The potential use of filamentous bacterial growth on stream macroinvertebrates as an indicator of nutrient enrichment

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Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

The influence of environmental stressors, such as nutrient enrichment and physical habitat degradation, can lead to a loss of biological integrity in streams. Nutrient enrichment in streams is a major concern in agriculturally dominated watersheds as fertilizers and animal waste runoff contaminate and pollute these systems through non-point source means. The impacts of nutrient enrichment on stream health requires further attention as well as the methods used to determine the source of contamination. One of the notable effects of nutrient addition in streams is the increased growth of aquatic, filamentous bacteria such as *Sphaerotilus* and *Leptothrix*. These bacteria have been known to colonize and grow on aquatic insects in nutrient enriched streams, and cases of high coverage on the insect have been found to be fatal. This study aims to analyze the differences in stream macroinvertebrate communities by looking at community composition, diversity, taxa richness, and biomass in nutrient enriched and non-enriched streams as well as evaluating the growth of filamentous bacteria on macroinvertebrates and its potential use as a bioindicator for nutrient pollution that is beyond extreme levels. Seasonal field measurements were taken of macroinvertebrate abundances, nutrient concentrations, and degree of bacterial growth on insects in six streams located in Western New York that were categorized as nutrient enriched or not based on nitrogen and phosphorus concentrations. Using non-metric multi-dimensional scaling (NMDS), analysis of similarities (ANOSIM), and similarity percentages (SIMPER), macroinvertebrate abundances in the enriched streams were compared to the non-enriched streams. The enriched and non-enriched streams were found to have significantly different macroinvertebrate communities, where the enriched streams had an abundance of pollution--tolerant organisms such as oligochaetes and leeches, while the non-enriched streams were mainly composed of pollution-sensitive insect species, and the non-

enriched streams were also significantly more diverse and had higher family richness. While the biological data indicate significant differences in stream integrity between the enriched and the non-enriched sites, bacterial growth and coverage did not follow similar expectations. There was no difference in the presence of bacteria between the enriched and non-enriched sites and the percentage of colonization was not greater in the enriched sites. Based on the data in this study, the use of filamentous bacteria growth on aquatic macroinvertebrates as a rapid bioindicator for nutrient enrichment in streams should be re-evaluated as nutrient levels in streams may not be the only contributing factor for bacterial growth to occur, and interpretation may not be broadly valid compared with traditional monitoring techniques.

Introduction

Anthropogenic changes in land associated with intensive agricultural practices such as tilling, fertilizer application, and deforestation have had significant negative impacts on aquatic ecosystems and have led to declines in stream health (Richards et al. 1996, Maloney and Weller 2011, Connolly et al. 2016). A healthy ecosystem is defined by the "absence of danger signals in the ecosystem, the ability of an ecosystem to quickly and completely recover (resilience), and having the lack of threats that could change the structure and/or function of the ecosystem" (Fierro et al. 2017). Land alteration and fertilizer application within a watershed heavily alter the hydrology, chemistry, and physical habitat within a stream, and these changes often result in decreased habitat quality and declines in the taxonomic diversity and abundance of aquatic organisms (Olson et al. 2016). Manure is an agriculture by-product that is utilized as a fertilizer and applied to crop fields. Spreading manure on agricultural fields is a cost effective, fertilization practice that is used to enhance soil productivity and is common among farmers in North America (Lewis and Makarewicz 2009). When applied in excess, manure can pollute waterways and infiltrate groundwater systems, introducing high concentrations of nutrients such as nitrogen and phosphorus.

Runoff from areas that are dominated by agricultural fields often contains a variety of nonpoint source pollutants such as sediments, nutrients, and pesticides (Lenat 1984). The influx of these pollutants into streams and other waterways can cause a variety of adverse effects on the water quality and aquatic organisms. The structure of many aquatic macroinvertebrate and fish communities are significantly different depending on nitrogen and phosphorus concentrations as these nutrients have direct and indirect impacts on biological assemblages. Nutrients directly

affect the primary productivity within a stream and are indirectly linked to primary and secondary consumers. Miltner and Rankin (1998) found that the biological integrity of streams is often negatively correlated with increasing nutrient concentrations, especially phosphorus. With increasing nutrient concentrations, they saw a loss in the abundance of sensitive fish species, decrease in abundance of top carnivores, and an increase in the proportion of tolerant or omnivorous fish species. In the macroinvertebrate communities, they saw high abundances of grazers present in headwater streams that had elevated nutrient concentrations. The river continuum concept (Vannote et al. 1980) suggests that the abundances of grazers should be lower in headwater streams due to low autochthonous production. In Miltner and Rankin (1998) the high abundance of grazers in the headwaters was a result of high algal production that was stimulated by nutrient enrichment.

