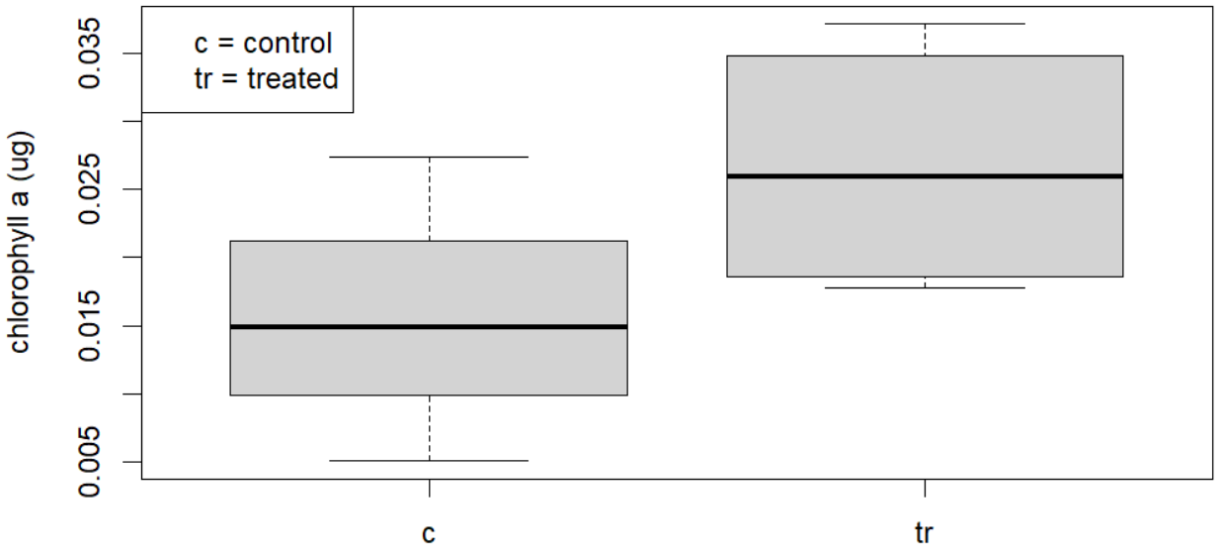


Figure C.10e: Total chlorophyll a boxplots for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

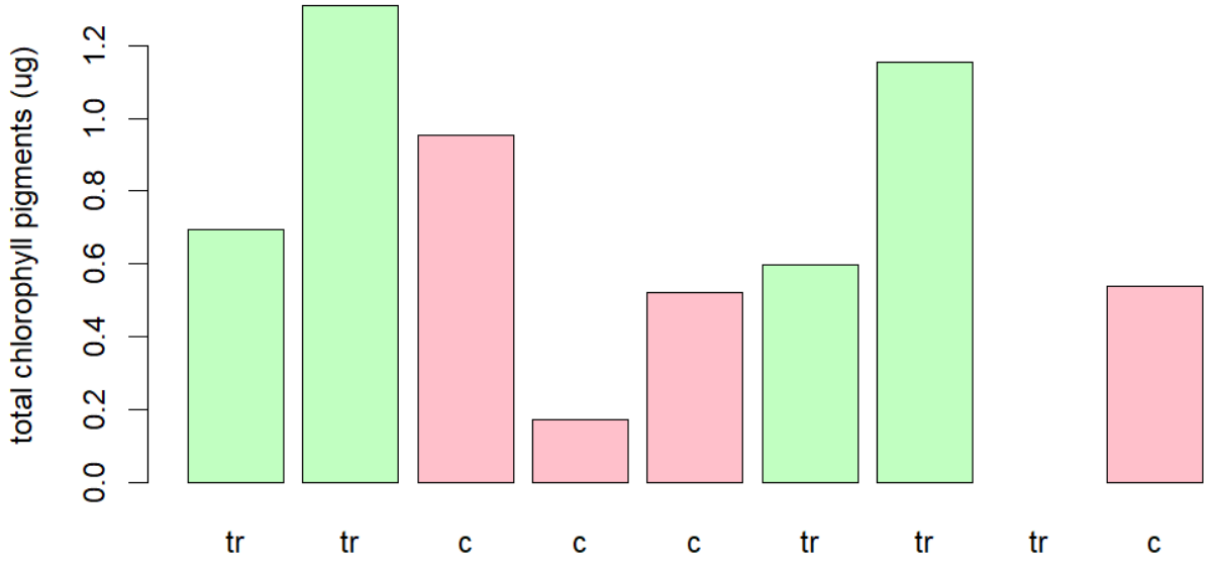


Figure C.10f: Total chlorophyll pigments for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

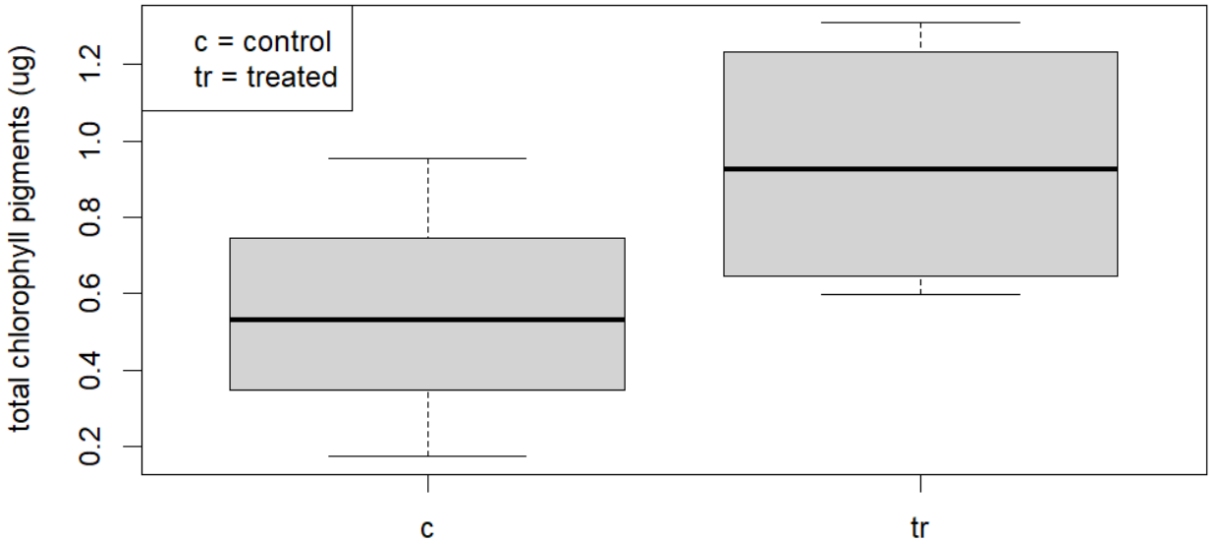


Figure C.10g: Total chlorophyll pigments boxplot for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

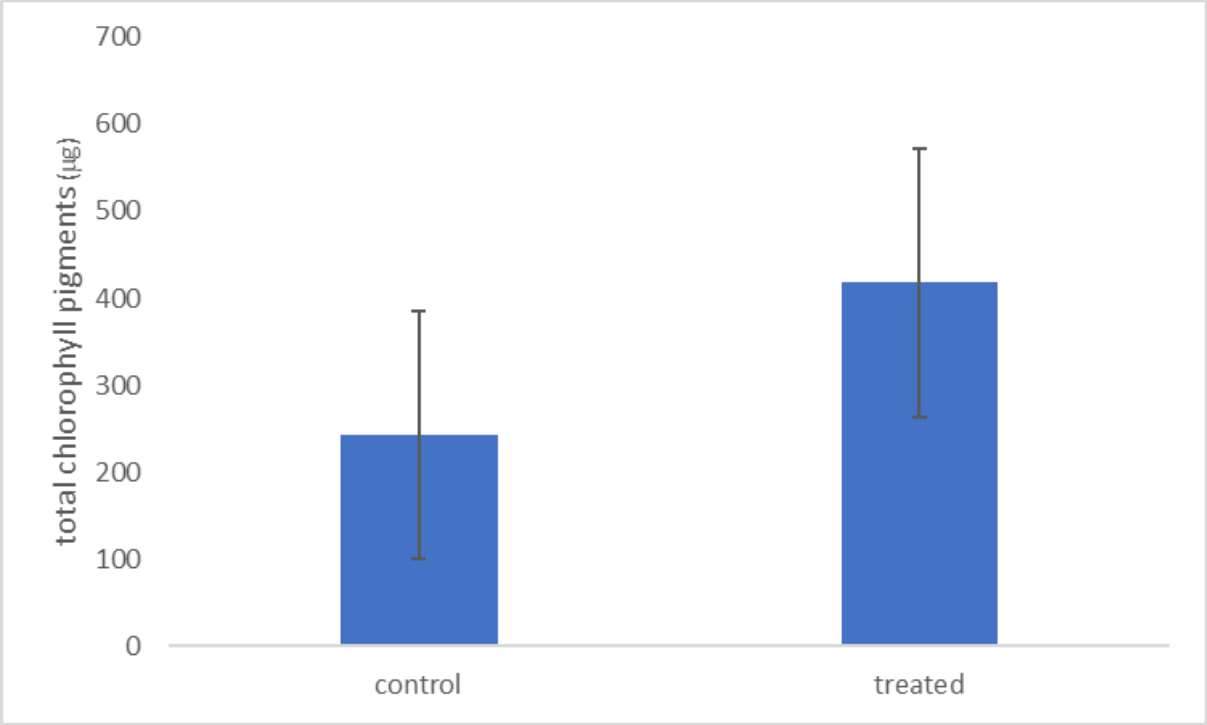


Figure C.10h: Mean total chlorophyll pigments with standard deviation bars for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste

*Table C.10a: Placement set-up for experiment testing the effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with place in each 3x3 section of the chart simulating 3x3 placement on the actual rack going from top to bottom (a *indicates removal of some or all of this system's data from analysis, "tr" indicates a treated system, and*

"c" indicates a control system; for this trial only, "np" indicates that the pump for that system is a new one)

Lids			Systems			Substrata		
4	8	6	3 (tr)	1 (c)	7 (tr,np)	1	5	8
5	2	7	9 (c)	5 (c,np)	8 (tr)	9	4	7
9	10	1	4 (tr)	6 (tr)	2 (c)	6	2	3

Appendix D: Environmental conditions

Appendix D.1 Environmental conditions for Trials 1-3: The effect of Tallapoosa River water bacteria biofilm on attachment in low nutrient water

Appendix D.1.1: Trial 1 environmental conditions

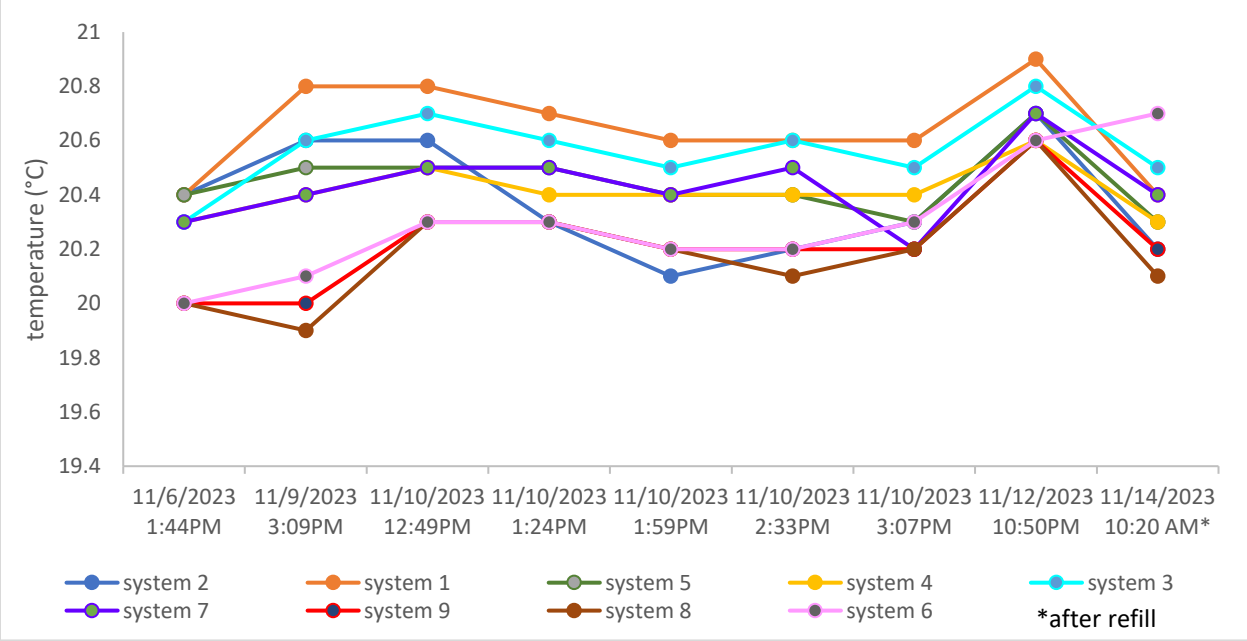


Figure D.1.1a: Temperatures in °C for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

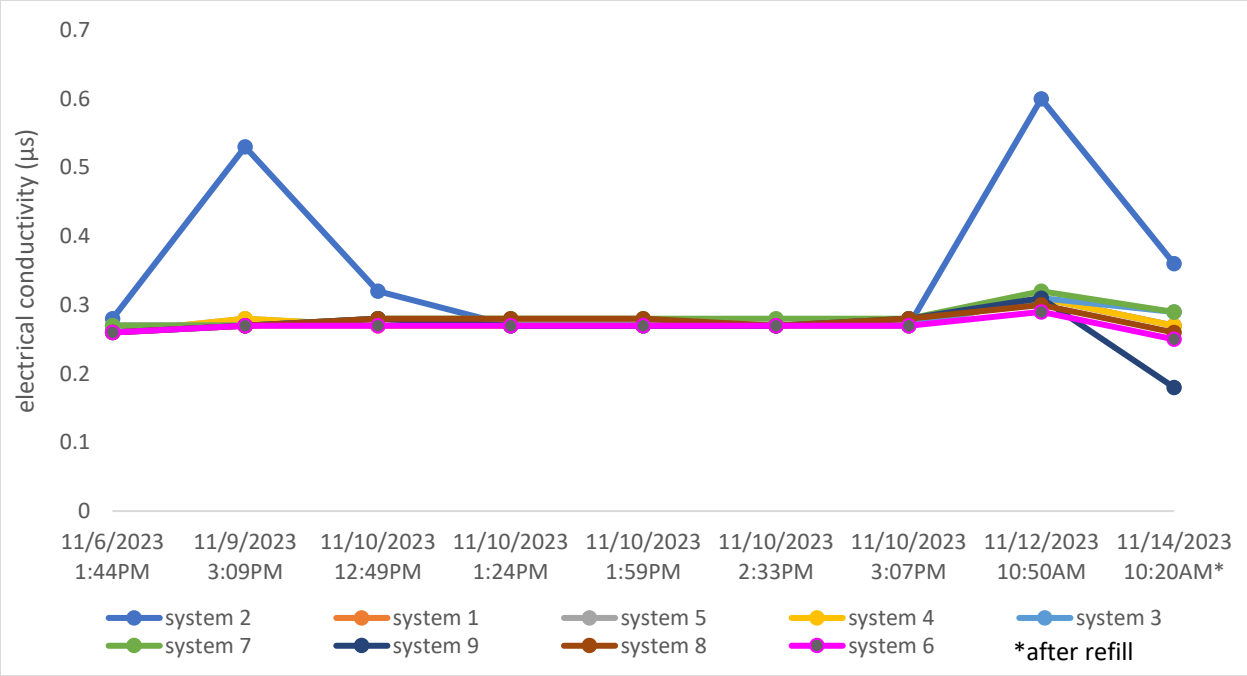


Figure D.1.1b: Electrical conductivities in (µS) for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

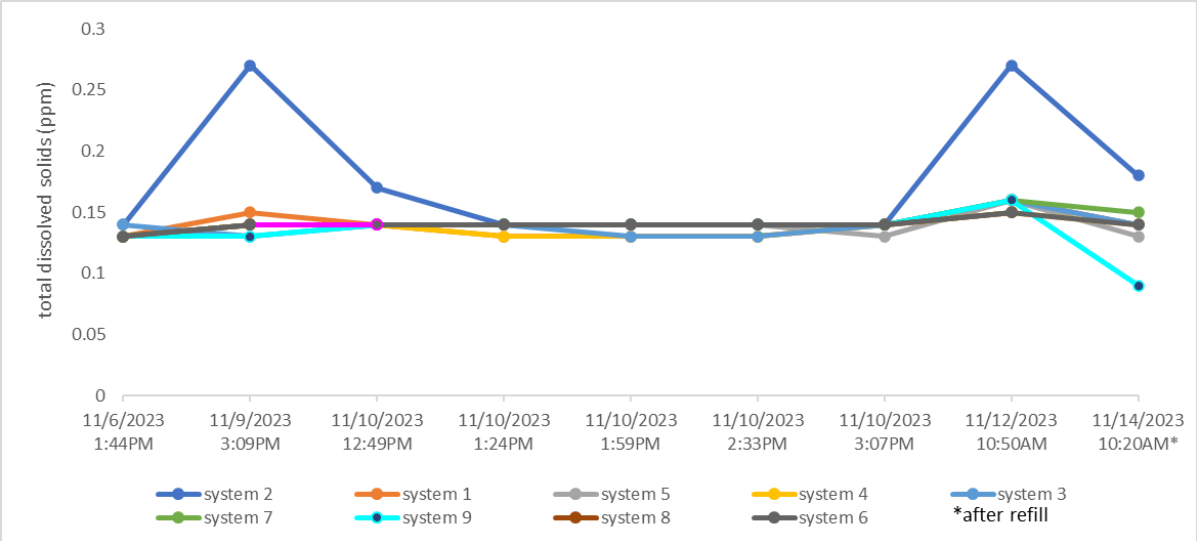


Figure D.1.1c: Total dissolved solids in ppm for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

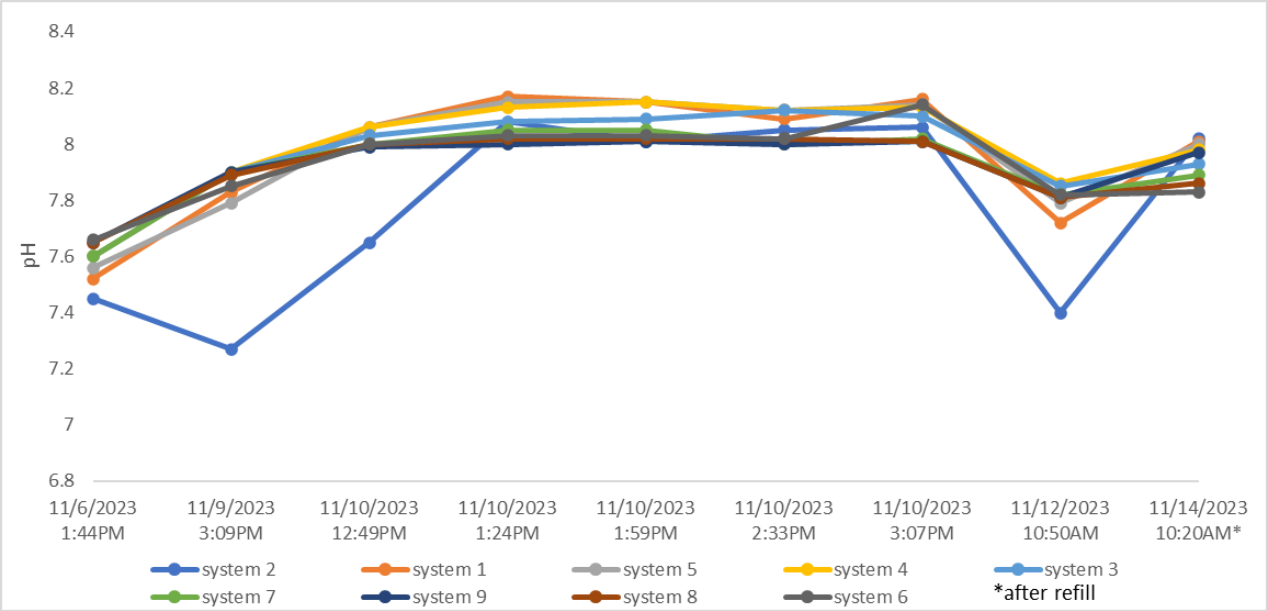


Figure D.1.1d: pH for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

Figure D.1.1e: Decrease in nitrate concentration over Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

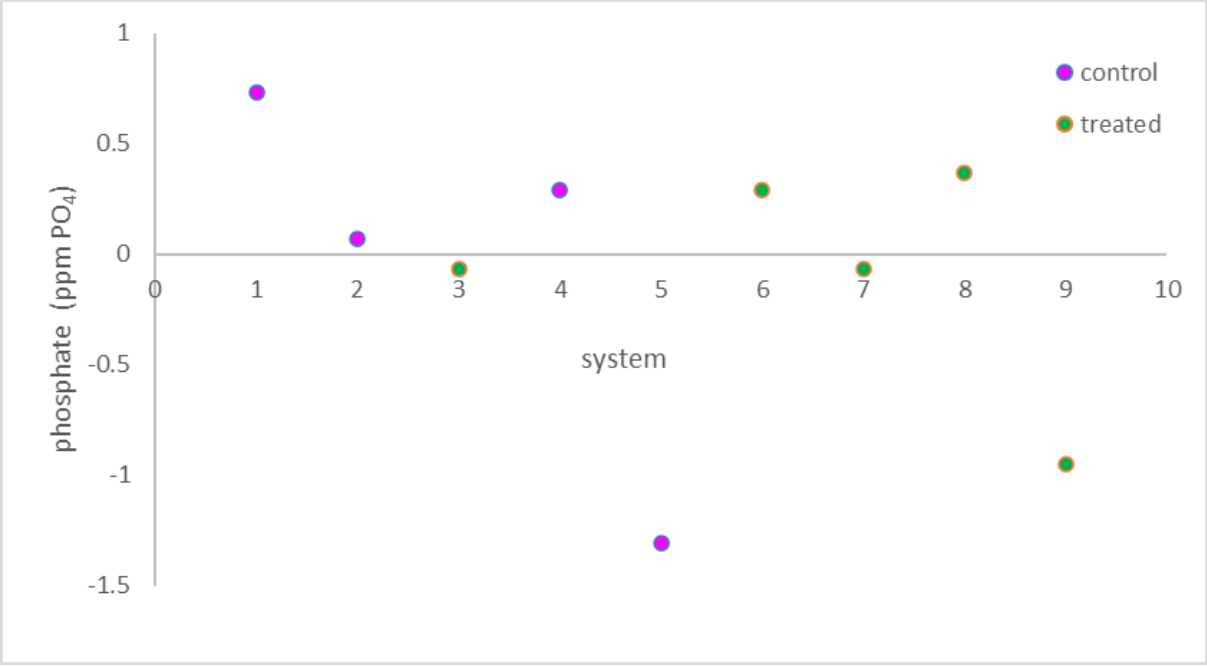


Figure D.1.1f: Decrease in phosphate concentration over Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

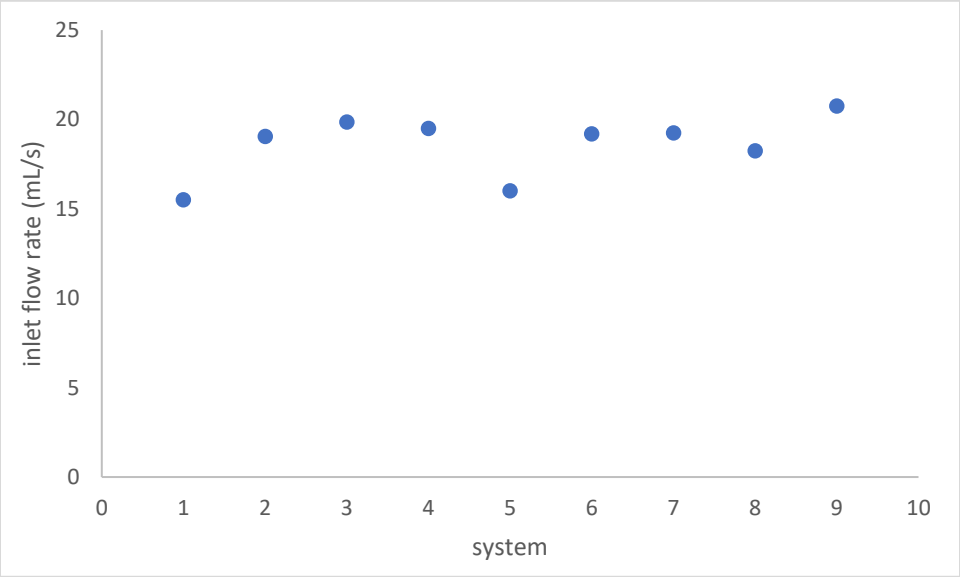


Figure D.1.1g: Average inlet flow rate in mL/s for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

Table D.1.1a: Channel travel time in s for Trial 1 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

System	Channel Travel Time (s)
1	1
2	2
3	1
4	1

5	1
6	1
7	1
8	2
9	2

Appendix D.1.2: Trial 2 environmental conditions

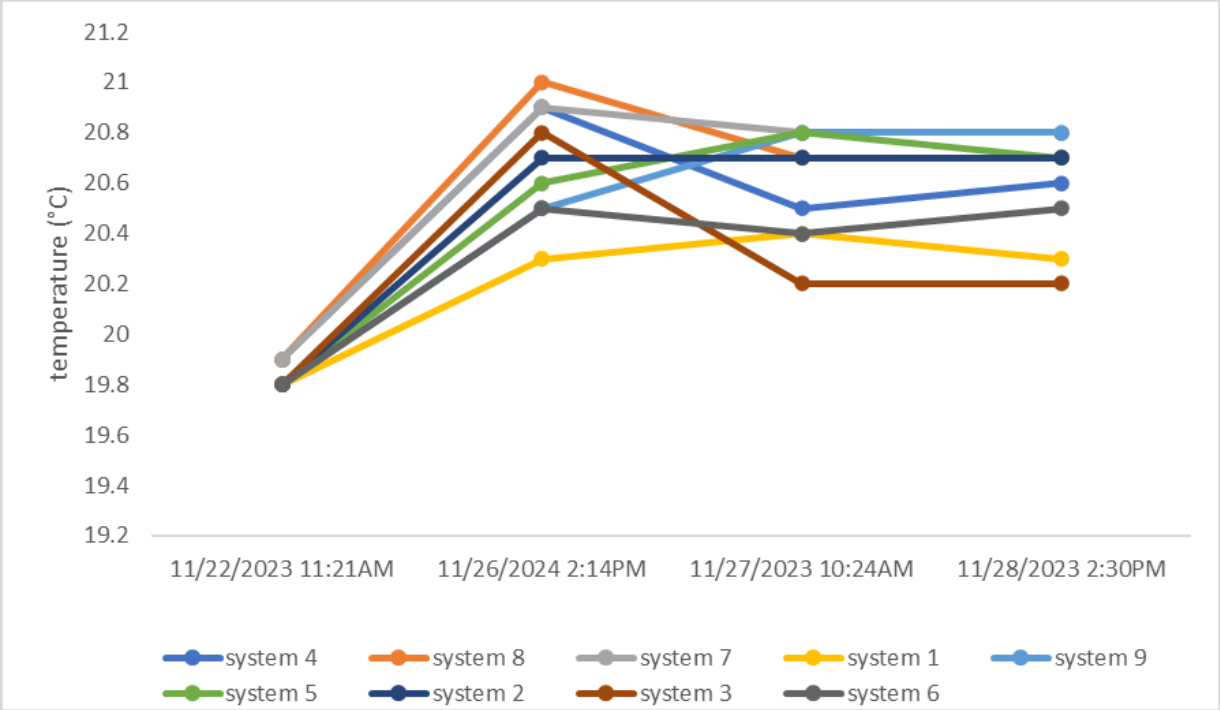


Figure D.1.2a: Temperatures in °C for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

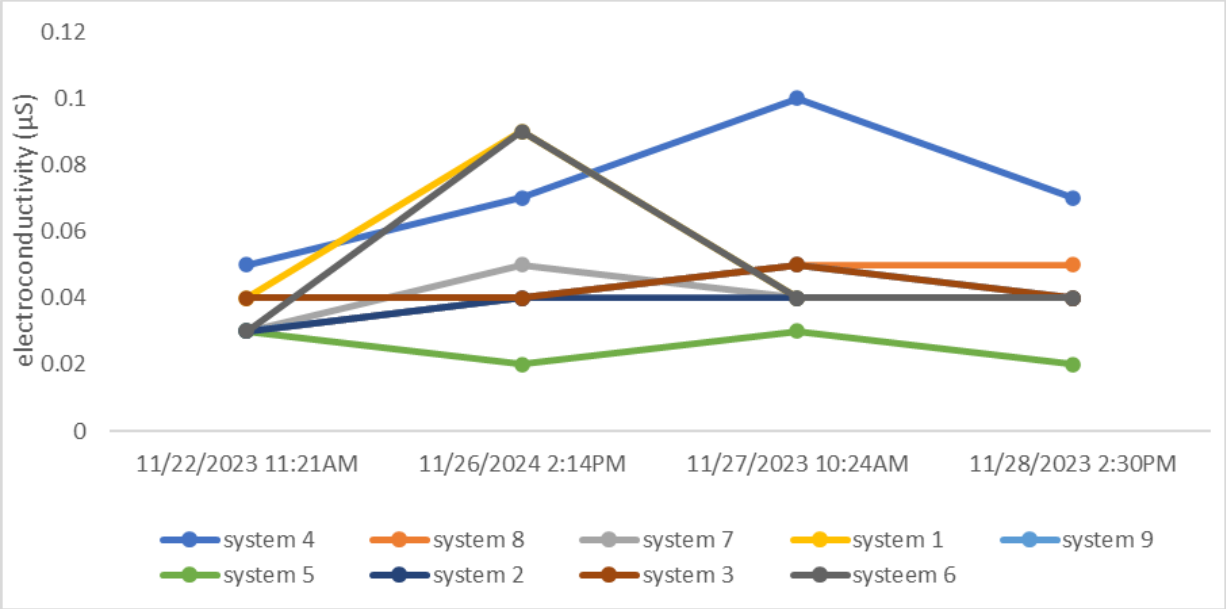


Figure D.1.2b: Electrical conductivities in (µS) for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

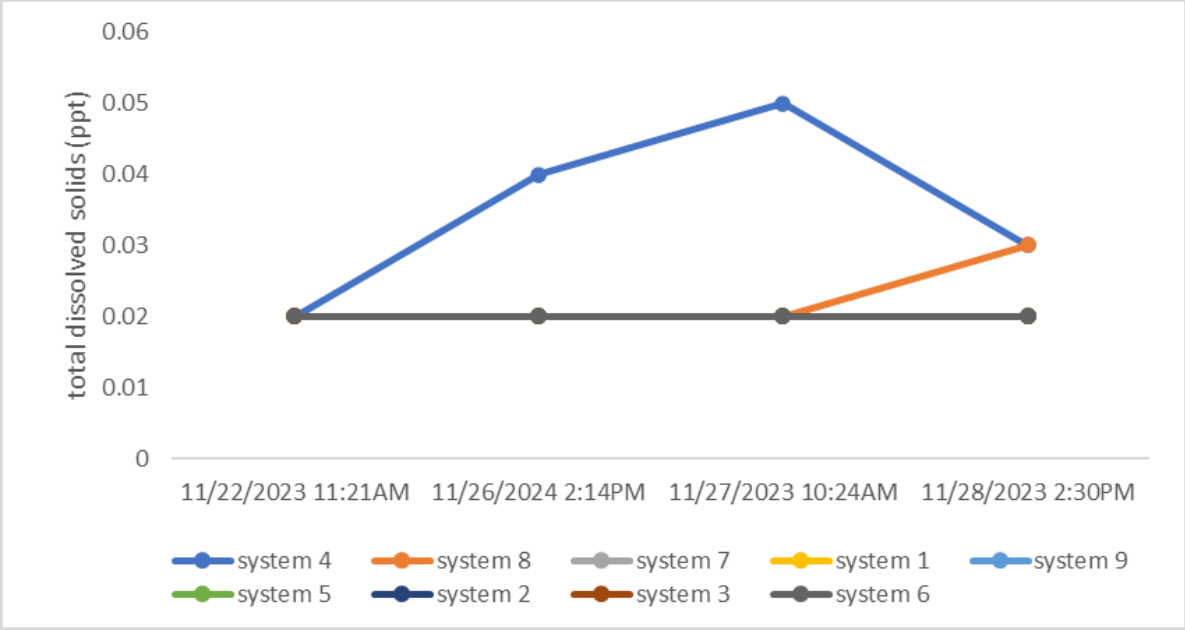


Figure D.1.2c: Total dissolved solids in ppm for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

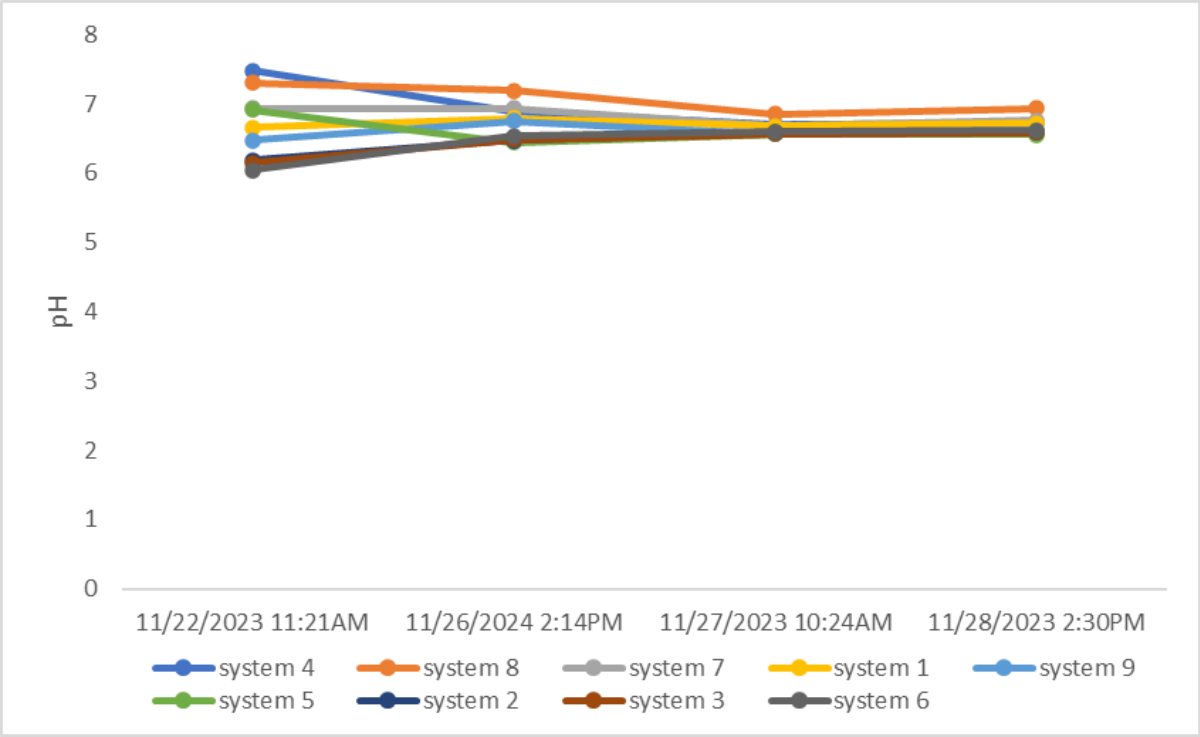


Figure D.1.2d: pH for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

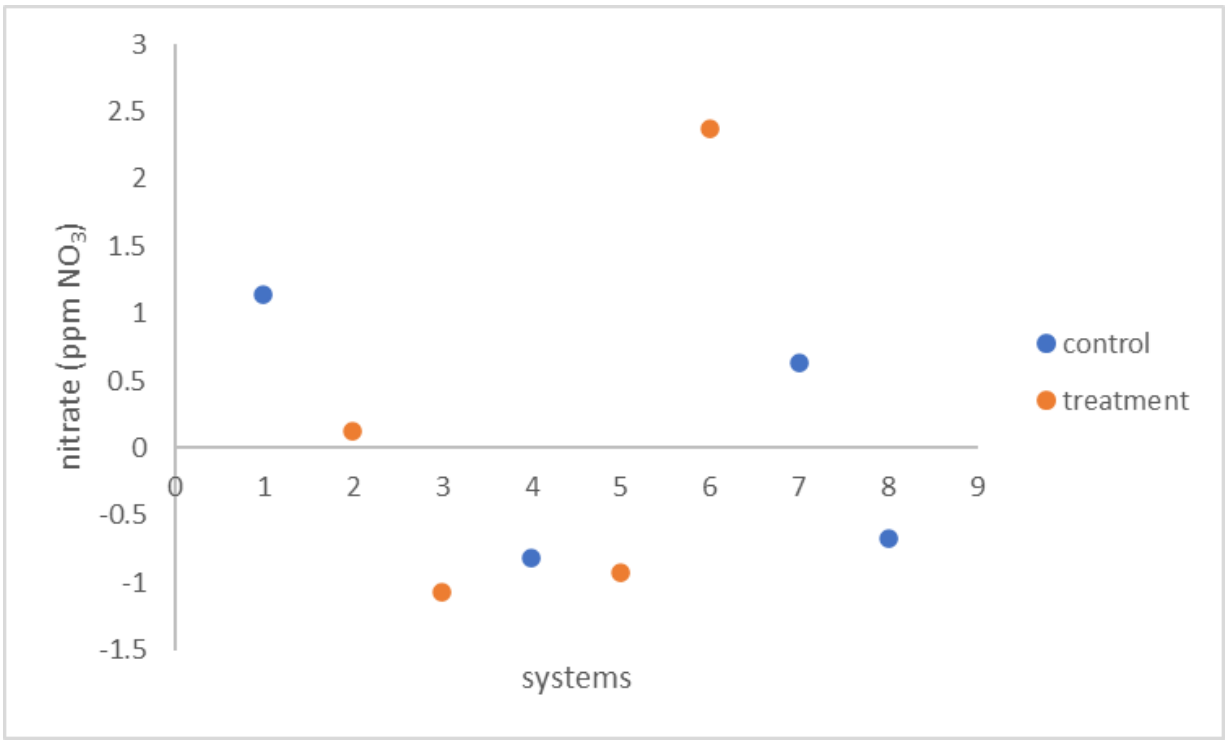


Figure D.1.2e: Decrease in nitrate concentration over Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

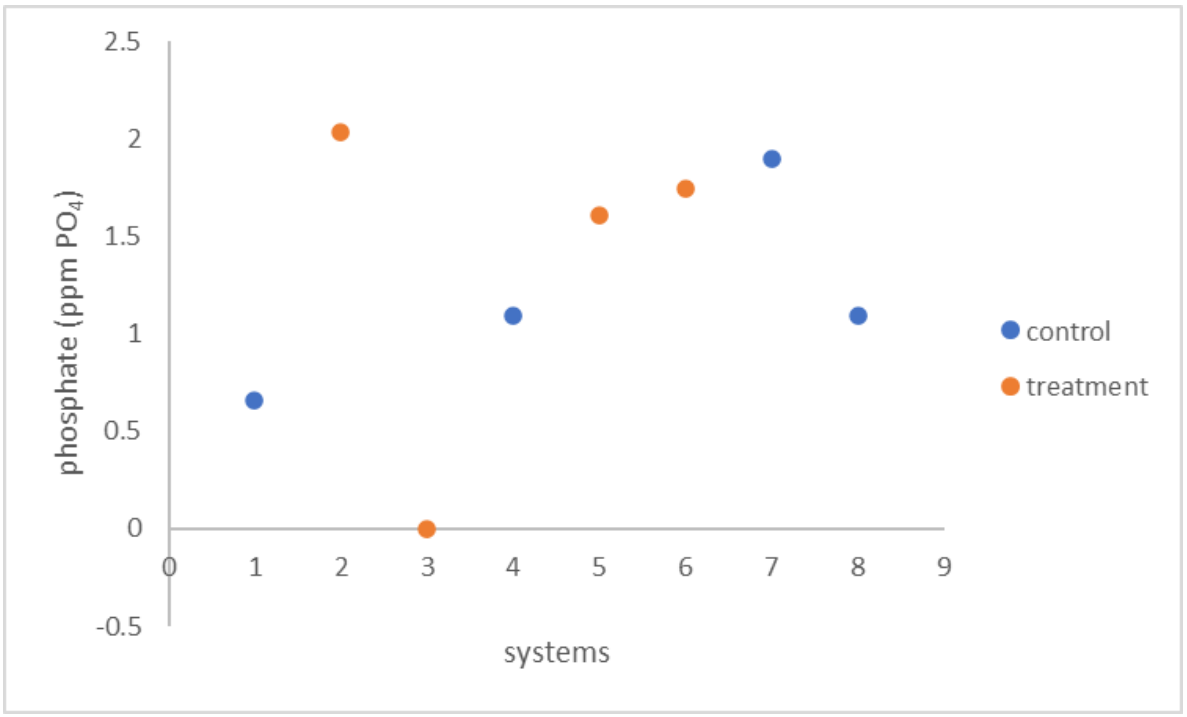


Figure D.1.2f: Decrease in phosphate concentration over Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