Increased and excessive nutrient loading in waterways is one of the leading causes of impairment in rivers and streams in the U.S. There is often an overgrowth of benthic algae (Wang et al. 2007) and can cause changes in the structure of benthic aquatic macroinvertebrate communities (Sponseller et al. 2001) as there is a shift in the dominant functional feeding group due to a change in food source and a decrease in drift rates. Kerans (1996) found that the larvae of *Hydropsyche slossonae* drifted less frequently when rocks were covered with periphyton compared to when there was no periphyton present. An increase in benthic periphyton growth can also contribute towards a depletion in the oxygen concentration through nocturnal respiration, which can negatively affect the sensitive species that require high levels of dissolved oxygen (Wang et al. 2007). Most lotic macroinvertebrates are directly dependent on the dissolved oxygen in streams as they possess gills or other underwater respiratory mechanisms (Verberk et al. 2016). High algal and macrophyte biomass in streams can generate high amounts

of harmful organic matter when they decompose and can also lead to extreme diurnal variations in dissolved oxygen and pH levels (Wang et al. 2007).

Row crop agriculture and grazing have also significantly disturbed and degraded riparian and aquatic ecosystems (Stevens and Cummins 1999). Riparian zones along streams act as a buffer from adjacent landscapes by trapping and preventing excess sediment, nutrients, and other contaminants from entering the stream (Rios and Bailey 2006). Vegetation along streambanks help regulate water temperature (Rios and Bailey 2006) and light availability (Astudillo et al. 2016) by providing shade and also adds important allochthonous material to the streams which provide habitat and a principal energy source for aquatic macroinvertebrates (Fierro et al. 2017). Degraded stream channels with poorly developed riparian habitat often intensify the effects of nutrient enrichment through decreased nutrient uptake, increased retention time due to siltation, and by allowing increased sunlight to reach the stream (Miltner and Rankin 1998). In contrast, streams with high quality (i.e., mature, intact) riparian zones experience an uptake in nutrients before they enter the stream and are associated with stream insect communities consisting of higher densities of scrapers, shredders, collector-gatherers, and predators (Mesa 2014).

The structure of macroinvertebrate assemblages in streams is a function of the quality of the stream habitat, and environmental stressors are a major factor in driving benthic community composition (Rios and Bailey 2006). To assess and monitor the quality of aquatic environments, there are several strategies that are implemented to gauge the health of a habitat. Traditionally, stream assessments are based primarily on water chemistry analyses. However, relying on only these parameters often do not provide a thorough assessment of the habitat and stream function so it is important to analyze the biological composition as well (Herman and Nejadhashemi

2015). Biological monitoring, or biomonitoring, uses a wide array of aquatic organisms such as diatoms, fish, and macroinvertebrates as indicators to help determine the health of stream habitats (Deborde et al. 2016).

Macroinvertebrate communities are shaped by environmental factors at both local and regional scales. Invertebrate communities are commonly chosen for aquatic bioassessment investigations as they respond to a variety of different environmental stressors, are found in a wide variety of habitat types, are relatively sedentary and show localized disturbances, and are relatively simple to sample and process (Whiles at al. 2000). Since their communities are often heterogeneous, and are usually comprised of several different taxa, there are high chances that some of the groups (and the community as a whole) will respond to environmental perturbations (Berkman et al. 1986). Aquatic invertebrate bioassessment is an effective means for detecting stream reaches that are impaired by point-source and nonpoint-source pollutants due to their varied sensitivities to organic pollution (Fierro et al. 2017). Most studies on stream health and integrity include analyses on the community of taxa within the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). Taxa within EPT are significant components of headwater habitats and streams and fill important trophic roles in the ecosystem. They function as important detritivores, algivores, and predators and fulfill intermediate pathways of nutrient cycling and food web support while also serving as an important food source for higher trophic organisms such as fish, salamanders, and birds (Ponds 2011). Most EPT are known to be very intolerant to pollution in streams and thus are crucial biological indicators in determining stream health and in many cases, anthropogenic activities can have a profound effect on their abundance and diversity (Hamid and Rawi 2017). The presence of EPT taxa in streams indicates that the water chemistry and physicochemical parameters are within the tolerance zones of the species. EPT are useful as

ecological indicators because their nymphs/larvae can only survive in streams with high water quality, and their distribution in streams is strongly dependent on environmental factors.