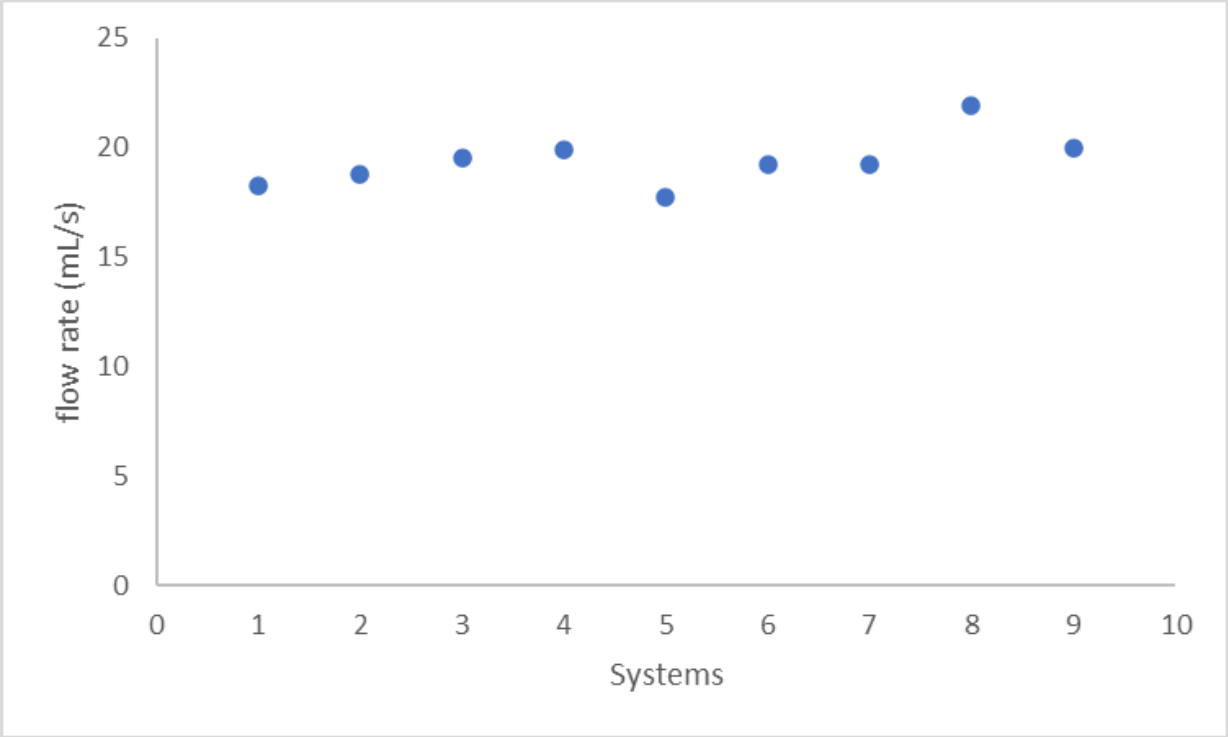


Figure D.1.2g: Average inlet flow rate in mL/s for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

Table D.1.2a: Channel travel time in s for Trial 2 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

System	Channel Travel Time (s)
1	2
2	1
3	1
4	1
5	1

6	1
7	1
8	1
9	2

Appendix D.1.3: Trial 3 environmental conditions

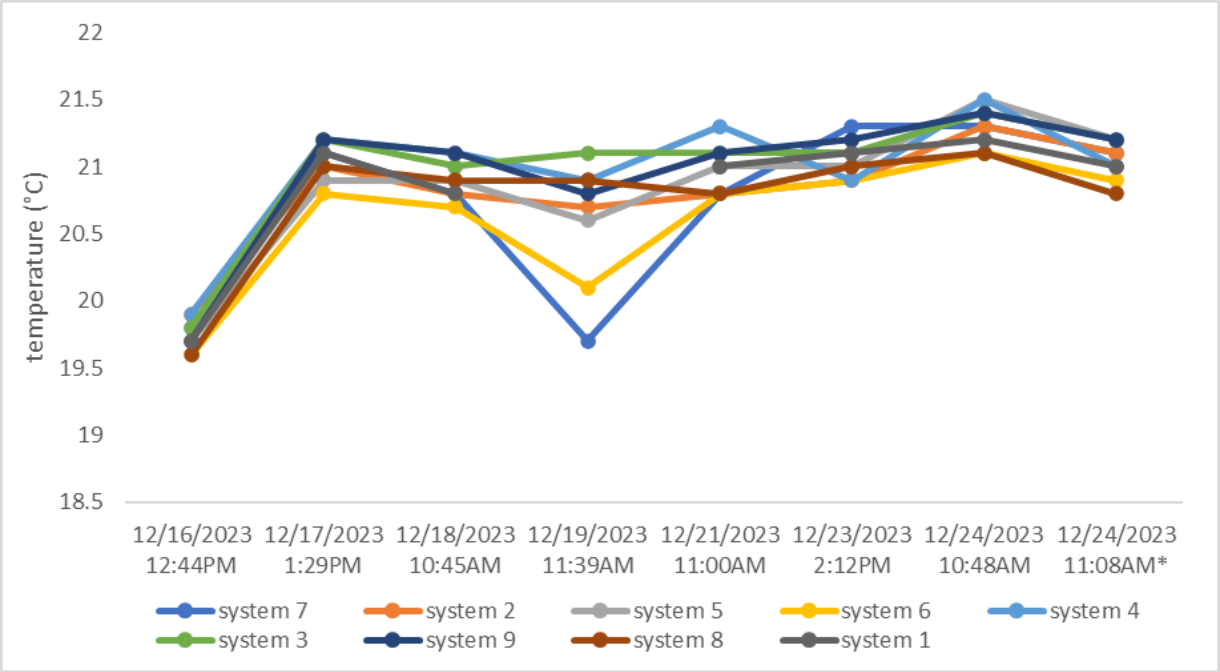


Figure D.1.3a: Temperatures in °C for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

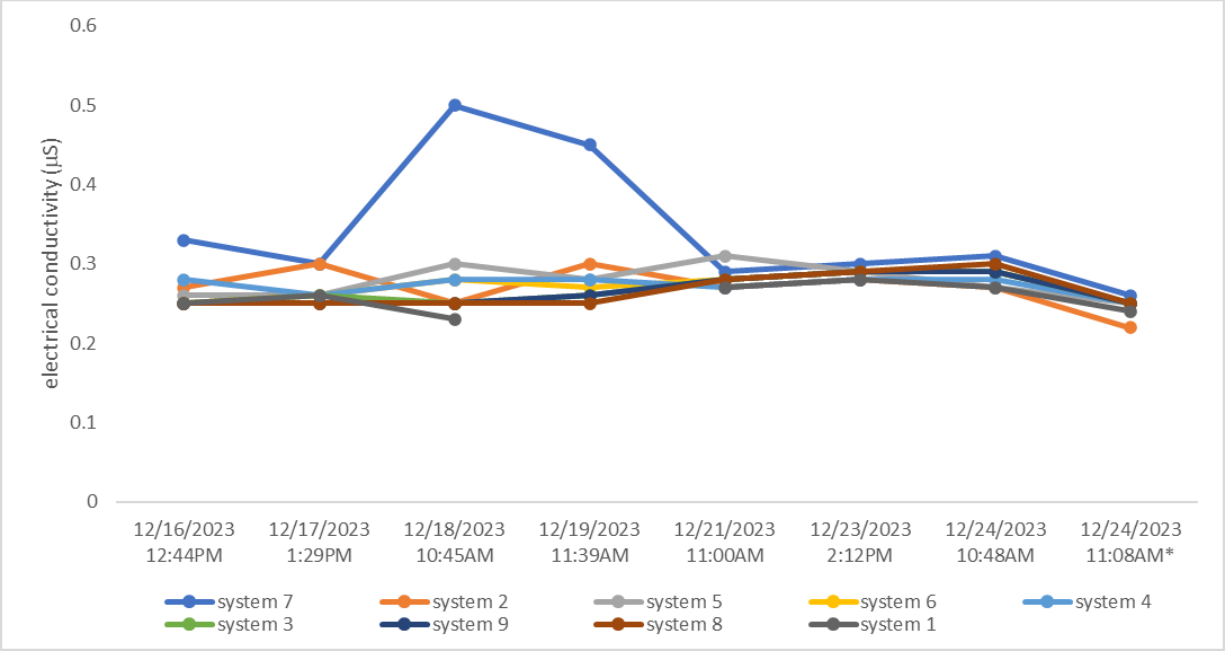


Figure D.1.3b: Electrical conductivities in (µS) for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

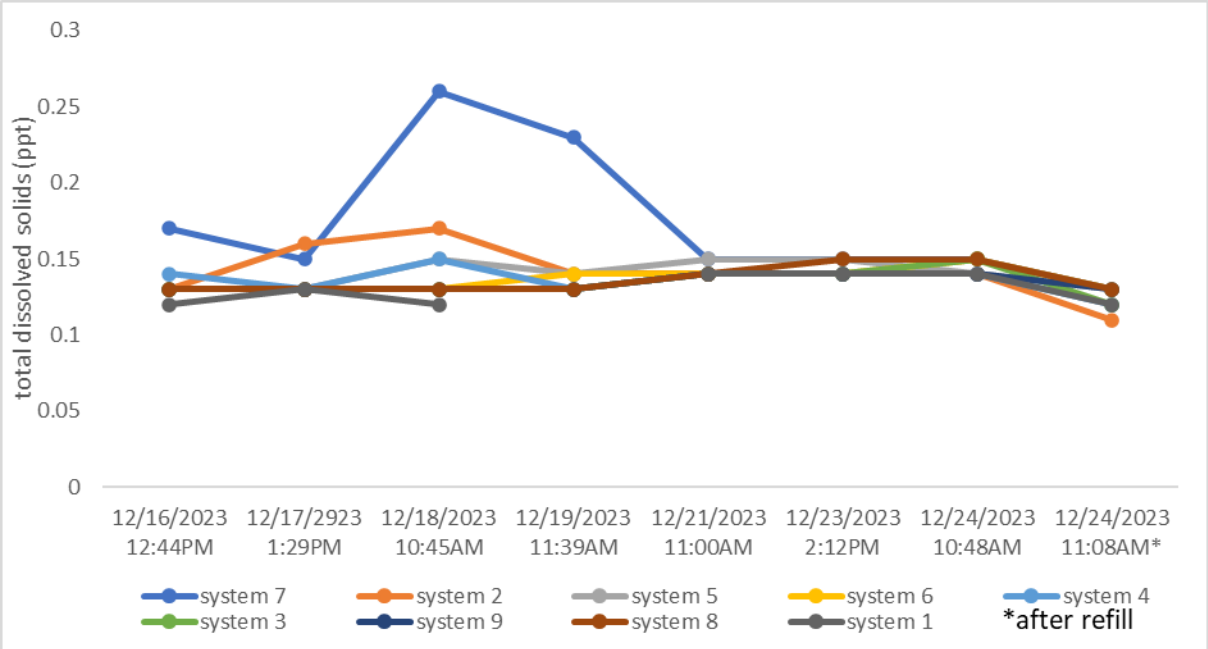


Figure D.1.3c: Total dissolved solids in ppm for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

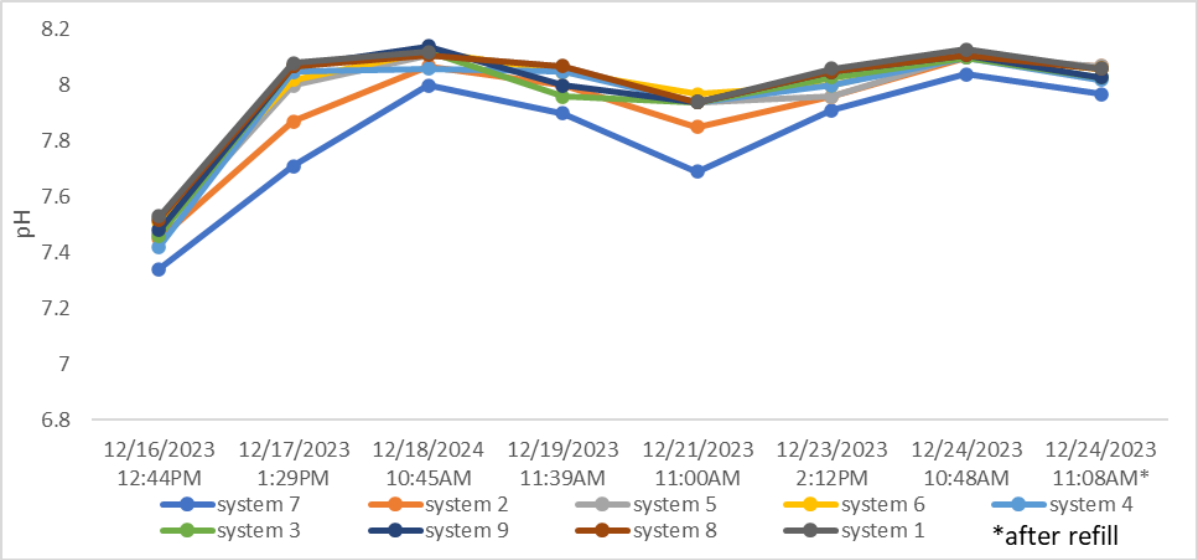


Figure D.1.3d: pH for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

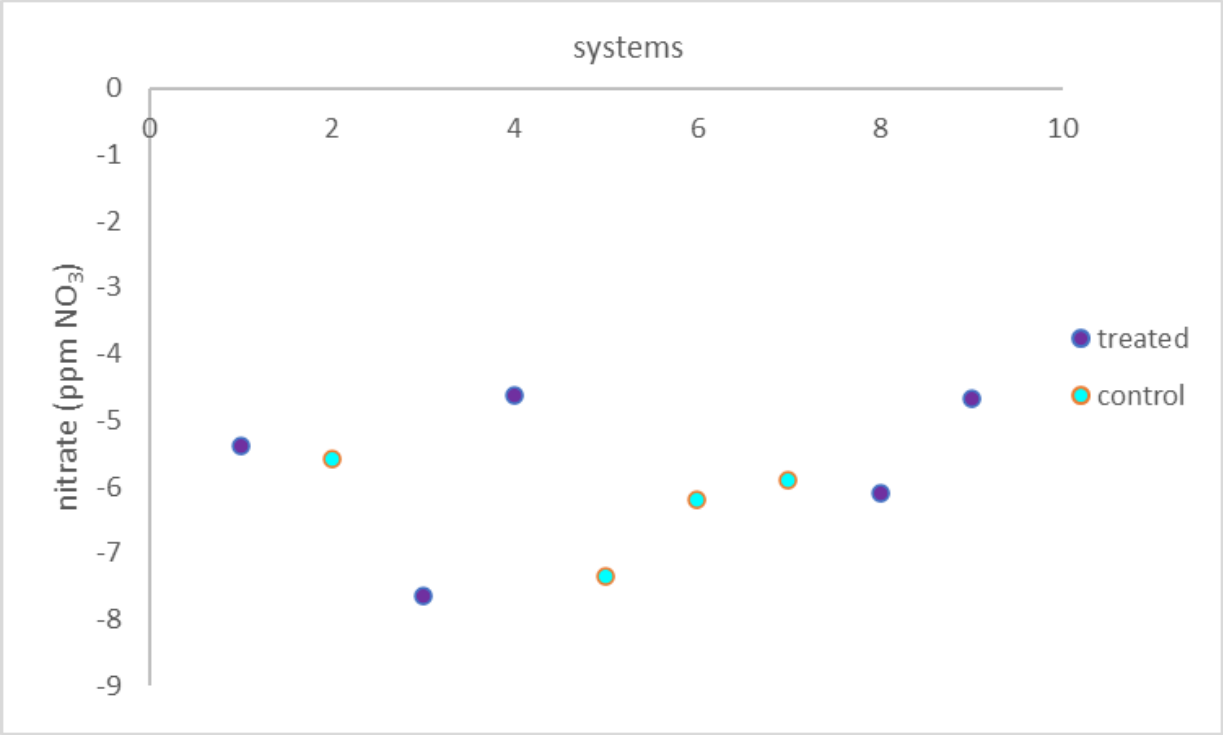


Figure D.1.3e: Decrease in nitrate concentration over Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

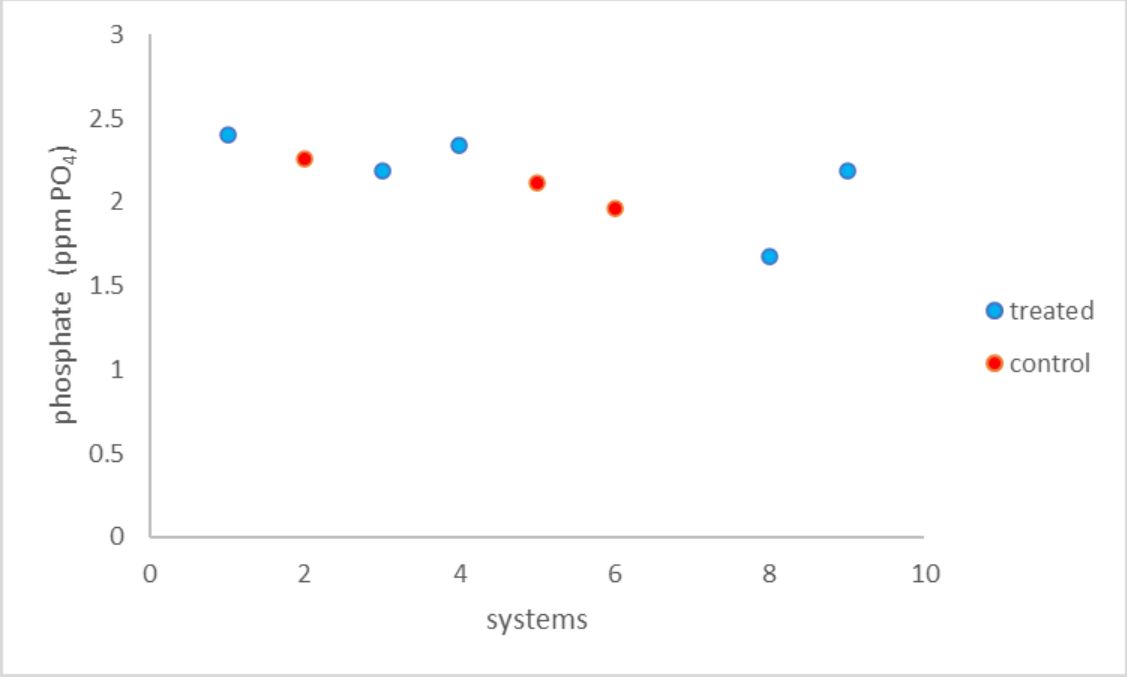


Figure D.1.3f: Decrease in phosphate concentration over Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

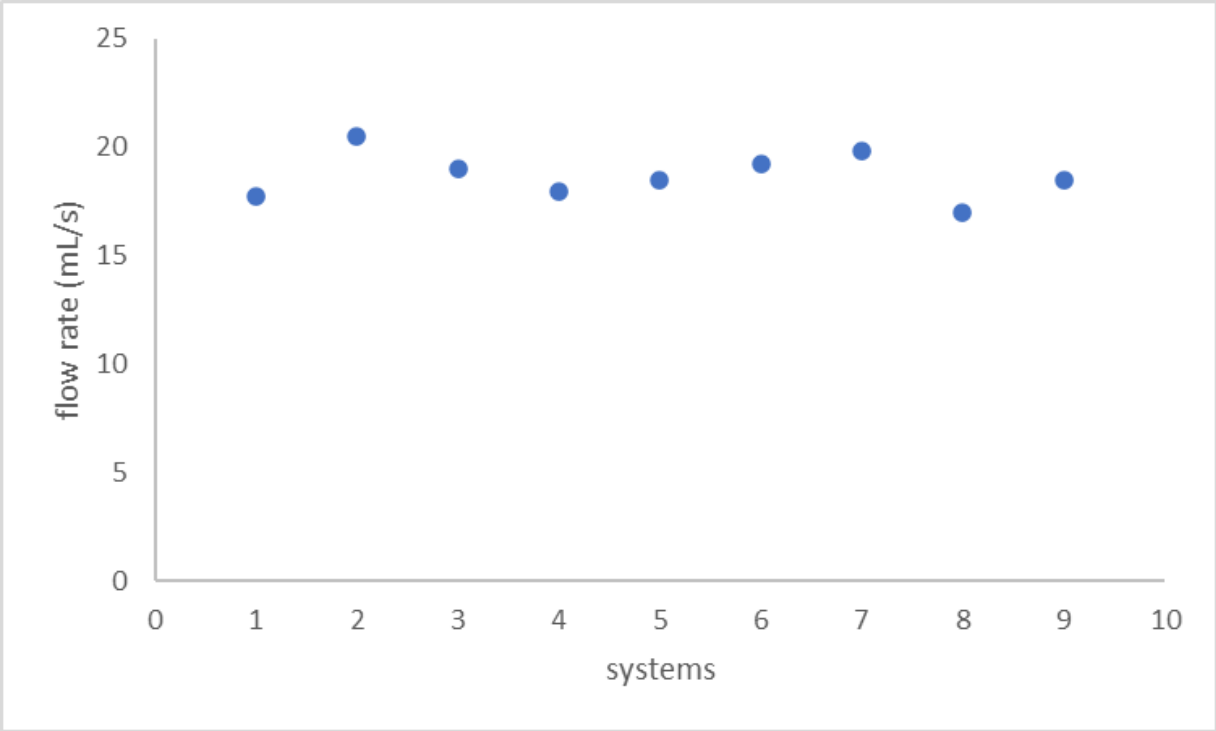


Figure D.1.3g: Average inlet flow rate in mL/s for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

Table D.1.3a: Channel travel time in s for Trial 3 of experiment testing the effect of the Tallapoosa River bacteria biofilm on attachment in low nutrient synthetic media

System	Channel Travel Time (s)
1	1
2	1

3	2
4	1
5	1
6	2
7	1
8	1
9	1

Appendix D.2 Environmental conditions for Trial 4: The effect of Tallapoosa River water bacteria biofilm on attachment in high nutrient synthetic media

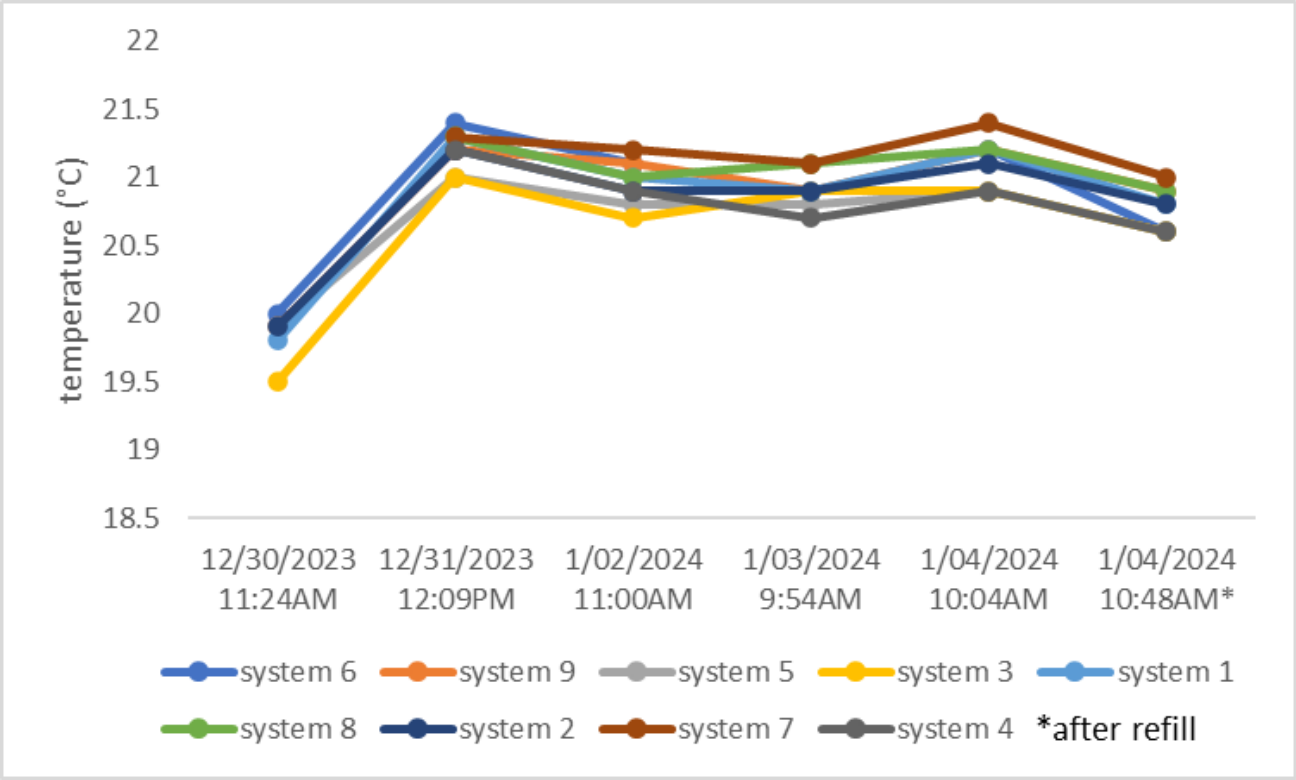


Figure D.2a: Temperatures in °C for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

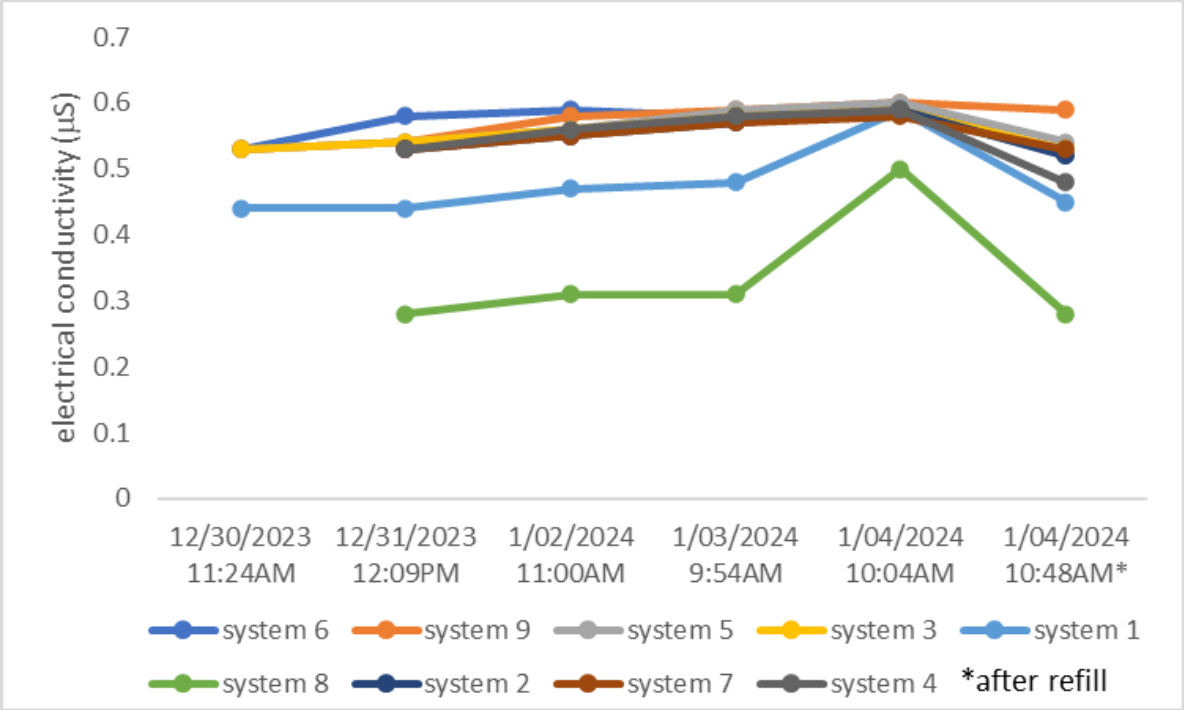


Figure D.2b: Electrical conductivities in (μS) for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

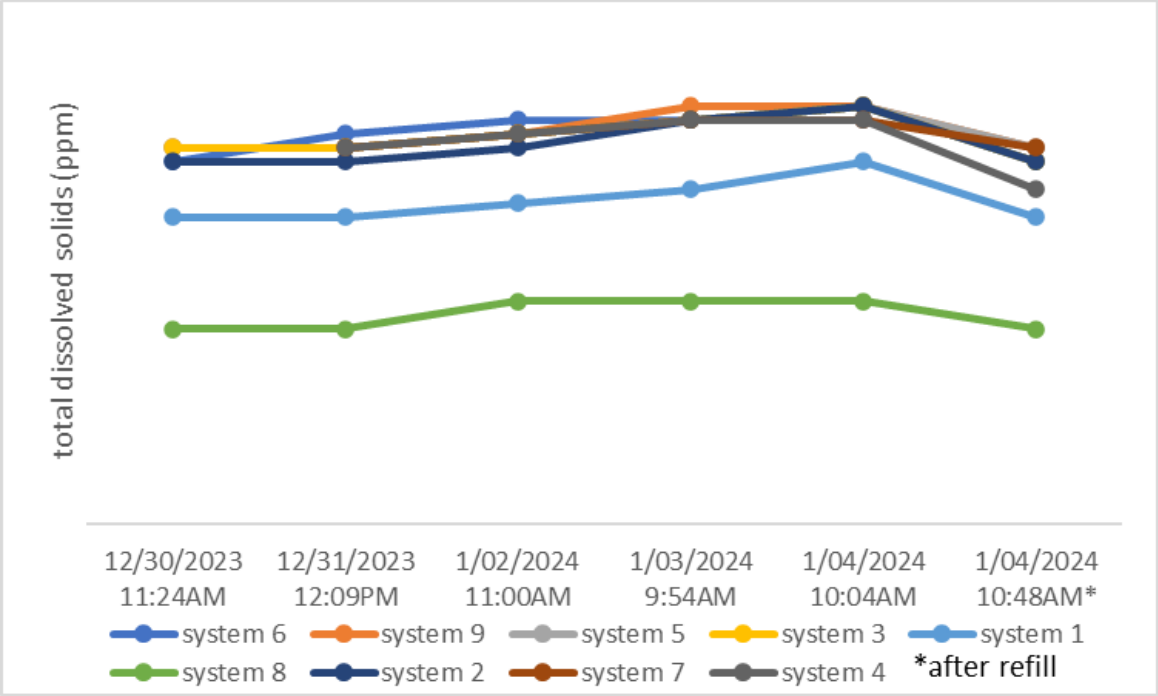


Figure D.2c: Total dissolved solids in ppm for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

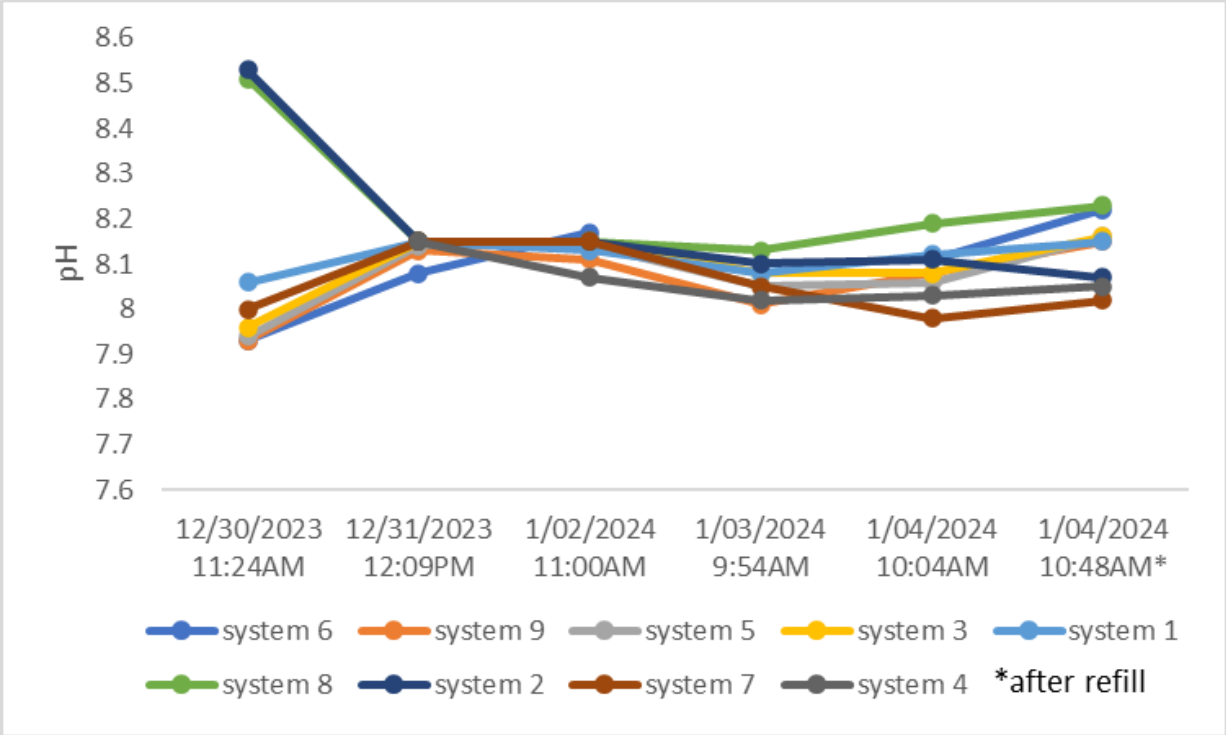


Figure D.2d: pH for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

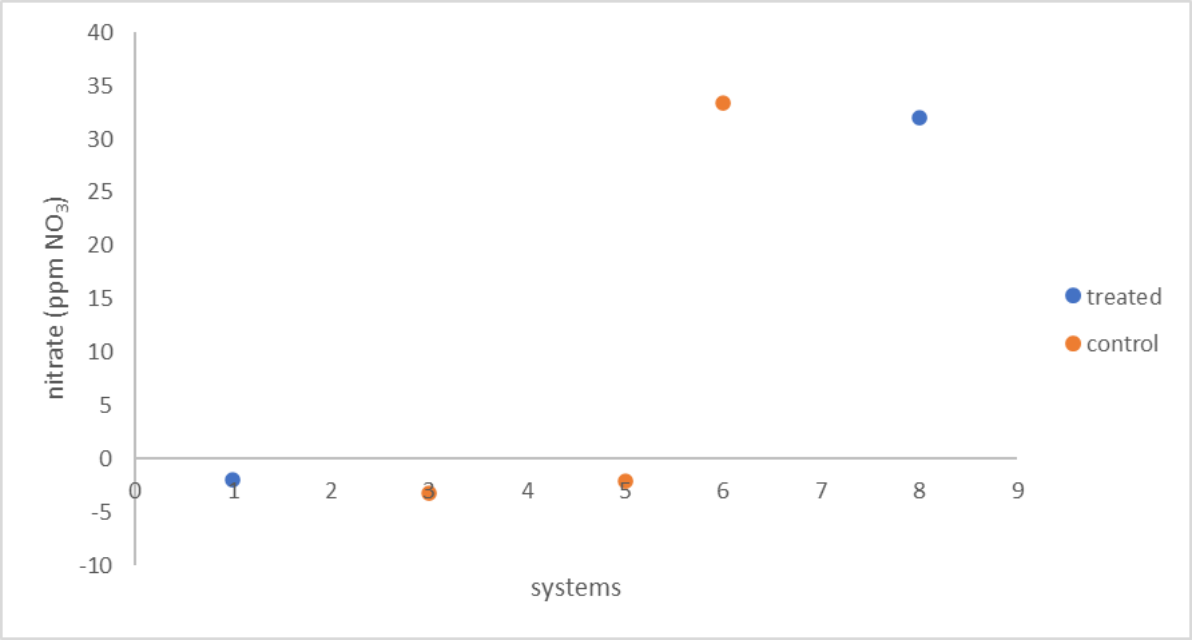


Figure D.2e: Decrease in nitrate concentration over Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media (data for systems 2, 4, 6, and 7 unavailable)

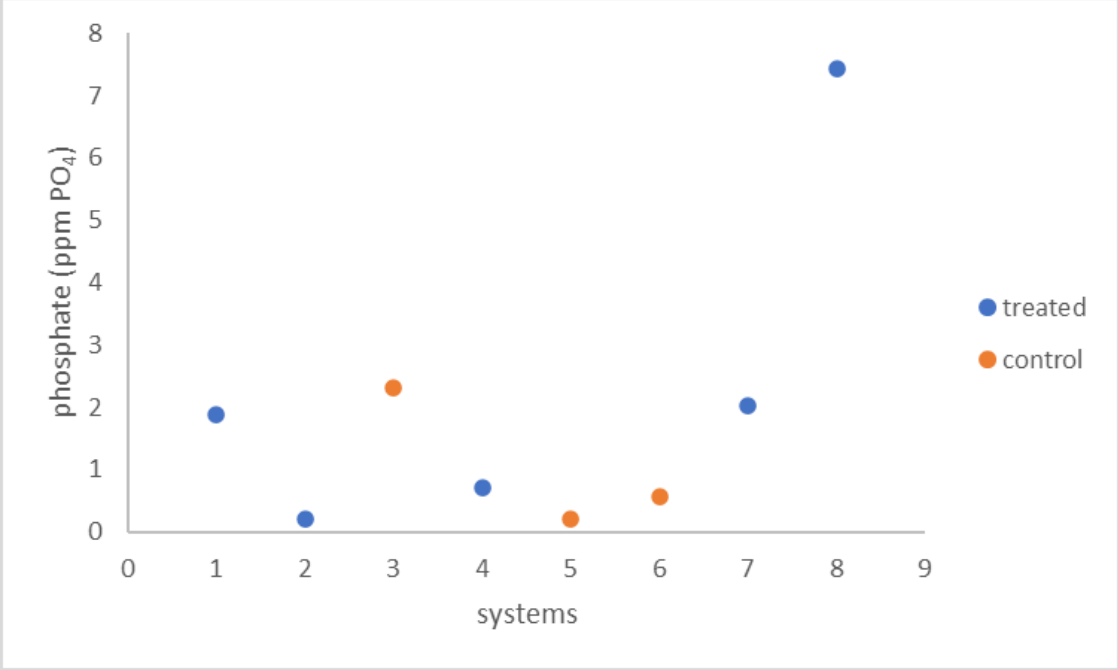


Figure D.2f: Decrease in phosphate concentration over Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