When looking at macroinvertebrate communities to evaluate stream health, metrics such as abundance, density, taxa richness, and diversity are commonly used (Fierro et al. 2015). Taxa richness is a measurement of the number of macroinvertebrate taxa in the community assemblage within the stream; density is a measurement of total macroinvertebrate abundance per unit area, and diversity can be measured via several different diversity indices, the most common being the Shannon-Weiner Diversity index which incorporates both taxa richness and evenness (Brand and Miserendino 2015). These metrics help provide an overview of what is found within the stream while also indicating the health of the stream based on species distributions (Herman and Nejadhashemi 2015). In streams where there is high water quality and habitat quality, there is generally high macroinvertebrate diversity and richness, whereas impaired streams show altered macroinvertebrate communities with high abundances of tolerant species such as dipterans and oligochaetes. Brand and Miserendino (2015) found a strong decrease in richness (total taxa and EPT) and diversity when they compared macroinvertebrate communities from pasture dominated streams to their reference sites (forested streams). They also found that sensitive, non-tolerant taxa (EPT) were the most negatively affected, resulting in low abundances in the pastured streams. In their study, the metrics that effectively showed habitat degradation and impairment were richness, including total taxa richness and EPT richness, and composition metrics such as diversity.

While using aquatic macroinvertebrates is a useful approach to assess stream health, this method requires time identifying taxa, as there are usually high numbers of individuals in the

samples, and knowledge of invertebrate taxonomy. Using macroinvertebrates also does not identify the cause of impairment, such as high sedimentation, chemical pollution, nutrient enrichment, or other disturbances, and due to this, it is important to have other assessment methods alongside benthic sampling. Lemly (1998 and 2000) proposed the method of using the growth of filamentous bacteria (*Leptothrix* spp. and *Sphaerotilus* spp.) on aquatic insects as a rapid bioassessment tool that can be used as a bioindicator specifically for nutrient enrichment in streams. The addition of nutrients in streams can cause increased growth of these bacteria which can lead to the formation of large colonies on stream substrate and macroinvertebrates (Lemly 1998).

Bacterial assemblages on aquatic insects can be composed of several different growth stages, ranging from early colonization to mature forms with well-developed sheaths. The areas of heaviest infestation are typically found on the gills and caudal filaments (Lemly 1998). Heavy infestations can occur where over 25% of the insect's body is colonized by the bacteria; this level of coverage is associated with 100% mortality of the insect (Lemly 2000). Bacterial infection on the insects can be identified in the field using a hand lens with 10-15x magnification. Preservation of the insects in ethanol does not dislodge the bacteria, which means samples can be archived with no loss of data. Using bacterial growth on aquatic macroinvertebrates can be a potentially valuable method for identifying non-point source runoff and nutrient enrichment in streams and evaluating the severity of this impairment.

Leptothrix and *Sphaerotilus* are both found in stream ecosystems and typically thrive in polluted waters (Buchanan and Gibbons 1974) that are characterized by having a neutral pH, an oxygen gradient, and a source of reduced Fe and Mn minerals (Kunoh et al. 2016). They are both

sheath forming bacteria and can be identified by their sheaths and the presence of iron or manganese oxide crusts as well as the presence of swollen sheath tips (Lemly 2000). The color of the sheaths can range from yellow to brown and is dependent on environmental factors such as the presence and deposition of iron oxide. Individual cells of *Sphaerotilus* spp. swim freely until they attach onto solid objects where they can develop into delicate trichomes and eventually into longer tassels that are held together by tubular sheaths (Buchanan and Gibbons 1974). When *Sphaerotilus* spp. become well established in streams containing high pollution, they are known to line the riverbed for long distances, forming a woolly, filamentous bed (Dondero 1975). The *Leptothrix* spp. use copious amounts of oxidized Fe or Mn to produce extracellular, tubular sheaths. When multiplying, the cells divide and secrete exopolymers which provide a platform for the sheaths to form and become enriched with metals (Kunoh et al. 2016). Sheldon and Shelly (1990) found high abundance of *Leptothrix* in streams with high concentrations of Fe and Mn, which suggests these nutrients might be important in the ability of these bacteria to colonize stream substrate and dominate the periphytic community.