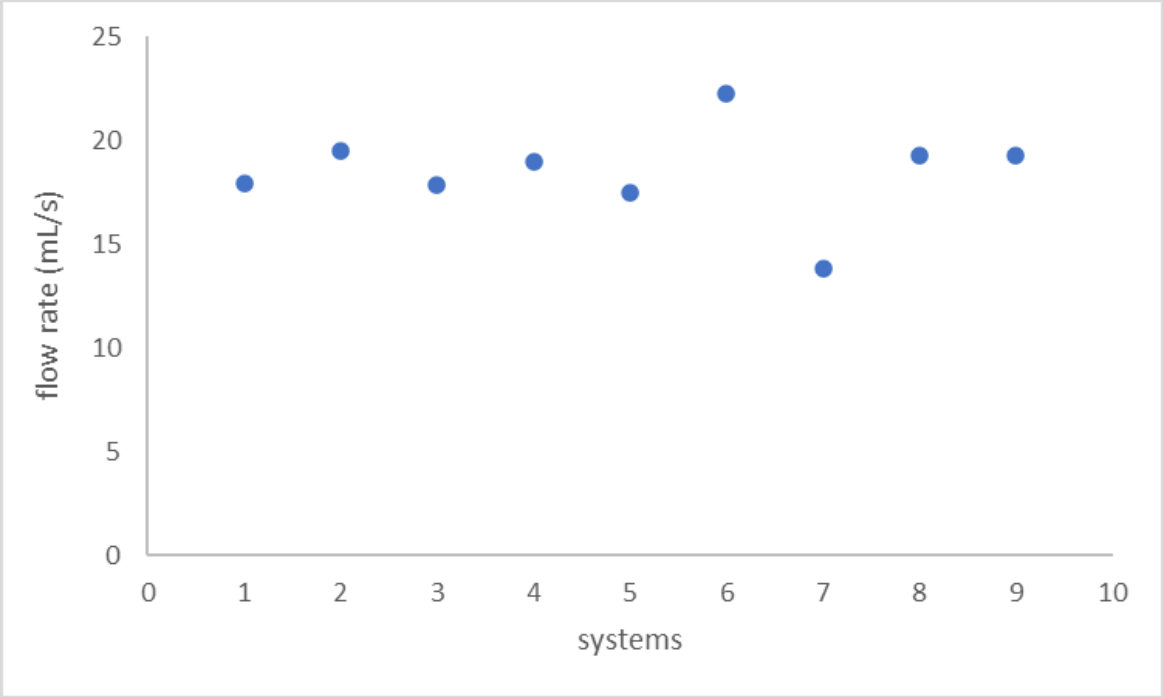


Figure D.2g: Average inlet flow rate in mL/s for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

Table D.2a: Channel travel time in s for Trial 4: The effect of the Tallapoosa River bacteria biofilm on attachment in high nutrient synthetic media

System	Channel Travel Time (s)
1	2
2	2
3	2
4	1

5	2
6	2
7	2
8	2
9	2

Appendix D.3 Environmental conditions for Trial 5: The effect of Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

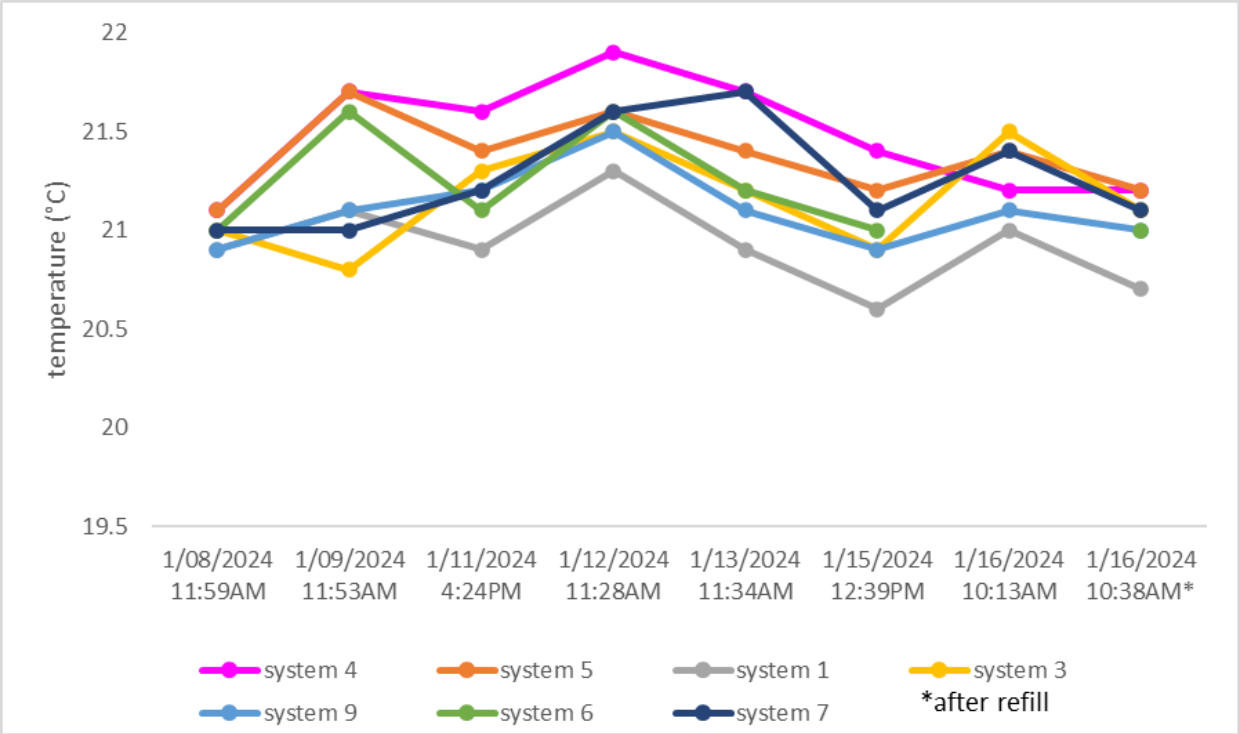


Figure D.3a: Temperatures in °C for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

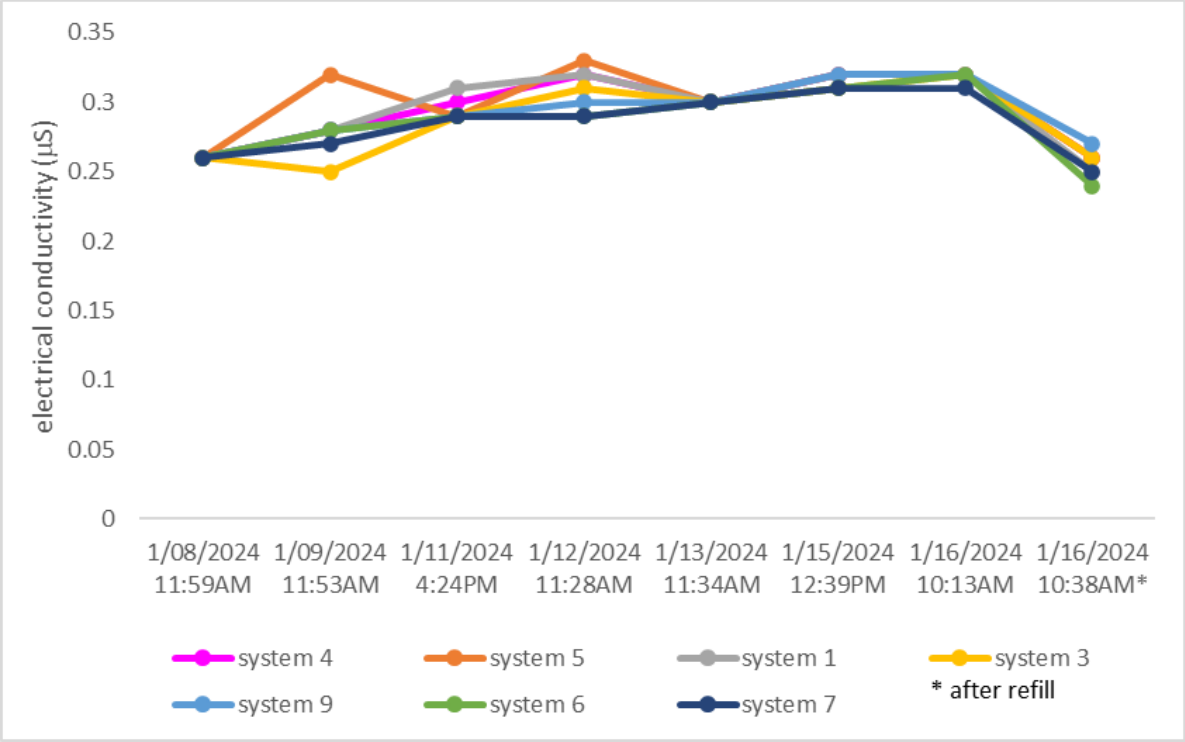


Figure D.3b: Electrical conductivities in (μS) for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

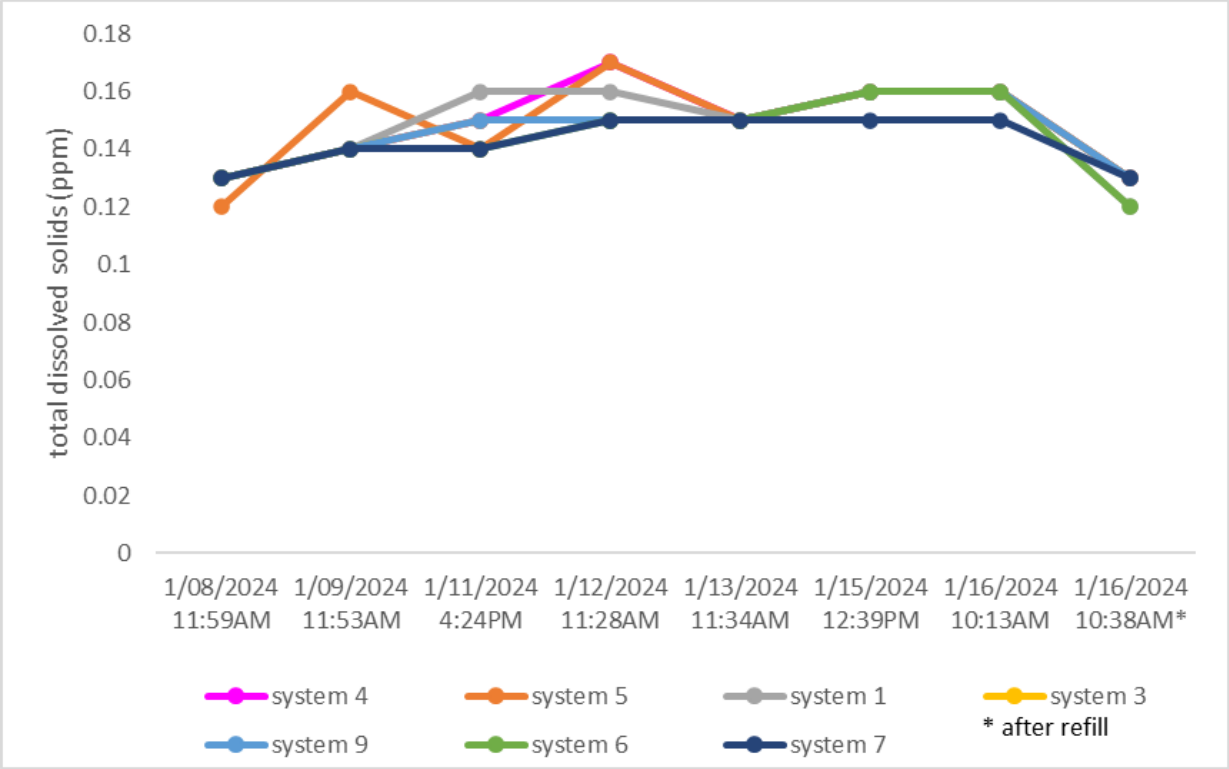


Figure D.3c: Total dissolved solids in ppm for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

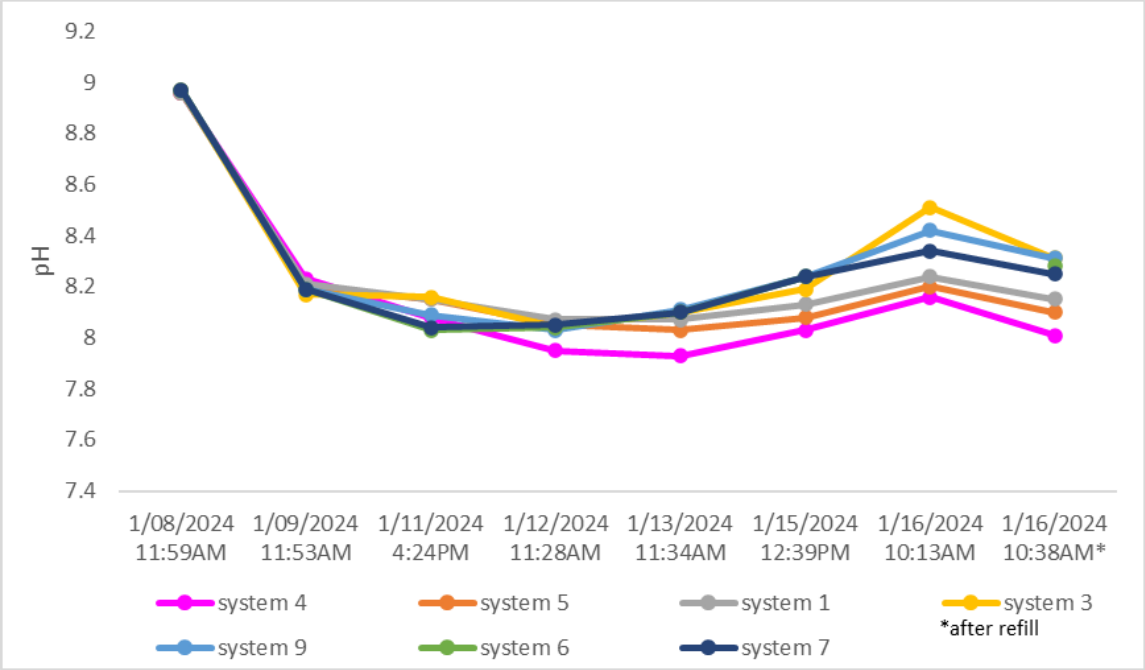


Figure D.3d: pH for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

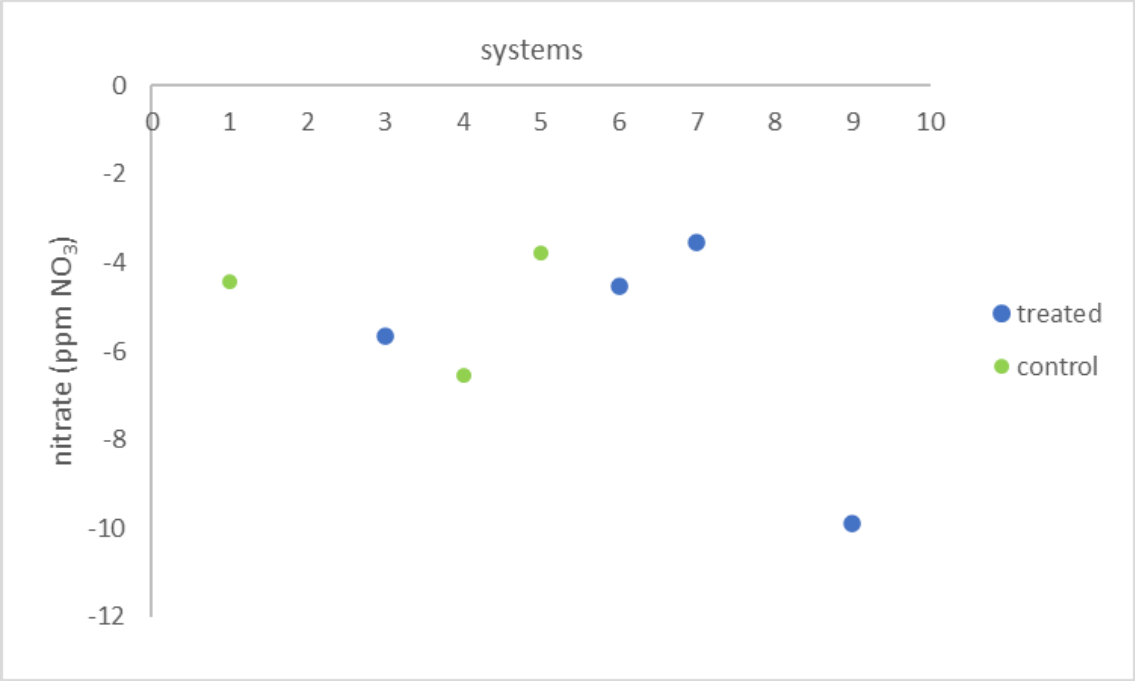


Figure D.3e: Decrease in nitrate concentration over Trial 5: The effect of the Town Creek Park bacteria biofilm on attachment in low nutrient synthetic media

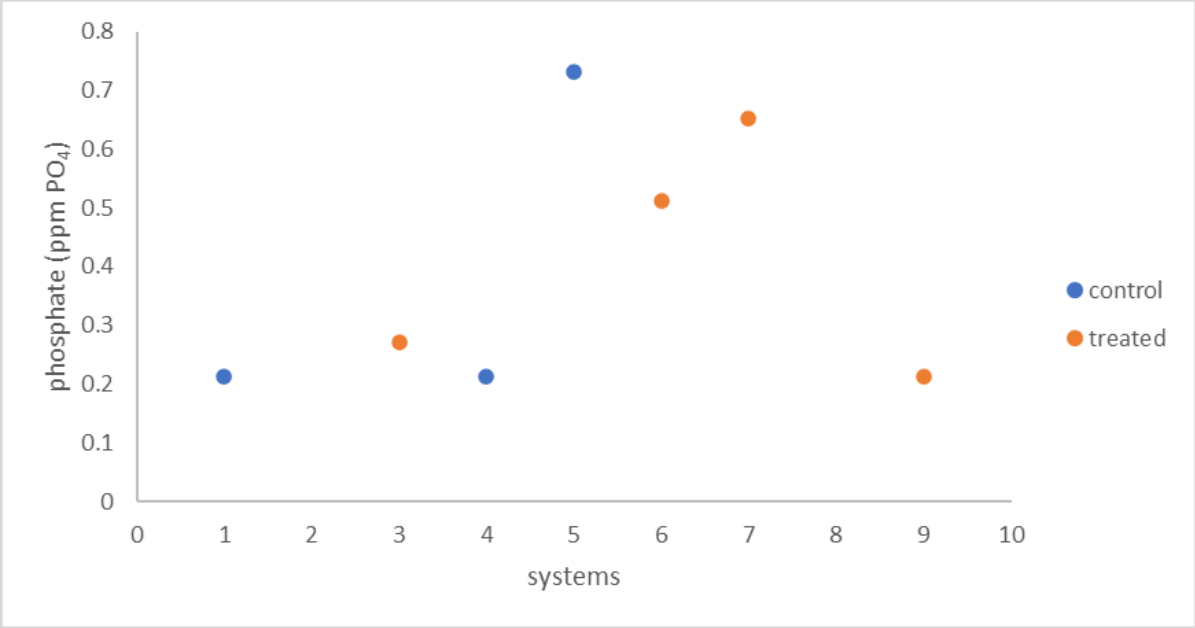


Figure D.3f: Decrease in phosphate concentration over Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

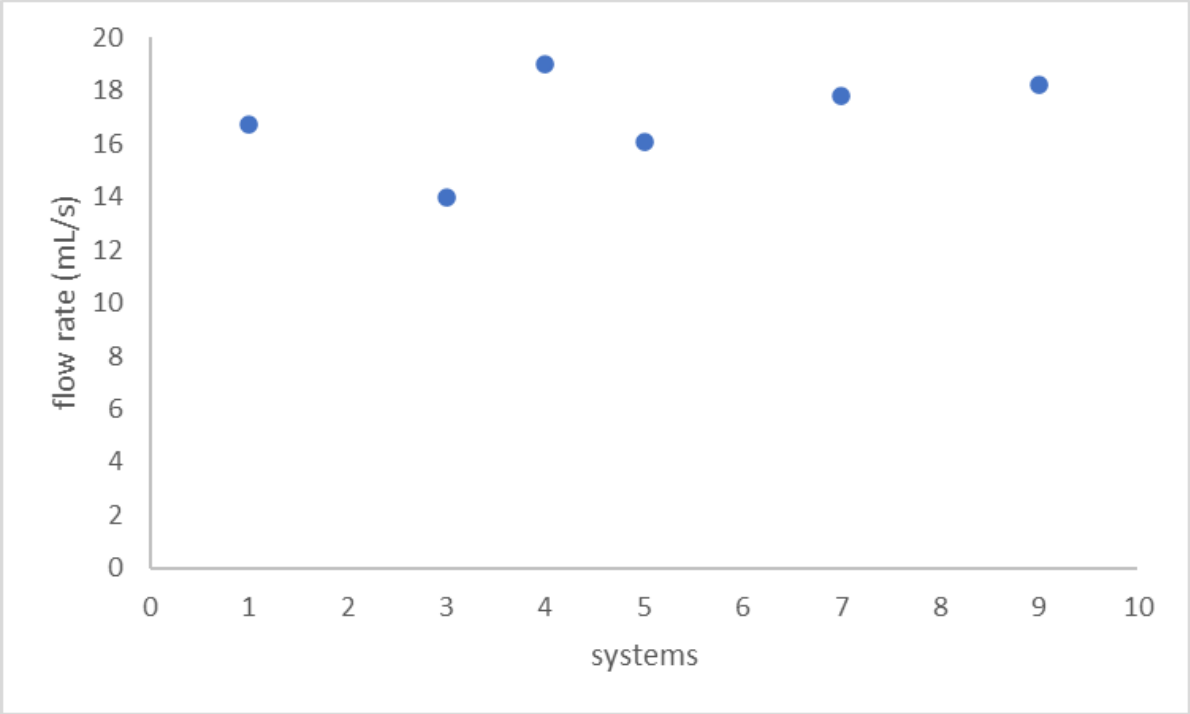


Figure D.3g: Average inlet flow rate in mL/s for Trial 5: The effect of the Town Creek Park bacteria biofilm on attachment in low nutrient synthetic media

Table D.3a: Channel travel time in s for Trial 5: The effect of the Town Creek Park stream bacteria biofilm on attachment in low nutrient synthetic media

System	Channel Travel Time (s)
1	1
3	1
4	1

5	2
6	1
7	1
9	2

Appendix D.4 Environmental conditions for Trial 6: The effect of Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

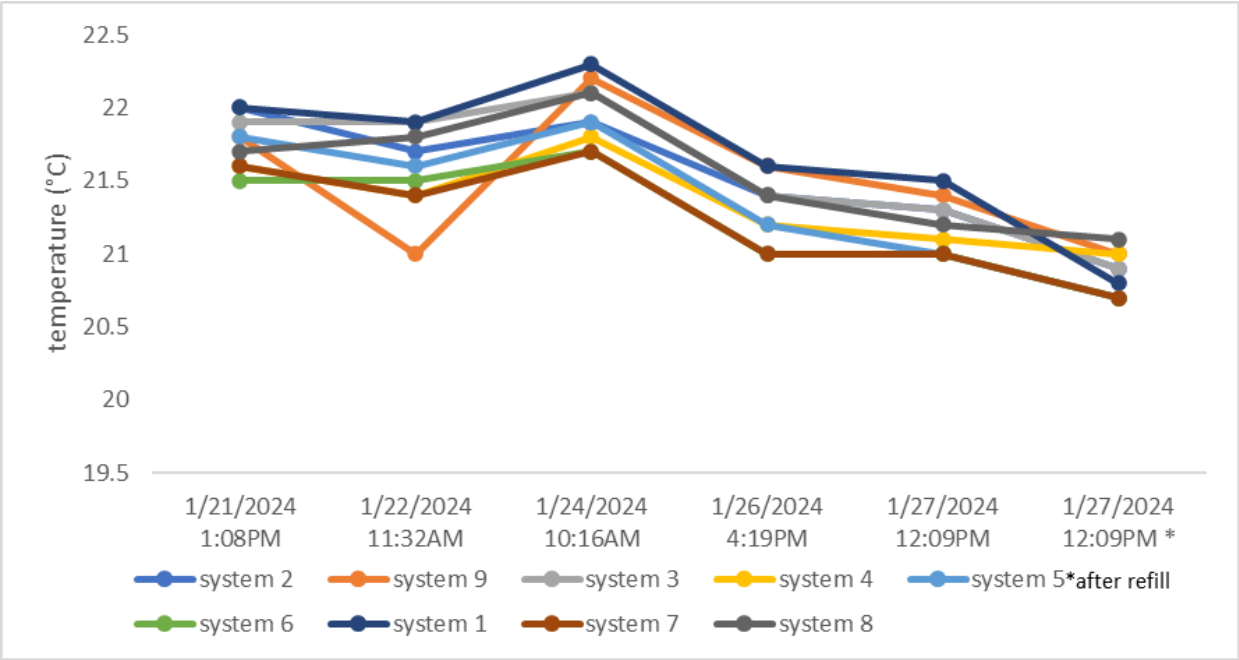


Figure D.4a: Temperatures in °C for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

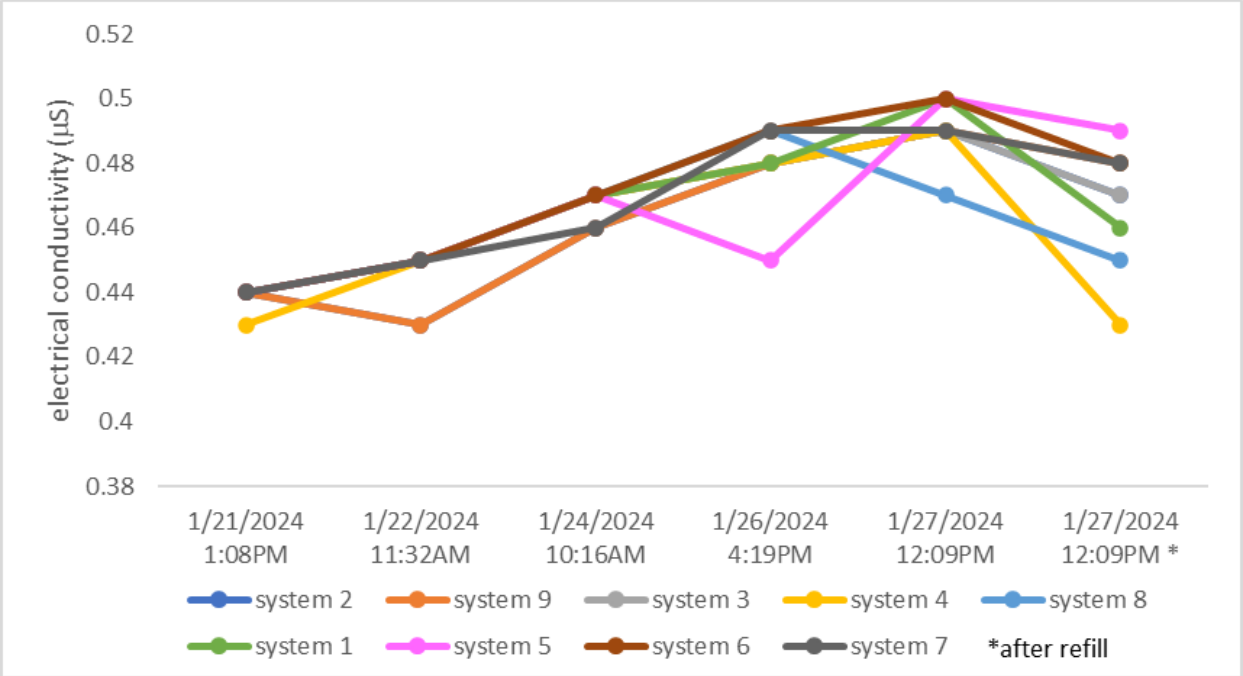


Figure D.4b: Electrical conductivities in µS for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

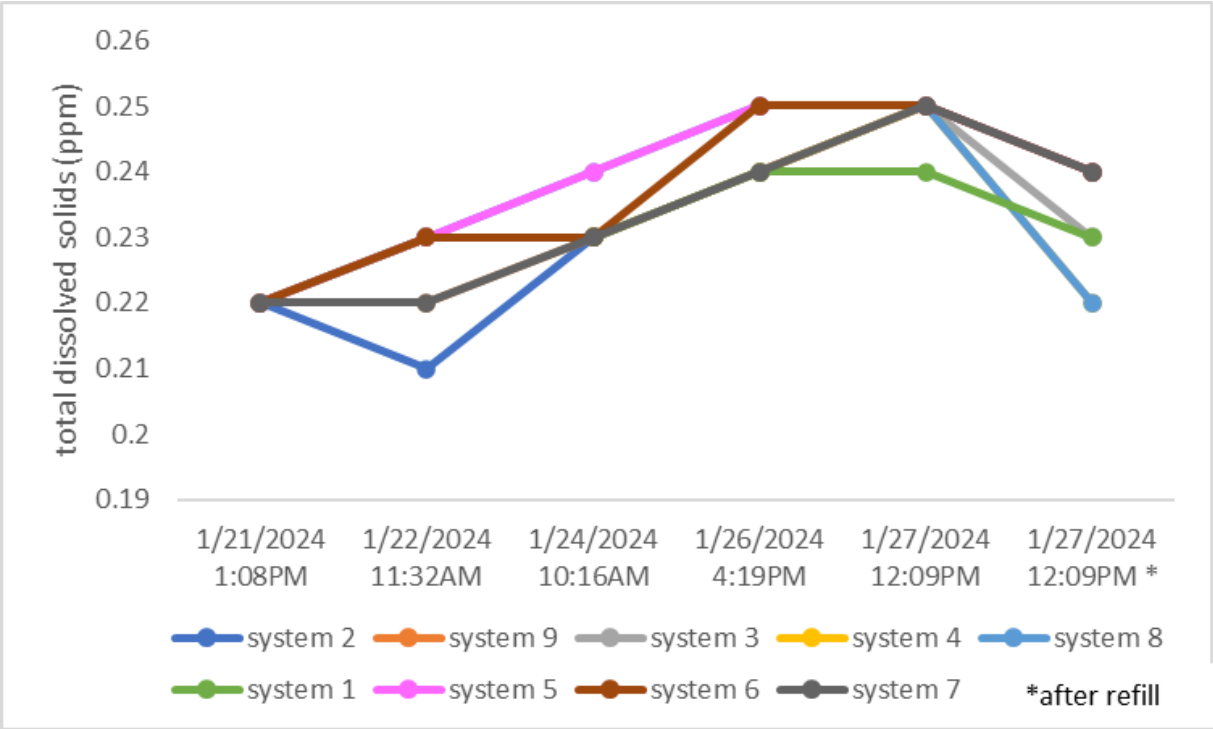


Figure D.4c: Total dissolved solids in ppm for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

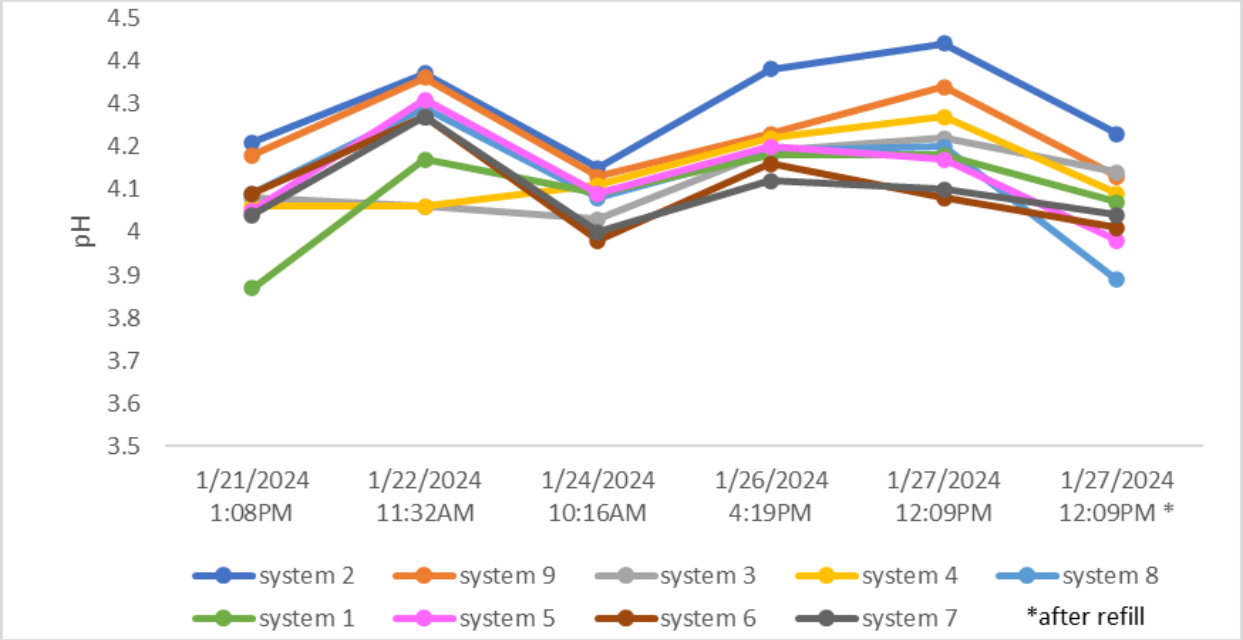


Figure D.4d: pH for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

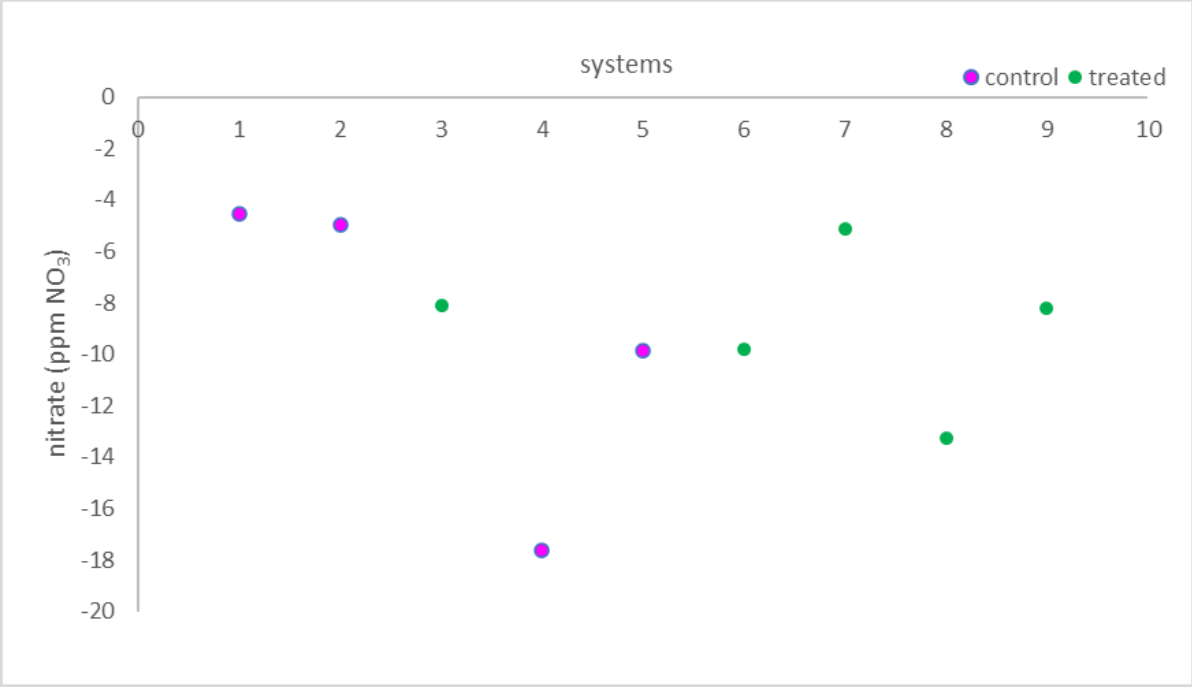


Figure D.4e: Decrease in nitrate concentration over Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

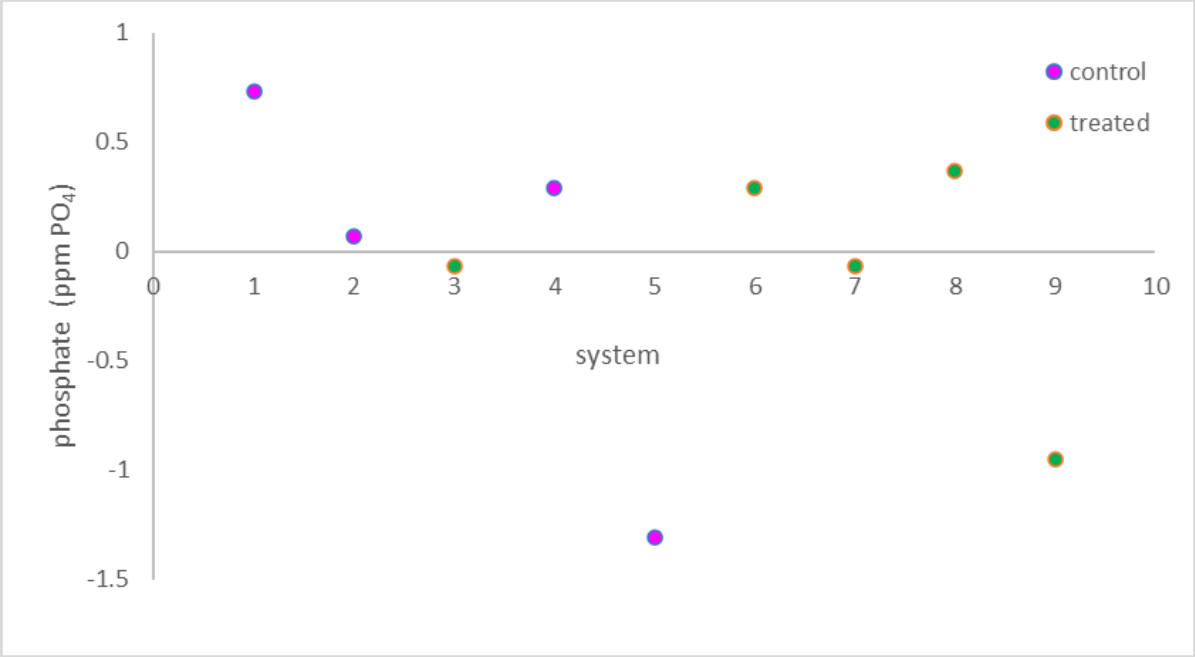


Figure D.4f: Decrease in phosphate concentration over Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

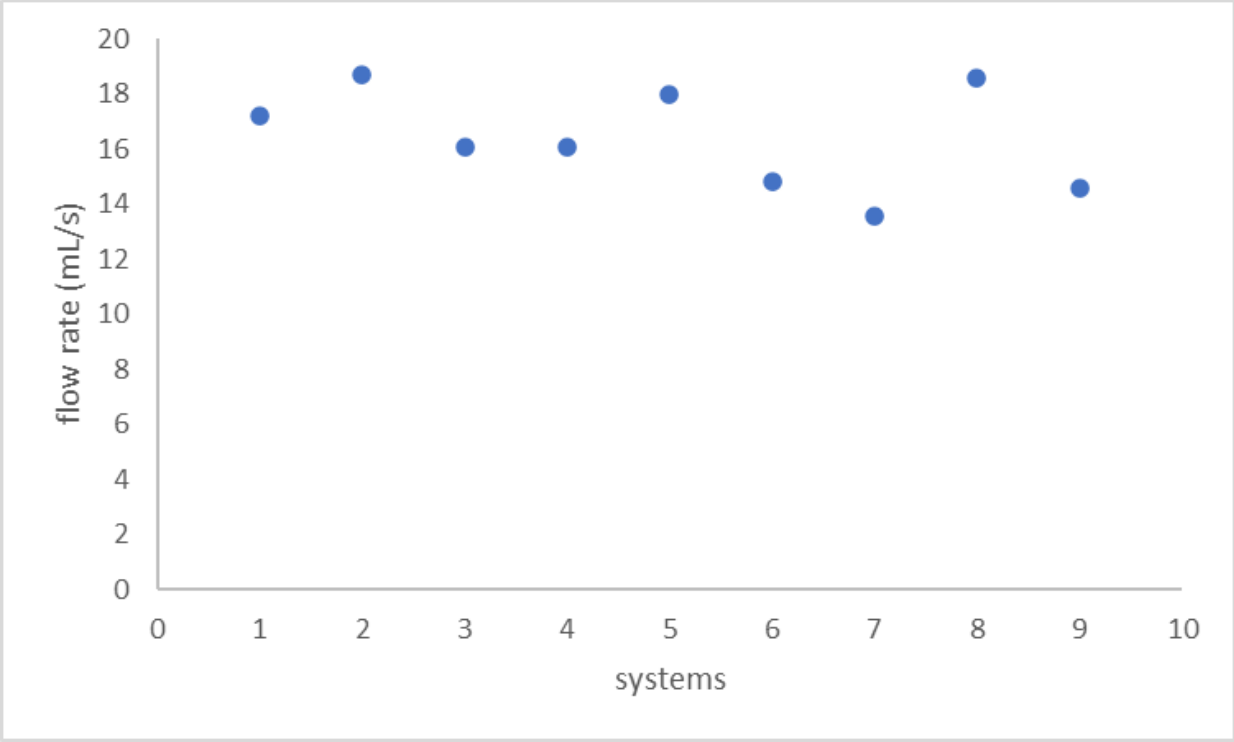


Figure D.4g: Average inlet flow rate in mL/s for Trial 6: The effect of the Town Creek Park stream bacteria biofilm on attachment in high nutrient synthetic media