Objectives and Hypotheses

The goals of this study were to 1) determine and characterize the differences in macroinvertebrate communities between nutrient enriched and non-enriched streams by analyzing diversity, richness, community composition, and biomass, 2) asses differences in water quality parameters such as water chemistry (concentrations of dissolved nitrogen and total phosphorus) and physicochemical properties between enriched and non-enriched streams, 3) assess bacterial growth and coverage on aquatic macroinvertebrates and evaluate seasonal patterns and differences, and 4) determine which nutrient (N or P) leads to more bacterial growth

on damselflies in a controlled, microcosm experiment. I hypothesized that the non-enriched streams would have higher diversity, richness, and biomass as the non-enriched streams would be able to support a higher abundance of sensitive species. The enriched and non-enriched sites would be composed of different macroinvertebrate communities, and I hypothesized the non-enriched streams would contain more species belonging to EPT while the enriched streams would have more non-insect taxa and tolerant groups such as Diptera. The nutrient enriched streams were predicted to have higher concentrations of N and P due to having a more agriculturally dominated watershed compared to the non-enriched streams which were characterized by having wider riparian zones and I hypothesized that the nutrient enriched streams would have more invertebrates with bacterial colonization and have a higher percent coverage compared to the non-enriched streams. I expected to see seasonal differences in the presence and coverage of the filamentous bacteria in the enriched streams. Finally, I predicted that there would be a difference in bacterial growth between the microcosm tanks containing high nitrogen and high phosphorus concentrations.

Methods

Study Area

The six streams chosen for this study were Sandy Creek, Oatka Creek, Springwater Creek, Canaseraga Creek, Stony Brook, and Conesus Inlet (Figure 1). These streams are all located within Western New York (Livingston, Genesee, Steuben, and Monroe County) and are a part of the Lake Ontario and Minor Tributaries Watershed and the Genesee River Watershed. Western New York was chosen as the area for site selection due to the presence of many streams and the heavy influence that agriculture has on the area. The region of New York that includes

Monroe, Genesee, Steuben, and Livingston Counties has the largest amount of farmland in the state and had a total of 3,215 farms with a combined total of 352,224.6 ha (870,366 acres) of farmland in 2017 (USDA 2017). Livingston, Steuben, and Genesee County are all within the top ten counties for farmland in New York State. In 2017, Livingston County had 76,683.1 ha (189,488 acres) of land being used for agriculture which contributed to about 40%-49.9% of the county being made up of farmland (USDA 2017). The majority use of the land (78%) in Livingston County is cropland which includes corn, soybeans, and wheat. Genesee County had 71,606.3 ha (176,943 acres) of farmland in 2017 which made up more than 50% of the total land use in the county. Most of the farmland is used to grow crops like Livingston County and cropland makes up 84% of the land use by farms. Steuben County is the leading producer of hay and had 160,723.7 ha (397,157 acres) of farmland which made up about 44.6% of county land. Monroe County had 43,211.5 ha (106,778 acres) used for farmland in 2017 which makes up about 20%-29% of the county (USDA 2017). Like Livingston and Genesee County, most of the farmland (80%) is used for crops that include corn, soybeans, and wheat.

Sampling Site Selection

The six streams that I chose to sample were Sandy Creek, Oatka Creek, Springwater Creek, Canaseraga Creek, Stony Brook, and Conesus Inlet. They were selected as study sites based on preliminary water quality measurements and the presence/absence of agricultural farmland within their vicinity. The streams were also consistent with having riffle/run habitat areas made up of cobble and gravel substrate in which benthic macroinvertebrate samples were taken. Three riffle/run sections greater than 50 meters apart were selected to achieve at least a 100-meter representation of the stream and used as subsampling locations. Three streams with

more dense agricultural landscapes and higher amounts of total phosphorus and nitrates/nitrites were selected to be the nutrient enriched streams and included Sandy Creek, Oatka Creek, and Springwater Creek. The other three streams, Canaseraga Creek, Stony Brook, and Conesus Inlet were selected as the control/non-enriched sites based on having less of an agricultural presence within the stream's vicinity and total phosphorus and nitrates/nitrites concentrations around natural background levels (10 µg P/L and below 1 mg N/L).

Sandy Creek

The section of Sandy Creek that I sampled is located in Hamlin, New York (Monroe County). Sandy Creek is part of the Western Lake Ontario Basin and is one of ten major tributaries to Lake Ontario. It is a relatively large stream with substrate composed of sand and small to medium sized gravel. Agriculture is the dominant land use in the watershed and the New York State Department of Environmental Conservation (NYSDEC 2007) has listed Sandy Creek as stressed by high levels of nutrient pollution, with the suspected causes listed as agriculture and non-point source pollutants (NYSDEC 2007). Sandy Creek is an important water body as it provides many recreational opportunities and experiences heavy fishing during coho (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tshawytscha*) salmon runs in the fall.

Oatka Creek

The area of Oatka Creek that I used for sampling is located near Le Roy, New York (Genesee County). Oatka Creek is part of the Lower Genesee River Basin and is a major tributary to the Genesee River. It is a medium sized creek with sandy to medium sized gravel substrate. There are also many public fishing areas along the creek that allow for fly fishing for brook (*Salvelinus fontinalis*) and brown (*Salmo trutta*) trout. The majority of the Oatka Creek

watershed is comprised of active or inactive agricultural land and has been listed as threatened due to suspended solids and high nutrient concentrations from non-point source pollution (Tatakis 2002).