Table D.4a: Channel travel time in s for Trial 6: The effect of the Town Creek Park bacteria biofilm on attachment in high nutrient synthetic media

System	Channel Travel Time (s)
1	1
2	1
3	2

4	1
5	1
6	2
7	1
8	1
9	2

Appendix D.5 Environmental conditions for Trial 7: The effect of Town Creek Park water bacteria biofilm in medium strength diluted, filtered aquaculture waste

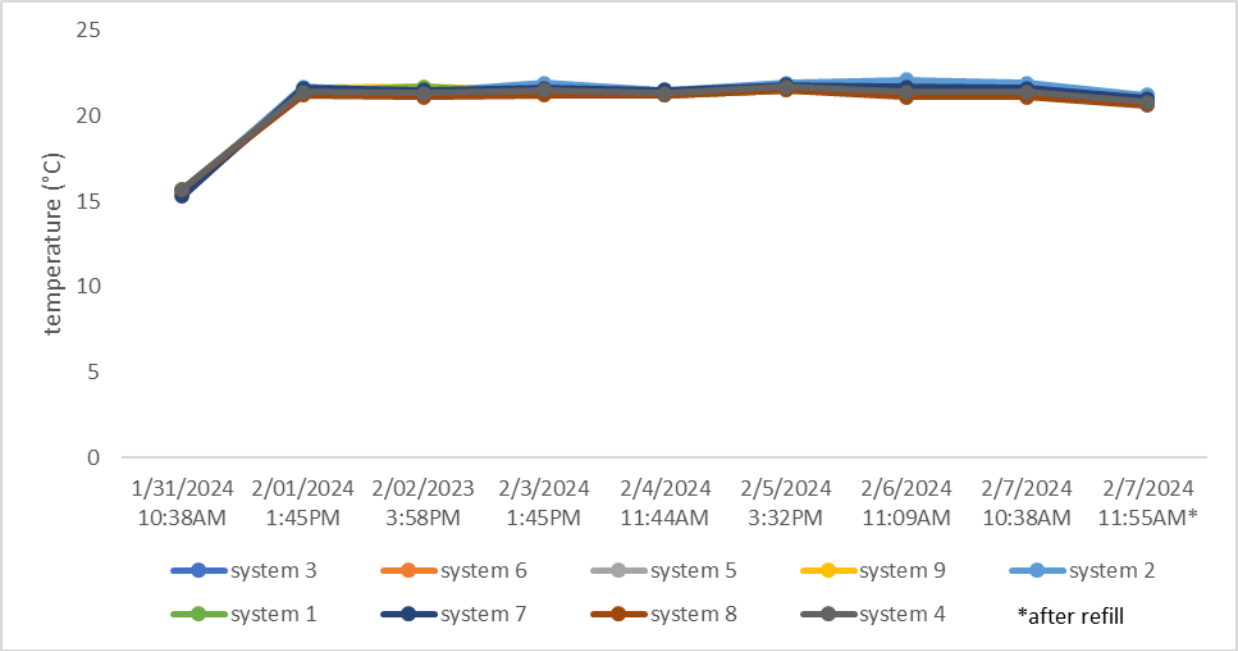


Figure D.5a: Temperatures in °C for Trial 7: The effect of the Town Creek Park stream biofilm on attachment in medium strength diluted, filtered aquaculture waste

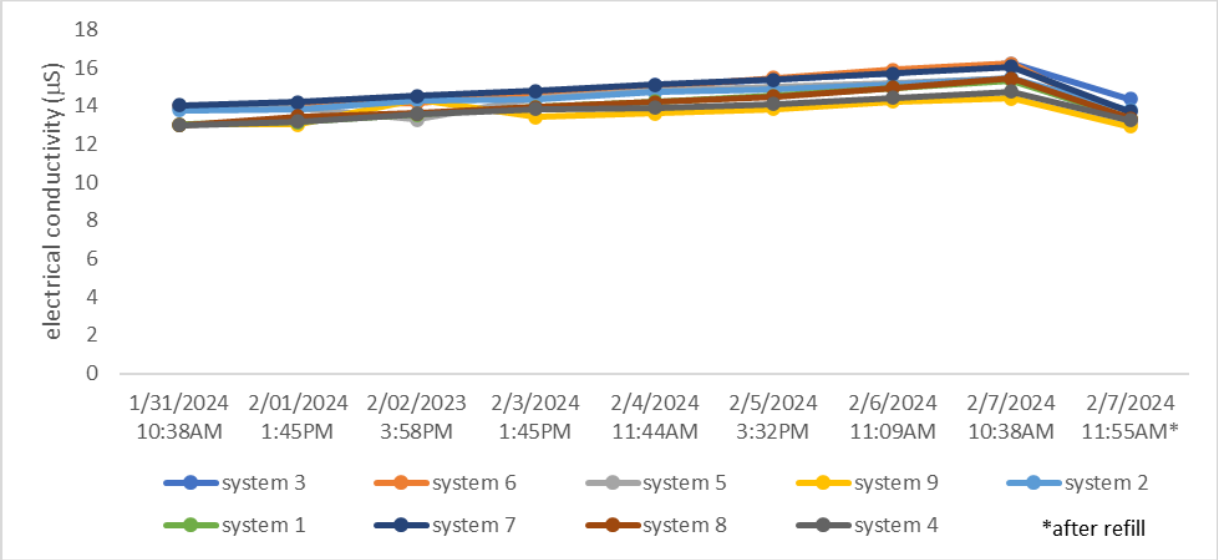


Figure D.5b: Electrical conductivities in µS for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

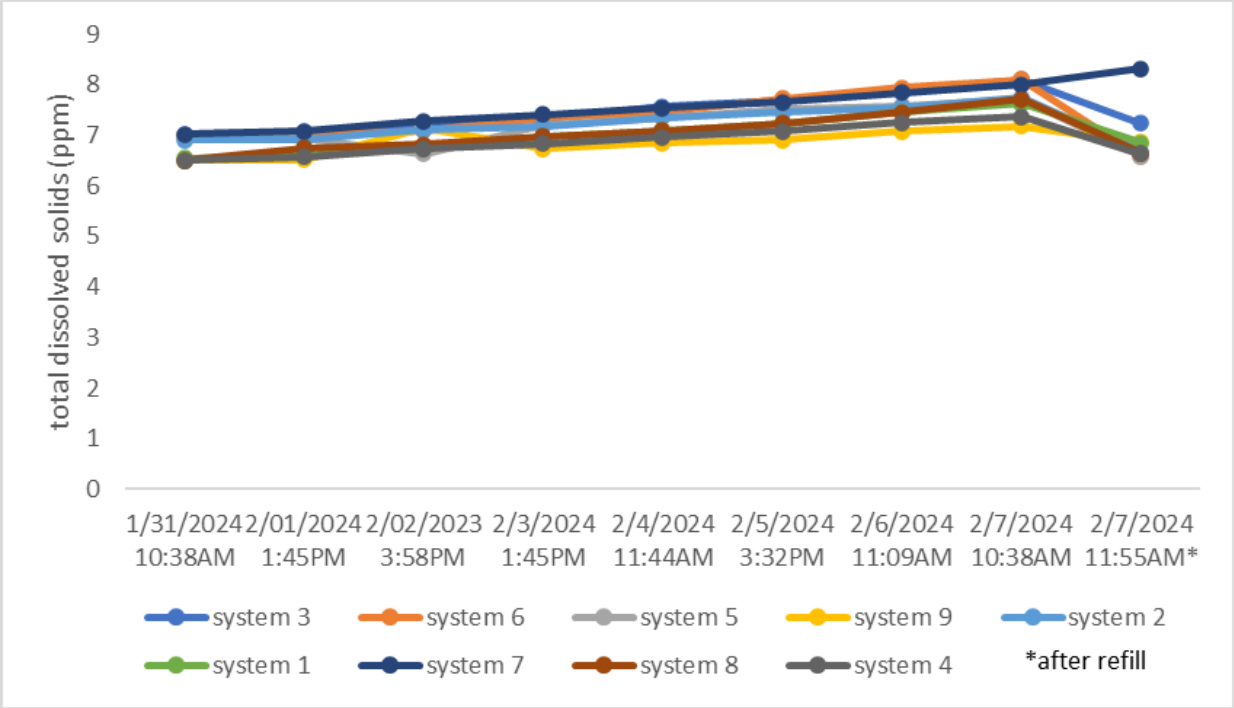


Figure D.5c: Total dissolved solids in ppm for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

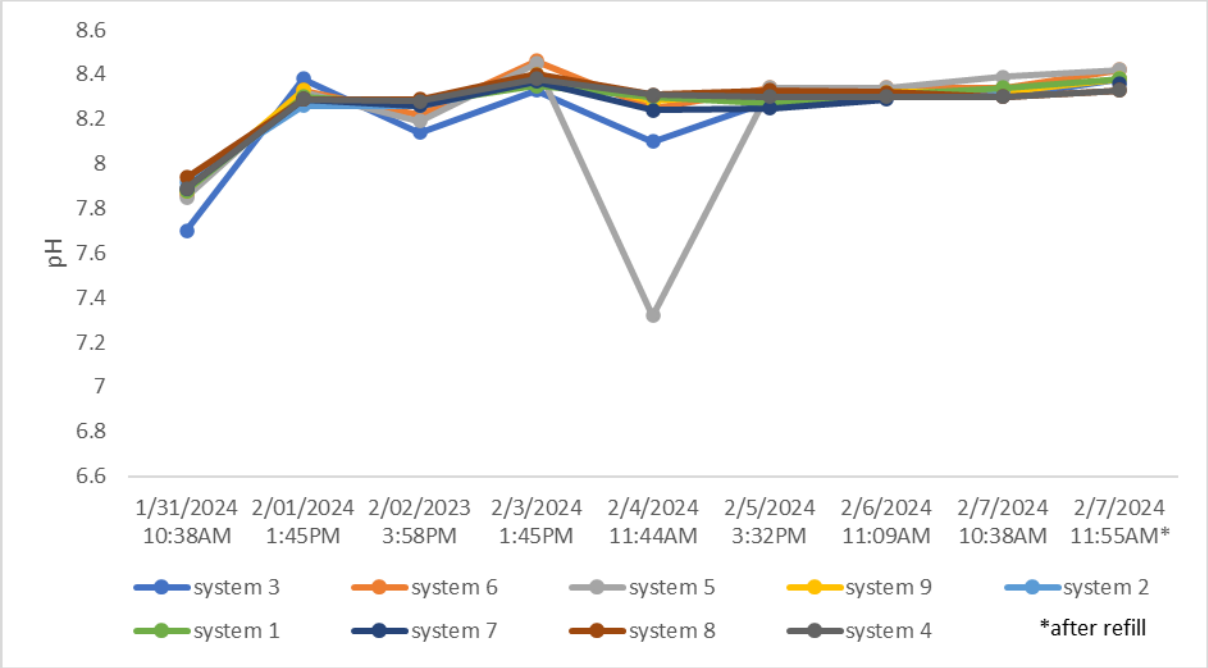


Figure D.5d: pH for Trial 7: The Town Creek Park bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

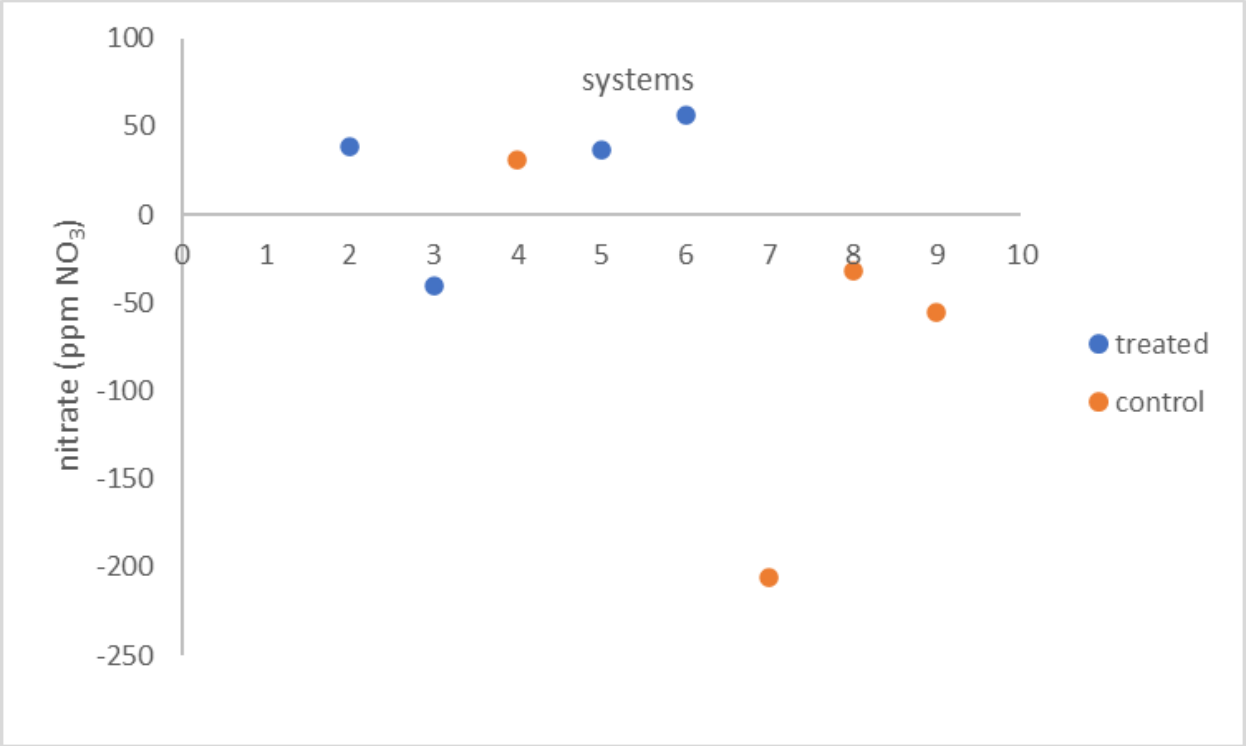


Figure D.5e: Decrease in nitrate concentration over Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

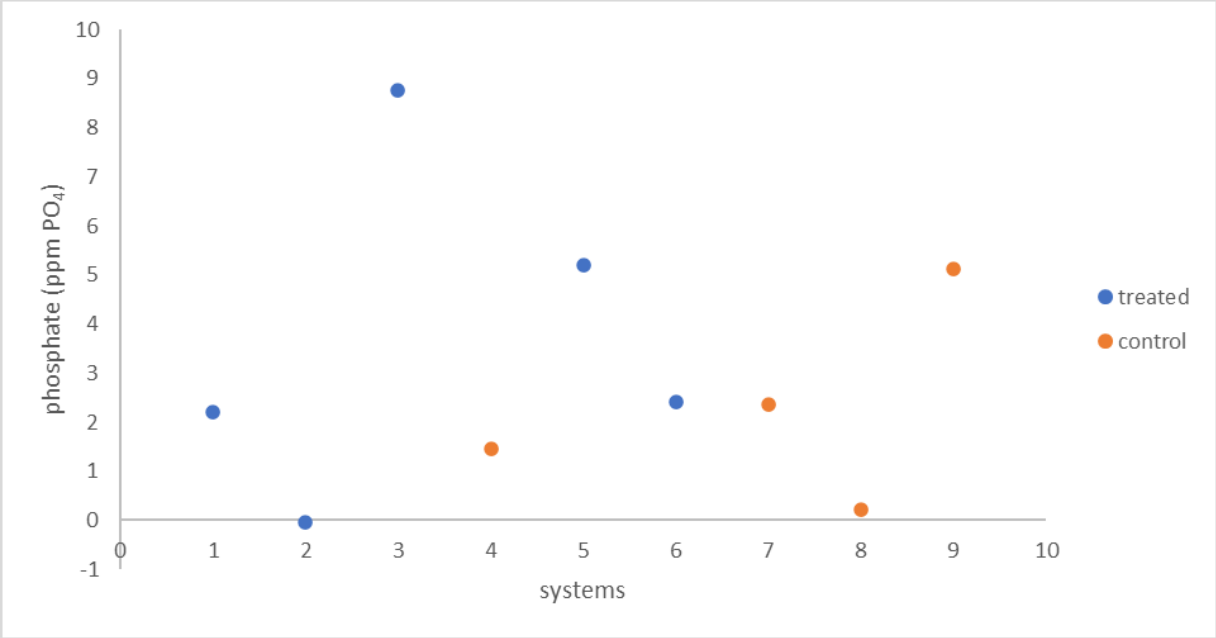


Figure D.5f: Decrease in phosphate concentration over Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

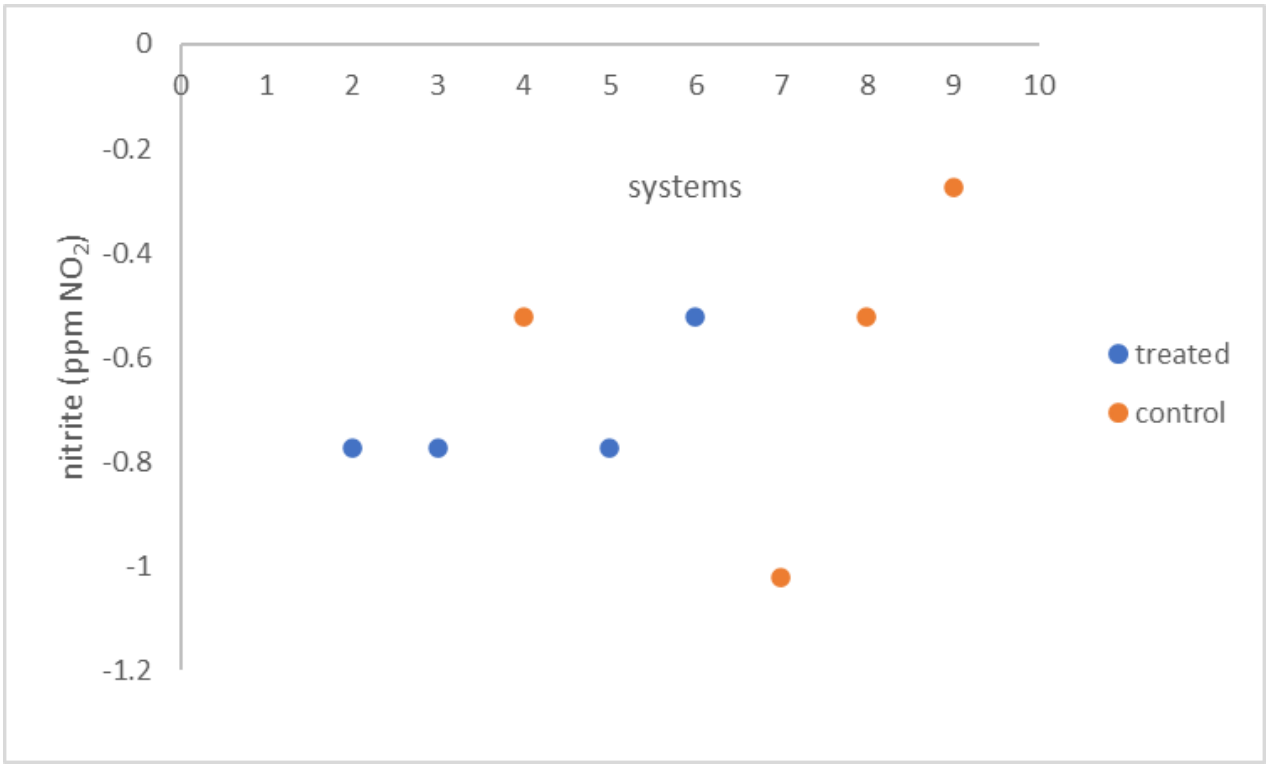


Figure D.5g: Decrease in nitrite concentration over Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

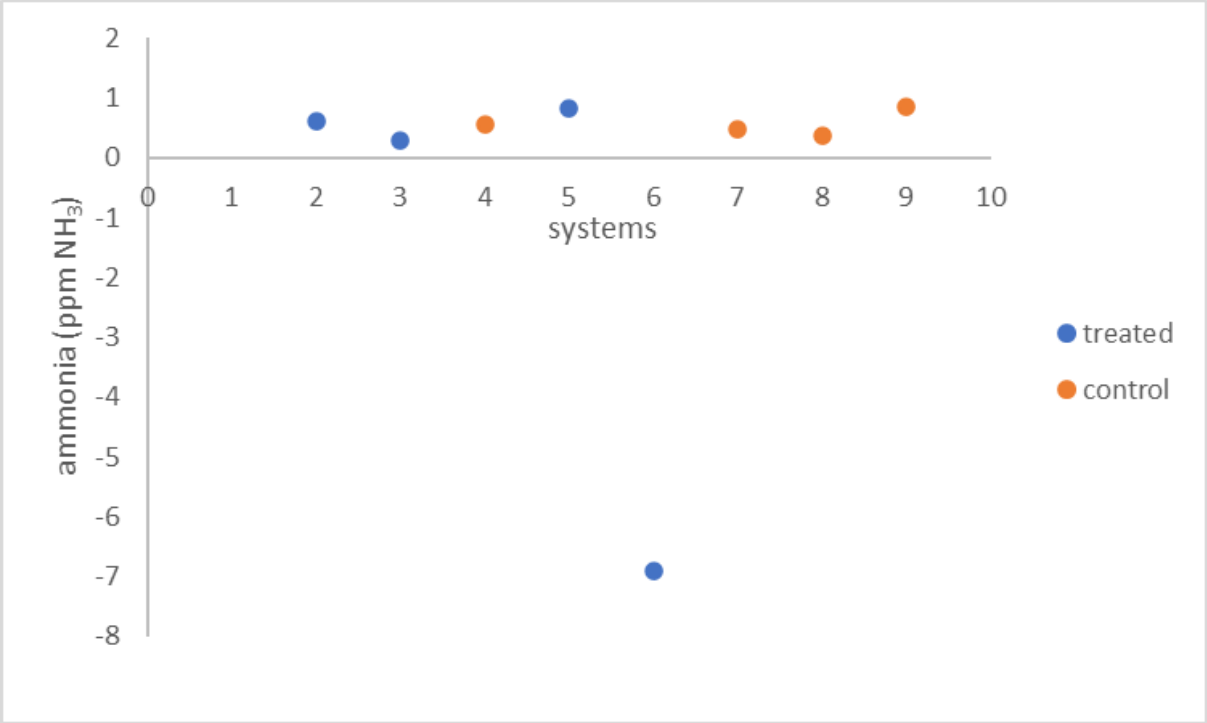


Figure D.5h: Decrease in ammonia concentration over Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

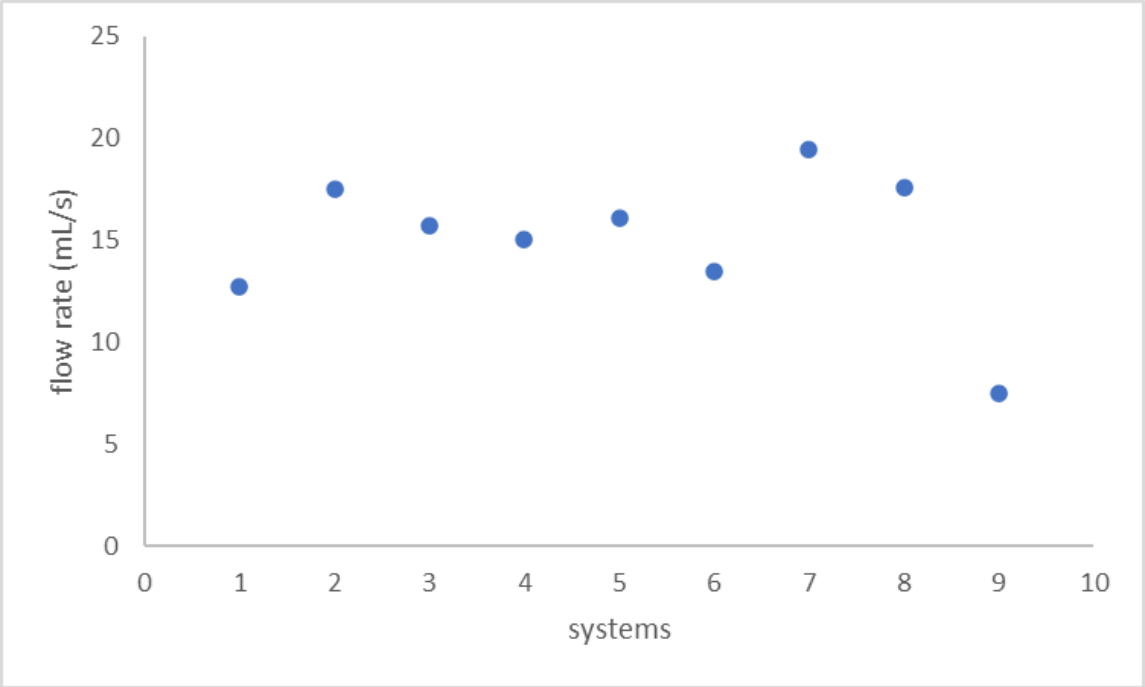


Figure D.5i: Average inlet flow rate in mL/s for Trial 7: The effect of the Town Creek Park stream bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

Table D.5a: Channel travel time in s for Trial 7: The effect of the Town Creek Park bacteria biofilm on attachment in medium strength diluted, filtered aquaculture waste

System	Channel Travel Time (s)
1	2
2	1
3	2

4	1
5	2
6	2
7	2
8	2
9	2

Appendix D.6 Environmental conditions for Trial 8: The effect of the mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with reduced slope

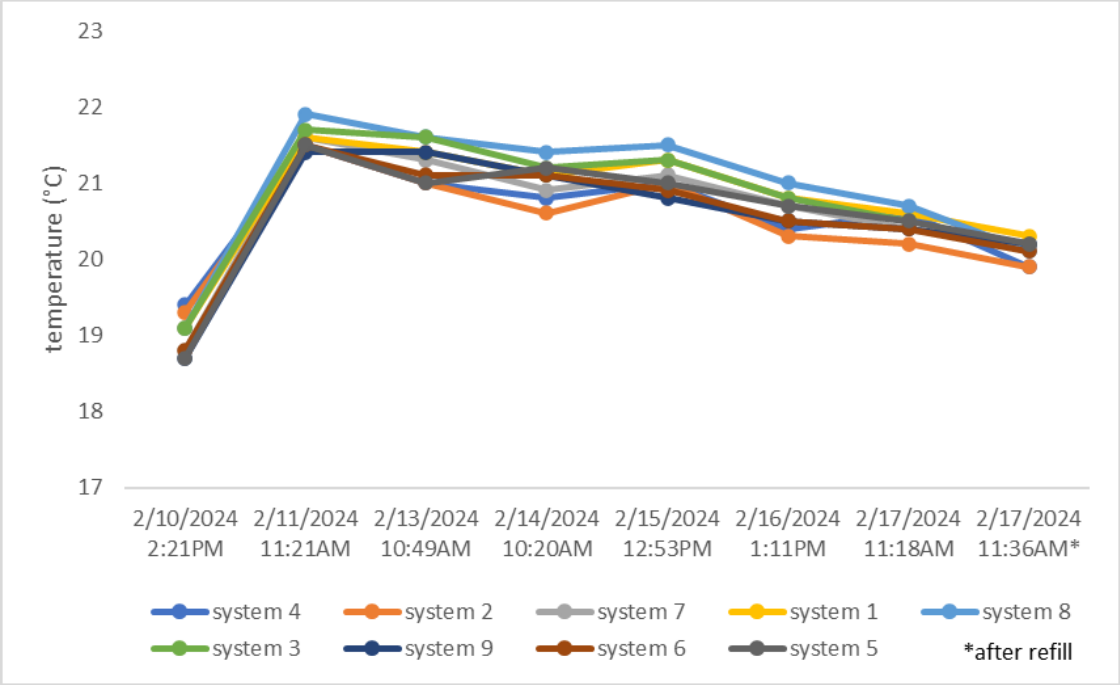


Figure D.6a: Temperatures in °C for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

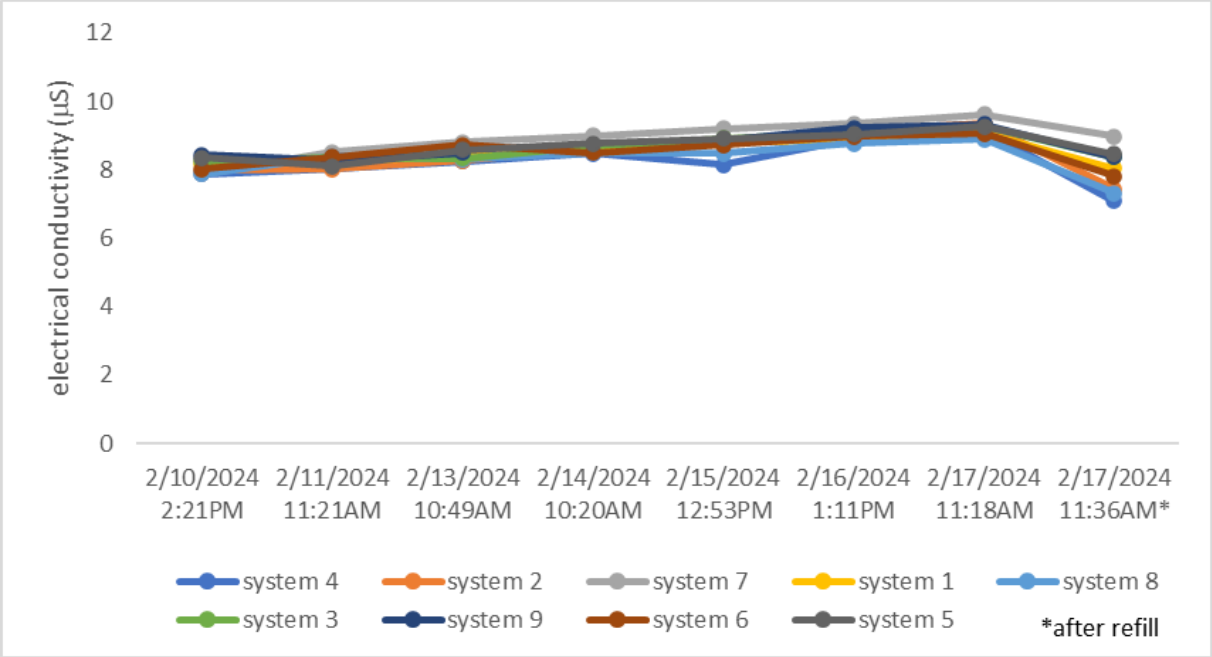


Figure D.6b: Electrical conductivities in µS for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

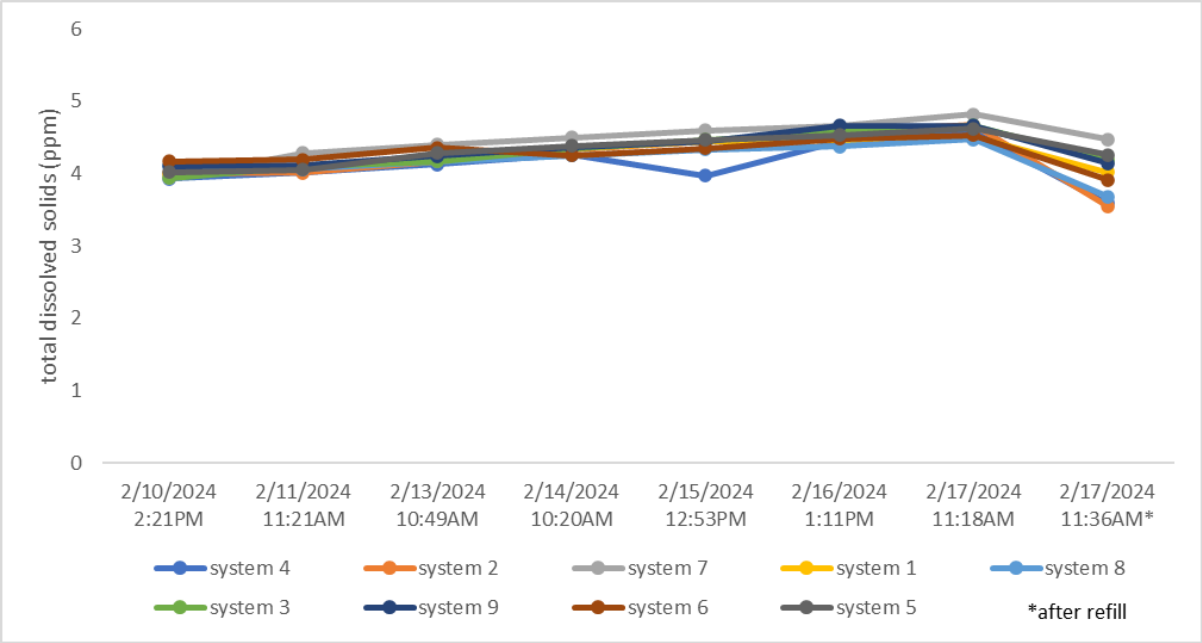


Figure D.6c: Total dissolved solids in ppm for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

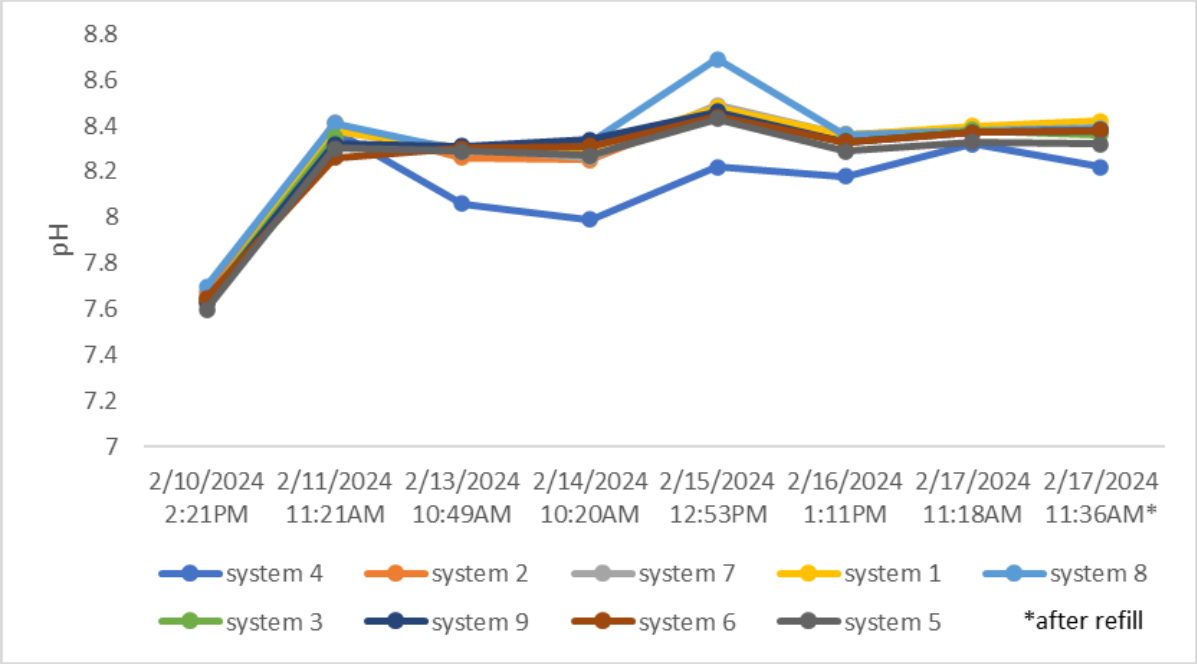


Figure D.6d: pH for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

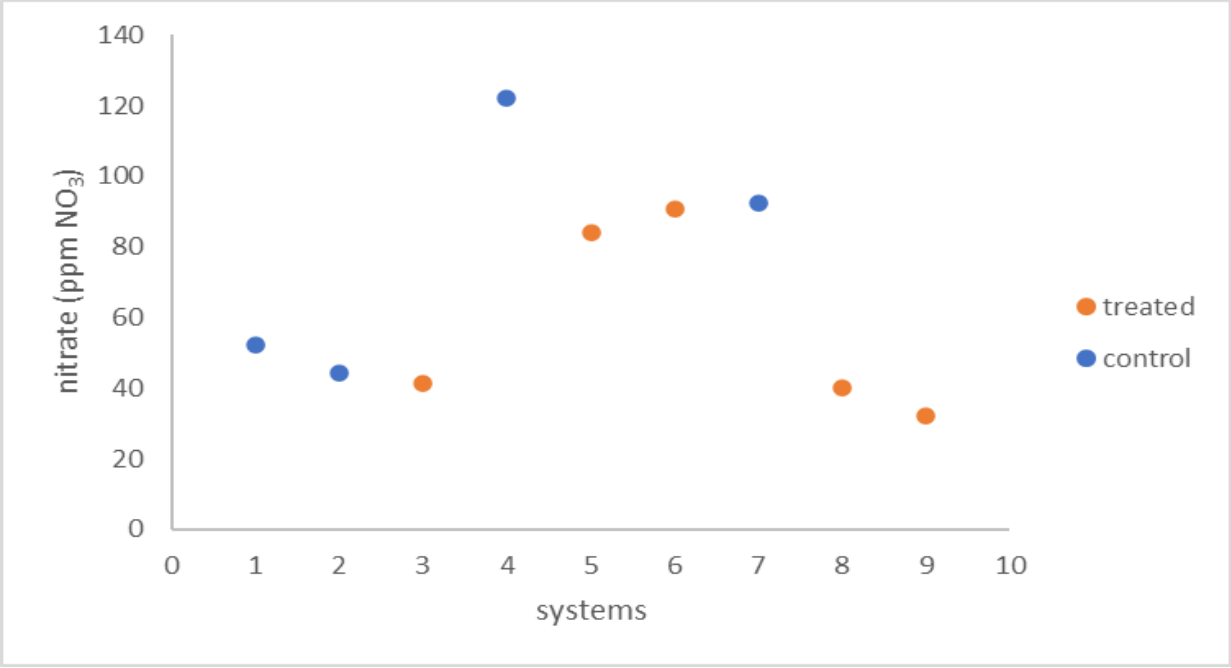


Figure D.6e: Decrease in nitrate concentration over Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

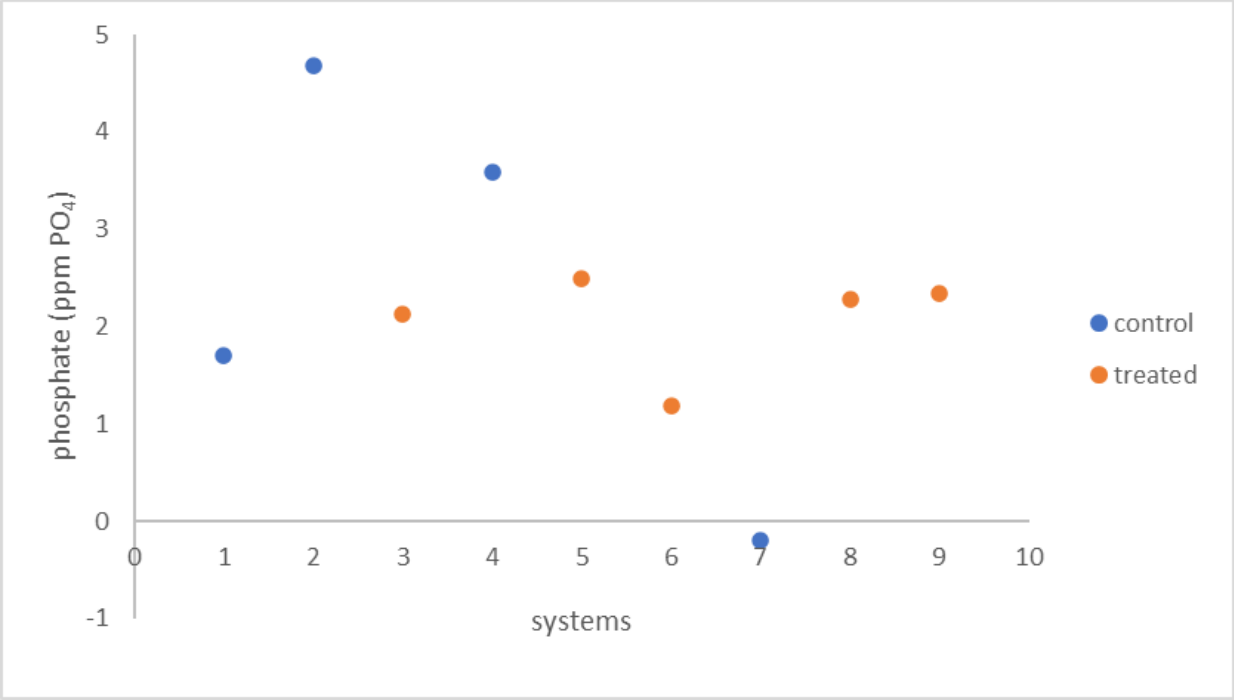


Figure D.6g: Decrease in phosphate concentration over Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

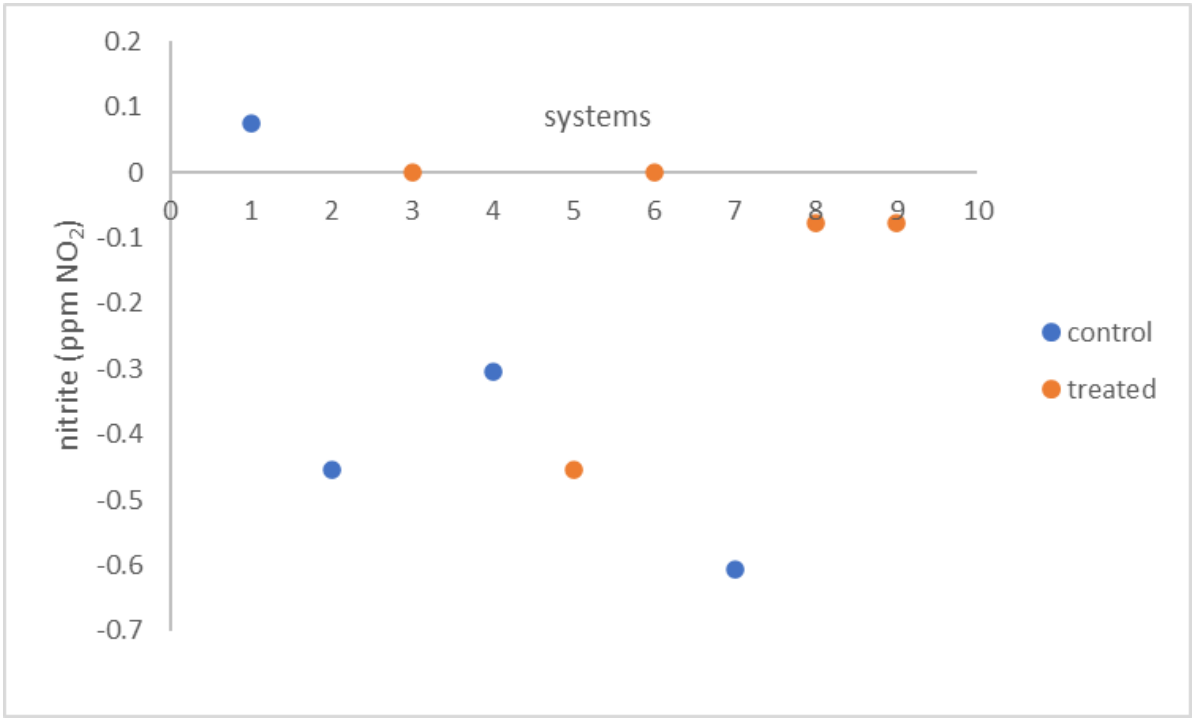


Figure D.6h: Decrease in nitrite concentration over Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

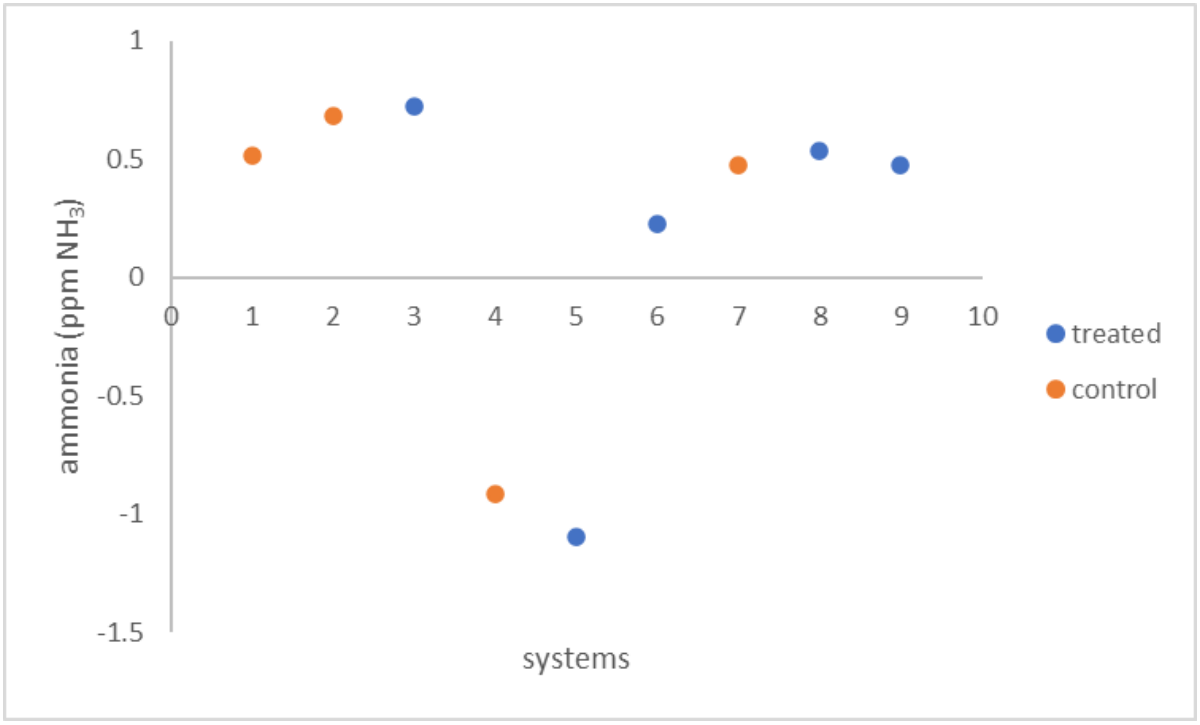


Figure D.6i: Decrease in ammonia concentration over Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

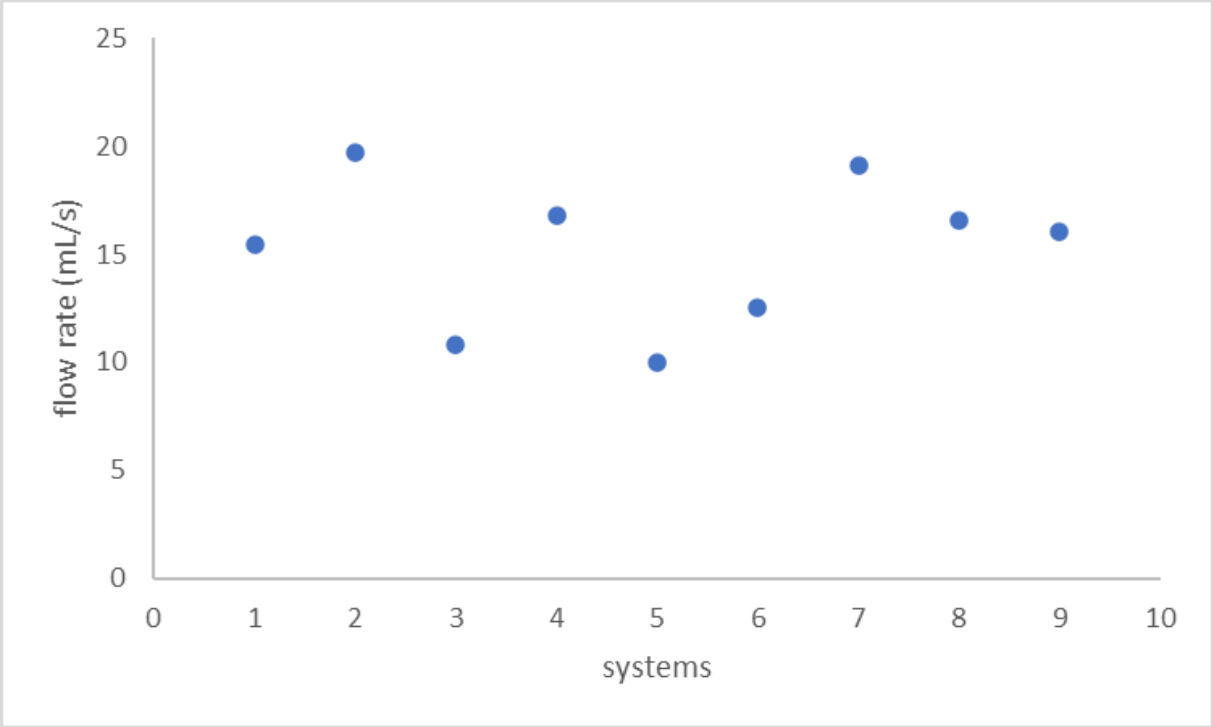


Figure D.6g: Average inlet flow rate in mL/s for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

Table D.6a: Channel travel time in s for Trial 8: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

System	Channel Travel Time (s)
1	8
2	2
3	4

4	2
5	7
6	2
7	1
8	2
9	2

Appendix D.7 Environmental conditions for Trial 9: The effect of the mixed bacteria biofilm in undiluted, unfiltered aquaculture waste with reduced slope

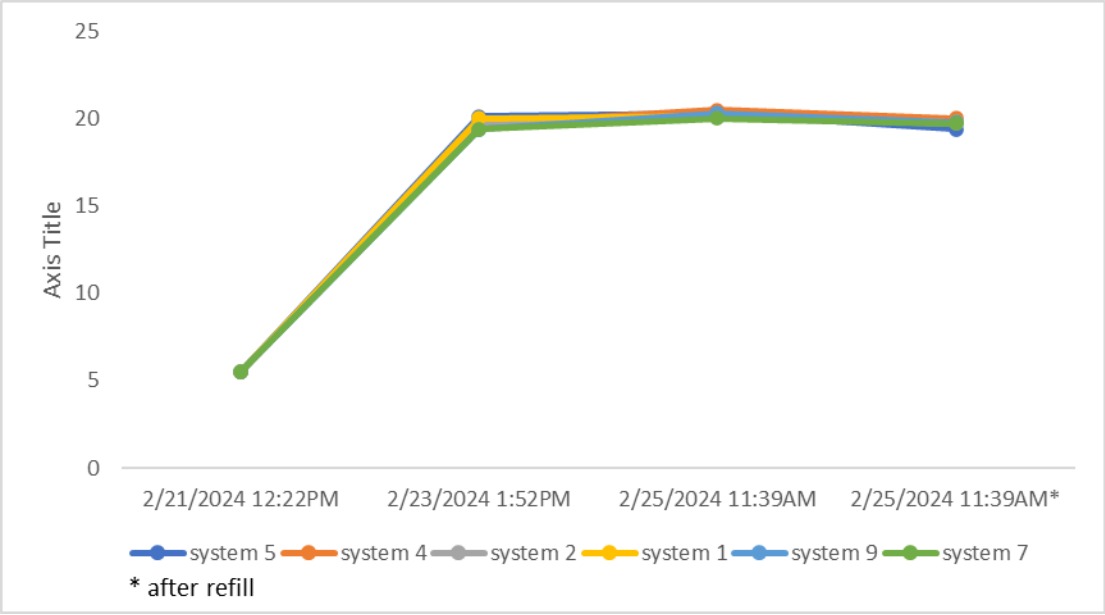


Figure D.7a: Temperatures in °C for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

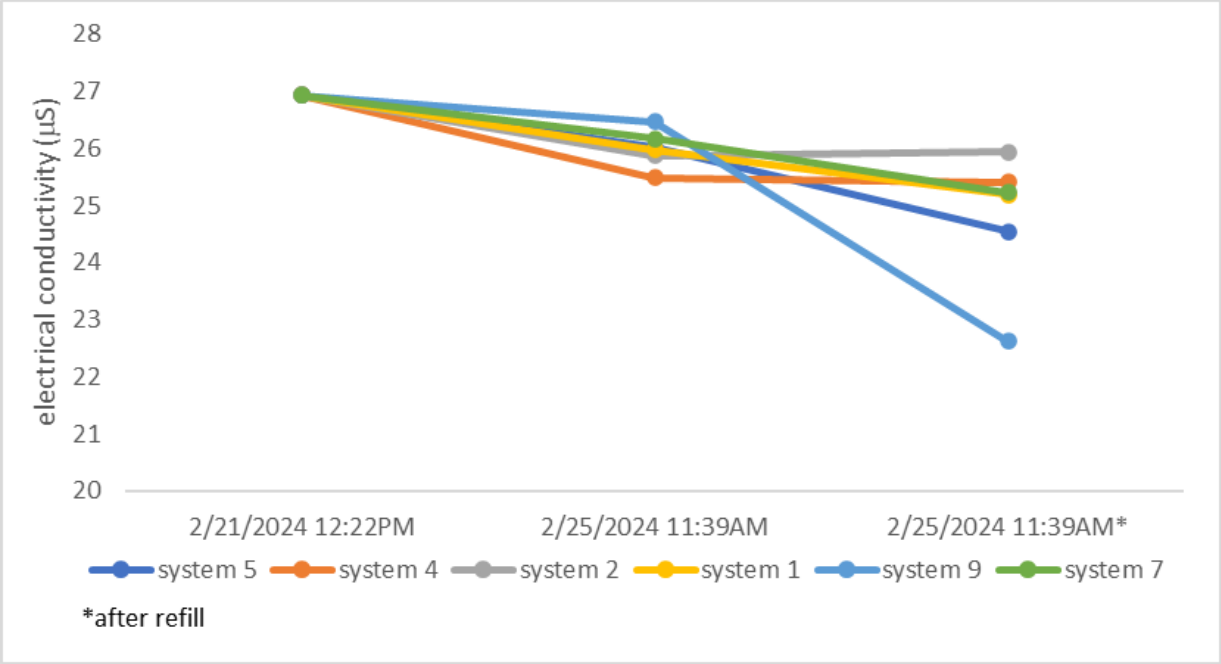


Figure D.7b: Electrical conductivities in µS for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

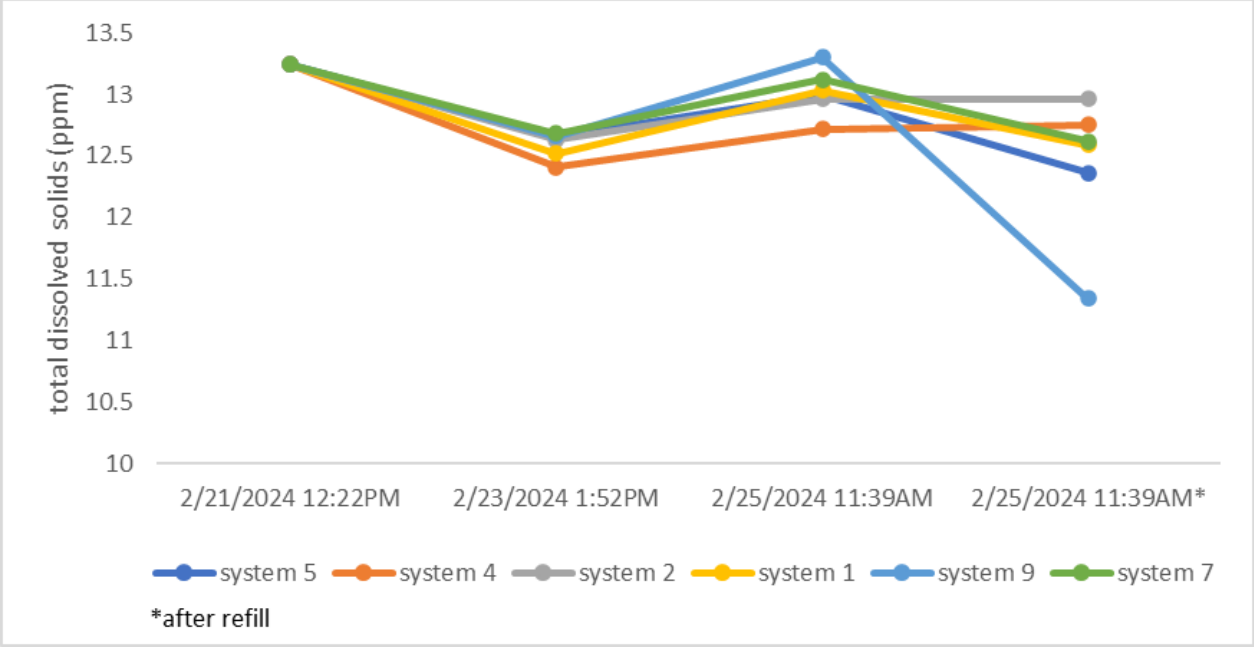


Figure D.7c: Total dissolved solids in ppm for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

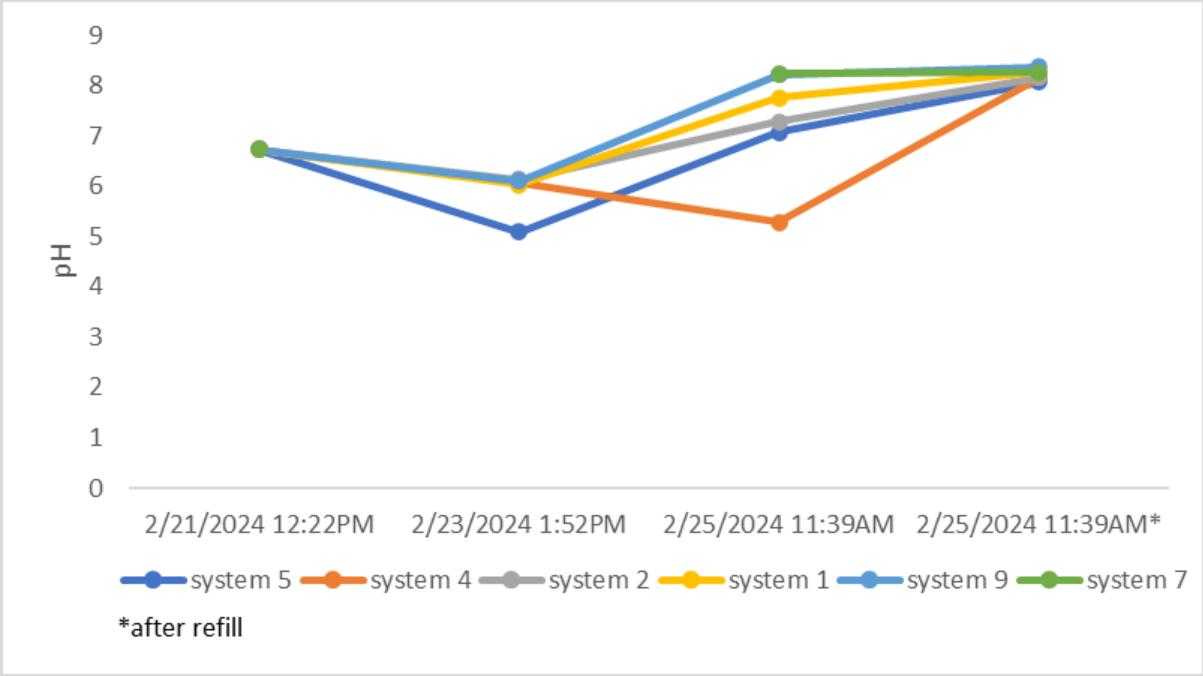


Figure D.7d: pH for Trial 9: The mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

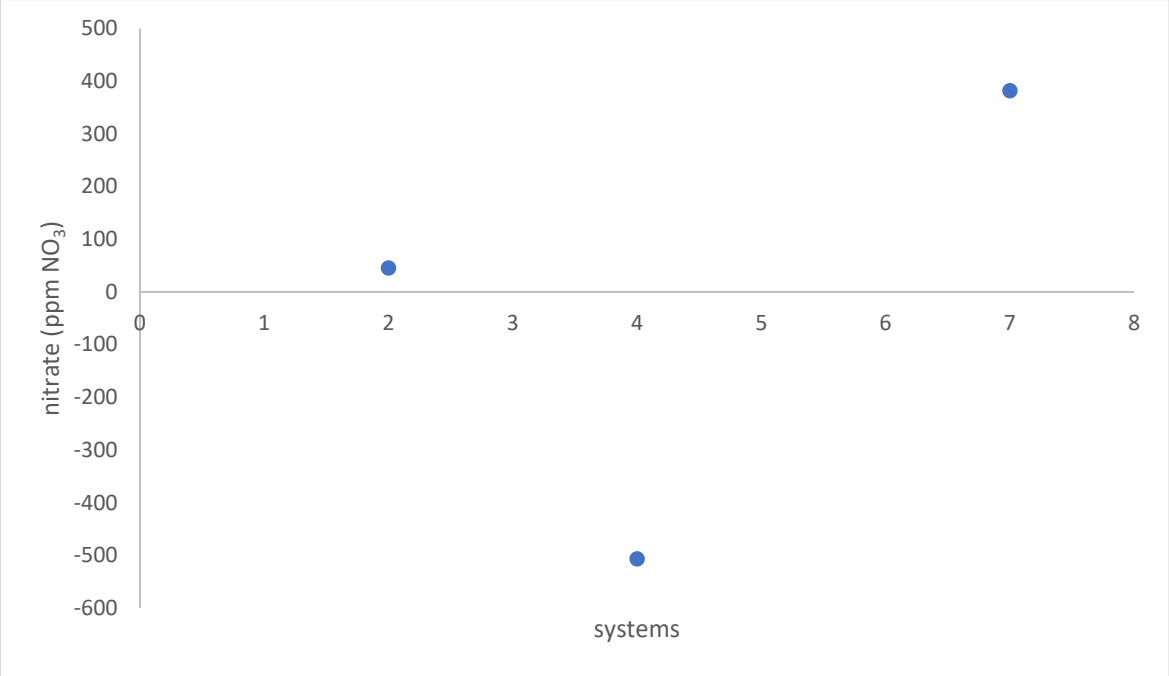


Figure D.7e: Decrease in nitrate concentration over Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope (note: the

data for systems 1, 5, and 9 is not available; the systems for which data was available are control systems)

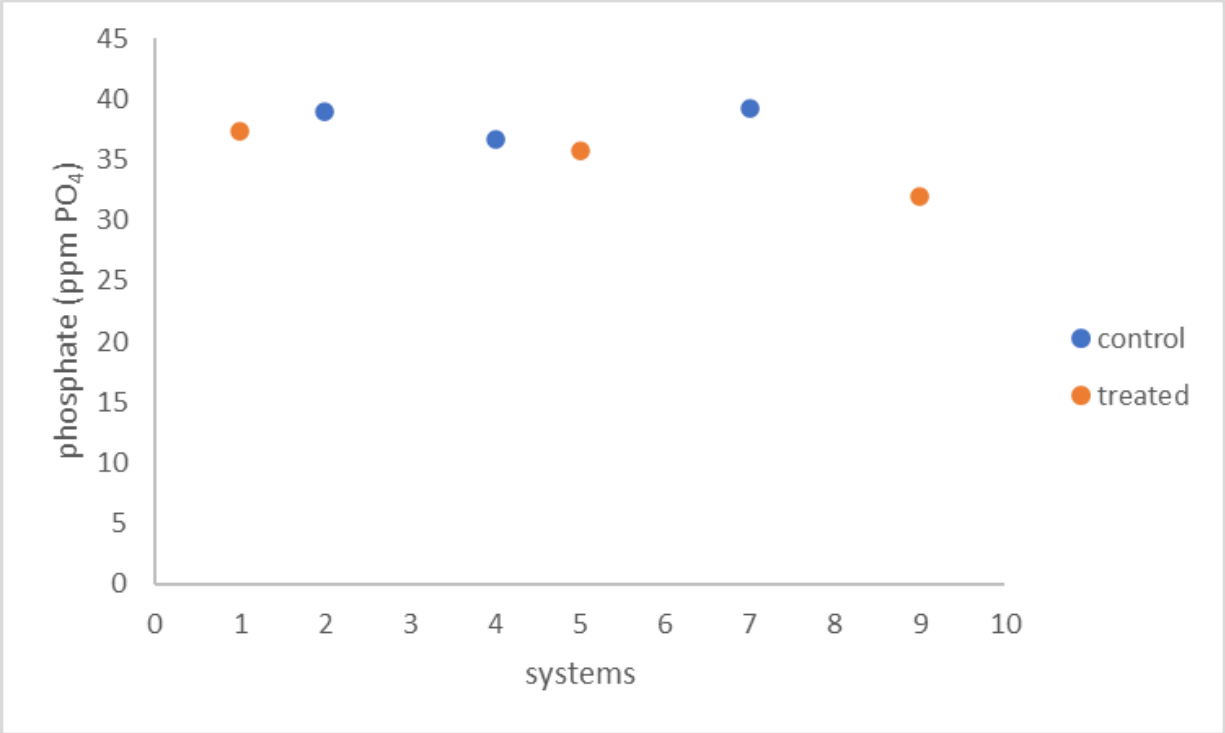


Figure D.7f: Decrease in phosphate concentration over Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

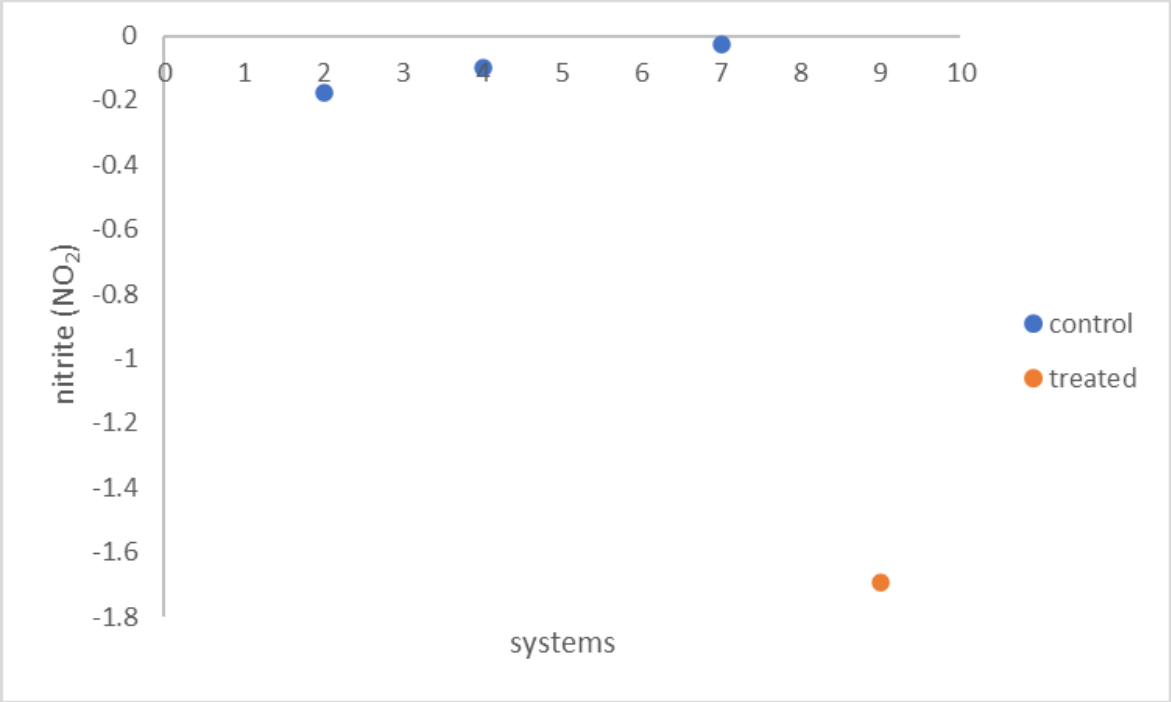


Figure D.7g: Decrease in nitrite concentration over Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope (note: the data for systems 1 and 5 is not available)

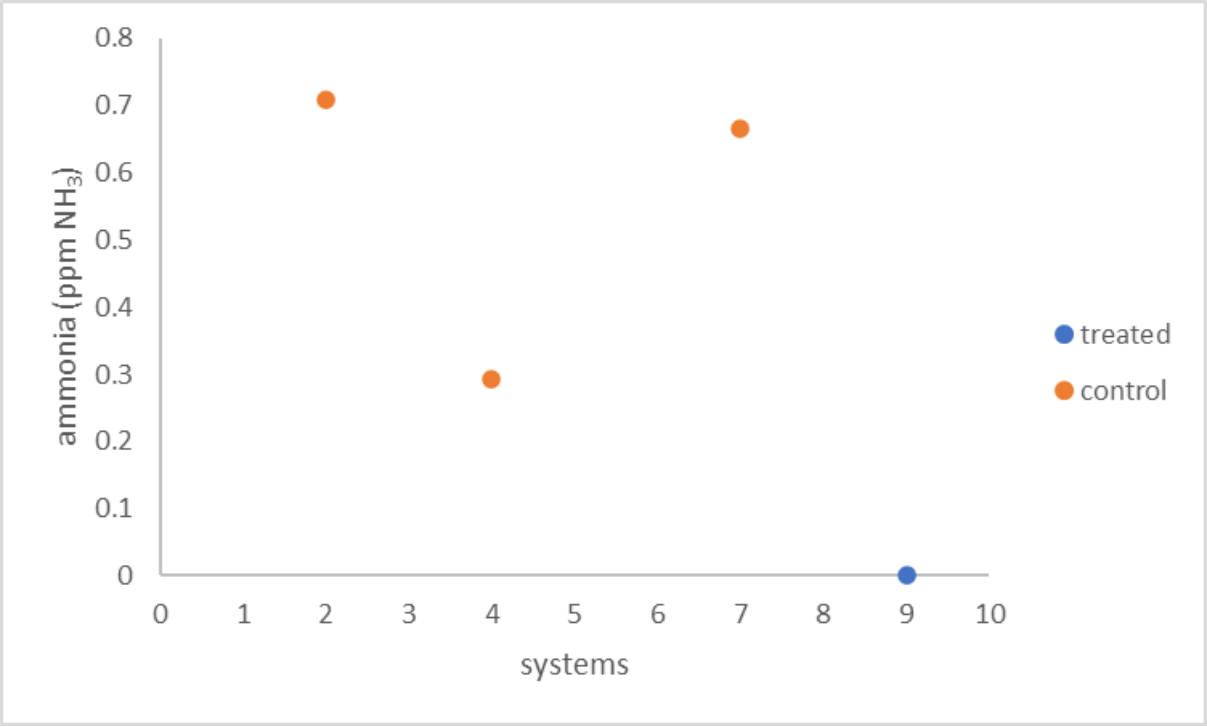


Figure D.7h: Decrease in ammonia concentration over Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope (note: the data for systems 1 and 5 is not available)

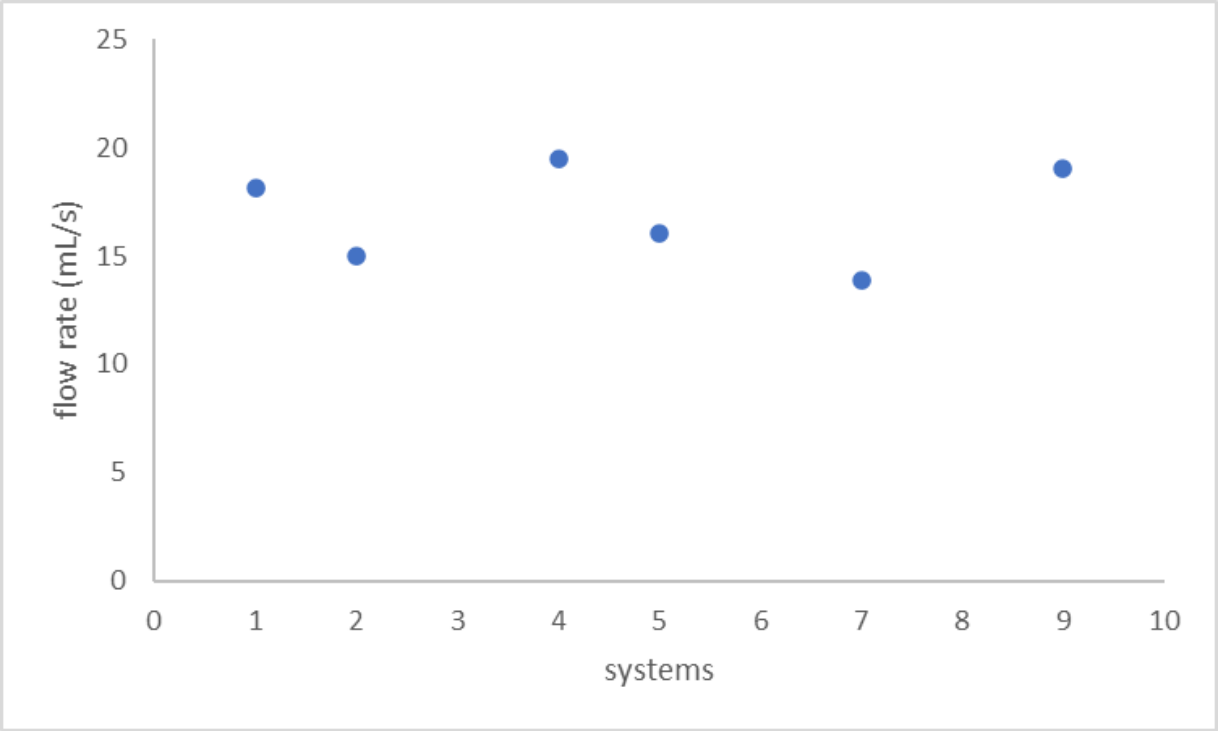


Figure D.7i: Average inlet flow rate in mL/s for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

Table D.7a: Channel travel time in s for Trial 9: The effect of the mixed bacteria biofilm on attachment in undiluted, unfiltered aquaculture waste with reduced slope

System	Channel Travel Time (s)
1	3
2	3
4	2
5	8
7	1

9	6

Appendix D.8 Environmental conditions for Trial 10: The effect of the mixed bacteria biofilm in half-diluted, unfiltered aquaculture waste with reduced slope

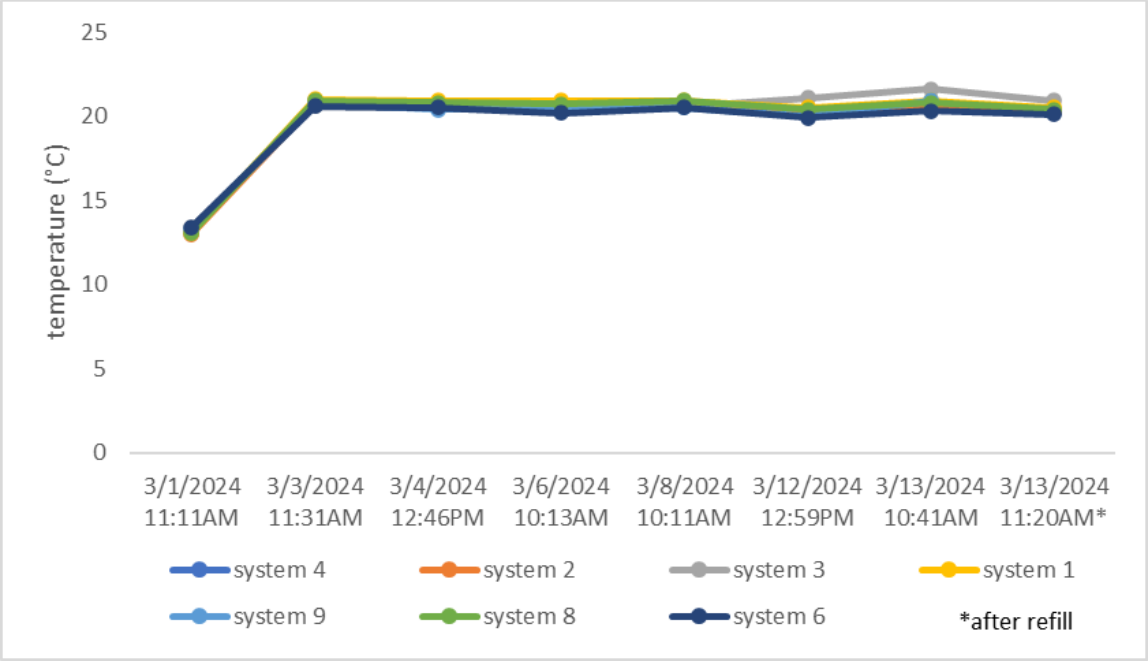


Figure D.8a: Temperatures in °C for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

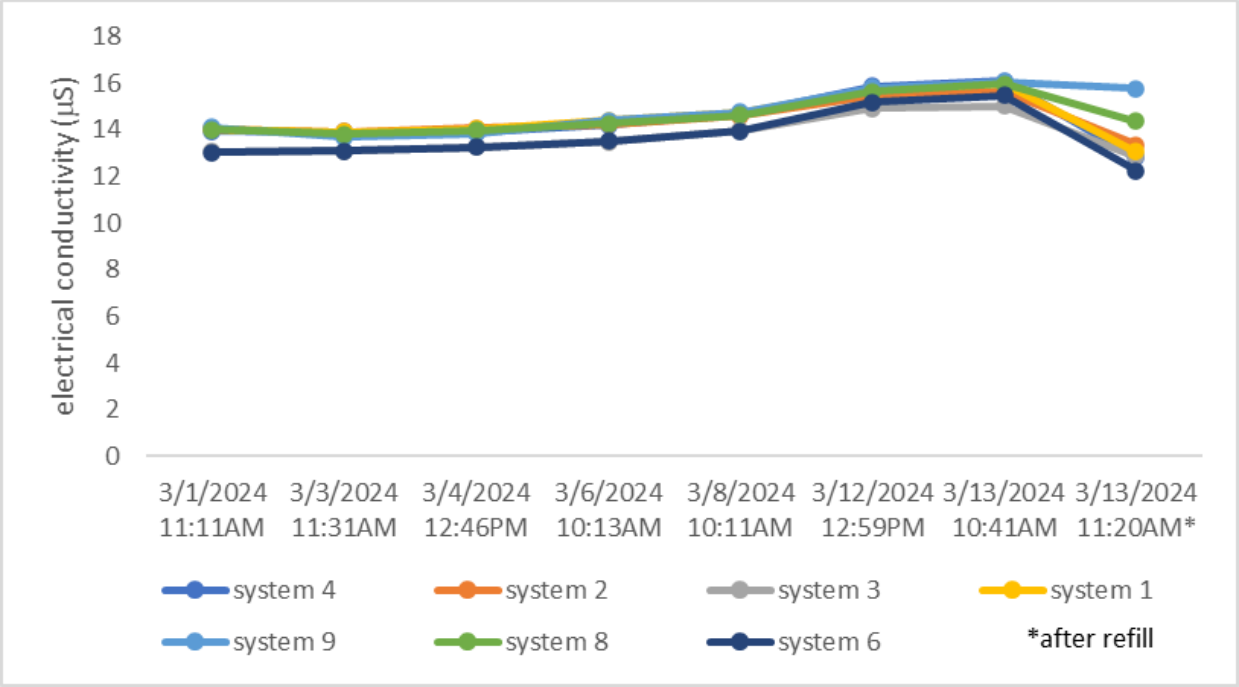


Figure D.8b: Electrical conductivities in µS for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