Springwater Creek

Springwater Creek is located in the town of Springwater, New York in eastern Livingston County. Springwater Creek is a major tributary to Hemlock Lake and is an important spawning and nursery habitat for lake trout (*Salvelinus namaycush*) and rainbow trout (*Oncorhynchus mykis*) (NYSDEC 2010). The watershed of Hemlock Lake is relatively pristine as Hemlock Lake is a source of water for the City of Rochester. Development is limited within the watershed. Only 11% of the land is used for agriculture, and 5% contains urban development (Eckhardt and Burke 1999). Springwater Creek has a drainage area of approximately 16.25 km^2 and flows through a high-density agricultural land use which has introduced pollutants to the water due to non-point source runoff (Eckhardt and Burke 1999).

Canaseraga Creek

Canaseraga Creek is one of the largest subbasins of the Genesee River with a land area of about 86,505 ha. The topography varies within the watershed where the upper reaches have a slope of about 211 m/km above the town of Dansville. North of Dansville, the slope decreases to about 16 m/km and eventually broadens into an alluvial plain (Rea et al. 2013). The dominant land usages in the Canaseraga Creek watershed are agriculture (46.8%), followed by a mixture of forests that include deciduous, evergreen, and mixed (44.4%), and developed land (5.7%) (Beers et al. 2004). The portion of Canaseraga Creek that I chose to sample is located within the Rattlesnake Hill Wildlife Management Area in southern Livingston County and is part of the

upper reach of the creek. Rattlesnake Hill Wildlife Management Area is made up of a blend of upland habitats that include deciduous and conifer forests.

Stony Brook

Stony Brook Creek is located in eastern Livingston County near the town of Dansville, New York. The creek begins south of Stony Brook State Park. The state park as well as the upper reaches of the creek are surrounded by dense vegetation and a mix of deciduous and coniferous forests. The lower reaches of the creek are used for recreational purposes such as swimming and wading while the upper reaches outside of the park are used infrequently. The sampling area consisted of medium sized gravel substrate.

Conesus Inlet

Conesus Inlet is a tributary located directly south of Conesus Lake in Livingston County. Conesus Inlet is primarily a Wildlife Management Area that is made up of marshes, swamps, and open land. The topography of Conesus Inlet consists of a flat valley floodplain with steep sloping hills on the east and west sides of the inlet (NYSDEC 2018). Before the marsh begins, Conesus Inlet flows as a creek that begins south of the town of Scottsburg. South of Scottsburg, the creek flows through valleys surrounded by upland deciduous forests while north of Scottsburg consists of intensely developed agricultural fields. The sampling area is 0.8 km north of Scottsburg on East Swamp Rd. and is surrounded by a vegetation buffer zone in an agricultural field.

Aquatic Macroinvertebrate Sampling

Aquatic macroinvertebrate samples were collected seasonally during the summer (June 17-18, 2019), fall (November 1-2, 2019), winter (February 25-26, 2020), and spring (April 27-

28, 2020). The samples were collected in riffle habitats (runs were selected when riffles were not present) using a D-frame, 500 µm mesh net with a net diameter of 30.48 cm and a net depth of 55.88 cm. Sampling began at the farthest subsampling location downstream, and then continued upstream to the next riffle/run that was at least 50 meters from the previous location. The D-net was placed so that the frame was perpendicular to the stream bottom in the center of the riffle/run facing upstream. Facing downstream, I agitated the stream bottom to disturb the substrate and dislodge any benthic macroinvertebrates on the substrate. I sampled for a total length of one meter upstream of the net. Large pieces of debris and rocks were removed after being inspected for any attached invertebrates. The remaining contents of the net were transferred to gallon sized Ziplock bags and preserved using 95% ethanol. This process was repeated at each subsampling location, resulting in three subsamples per stream per sampling date. Preserved samples were then transported to the macroinvertebrate lab at The College at Brockport, State University at New York (SUNY) and stored for later identification.

Field Water Quality Analysis

Field water quality measurements were taken at every subsampling location in each stream using a YSI ProPlus Water Quality Meter. Measurements included temperature (°C), dissolved oxygen (% and mg/L), specific conductance (µS/cm), and pH. Before every sampling session, the instrument was calibrated. The sensor remained submerged until all readings stabilized.