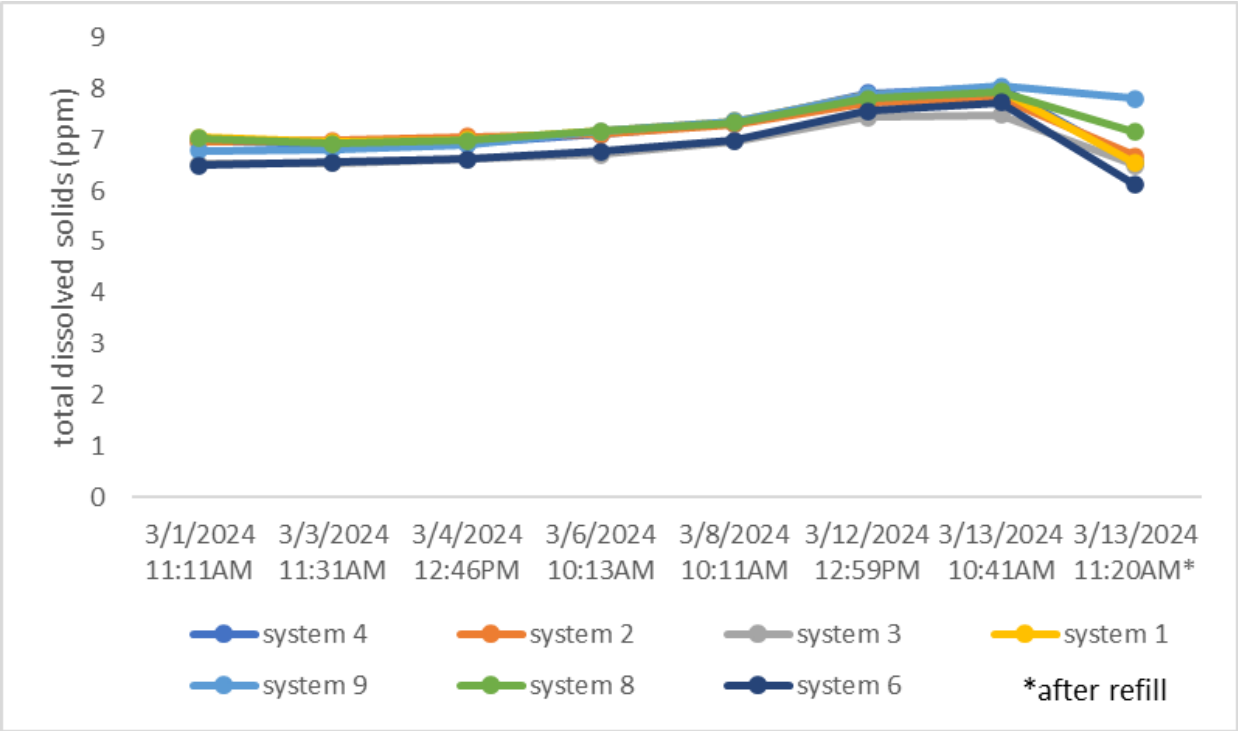


Figure D.8c: Total dissolved solids in ppm for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

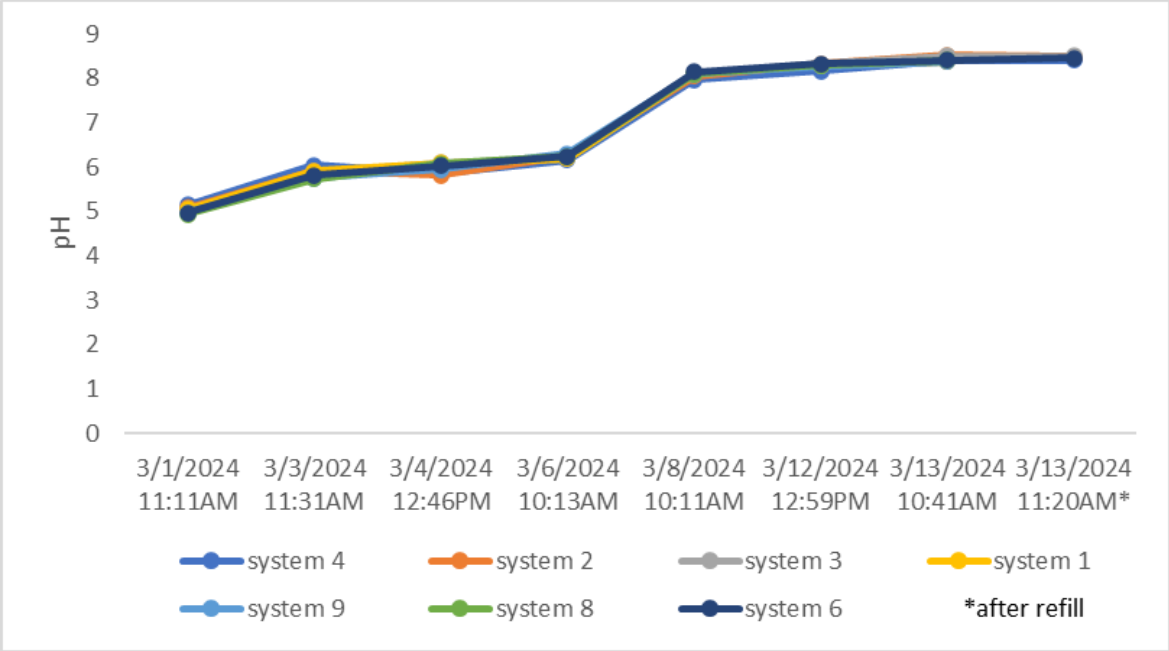


Figure D.8d: pH for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

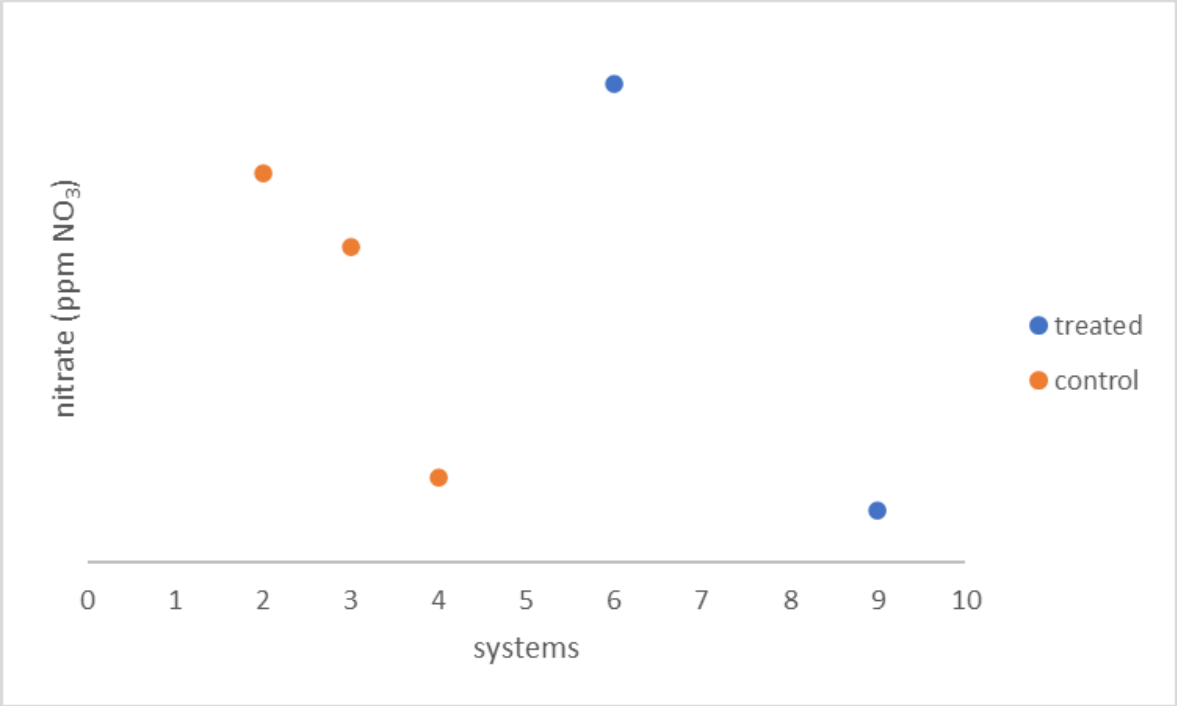


Figure D.8e: Decrease in nitrate concentration over Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope (nitrate data for systems 1 and 8 is unavailable)

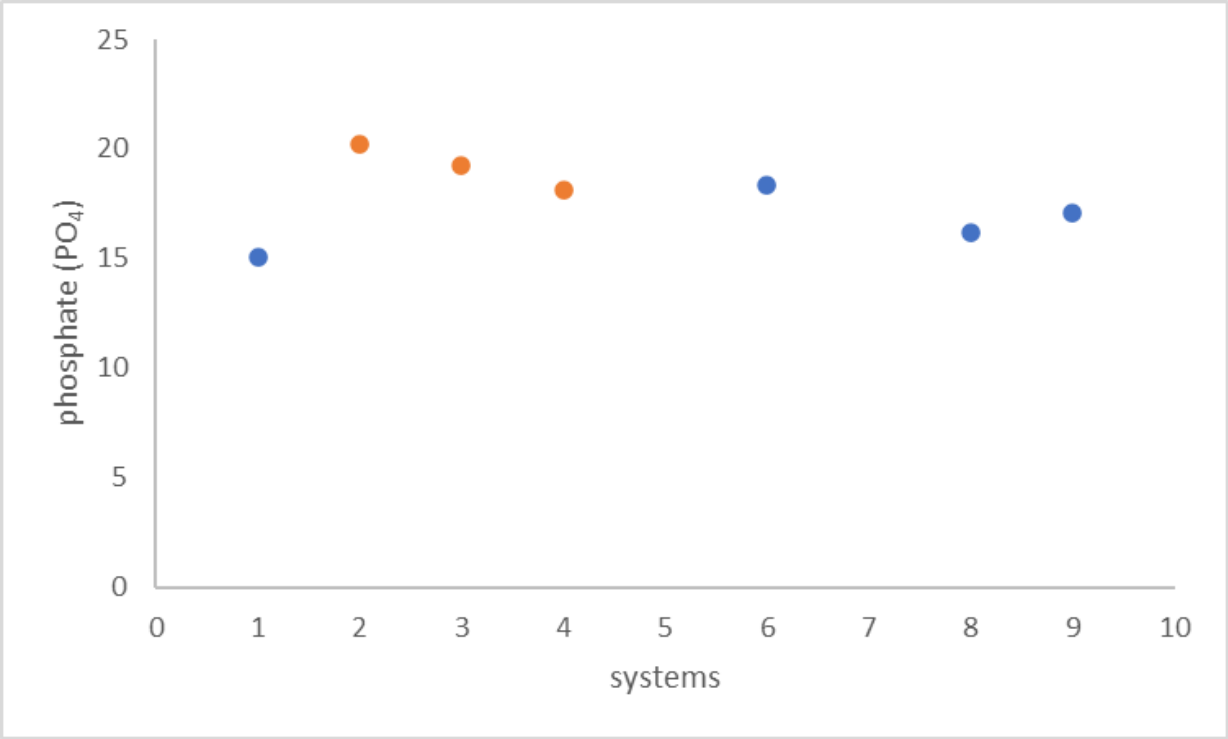


Figure D.8f: Decrease in phosphate concentration over Trial 10: The effect of the mixed bacteria biofilm on attachment in l half-diluted, unfiltered aquaculture waste with reduced slope

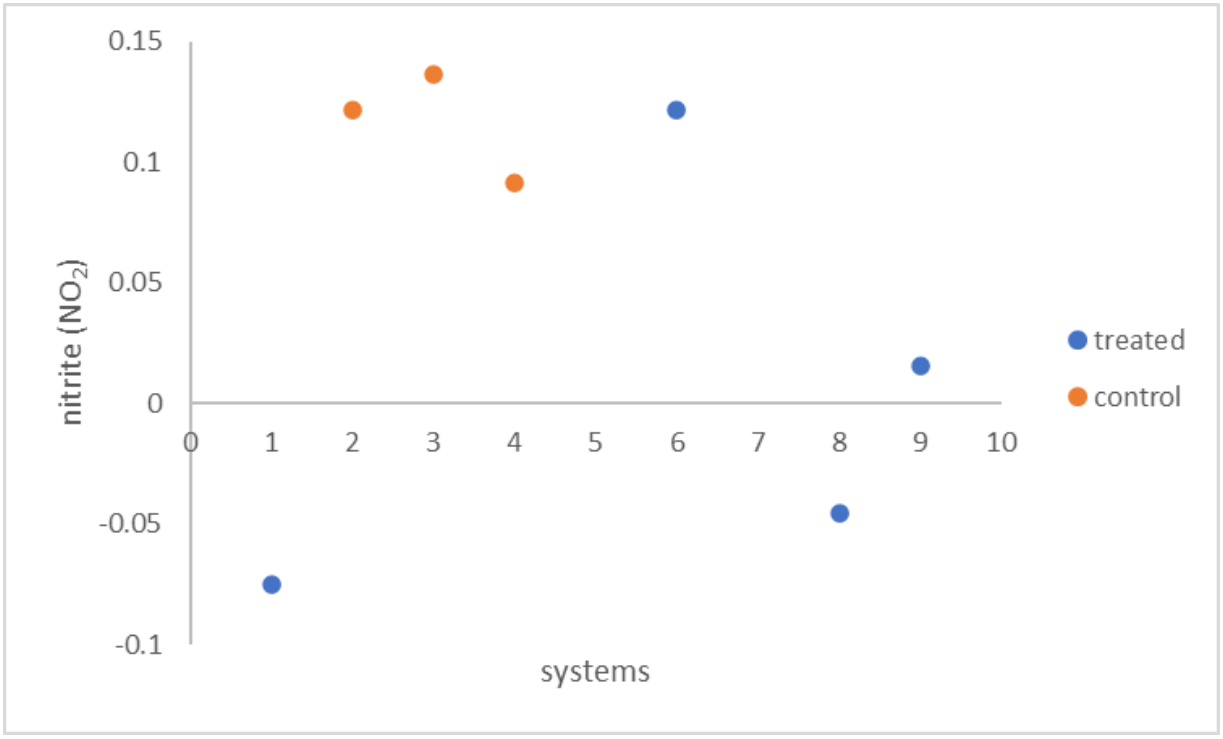


Figure D.8g: Decrease in nitrite concentration over Trial 10: The effect of the mixed bacteria biofilm on attachment in l half-diluted, unfiltered aquaculture waste with reduced slope

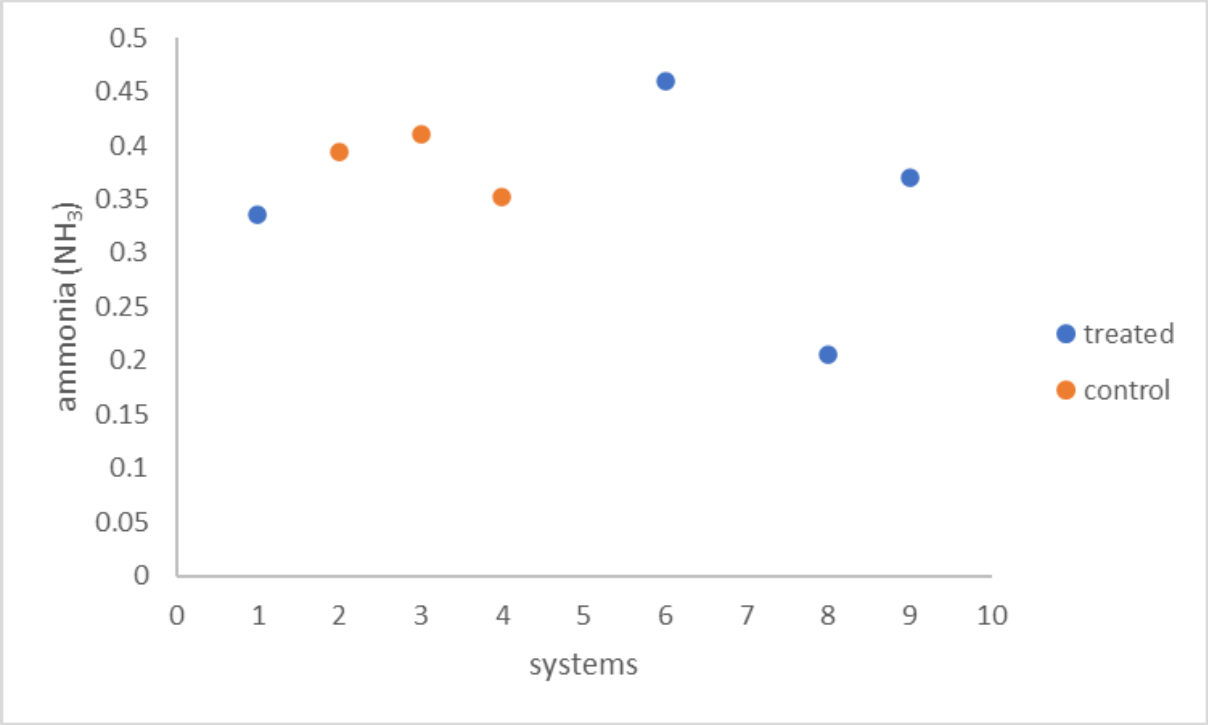


Figure D.8h: Decrease in ammonia concentration over Trial 10: The effect of the mixed bacteria biofilm on attachment in l half-diluted, unfiltered aquaculture waste with reduced slope

Table D.8a: Channel travel time in s for Trial 10: The effect of the mixed bacteria biofilm on attachment in half-diluted, unfiltered aquaculture waste with reduced slope

System	Channel Travel Time (s)
1	3
2	3
3	1

4	2
6	3
8	2
9	2

Appendix D.9 Environmental conditions for Trial 11: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with reduced slope

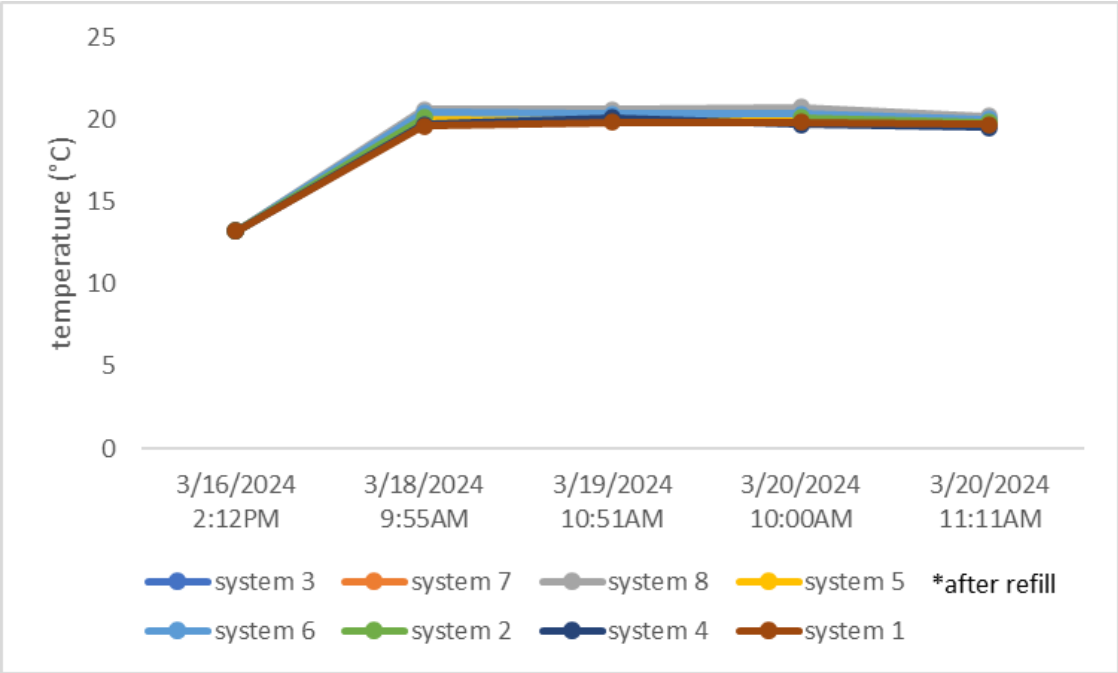


Figure D.9a: Temperatures in °C for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

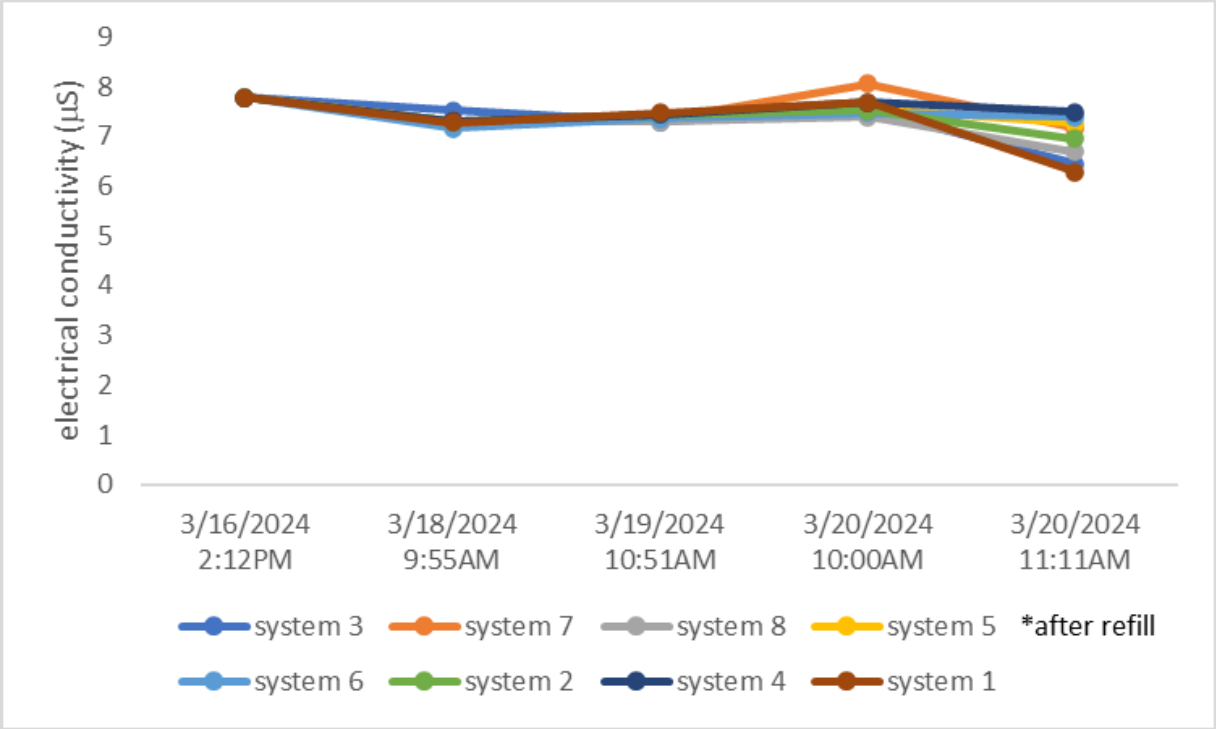


Figure D.9b: Electrical conductivities in µS for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

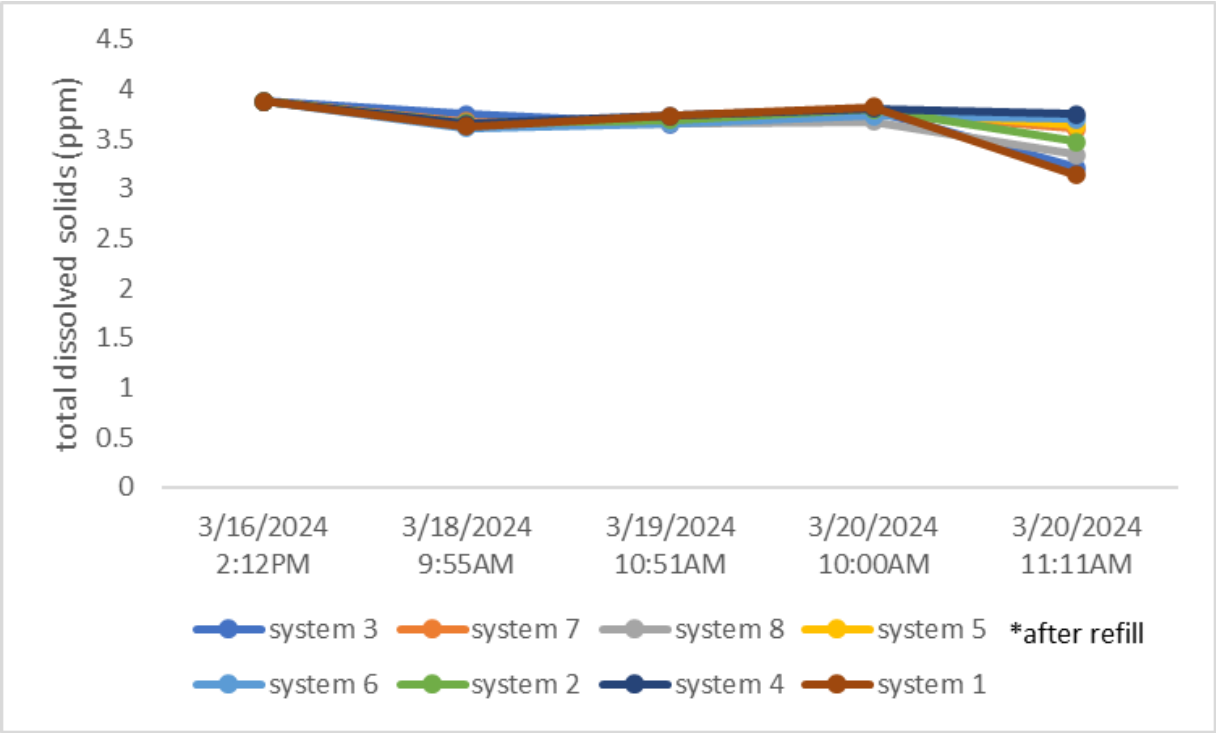


Figure D.9c: Total dissolved solids in ppm for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

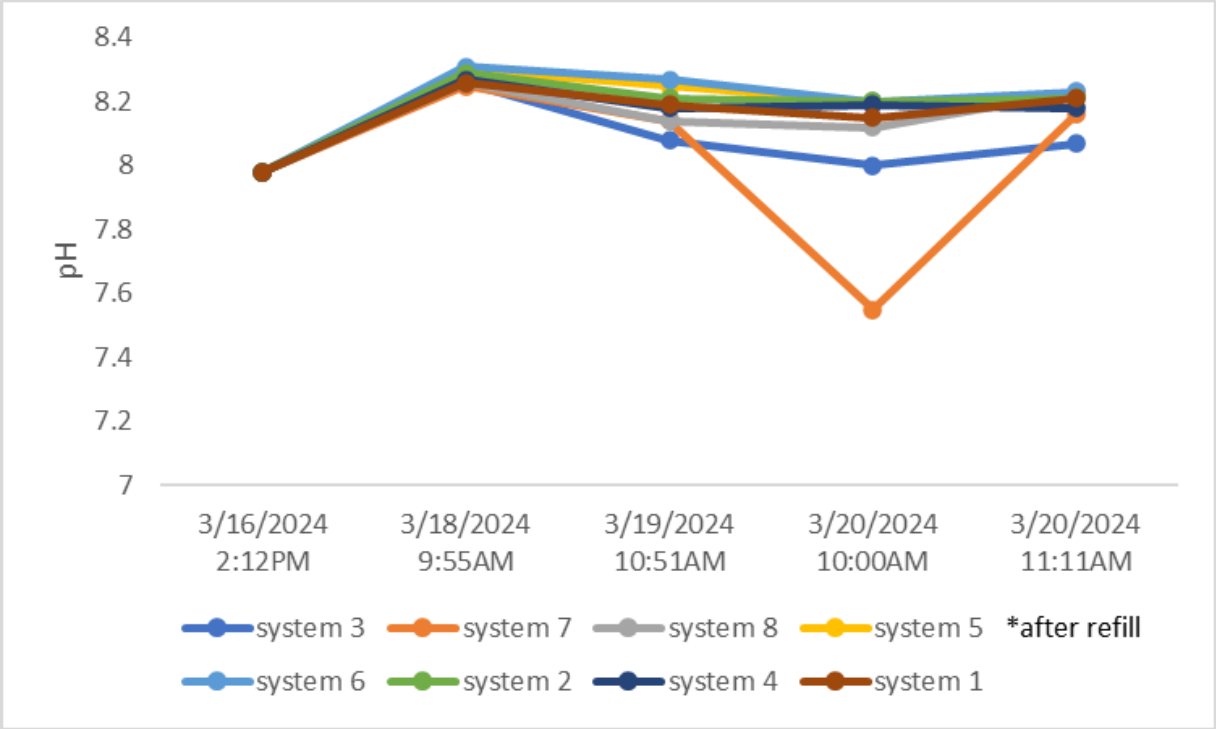


Figure D.9d: pH for Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

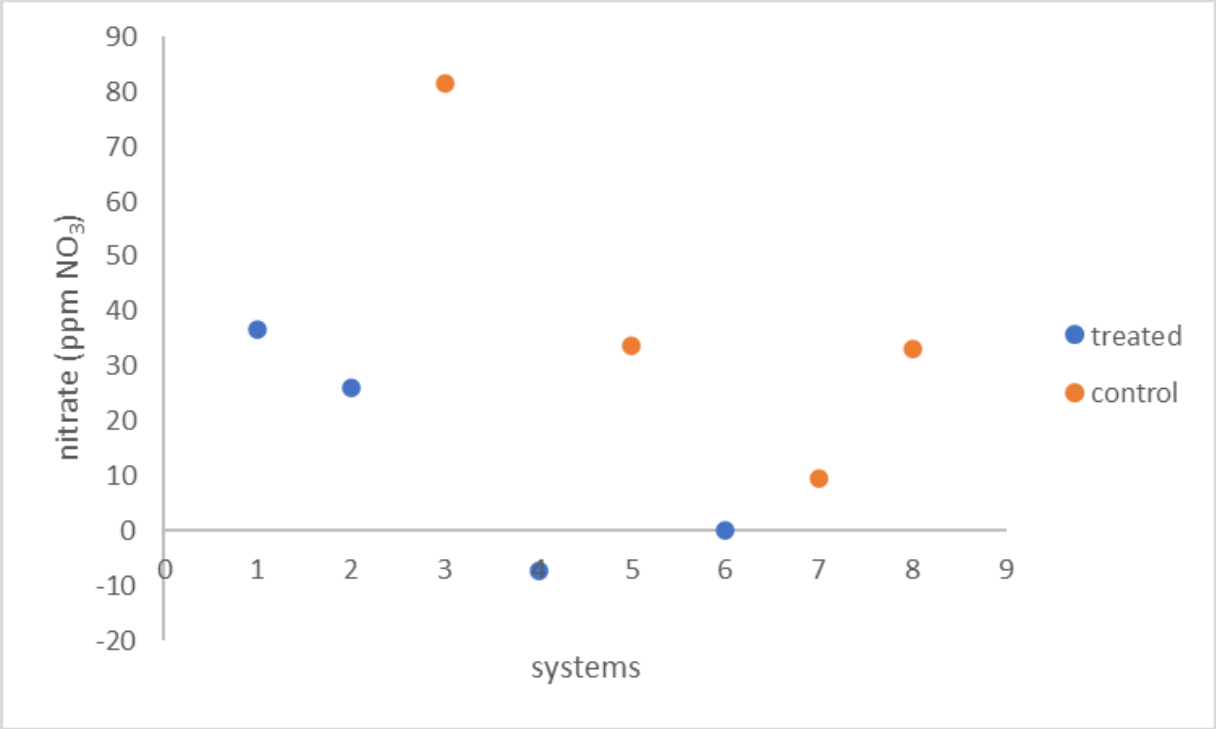


Figure D.9e: Decrease in nitrate concentration over Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

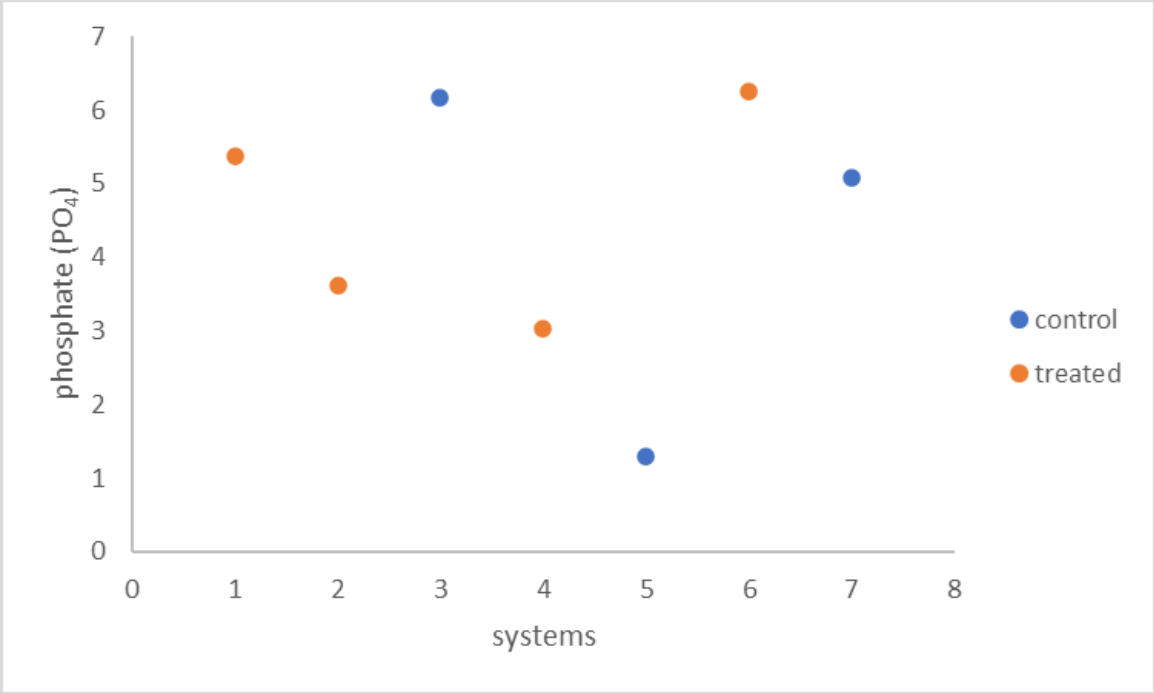


Figure D.9f: Decrease in phosphate concentration over Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

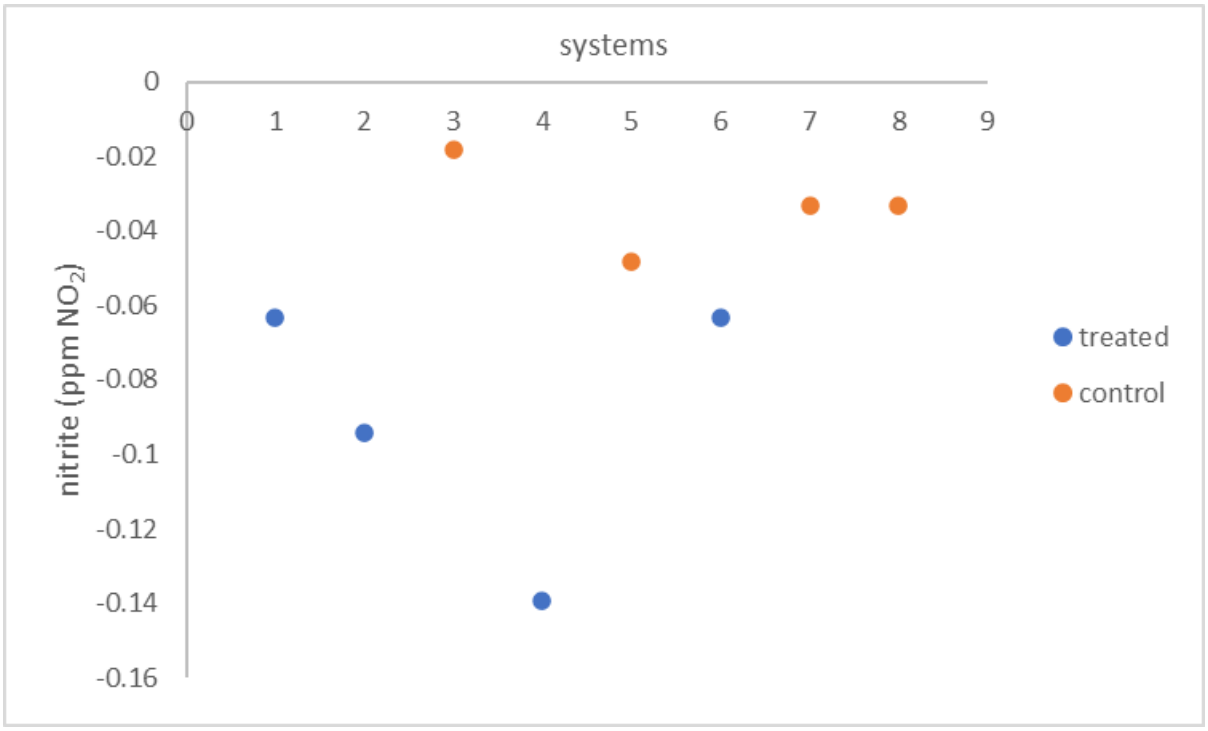


Figure D.9g: Decrease in nitrite concentration over Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

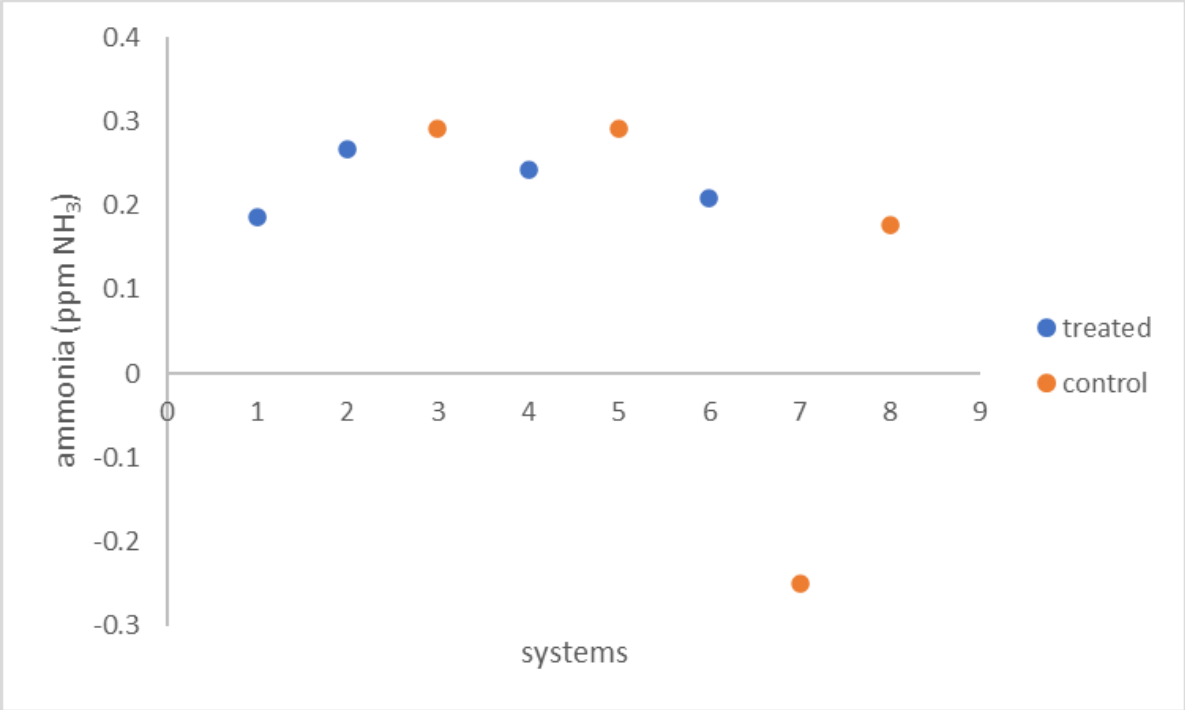


Figure D.9h: Decrease in ammonia concentration over Trial 11: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

Table D.9a: Channel travel time in s for Trial 9: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with reduced slope

System	Channel Travel Time (s)
1	8
2	13
3	6

4	1
5	7
6	3
7	5
8	4

Appendix D.10 Environmental conditions for Trial 12: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with increased slope