Water Chemistry Sampling

To provide an estimate of the average water quality conditions of the streams during each season, a composite water sample was taken by mixing one liter of stream water from each

subsample location. These water samples were collected before any other sampling began to prevent any mixing from streambed disturbances. The water samples were collected starting from the first subsample location farthest downstream and then proceeding upstream towards the next sample location. The composite water sample was thoroughly mixed before taking a raw and filtered water sample. Samples were filtered on site with 0.45 µm MCI Magna Nylon 66 filters. To prevent contamination, sample bottles for water collection and raw water storage were pre-rinsed with deionized water and stream water and the storage bottle for the filtered water sample was rinsed with deionized water and filtered water. The samples were placed in a cooler on ice during transport and then placed in a freezer until they were analyzed at SUNY Brockport's Water Chemistry Laboratory.

Water Quality Analysis

Samples were thawed 24 hours before analysis to ensure all ice had melted. The filtered samples were analyzed for nitrate+nitrite (NO_3^-+NO_2^-) and the raw samples were analyzed for total phosphorus (TP). Concentrations of nitrate/nitrite were determined using a cadmium reduction flow injection method (EPA Method 353.2, ELAP #2281) Total Phosphorus concentrations were determined by using manual acid persulfate digestion and flow injection analysis (APHA 4500-P H-2011, ELAP #9964).

Analysis of Field Specimens

The preserved macroinvertebrate specimens were analyzed in SUNY Brockport's Macroinvertebrate Lab. In the laboratory, samples were identified to genus (ideally) or the lowest possible classification using taxonomic keys (Peckarsky et al. 1990, Merritt et al. 2008) and an Olympus SZX7 (8x - 56x zoom) stereomicroscope. Each sample was enumerated to

provide the total number of specimens collected. Any insects with bacterial coverage were placed in a separate vial for further analysis (see below).

Assessing Bacterial Coverage

Filamentous bacteria on the organisms were identified to genus using identification keys that use external morphological features of the sheaths (Buchanan and Gibbons 1974). The sheaths are identified using characteristics such as the presence or absence of manganese or iron oxide crusts as well as swollen sheath tips. These characteristics are easily identifiable using a dissecting scope, and there is no need for culturing or staining. The bacteria remain on the specimen even when preserved in ethanol.

Bacterial coverage on the specimens was quantified using a block-grid recording technique similar to Lemly and King (2000). A general outline of the macroinvertebrate families that showed bacterial coverage were sketched onto quad-ruled engineering paper. The family outlines were taken from Peckarsky et al. (1990). The specimen was viewed under a Laxco dissecting microscope (6.7x - 45x zoom) with an attached camera. Dorsal and ventral images of each insect were taken to provide high resolution images of how and where the bacteria colonize different orders of insects and to provide a visual for cover percentage. Percentage of bacterial coverage was determined by shading the corresponding body part that had bacteria on the gridded outline using a highlighter pen. The highlighted squares were counted and then compared to the total number of squares within the insect outline to calculate the percentage of body covered by bacteria (Lemly and King 2000).

Assessing Biomass of Field Specimens

The preserved samples were dried to constant weight at 60˚C for at least 48 hours and allowed to cool before weighing on an analytical scale. Each subsample was kept together for drying and weighing to determine the average biomass of aquatic macroinvertebrates present in a square meter of the stream.

Controlled Microcosm Experiment

The microcosm experiment used 12 aquaria that were each filled with 11 liters of water that was collected from Lake Ontario, a low nutrient, oligotrophic lake. There were four treatments (with three tanks per treatment) that consisted of "high" and "low" concentration of nitrogen and phosphorus. The nitrogen treatments were made using sodium nitrate and the phosphorus treatments were made using sodium phosphate. Target values for the high and low nitrogen treatment were 3.0 mg N/L and 0.2 mg N/L, respectively. Phosphorus treatment target values were 400 µg P/L (high) and 5 µg P/L (low). These values which were determined using water chemistry values from previous studies (Lemly 1998, 2000), where there were high amounts of bacteria recorded. The study insect for this experiment were damselflies in the Coenagrionidae family as they are relatively easy to acquire and maintain in a lab setting, and they have been previously observed to support filamentous bacterial growth. The damselflies were ordered from Carolina Biological Supply and the bacteria (*Leptothrix discophora*) was ordered freeze dried from Bigelow (National Center for Marine Algae and Microbiota). Damselflies were inoculated with the cultured bacteria using sterile swabs to coat their exoskeleton. After external inoculation, each damselfly was placed in an individual 2 oz. chamber, that contained a lid and holes to allow for water circulation. Each chamber was placed

into the treatment tanks discussed above. Each treatment tank contained seven damselflies in these individual chambers. Water samples were collected every week during the 21-day experiment time to monitor tank concentrations, and environmental parameters were recorded weekly using a YSI Pro DSS and a Vernier Labquest Pyranometer. After the three weeks, the damselflies were taken out of their containers, placed in 95% ethanol, and examined for bacterial growth.

Alongside the microcosm aquaria experiment, another microcosm experiment was set up using 10 mL glass tubes with lids (n=36). These tubes were filled with corresponding tank water (3 reps per tank) and inoculated with the bacteria. The tubes were left undisturbed for the same duration as the damselfly microcosm experiment. Upon completion, the presence/absence of bacterial growth was observed in each tube.

Statistical Analyses

For each season, the macroinvertebrate subsamples were analyzed separately to get a subsample average and then pooled to get a total site average during that season. Prior to analyses, variance equality was tested and the Anderson-Darling normality test was used to determine if the data were normal before testing for significance. If the data were not normal, log(x+1) transformations were used, and an arc sine square root transformation was used on percentage data.

The preserved macroinvertebrate samples were analyzed to determine if there were differences in the invertebrate taxa composition among the streams. T-tests were used to determine if there were differences in macroinvertebrate biomass, macroinvertebrate family richness, insect family richness, EPT density and abundance, and EPT family richness between

the enriched and non-enriched sites. A two-way ANOVA was also used for biomass to see if there were differences between treatments and season and if there was an interaction between the two factors. To determine if there were differences in genera diversity between the enriched and non-enriched streams, the Shannon Wiener Diversity Index (H') was calculated using the equation:

$$H' = -\sum[(p_i) \times ln(p_i)]$$

Where:

P_i = the proportion of each species in the sample

The average H' was calculated for each stream and a T-test was used to determine if there was a difference in diversity between the enriched and non-enriched streams.

Multivariate analyses, including Analysis of Similarities (ANOSIM), Similarity Percentages-Species Contributions (SIMPER), and non-metric multidimensional scaling (nMDS), were used to detect similarities and differences in the macroinvertebrate community assemblages among sites. All abundance data in Primer v7 software (Primer-e, Quest Research Limited, Auckland, NZ) were log (x+1) transformed and transcribed into a resemblance matrix to display the Bray-Curtis dissimilarity between the enriched and non-enriched sites

Analysis of Similarities (ANOSIM) and Similarity Percentages (SIMPER) are techniques that use permutation methods applied on Bray-Curtis dissimilarity matrices to identify differences among samples. ANOSIM gives a significance level (P value) and a test statistic, R, which ranges between 0 and 1. An R value that is close to 0 indicates that there is no separation between samples while a value close to 1 indicates high separation. Similarity Percentages

(SIMPER) incorporates the contributions that individual species have on the separation/closeness between the groups of samples and provides a species list with percent contributions from each species and is ordered from highest to lowest. Non-metric multi-dimensional scaling (nMDS) places samples as points in a 2 or 3-dimensional space. Points that are close together on the ordination indicate macroinvertebrate communities are similar in species composition and relative abundance, while points that are far apart represent communities that are different. I chose to run a 2-dimensional ordination plot with a a stress level of 0.2 to be the cut off for representation (Clarke 1993).

Water chemistry and physicochemical data were tested for normality using the Anderson-Darling normality test and their variances were tested for equality. The data were compared between the treatments (nutrient enriched and non-nutrient enriched sites) and season using two-way ANOVA's to determine if there were differences in $[NO_2^-+NO_3^-]$, [TP], temperature, dissolved oxygen, pH, and specific conductivity. The factors were treatment and season and interactions between the two factors were also tested.

The specimens with bacterial coverage were removed from the macroinvertebrate samples and analyzed for further analysis. A one-way ANOVA was used to determine if there were differences in bacterial presence among invertebrate orders and compare differences in bacterial coverage among EPT. To determine if there were seasonal differences in bacterial coverage, a two-way ANOVA was used with bacterial coverage and seasons as the factors. T-tests were used to compare the presence of bacteria between the enriched and non-enriched streams and to compare the amount of EPT with bacterial coverage to the amount of EPT without bacterial coverage.

All significance testing used $\alpha = 0.05$. NDMS, ANOSIM, and SIMPER analyses were performed using Primer v7 software. All other statistical analyses were performed using R (R Core Team, 2018).