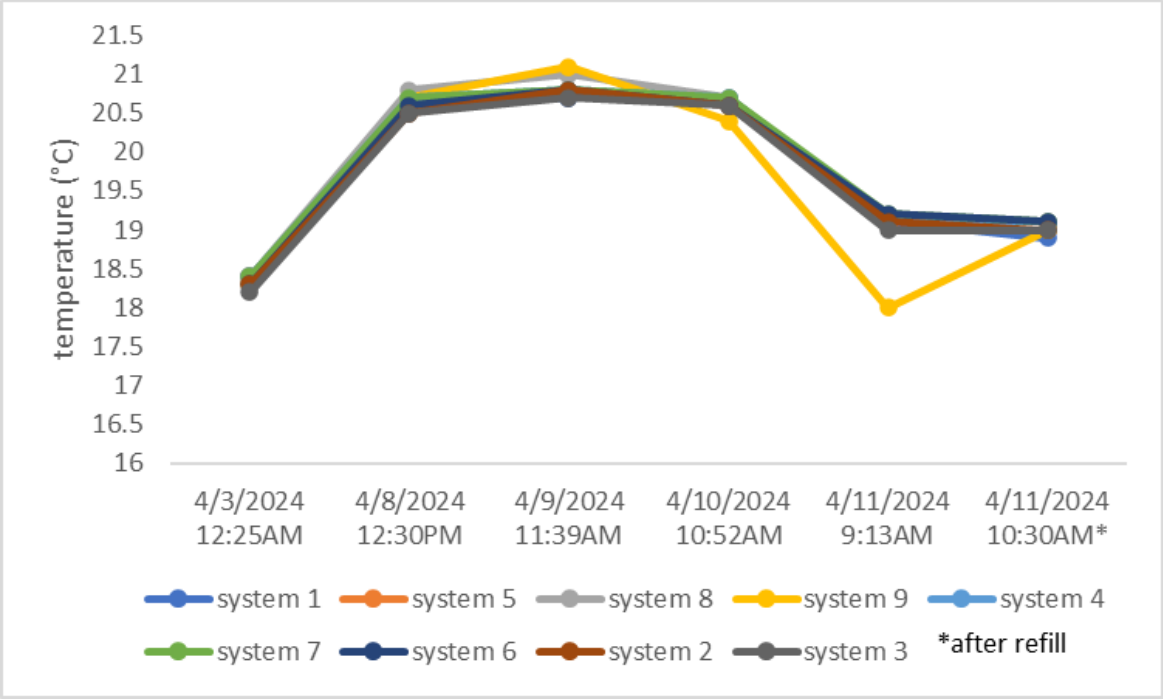


Figure D.10a: Temperatures in °C for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

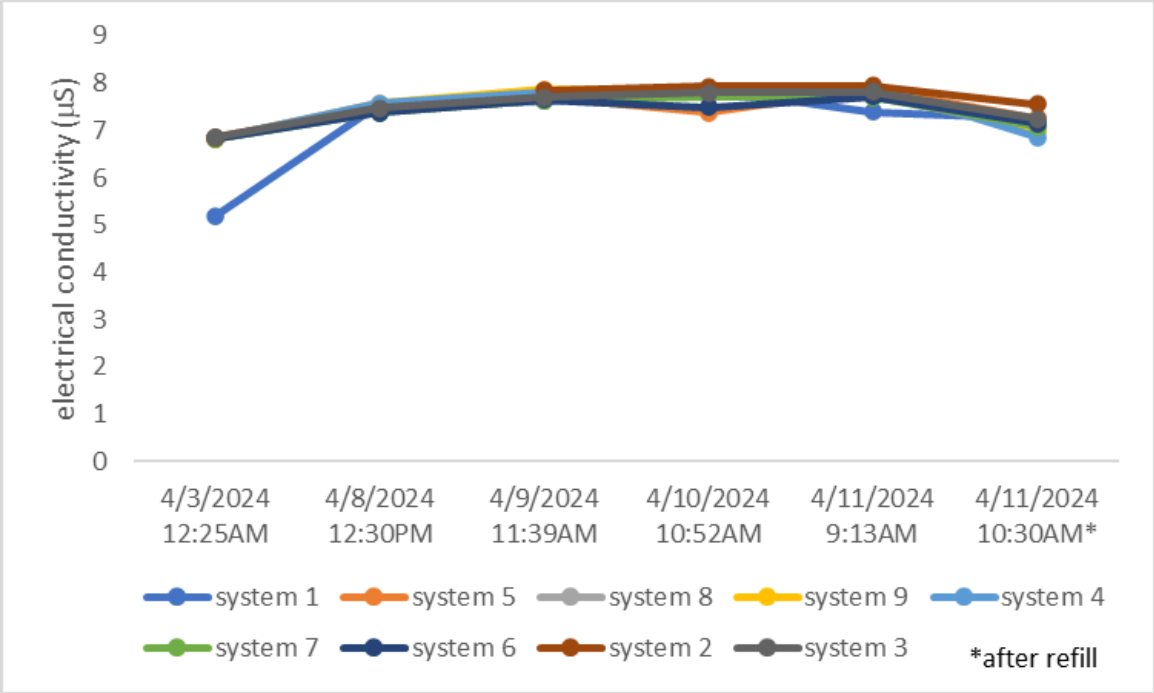


Figure D.10b: Electrical conductivities in µS for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

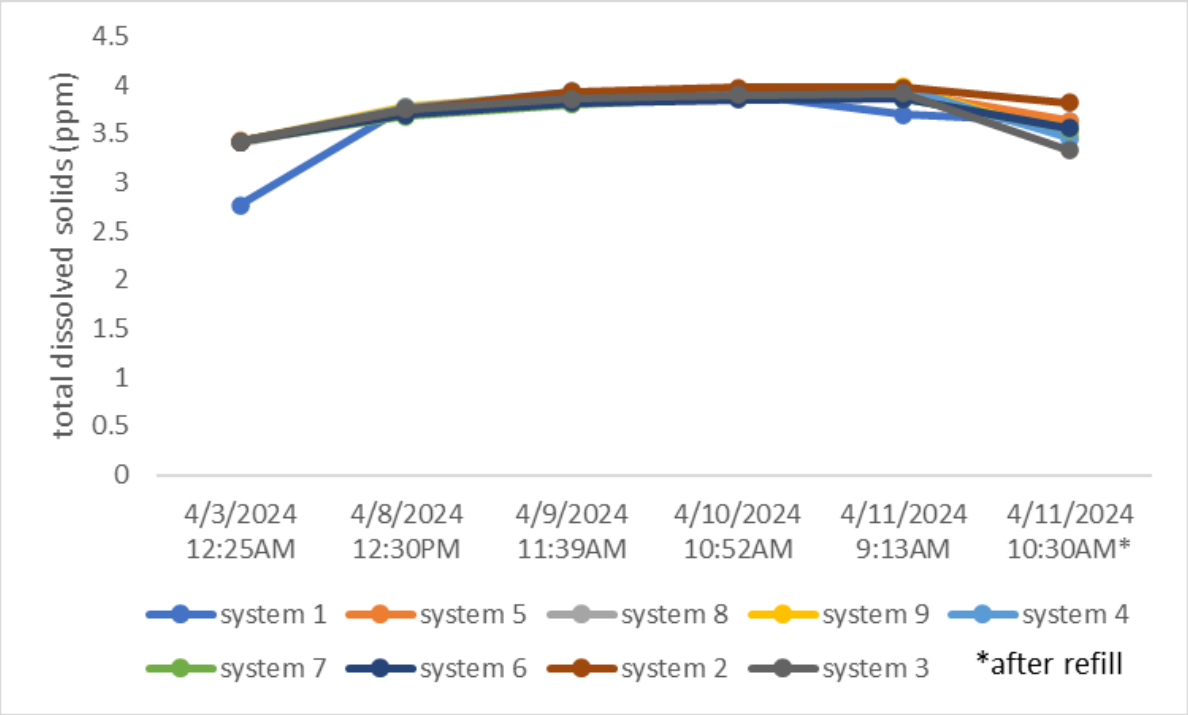


Figure D.10c: Total dissolved solids in ppm for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

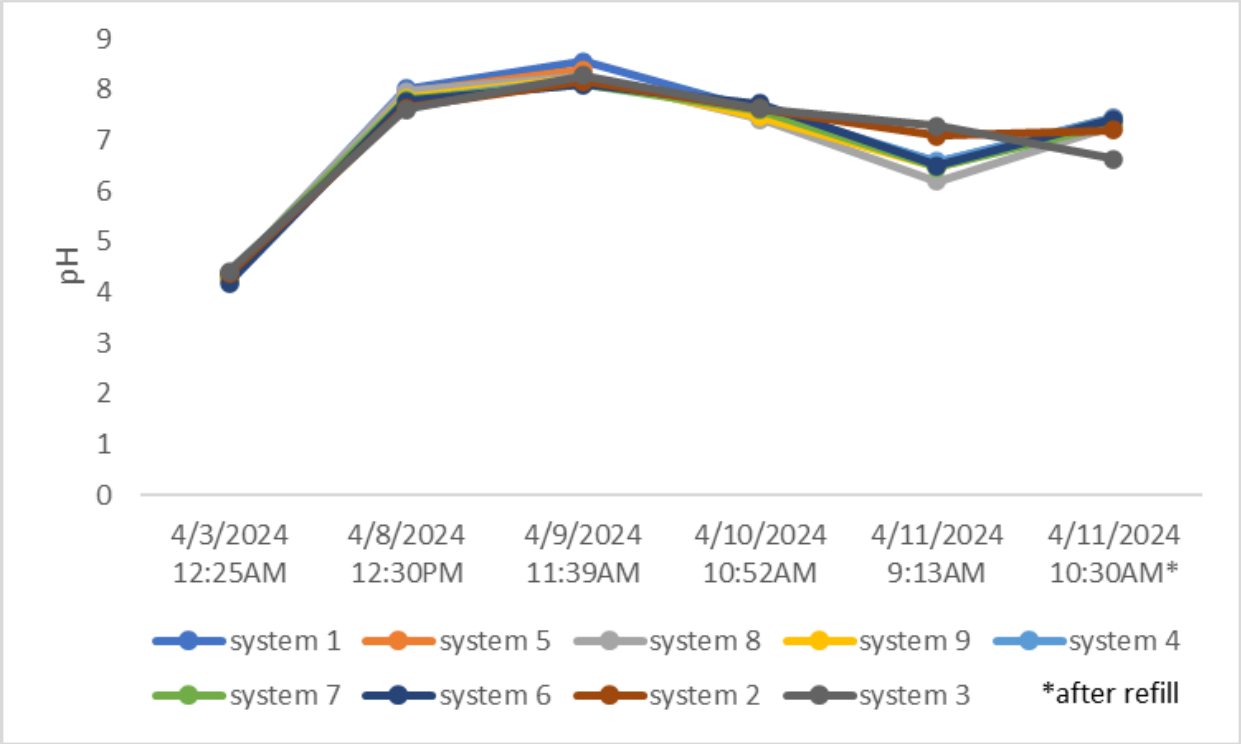


Figure D.10d: pH for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

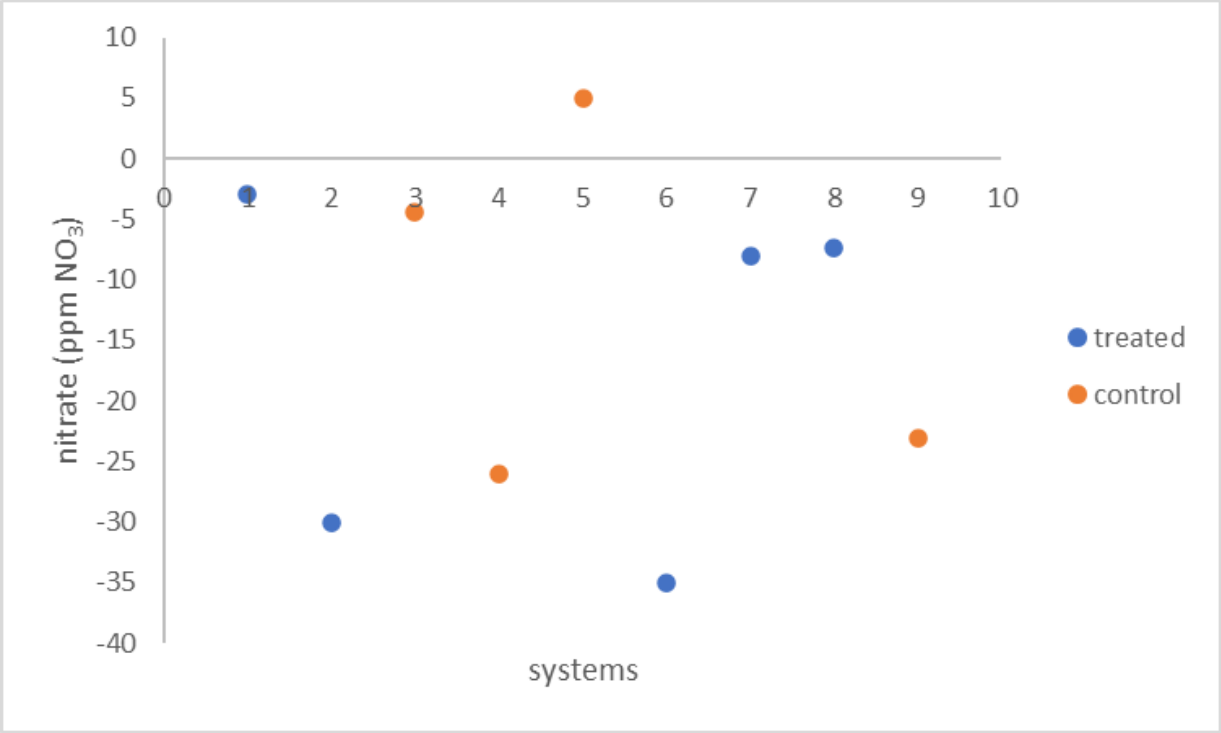


Figure D.10e: Decrease in nitrate concentration over Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

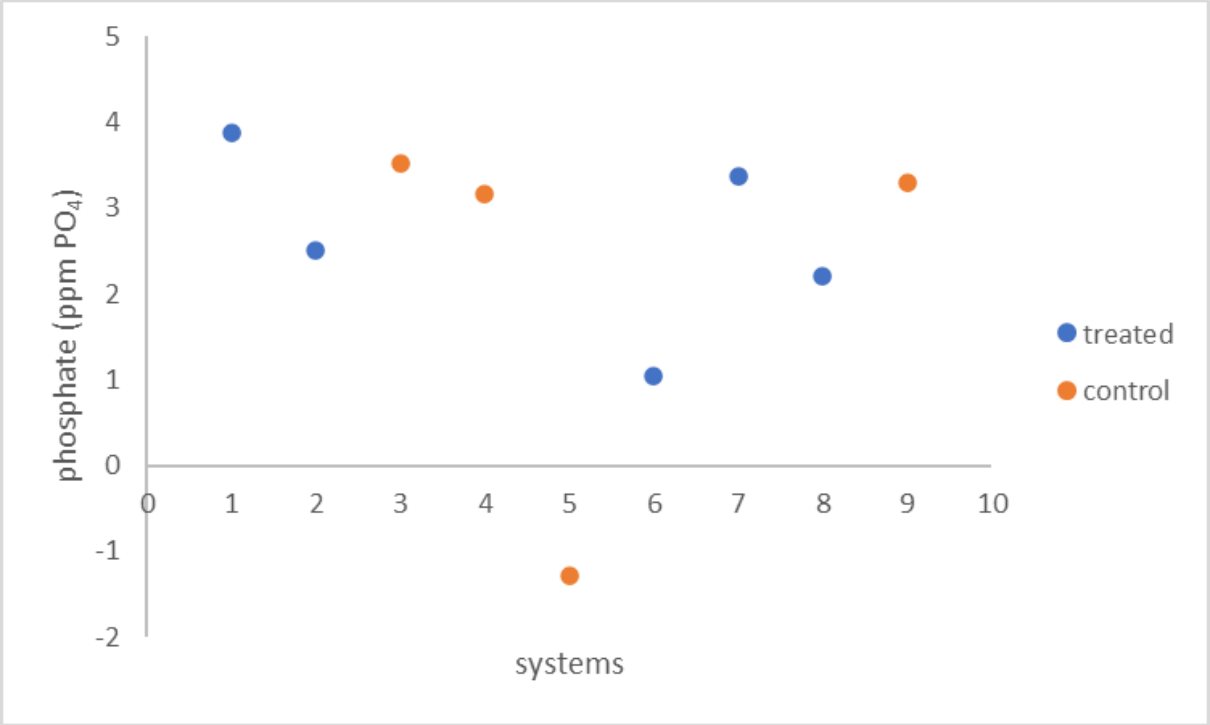


Figure D.10f: Decrease in phosphate concentration over Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

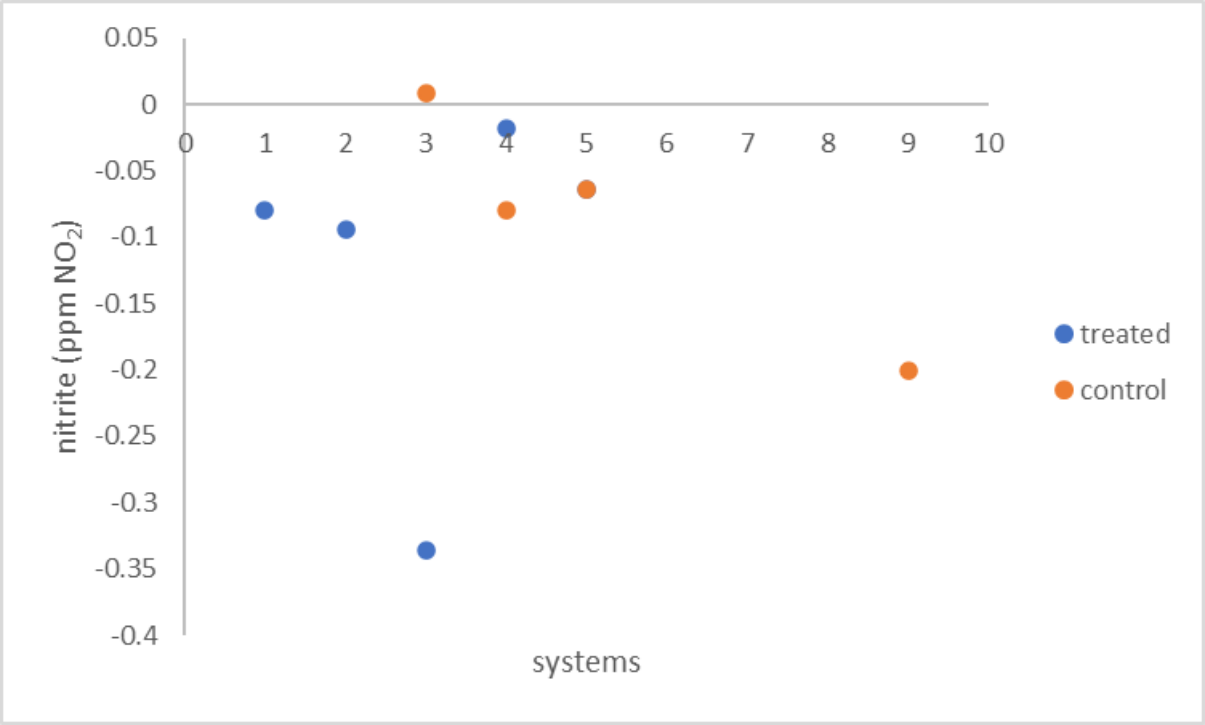


Figure D.10g: Decrease in nitrite concentration over Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

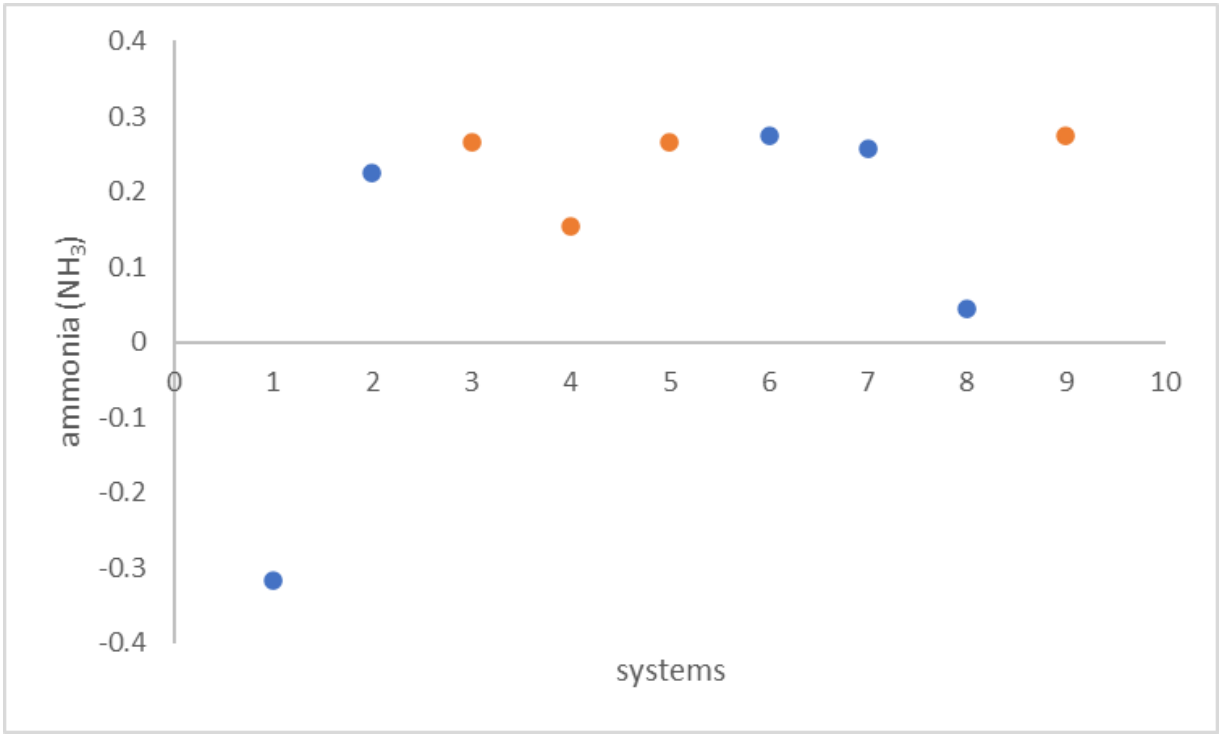


Figure D.10h: Decrease in ammonia concentration over Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

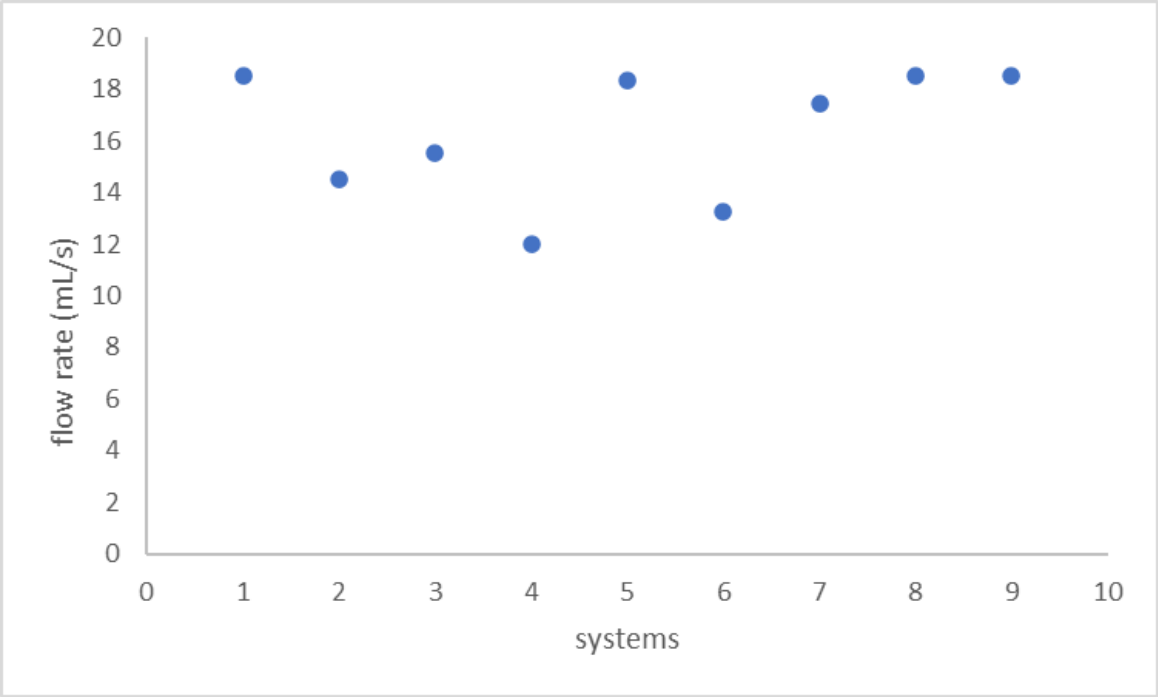


Figure D.10i: Average inlet flow rate in mL/s for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

Table D.10a: Channel travel time in s for Trial 12: The effect of the mixed bacteria biofilm on attachment in heavily diluted, unfiltered aquaculture waste with increased slope

System	Channel Travel Time (s)
1	2
2	1
3	2

4	2
5	2
6	2
7	2
8	2
9	2

Appendix E: Miscellaneous

Appendix E.1: Derivation of the total chlorophyll pigment equation

ORIGINAL EQUATIONS FROM GOLTERMANN, 1969 (Page 120)

$$^{U}E^{1}_{663}{}^{cm} = (^{U}E_{663} - {}^{U}E_{750})/\text{light path (cm)}$$

$$^{A}E^{1}_{663}{}^{cm} = (^{A}E_{663} - {}^{A}E_{750})/\text{light path (cm)}$$

$$P_t = {}^{U}E^{1}_{663}{}^{cm} \text{ x } (1003/K) \text{ x [vol. extract (ml)/vol. filtrate (ml)]} = \text{ug/L}$$

$$E^{1\ cm}_{chl} = 2*43(^{U}E^{1}_{663}{}^{cm} - {}^{A}E^{1}_{663}{}^{cm})$$

$$P_{chl} = E^{1\ cm}_{chl} \text{ x } (1000/K_{chl}) \text{ x [vol. extract (ml)/vol. filtrate (ml)]} = \text{ug/L}$$

where,

$^{U}E^{1}_{663}{}^{cm}$ = unacidified corrected extinction

$^{A}E^{1}_{663}{}^{cm}$ = acidified corrected extinction

P_t = total pigments (chlorophyll + phaeophytin)

P_{chl} = total chlorophyll pigments

MODIFICATION (SUBSTITUTING 665 NM FOR 663 NM AND REMOVING VOL. FILTRATE)

$$P_{chl} = E^{1\ cm}_{chl} \text{ x } (1000/K_{chl}) \text{ x vol. extract (ml)} = \text{ug}$$

$$P_{chl} = 2*43(^{U}E^{1}_{665}{}^{cm} - {}^{A}E^{1}_{665}{}^{cm}) \text{ x } (1000/K_{chl}) \text{ x vol. extract} = \text{ug}$$

P_{chl} = 2*43[{($^{U}E_{665}$ – $^{U}E_{750}$)/light path (cm)}-{($^{A}E_{665}$ – $^{A}E_{750}$)/light path (cm)}] x (1000/89) x vol. extract (ml) = ug

P_{chl} = 966.2921($^{U}E_{665}$ – $^{U}E_{750}$ – $^{A}E_{665}$ + $^{A}E_{750}$) x [vol. extract (ml)/light path (cm)] = ug

where,

89 = Parsons and Strickland, 1963 extinction coefficient at 665 nm, K from Goltermann (1969), Table 7.1, Page 121

Appendix E.2: Schedule of main experiments

Trial	Start Date	End (Harvest) Date	Duration (days)
1	11/06/2023	11/14/2023	8
2	11/22/2023	11/30/2023	8
3	12/16/2024	12/24/2023	8
4	12/30/2024	1/04/2024	5
5	1/08/2024	1/16/2024	8
6	1/21/2024	1/27/2024	6
7	1/31/2024	2/07/2024	7
8	2/10/2024	2/17/2024	7
9	2/21/2024	2/25/2024	4
10	3/01/2024	3/13/2024	12
11	3/16/2024	3/20/2024	4
12	4/03/2024	4/10/2024	7

Appendix E.3: Table of F-test results

Table E.3: F-test results for the trial where column 1 ("condition") indicates the bacteria ("TR" = Tallapoosa River, "TC" = Town Creek Park, "MB" = mixed bacteria), the media ("LN" = low nutrient, "HN" = high nutrient", "S" = synthetic, "ND" = non-diluted, "MD" = medium/half-diluted, "QD" = quarter-diluted, and "F" = fish/aquaculture waste), trial number, and % slope, column two indicates the measurement (where "DW" = total dry weight, "DW SAI" = dry weight SA index, "Tchla" = total chlorophyll a, "Tchl" = total chlorophyll pigments, "ChlI" = chlorophyll a SA index, and "TchlI" = total chlorophyll pigments SA index), the third column indicates the F-test p-values, and the fourth column gives "Yes" if the variances equal, "No" if they do not, and "NA" for those trials where that measurement was not available

Conditions	Measurement	F-test P-value	Equal Variances
TR LN S 1 3	DW	0.860	Yes
TR LN S 1 3	DW SAI	0.560	Yes
TR LN S 1 3	Chl a	0.960	Yes
TR LN S 1 3	Chl a SAI	0.510	Yes
TR LN S 2 3	DW	0.200	Yes
TR LN S 2 3	DW SAI	0.820	Yes
TR LN S 2 3	Chl a	0.620	Yes
TR LN S 2 3	Chl a SAI	0.110	Yes
TR LN S 3 3	DW	0.470	Yes

TR LN S 3 3	DW SAI	0.300	Yes
TR LN S 3 3	Chl a	0.320	Yes
TR LN S 3 3	Chl a SAI	0.280	Yes
TR LN S 3 3	Tchl	0.280	Yes
TR LN S 3 3	TchlSAI	0.310	Yes
TR HN S 4 3	DW	0.185	Yes
TR HN S 4 3	DW SAI	0.885	Yes
TR HN S 4 3	Chl a	0.743	Yes
TR HN S 4 3	Chl a SAI	0.301	Yes
TR HN S 4 3	Tchl	0.624	Yes
TR HN S 4 3	TchlSAI	0.137	Yes
TC LN S 5 3	DW	0.286	Yes
TC LN S 5 3	DW SAI	0.00498	No
TC LN S 5 3	Chl a	0.911	Yes
TC LN S 5 3	Chl a SAI	0.137	Yes
TC LN S 5 3	Tchl	0.886	Yes
TC LN S 5 3	TchlSAI	0.0141	No
TC HN S 6 3	DW	NA	NA
TC HN S 6 3	DW SAI	NA	NA
TC HN S 6 3	Chl a	0.462	Yes
TC HN S 6 3	Chl a SAI	0.803	Yes

TC HN S 6 3	Tchl	0.469	Yes
TC HN S 6 3	TchlSAI	0.105	Yes
TC MD F 7 3	DW	0.106	Yes
TC MD F 7 3	DW SAI	0.0566	Yes
TC MD F 7 3	Chl a	0.00301	No
TC MD F 7 3	Chl a SAI	0.920	Yes
TC MD F 7 3	Tchl	0.00519	No
TC MD F 7 3	TchlSAI	0.953	Yes
MB QD F 8 2	DW	NA	NA
MB QD F 8 2	DW SAI	NA	NA
MB QD F 8 2	Chl a	0.410	Yes
MB QD F 8 2	Chl a SAI	0.175	Yes
MB QD F 8 2	Tchl	0.816	Yes
MB QD F 8 2	TchlSAI	0.00738	No
MB ND F 9 2	DW	0.887	Yes
MB ND F 9 2	DW SAI	0.193	Yes
MB ND F 9 2	Chl a	0.701	Yes
MB ND F 9 2	Chl a SAI	0.587	Yes
MB ND F 9 2	Tchl	NA	NA
MB ND F 9 2	TchlSAI	NA	NA
MB MD F 10 2	DW	0.547	Yes

MB MD F 10 2	DW SAI	0.165	Yes
MB MD F 10 2	Chl a	0.529	Yes
MB MD F 10 2	Chl a SAI	0.00176	No
MB MD F 10 2	Tchl	0.528	Yes
MB MD F 10 2	TchlSAI	0.00154	No
MB QD F 11 1	DW	0.851	Yes
MB QD F 11 1	DW SAI	0.0105	No
MB QD F 11 1	Chl a	0.0153	No
MB QD F 11 1	Chl a SAI	0.00424	No
MB QD F 11 1	Tchl	0.0139	No
MB QD F 11 1	TchlSAI	0.00196	No
MB QD F 12 3	DW	0.816	Yes
MB QD F 12 3	DW SAI	NA	NA
MB QD F 12 3	Chl a	0.941	Yes
MB QD F 12 3	Chl a SAI	0.898	Yes
MB QD F 12 3	Tchl	0.291	Yes
MB QD F 12 3	TchlSAI	0.100	Yes

Appendix E.4: TOST results

Appendix E.4.1 TOST results for Trials 1-3: The effect of the Tallapoosa River biofilm in low nutrient concentration synthetic media

Appendix E.4.1.1: Trial 1 TOST results

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 1.780, p = 9.41e-01
The null hypothesis test was significant, t(7) = 2.571, p = 3.69e-02
NHST: reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	2.571124	7	0.037
TOST Lower	3.362239	7	0.006
TOST Upper	1.780009	7	0.941

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.001300	0.0005056155	[3e-04, 0.0023]	0.9
Hedges's g	1.531939	0.6966970491	[0.3071, 2.6707]	0.9

Figure E.4.1.1a: Trial 1 TOST results for total dry weight

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.415, p = 6.55e-01
The null hypothesis test was non-significant, t(7) = 1.160, p = 2.84e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.1603620	7	0.284
TOST Lower	0.8647284	7	0.208
TOST Upper	0.4150696	7	0.655

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	3.9250000	3.3825650	[-2.4835, 10.3335]	0.9
Hedges's g	0.6913723	0.6177073	[-0.3559, 1.6928]	0.9

Figure E.4.1.1b: Trial 1 TOST results for total chlorophyll a

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.513, p = 6.88e-01
The null hypothesis test was non-significant, t(7) = 0.811, p = 4.44e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.8107080	7	0.444
TOST Lower	1.1081144	7	0.152
TOST Upper	0.5133015	7	0.688

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	3.6800000	4.539242	[-4.92, 12.28]	0.9
Hedges's g	0.4830398	0.606605	[-0.5353, 1.4685]	0.9

Figure E.4.1.1c: Trial 1 TOST results for dry weight SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.262, p = 4e-01
The null hypothesis test was non-significant, t(7) = -0.427, p = 6.82e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.4270395	7	0.682
TOST Lower	0.2623516	7	0.400
TOST Upper	-1.1164307	7	0.151

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-2.2300000	5.2219990	[-12.1235, 7.6635]	0.9
Hedges's g	-0.2544407	0.5988352	[-1.2318, 0.7406]	0.9

Figure E.4.1.1c: Trial 1 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.262, p = 4e-01
The null hypothesis test was non-significant, t(7) = -0.427, p = 6.82e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.4270395	7	0.682
TOST Lower	0.2623516	7	0.400
TOST Upper	-1.1164307	7	0.151

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-2.2300000	5.2219990	[-12.1235, 7.6635]	0.9
Hedges's g	-0.2544407	0.5988352	[-1.2318, 0.7406]	0.9

Appendix E.4.1.2: Trial 2 TOST results

```
Two Sample t-test

The equivalence test was non-significant, t(6) = 2.631, p = 9.81e-01
The null hypothesis test was significant, t(6) = 2.925, p = 2.65e-02
NHST: reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	2.924702	6	0.026
TOST Lower	3.218100	6	0.009
TOST Upper	2.631303	6	0.981

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.001575	0.0005385165	[5e-04, 0.0026]	0.9
Hedges's g	1.796386	0.7608836251	[0.4326, 3.052]	0.9

Figure E.4.1.2a: Trial 2 TOST results for total dry weight

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.850, p = 7.83e-01
The null hypothesis test was non-significant, t(5) = 1.448, p = 2.07e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.4475681	5	0.207
TOST Lower	2.0451900	5	0.048
TOST Upper	0.8499461	5	0.783

Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	12.7360000	8.7982046	[-4.9928, 30.4648]	0.9
Hedges's g	0.9295304	0.6885131	[-0.2669, 2.0454]	0.9

Figure E.4.1.2b: Trial 2 TOST results for total chlorophyll a

Two Sample t-test

The equivalence test was non-significant, t(6) = 0.206, p = 4.22e-01
The null hypothesis test was non-significant, t(6) = -0.502, p = 6.33e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.5023536	6	0.633
TOST Lower	0.2060938	6	0.422
TOST Upper	-1.2108010	6	0.136

Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-0.7800000	1.5526912	[-3.7972, 2.2372]	0.9
Hedges's g	-0.3085515	0.6190367	[-1.3166, 0.7242]	0.9

Figure E.4.1.2c: Trial 2 TOST results for dry weight SA index

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.0636, p = 4.76e-01
The null hypothesis test was non-significant, t(5) = -0.591, p = 5.8e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.59076280	5	0.580
TOST Lower	0.06360402	5	0.476
TOST Upper	-1.24512962	5	0.134

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-5.4800000	9.2761427	[-24.1719, 13.2119]	0.9
Hedges's g	-0.3793479	0.6500869	[-1.4348, 0.7122]	0.9

Figure E.4.1.2c: Trial 2 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.0636, p = 4.76e-01
The null hypothesis test was non-significant, t(5) = -0.591, p = 5.8e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.59076280	5	0.580
TOST Lower	0.06360402	5	0.476
TOST Upper	-1.24512962	5	0.134

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-5.4800000	9.2761427	[-24.1719, 13.2119]	0.9
Hedges's g	-0.3793479	0.6500869	[-1.4348, 0.7122]	0.9

Appendix E.4.1.3: Trial 3 TOST results

```
Two Sample t-test

The equivalence test was non-significant, t(6) = 0.660, p = 7.33e-01
The null hypothesis test was non-significant, t(6) = 1.368, p = 2.2e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.3681637	6	0.220
TOST Lower	2.0762017	6	0.042
TOST Upper	0.6601257	6	0.733

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.0025700	0.00187843	[-0.0011, 0.0062]	0.9
Hedges's g	0.8403423	0.64914722	[-0.274, 1.8919]	0.9

Figure E.4.1.3a: Trial 3 TOST results for total dry weight

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.000, p = 5e-01
The null hypothesis test was non-significant, t(7) = 0.746, p = 4.8e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.7456285	7	0.48
TOST Lower	1.4912569	7	0.09
TOST Upper	0.0000000	7	0.50

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	2.7400000	3.6747525	[-4.2221, 9.7021]	0.9
Hedges's g	0.4442638	0.6049562	[-0.5695, 1.4277]	0.9

Figure E.4.1.3b: Trial 3 TOST results for total chlorophyll a

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -0.00421, p = 4.98e-01
The null hypothesis test was non-significant, t(7) = 0.741, p = 4.83e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.741154943	7	0.483
TOST Lower	1.486515729	7	0.090
TOST Upper	-0.004205842	7	0.498

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	103.9700000	140.2810586	[-161.8035, 369.7435]	0.9
Hedges's g	0.4415984	0.6048478	[-0.5719, 1.4249]	0.9

Figure E.4.1.3c: Trial 3 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -1.441, p = 9.04e-01
The null hypothesis test was non-significant, t(7) = -2.187, p = 6.5e-02
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-2.187116	7	0.065
TOST Lower	-1.441463	7	0.904
TOST Upper	-2.932770	7	0.011

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-3.247000	1.4846032	[-6.0597, -0.4343]	0.9
Hedges's g	-1.303138	0.6703355	[-2.3943, -0.135]	0.9

Figure E.4.1.3d: Trial 3 TOST results for dry weight SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.506, p = 3.14e-01
The null hypothesis test was non-significant, t(7) = -0.238, p = 8.19e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.2381517	7	0.819
TOST Lower	0.5057888	7	0.314
TOST Upper	-0.9820921	7	0.179

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-0.2100000	0.8817910	[-1.8806, 1.4606]	0.9
Hedges's g	-0.1418966	0.5967626	[-1.1189, 0.845]	0.9

Figure E.4.1.3e: Trial 3 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.659, p = 2.66e-01
The null hypothesis test was non-significant, t(7) = -0.0862, p = 9.34e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-2.187116	7	0.065
TOST Lower	-1.441463	7	0.904
TOST Upper	-2.932770	7	0.011

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-3.247000	1.4846032	[-6.0597, -0.4343]	0.9
Hedges's g	-1.303138	0.6703355	[-2.3943, -0.135]	0.9

Figure E.4.1.3f: Trial 3 TOST results for total chlorophyll pigments SA index

Appendix E.4.2 TOST results for Trial 4: The effect of Tallapoosa River water bacteria biofilm

on attachment in high nutrient synthetic media

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -0.268, p = 3.98e-01
The null hypothesis test was non-significant, t(7) = 0.477, p = 6.48e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.4770280	7	0.648
TOST Lower	1.2218421	7	0.131
TOST Upper	-0.2677862	7	0.398

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.000440	0.0009223778	[-0.0013, 0.0022]	0.9
Hedges's g	0.284225	0.5995789975	[-0.7133, 1.2621]	0.9

Figure E.4.2a: Trial 4 TOST results for total dry weight

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.000, p = 5e-01
The null hypothesis test was non-significant, t(7) = 0.746, p = 4.8e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.7456285	7	0.48
TOST Lower	1.4912569	7	0.09
TOST Upper	0.0000000	7	0.50

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	2.7400000	3.6747525	[-4.2221, 9.7021]	0.9
Hedges's g	0.4442638	0.6049562	[-0.5695, 1.4277]	0.9

Figure E.4.2b: Trial 4 TOST results for total chlorophyll a

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -0.00421, p = 4.98e-01
The null hypothesis test was non-significant, t(7) = 0.741, p = 4.83e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.741154943	7	0.483
TOST Lower	1.486515729	7	0.090
TOST Upper	-0.004205842	7	0.498

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	103.9700000	140.2810586	[-161.8035, 369.7435]	0.9
Hedges's g	0.4415984	0.6048478	[-0.5719, 1.4249]	0.9

Figure E.4.2c: Trial 4 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -1.433, p = 9.02e-01
The null hypothesis test was non-significant, t(7) = -2.179, p = 6.57e-02
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-2.179253	7	0.066
TOST Lower	-1.432657	7	0.902
TOST Upper	-2.925849	7	0.011

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-3.240000	1.4867479	[-6.0568, -0.4232]	0.9
Hedges's g	-1.298453	0.6698302	[-2.3887, -0.1314]	0.9

Figure E.4.2d: Trial 4 TOST results for dry weight SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.335, p = 3.74e-01
The null hypothesis test was non-significant, t(7) = -0.147, p = 8.87e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.1469471	7	0.887
TOST Lower	0.3350395	7	0.374
TOST Upper	-0.6289338	7	0.275

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-0.20000000	1.3610336	[-2.7786, 2.3786]	0.9
Hedges's g	-0.08755472	0.5961819	[-1.0653, 0.8963]	0.9

Figure E.4.2e: Trial 4 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.659, p = 2.66e-01
The null hypothesis test was non-significant, t(7) = -0.0862, p = 9.34e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.08618011	7	0.934
TOST Lower	0.65879909	7	0.266
TOST Upper	-0.83115931	7	0.217

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-0.09000000	1.0443245	[-2.0686, 1.8886]	0.9
Hedges's g	-0.05134823	0.5959475	[-1.0298, 0.9307]	0.9

Figure E.4.2f: Trial 4 TOST results for total chlorophyll pigments SA index

Appendix E.4.3 TOST results for Trial 5: The effect of Town Creek Park Water bacteria biofilm on attachment in low nutrient water

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.803, p = 7.71e-01
The null hypothesis test was non-significant, t(5) = 1.452, p = 2.06e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.4516116	5	0.206
TOST Lower	2.1002041	5	0.045
TOST Upper	0.8030192	5	0.771

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.0047000	0.003237781	[-0.0018, 0.0112]	0.9
Hedges's g	0.9321269	0.688763749	[-0.2649, 2.0485]	0.9

Figure E.4.3a: Trial 5 TOST results for total dry weight

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.297, p = 6.11e-01
The null hypothesis test was non-significant, t(5) = 0.952, p = 3.85e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.5578926	5	0.180
TOST Lower	2.2125581	5	0.039
TOST Upper	0.9032272	5	0.796

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	41.335000	26.5326371	[-12.1295, 94.7995]	0.9
Hedges's g	1.000373	0.6955688	[-0.2134, 2.1289]	0.9

Figure E.43b: Trial 5 TOST results for total chlorophyll a

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.871, p = 7.88e-01
The null hypothesis test was non-significant, t(5) = 1.525, p = 1.88e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.5254091	5	0.188
TOST Lower	2.1800559	5	0.041
TOST Upper	0.8707622	5	0.788

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	1486.4800000	974.4795867	[-477.1435, 3450.1035]	0.9
Hedges's g	0.9795146	0.6934451	[-0.2291, 2.1042]	0.9

Figure E.4.3c: Trial 5 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.803, p = 7.71e-01
The null hypothesis test was non-significant, t(5) = 1.452, p = 2.06e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.5254091	5	0.188
TOST Lower	2.1800559	5	0.041
TOST Upper	0.8707622	5	0.788

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	22.1400000	21.500529	[-28.3232, 72.6032]	0.9
Hedges's g(av)	0.5281939	1.472629	[-0.4178, 1.3993]	0.9

Figure E.4.3d: Trial 5 TOST results for dry weight SA index

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.297, p = 6.11e-01
The null hypothesis test was non-significant, t(5) = 0.952, p = 3.85e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.9524369	5	0.385
TOST Lower	1.6075981	5	0.084
TOST Upper	0.2972758	5	0.611

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	3.3000000	3.4647963	[-3.6817, 10.2817]	0.9
Hedges's g	0.6115906	0.6626095	[-0.5171, 1.684]	0.9

Figure E.4.3e: Trial 5 TOST results for chlorophyll a SA index

```
Welch Two Sample t-test

The equivalence test was non-significant, t(2.08) = -0.377, p = 6.29e-01
The null hypothesis test was non-significant, t(2.08) = -0.931, p = 4.47e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.9307601	2.078418	0.447
TOST Lower	-0.3769194	2.078418	0.629
TOST Upper	-1.4846007	2.078418	0.136

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-1.2100000	1.300013	[-4.9101, 2.4901]	0.9
Hedges's g(av)	-0.4461122	4.154011	[-1.2587, 0.4527]	0.9

Figure E.4.3f: Trial 5 TOST results for total chlorophyll pigments SA index

Appendix E.4.4 TOST results for Trial 6: The effect of Town Creek Park water bacteria biofilm on attachment in high nutrient water

```
Two Sample t-test

The equivalence test was non-significant, t(6) = 0.590, p = 7.12e-01
The null hypothesis test was non-significant, t(6) = 1.297, p = 2.42e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.297270	6	0.242
TOST Lower	2.004871	6	0.046
TOST Upper	0.589668	6	0.712

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	9.1300000	7.0378583	[-4.5458, 22.8058]	0.9
Hedges's g	0.7967984	0.6457063	[-0.309, 1.8427]	0.9

Figure E.4.4a: Trial 6 TOST results for total chlorophyll a

```
Two Sample t-test

The equivalence test was non-significant, t(6) = 0.610, p = 7.18e-01
The null hypothesis test was non-significant, t(6) = 1.317, p = 2.36e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.3166787	6	0.236
TOST Lower	2.0237925	6	0.045
TOST Upper	0.6095649	6	0.718

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	340.1400000	258.3318150	[-161.8453, 842.1253]	0.9
Hedges's g	0.8087196	0.6466319	[-0.2994, 1.8561]	0.9

Figure E.4.4b: Trial 6 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(6) = -0.389, p = 3.55e-01
The null hypothesis test was non-significant, t(6) = 0.317, p = 7.62e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.3173135	6	0.762
TOST Lower	1.0239085	6	0.173
TOST Upper	-0.3892815	6	0.355

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	2.9100000	9.1707415	[-14.9104, 20.7304]	0.9
Hedges's g	0.1948977	0.6161414	[-0.8273, 1.2013]	0.9

Figure E.4.4c: Trial 6 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(6) = 0.119, p = 4.55e-01
The null hypothesis test was non-significant, t(6) = -0.588, p = 5.78e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-0.5881202	6	0.578
TOST Lower	0.1191320	6	0.455
TOST Upper	-1.2953725	6	0.121

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-7.8000000	13.2625940	[-33.5716, 17.9716]	0.9
Hedges's g	-0.3612304	0.6208153	[-1.3709, 0.6773]	0.9

Figure E.4.4d: Trial 6 TOST results for total chlorophyll pigments SA index

Appendix E.4.5 TOST results for Trial 7: The effect of Town Creek Park water bacteria biofilm in medium strength diluted, filtered aquaculture waste

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 0.511, p = 6.88e-01
The null hypothesis test was non-significant, t(7) = 1.256, p = 2.49e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.2563319	7	0.249
TOST Lower	2.0014938	7	0.043
TOST Upper	0.5111701	7	0.688

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.0001600	0.0001273549	[-1e-04, 4e-04]	0.9
Hedges's g	0.7485535	0.6213988708	[-0.3079, 1.7558]	0.9

Figure E.4.5a: Trial 7 TOST results for total dry weight

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.11) = 0.135, p = 5.5e-01
The null hypothesis test was non-significant, t(3.11) = 0.586, p = 5.98e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.5857864	3.105605	0.598
TOST Lower	1.0363914	3.105605	0.187
TOST Upper	0.1351815	3.105605	0.550

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.650000	1.109619	[-1.9255, 3.2255]	0.9
Hedges's g(av)	0.304363	1.258358	[-0.5936, 1.1578]	0.9

Figure E.4.5b: Trial 7 TOST results for total chlorophyll a

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.14) = 0.261, p = 5.95e-01
The null hypothesis test was non-significant, t(3.14) = 0.409, p = 7.09e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.4087999	3.139509	0.709
TOST Lower	0.5570901	3.139509	0.307
TOST Upper	0.2605098	3.139509	0.595

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs")
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	15.3000000	37.426622	[-71.2055, 101.8055]	0.9
Hedges's g(av)	0.2133738	1.200809	[-0.6711, 1.0665]	0.9

Figure E.4.5c: Trial 7 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -0.955, p = 8.14e-01
The null hypothesis test was non-significant, t(7) = -1.703, p = 1.32e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-1.7025660	7	0.132
TOST Lower	-0.9549175	7	0.814
TOST Upper	-2.4502146	7	0.022

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.0001600	0.0001273549	[-1e-04, 4e-04]	0.9
Hedges's g	0.7485535	0.6213988708	[-0.3079, 1.7558]	0.9

Figure E.4.5d: Trial 7 TOST results for dry weight SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -0.454, p = 3.32e-01
The null hypothesis test was non-significant, t(7) = 0.236, p = 8.2e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.2361907	7	0.820
TOST Lower	0.9262783	7	0.193
TOST Upper	-0.4538969	7	0.332

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.2300000	0.9737894	[-1.6149, 2.0749]	0.9
Hedges's g	0.1407282	0.5967472	[-0.8461, 1.1177]	0.9

Figure E.4.5e: Trial 7 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -0.289, p = 3.9e-01
The null hypothesis test was non-significant, t(7) = 0.449, p = 6.67e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.4486609	7	0.667
TOST Lower	1.1867804	7	0.137
TOST Upper	-0.2894586	7	0.390

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.3100000	0.690945	[-0.999, 1.619]	0.9
Hedges's g	0.2673232	0.599147	[-0.7287, 1.2449]	0.9

Figure E.4.5f: Trial 7 TOST results for total chlorophyll pigments SA index

Appendix E.4.6 TOST results for Trial 8: The effect of mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with reduced slope

```
Welch Two Sample t-test

The equivalence test was non-significant, t(6.58) = 1.861, p = 9.46e-01
The null hypothesis test was significant, t(6.58) = 2.655, p = 3.46e-02
NHST: reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	2.655157	6.576122	0.035
TOST Lower	3.448939	6.576122	0.006
TOST Upper	1.861376	6.576122	0.946

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	1.920000	0.723121	[0.5366, 3.3034]	0.9
Hedges's g(av)	1.514743	1.498212	[0.3047, 2.6329]	0.9

Figure E.4.6a: Trial 8 TOST results for total chlorophyll a

```
Welch Two Sample t-test

The equivalence test was non-significant, t(6.94) = 1.541, p = 9.16e-01
The null hypothesis test was non-significant, t(6.94) = 2.302, p = 5.51e-02
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	2.302049	6.944059	0.055
TOST Lower	3.063413	6.944059	0.009
TOST Upper	1.540685	6.944059	0.916

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	64.100000	27.844757	[11.2819, 116.9181]	0.9
Hedges's g(av)	1.358969	1.322063	[0.1852, 2.4535]	0.9

Figure E.4.6b: Trial 8 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(7) = -0.403, p = 3.49e-01
The null hypothesis test was non-significant, t(7) = 0.345, p = 7.41e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.3445233	7	0.741
TOST Lower	1.0922121	7	0.155
TOST Upper	-0.4031656	7	0.349

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.9400000	2.7284077	[-4.2292, 6.1092]	0.9
Hedges's g	0.2052755	0.5977859	[-0.7859, 1.1822]	0.9

Figure E.4.6c: Trial 8 TOST results for chlorophyll a SA index

```
Welch Two Sample t-test

The equivalence test was non-significant, t(4.18) = 0.120, p = 5.45e-01
The null hypothesis test was non-significant, t(4.18) = 0.962, p = 3.89e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.9615627	4.175319	0.389
TOST Lower	1.8033937	4.175319	0.071
TOST Upper	0.1197318	4.175319	0.545

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	3.6300000	3.775105	[-4.3218, 11.5818]	0.9
Hedges's g(av)	0.4907618	1.042516	[-0.4181, 1.3462]	0.9

Figure E.4.6d: Trial 8 TOST results for total chlorophyll pigments SA index

Appendix E.4.7 TOST results for Trial 9: The effect of the mixed bacteria biofilm in undiluted, unfiltered aquaculture waste with reduced slope

```
Two Sample t-test

The equivalence test was non-significant, t(4) = -0.821, p = 7.71e-01
The null hypothesis test was non-significant, t(4) = -1.433, p = 2.25e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-1.4330887	4	0.225
TOST Lower	-0.8210667	4	0.771
TOST Upper	-2.0451108	4	0.055

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-0.0056900	0.003970445	[-0.0142, 0.0028]	0.9
Hedges's g	-0.9336143	0.705017371	[-2.0775, 0.308]	0.9

Figure E.4.7a: Trial 9 TOST results for total dry weight

```
Two Sample t-test

The equivalence test was non-significant, t(3) = 0.399, p = 6.42e-01
The null hypothesis test was non-significant, t(3) = 1.349, p = 2.7e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.3493040	3	0.270
TOST Lower	2.3000736	3	0.052
TOST Upper	0.3985343	3	0.642

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.9920000	0.7351939	[-0.7382, 2.7222]	0.9
Hedges's g	0.8912889	0.7181726	[-0.3952, 2.0585]	0.9

Figure E.4.7b: Trial 9 TOST results for total chlorophyll a

```
Two Sample t-test

The equivalence test was non-significant, t(3) = 1.237, p = 8.48e-01
The null hypothesis test was non-significant, t(3) = 1.406, p = 2.54e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.405844	3	0.254
TOST Lower	1.574991	3	0.107
TOST Upper	1.236696	3	0.848

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	31.5000000	22.4064723	[-21.2306, 84.2306]	0.9
Hedges's g	0.9286365	0.7228893	[-0.3709, 2.1054]	0.9

Figure E.4.7c: Trial 9 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(4) = -2.112, p = 9.49e-01
The null hypothesis test was non-significant, t(4) = -1.498, p = 2.09e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-1.497552	4	0.209
TOST Lower	-2.112489	4	0.949
TOST Upper	-2.112489	4	0.051

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-2.4840000	1.6587068	[-6.0201, 1.0521]	0.9
Hedges's g	-0.9756103	0.7097402	[-2.1284, 0.2782]	0.9

Figure E.4.7d: Trial 9 TOST results for dry weight SA index

Appendix E.4.8 TOST results for Trial 10: The effect of mixed bacteria biofilm in medium diluted, unfiltered aquaculture waste with reduced slope

```
Two Sample t-test

The equivalence test was non-significant, t(5) = -0.360, p = 3.67e-01
The null hypothesis test was non-significant, t(5) = 0.294, p = 7.81e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.2939261	5	0.781
TOST Lower	0.9476891	5	0.193
TOST Upper	-0.3598368	5	0.367

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.2939261	5	0.781
TOST Lower	0.9476891	5	0.193
TOST Upper	-0.3598368	5	0.367

Figure E.4.8a: Trial 10 TOST results for total dry weight

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.37) = 0.0462, p = 5.17e-01
The null hypothesis test was non-significant, t(3.37) = 0.661, p = 5.51e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.66102651	3.374794	0.551
TOST Lower	1.27588610	3.374794	0.141
TOST Upper	0.04616693	3.374794	0.517

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	31.500000	47.653157	[-75.7455, 138.7455]	0.9
Hedges's g(av)	0.405969	1.249043	[-0.656, 1.418]	0.9

Figure E.4.8b: Trial 10 TOST results for total chlorophyll a

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.37) = 0.0441, p = 5.16e-01
The null hypothesis test was non-significant, t(3.37) = 0.659, p = 5.52e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.65871321	3.369029	0.552
TOST Lower	1.27335960	3.369029	0.142
TOST Upper	0.04406683	3.369029	0.516

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	1151.0000000	1747.346154	[-2783.8544, 5085.8544]	0.9
Hedges's g(av)	0.4043892	1.249796	[-0.657, 1.416]	0.9

Figure E.4.8c: Trial 10 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(5) = -0.0241, p = 4.91e-01
The null hypothesis test was non-significant, t(5) = 0.582, p = 5.86e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.5819483	5	0.586
TOST Lower	1.1880156	5	0.144
TOST Upper	-0.0241190	5	0.491

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	18.8200000	32.3396429	[-46.3459, 83.9859]	0.9
Hedges's g	0.3736879	0.6498527	[-0.7171, 1.4289]	0.9

Figure E.4.8d: Trial 10 TOST results for dry weight SA index

```
Two Sample t-test

The equivalence test was non-significant, t(5) = -0.744, p = 7.55e-01
The null hypothesis test was non-significant, t(5) = -1.398, p = 2.21e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-1.3984262	5	0.221
TOST Lower	-0.7436669	5	0.755
TOST Upper	-2.0531854	5	0.048

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-16.8300000	12.0349578	[-41.081, 7.421]	0.9
Hedges's g	-0.8979748	0.6855152	[-2.0086, 0.2909]	0.9

Figure E.4.8e: Trial 10 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(5) = -0.711, p = 7.45e-01
The null hypothesis test was non-significant, t(5) = -1.365, p = 2.31e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	-1.3647610	5	0.231
TOST Lower	-0.7106499	5	0.745
TOST Upper	-2.0188720	5	0.050

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	-15.6900000	11.496519	[-38.856, 7.476]	0.9
Hedges's g	-0.8763573	0.683514	[-1.9834, 0.3075]	0.9

Figure E.4.8f: Trial 10 TOST results for total chlorophyll pigments SA index

Appendix E.4.9 TOST results for Trial 11: The effect of the mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with reduced slope

```
Two Sample t-test

The equivalence test was non-significant, t(6) = -0.502, p = 3.17e-01
The null hypothesis test was non-significant, t(6) = 0.552, p = 6.01e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.5520730	6	0.601
TOST Lower	1.6065323	6	0.080
TOST Upper	-0.5023864	6	0.317

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.0004000	0.0007245419	[-0.001, 0.0018]	0.9
Hedges's g	0.3390897	0.6200342949	[-0.697, 1.348]	0.9

Figure E.4.9a: Trial 11 TOST results for total dry weight

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.16) = -0.156, p = 4.43e-01
The null hypothesis test was non-significant, t(3.16) = 0.553, p = 6.17e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.5525180	3.159009	0.617
TOST Lower	1.2607455	3.159009	0.146
TOST Upper	-0.1557096	3.159009	0.443

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	110.0000000	199.088548	[-349.0679, 569.0679]	0.9
Hedges's g(av)	0.2886591	1.242751	[-0.6104, 1.1457]	0.9

Figure E.4.9b: Trial 11 TOST results for total chlorophyll a

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.04) = 2.012, p = 9.32e-01
The null hypothesis test was non-significant, t(3.04) = 2.717, p = 7.16e-02
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	2.716898	3.041665	0.072
TOST Lower	3.421474	3.041665	0.020
TOST Upper	2.012323	3.041665	0.932

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	1.554000	0.5719757	[0.2154, 2.8926]	0.9
Hedges's g(av)	1.398135	2.4764169	[0.104, 2.5563]	0.9

Figure E.4.9c: Trial 11 TOST results for total chlorophyll pigments SA index

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.13) = 1.131, p = 8.31e-01
The null hypothesis test was non-significant, t(3.13) = 1.836, p = 1.6e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.836466	3.130743	0.160
TOST Lower	2.541573	3.130743	0.041
TOST Upper	1.131358	3.130743	0.831

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	1.3830000	0.753077	[-0.3595, 3.1255]	0.9
Hedges's g(av)	0.9560898	1.849268	[-0.1449, 1.9457]	0.9

Figure E.4.9d: Trial 11 TOST results for dry weight SA index

```
Welch Two Sample t-test

The equivalence test was non-significant, t(3.07) = 2.375, p = 9.52e-01
The null hypothesis test was non-significant, t(3.07) = 3.081, p = 5.25e-02
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	3.080942	3.070717	0.052
TOST Lower	3.787268	3.070717	0.015
TOST Upper	2.374617	3.070717	0.952

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	1.62700	0.5280852	[0.3958, 2.8582]	0.9
Hedges's g(av)	1.59164	2.7105478	[0.2041, 2.8365]	0.9

Figure E.4.9e: Trial 11 TOST results for chlorophyll a SA index

Appendix E.4.10 TOST results for Trial 12: The effect of the mixed bacteria biofilm in heavily diluted, unfiltered aquaculture waste with increased slope

```
Two Sample t-test

The equivalence test was non-significant, t(7) = 2.341, p = 9.74e-01
The null hypothesis test was significant, t(7) = 3.086, p = 1.77e-02
NHST: reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	3.086222	7	0.018
TOST Lower	3.831240	7	0.003
TOST Upper	2.341204	7	0.974

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	0.001570	0.0005087126	[6e-04, 0.0025]	0.9
Hedges's g	1.838847	0.7367904931	[0.5301, 3.051]	0.9

Figure E.4.10a: Trial 12 TOST results for total dry weight

```
Two Sample t-test

The equivalence test was non-significant, t(6) = 0.978, p = 8.17e-01
The null hypothesis test was non-significant, t(6) = 1.684, p = 1.43e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.6837156	6	0.143
TOST Lower	2.3897898	6	0.027
TOST Upper	0.9776413	6	0.817

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	4.960000	2.9458657	[-0.7643, 10.6843]	0.9
Hedges's g	1.034158	0.6664074	[-0.1214, 2.1155]	0.9

Figure E.4.10b: Trial 12 TOST results for total chlorophyll a

```
Two Sample t-test

The equivalence test was non-significant, t(6) = 1.658, p = 9.26e-01
The null hypothesis test was non-significant, t(6) = 1.667, p = 1.47e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	1.666512	6	0.147
TOST Lower	1.675069	6	0.072
TOST Upper	1.657956	6	0.926

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	174.900000	104.949726	[-29.0362, 378.8362]	0.9
Hedges's g	1.023592	0.665387	[-0.1296, 2.1032]	0.9

Figure E.4.10c: Trial 12 TOST results for total chlorophyll pigments

```
Two Sample t-test

The equivalence test was non-significant, t(5) = 0.0263, p = 5.1e-01
The null hypothesis test was non-significant, t(5) = 0.681, p = 5.26e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	t <dbl>	df <dbl>	p.value <dbl>
t-test	0.68093534	5	0.526
TOST Lower	1.33558093	5	0.120
TOST Upper	0.02628975	5	0.510

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	11.0080000	16.1659991	[-21.5673, 43.5833]	0.9
Hedges's g	0.4372507	0.6526793	[-0.6625, 1.4958]	0.9

Figure E.4.10d: Trial 12 TOST results for chlorophyll a SA index

```
Two Sample t-test

The equivalence test was non-significant, t(5) = -0.082, p = 4.69e-01
The null hypothesis test was non-significant, t(5) = 0.573, p = 5.92e-01
NHST: don't reject null significance hypothesis that the effect is equal to zero
TOST: don't reject null equivalence hypothesis

TOST Results
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	8.4790000	14.8063283	[-21.3565, 38.3145]	0.9
Hedges's g	0.3677239	0.6496096	[-0.7222, 1.4227]	0.9

```
Effect Sizes
Note: SMD confidence intervals are an approximation. See vignette("SMD_calcs").
```

	Estimate <dbl>	SE <dbl>	C.I. <chr>	Conf. Level <dbl>
Raw	8.4790000	14.8063283	[-21.3565, 38.3145]	0.9
Hedges's g	0.3677239	0.6496096	[-0.7222, 1.4227]	0.9

Figure E.4.10e: Trial 12 TOST results for total chlorophyll pigments SA index

Appendix E.5: Pie charts showing classes of analyzed microbial samples

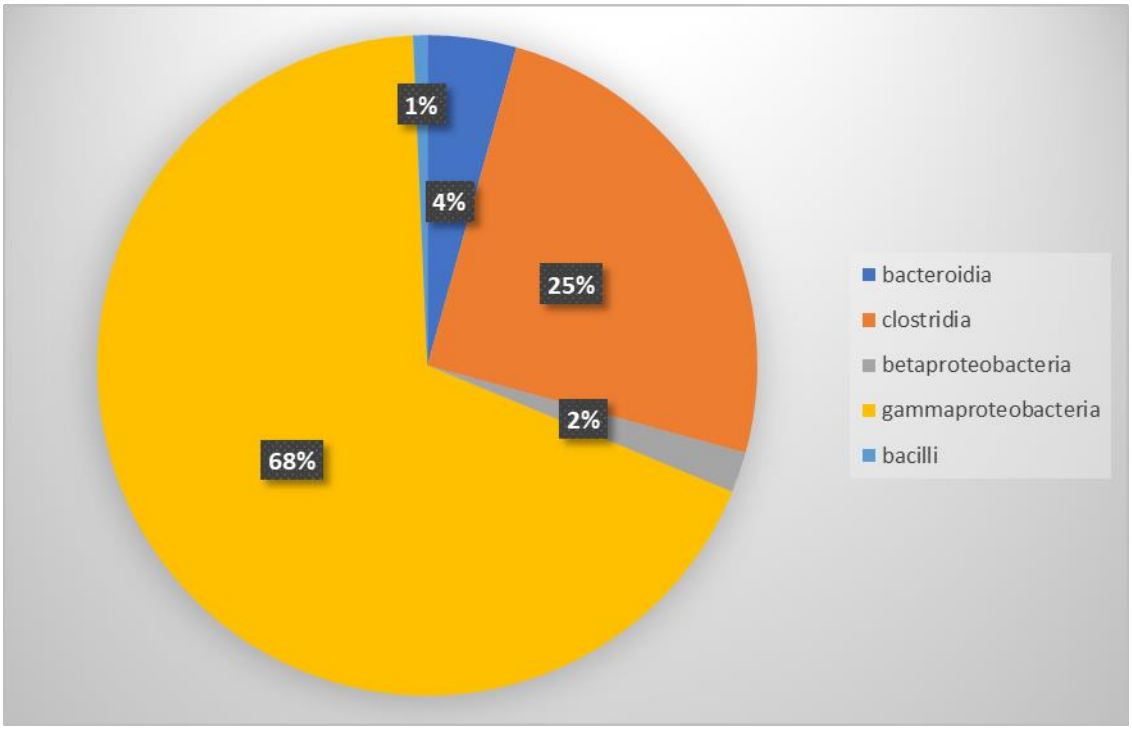


Figure E.5a: Percentages of classes present in Town Creek Park stream bacteria community

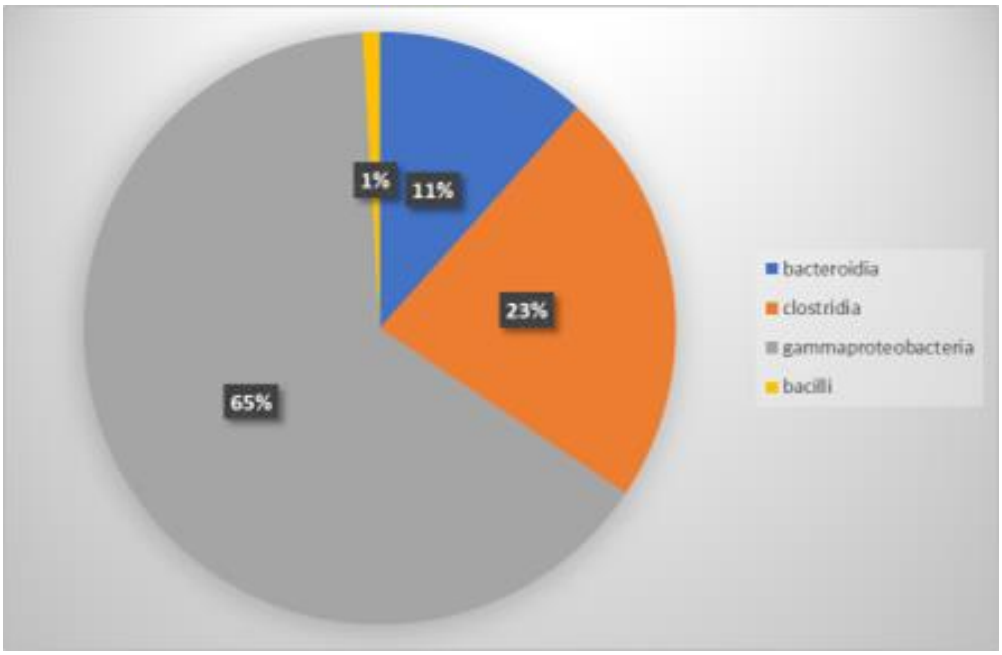
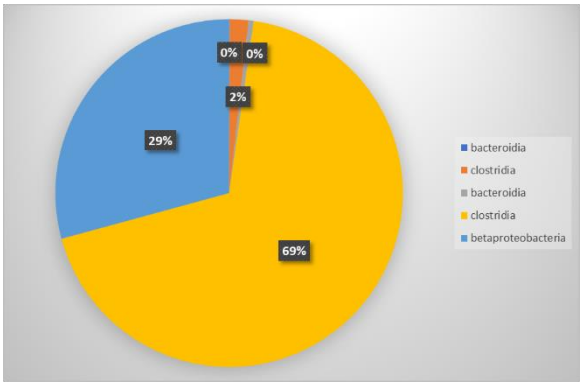


Figure E.5b: Percentages of classes present in Tallapoosa River bacteria community



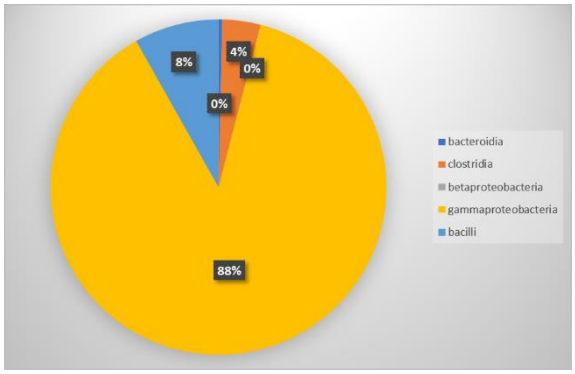


Figure E.5c: Percentages of classes present in mixed bacteria community (left: Trial 13, right: Trial 17; note that the 0% shown are not actually 0%, but significantly less than 1, and percentages are rounded up)

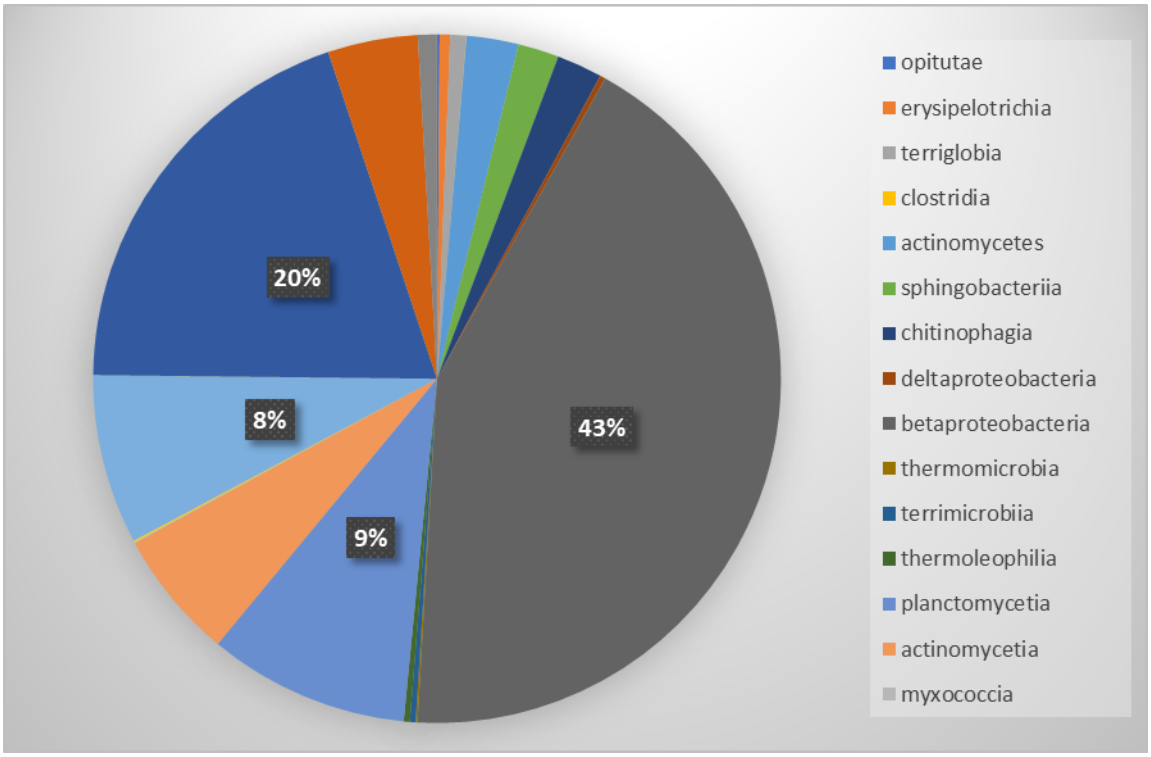


Figure E.5d: Percentages of classes present in fish waste background bacteria community (for visibility, only the percentages of the most dominant classes are shown)

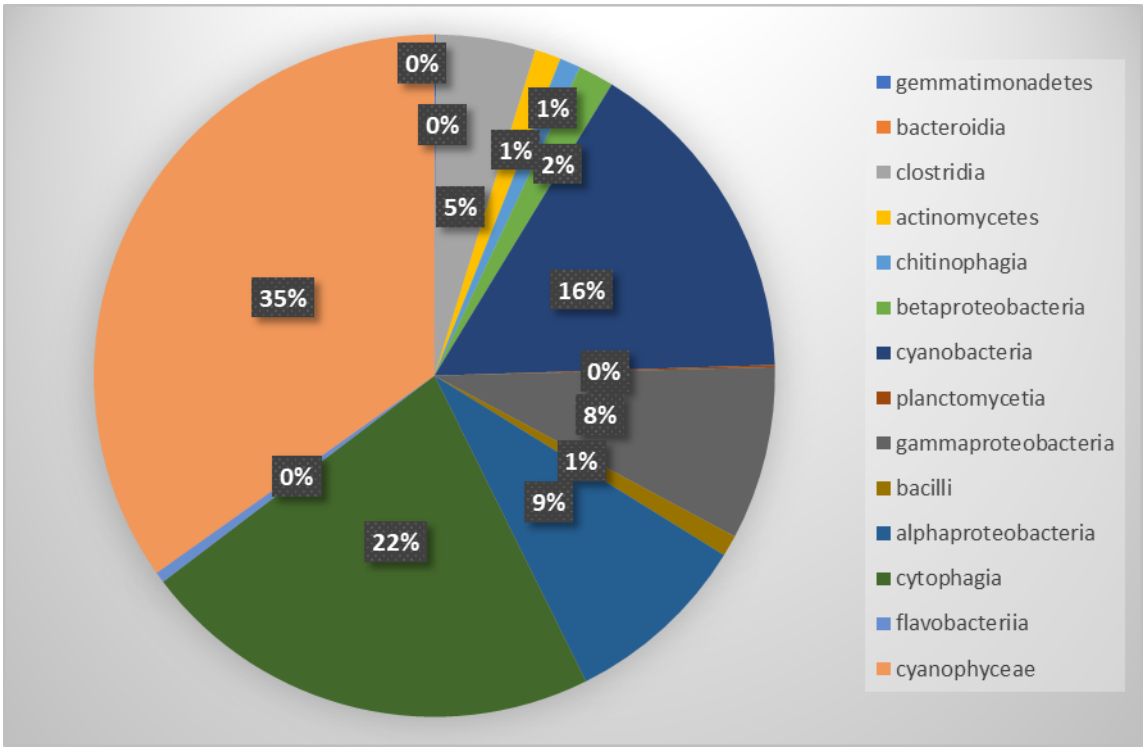


Figure E.5e: Percentages of classes present in algae inoculum background bacteria community (note that the 0% shown are not actually 0%, but significantly less than 1, and percentages are rounded up)

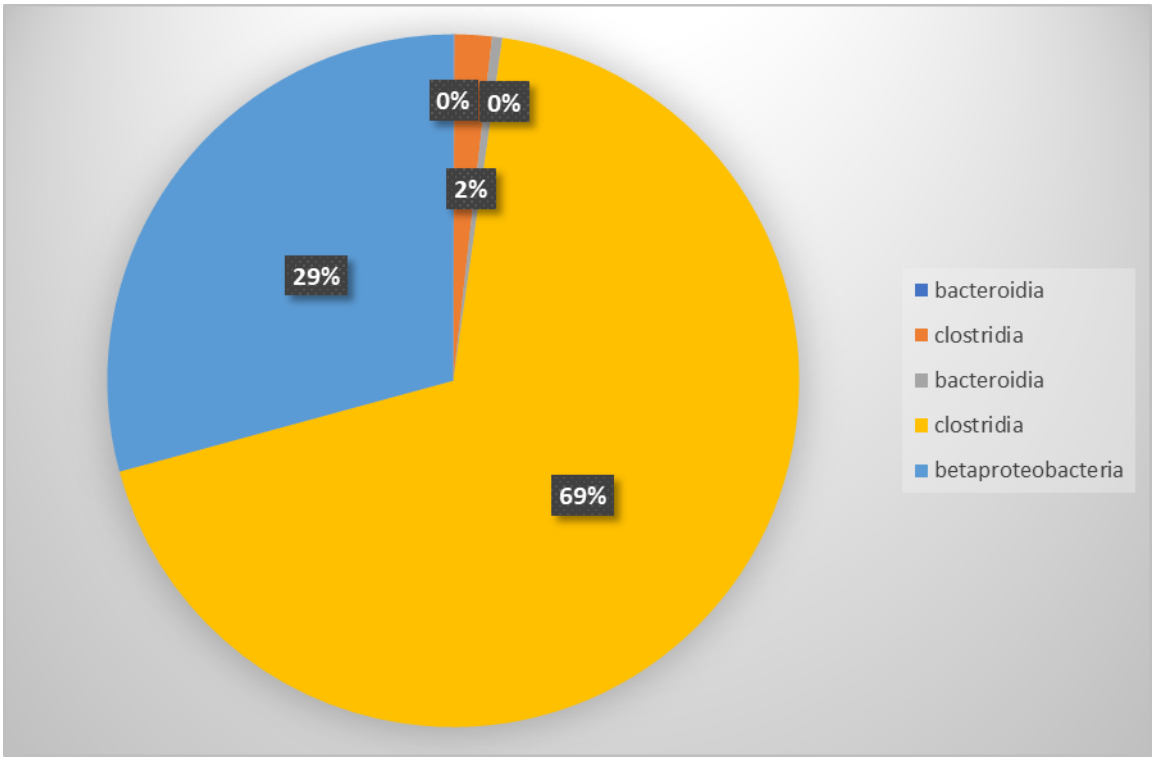


Figure E.5f: Percentages of classes present in synthetic media background bacteria community (note that the 0% shown are not actually 0%, but significantly less than 1, and percentages are rounded up)