Results

Macroinvertebrate Community Results

A total of 13,319 aquatic macroinvertebrates were collected during the sampling period. The enriched sites had a combined total of 4,561 invertebrates collected and the non-enriched sites had a combined total of 8,758 invertebrates (Appendix 1). The most invertebrates were collected during Spring 2020 (n = 4,415) sampling and the fewest were collected during the Fall 2020 (n = 2,476) sampling session. Of the enriched sites, Sandy Creek had a total of 37 taxa, 11 of which were non-insects, Oatka Creek had 35 taxa with seven belonging to non-insect groups, and Springwater Creek had a total of 40 taxa with four belonging to non-insect groups. Diptera was the most abundant group and made up 37% of the total abundance of macroinvertebrates and the next highest group were the non-insect taxa, making up 25.8% of the abundance (Figure 2). Of the non-enriched sites, Canaseraga Creek had a total of 50 different taxa with only one belonging to a non-insect group, Stony Brook had 58 different taxa and were all insects, and Conesus Inlet had a total of 47 different taxa with one non-insect taxon. Ephemeroptera was the most abundant group and made up 42.9% of the total abundance; Diptera was next highest at 31.3%. EPT taxa made up 64.3% of the total abundance (Figure 3).

Taxa Richness

There was a significant difference in average macroinvertebrate family richness between the enriched and non-enriched sites where the non-enriched sites had significantly higher

richness ($t = -2.78$, $p = 0.02$). The non-enriched sites had an average of 16.7 +/- 0.7 families present while the enriched sites had average of 12.3 +/- 1.4 families (Figure 4). There was also a significant difference in the average number of insect families present between the enriched and non-enriched sites, where the non-enriched sites had a significantly higher number of insect families ($t = -3.78$, $p = 0.01$). The non-enriched sites had an average of 16.6 +/- 0.7 insect families and the enriched sites had an average of 8.8 +/- 1.9 insect families (Figure 5).

The enriched sites had a combined total of 1,135 insects belonging to EPT which, out of the total number of macroinvertebrates collected ($n = 4,561$), made up about 25% of the sample. The nonenriched sites had a combined total of 5,629 insects that belonged to EPT and out of the total number of invertebrates collected ($n = 8,758$), made up about 64% of the total. This difference in EPT taxa abundance was significant ($t = -2.66$, $p = 0.03$). The average abundance of EPT (per site) for the enriched sites was 377.3 +/- 259.4 while the average EPT abundance for the non-enriched sites was 1871 +/- 499 (Figure 6). The non-enriched sites also had a significantly higher EPT density compared to the enriched sites ($t = -2.63$, $p = 0.03$). The average EPT density at the enriched sites was $31.5/m^2$ +/- 21.7 and the average EPT density at the non-enriched sites was $154.8/m^2$ +/- 41.3 (Figure 7).

The non-enriched sites also had significantly higher average EPT family richness compared to the enriched sites ($t = -6.2$, $p = 0.002$). The average number of families sampled in the non-enriched sites was 11.47 +/- 0.82 while the average number of EPT families sampled within the enriched sites was 3.83 +/- 0.92 (Figure 8).

Diversity (H') and Community Comparisons

The non-enriched sites had a significantly higher diversity index (H') compared to the enriched sites ($t = -2.47$, $p = 0.03$). The average diversity index for the non-enriched sites was 2.36 +/- 0.09 and the average diversity index for the enriched sites was 1.99 +/- 0.12 (Figure 9).

The analysis of similarity (ANOSIM) revealed significant differences in macroinvertebrate community composition between the enriched sites and the non-enriched sites ($R = 0.68$, $p = 0.001$; Figure 10). Table 1 provides details on which genera contributed most to the dissimilarity between the two macroinvertebrate communities (enriched vs. non-enriched sites). *Gammarus* spp. contributed the most towards the dissimilarity with a contribution of 5.61% and was only found in the enriched sites. *Baetis* spp. contributed the next highest in dissimilarity with a contribution of 5.33% and was more abundant in the non-enriched sites than in the enriched sites.

Biomass

The average biomass for the enriched sites was 0.216 g/m^2 +/- 0.012 and the average biomass for the non-enriched sites was 0.161 g/m^2 +/- 0.053 (Figure 11). The two-way ANOVA showed no significant differences in treatment (site) effects on biomass; however, it did show that were significant differences among seasons ($F = 6.3$, $p = 0.005$). The Tukey post-hoc test revealed that there were significant differences between the seasons of Spring and Fall ($p = 0.02$) and Spring and Summer ($p = 0.005$) (Figure 12). There was no interaction found between season and treatment. See Table 2 for seasonal biomass averages per treatment.

Water Quality and Environmental Parameters

Total phosphorus concentrations were found to be significantly different between treatments (nutrient enriched vs. non-nutrient enriched sites) ($F = 16.9$, $p = 0.001$, Figure 13), but not significantly different among seasons and there were no interaction effects between season and treatment. Similarly, nitrate/nitrite was also found to be significantly different between treatments ($F = 87.4$, $p = 0.0001$, Figure 14) and approaching significance when looking at seasonal differences (0.052) but there was no interaction between treatment and season. For temperature, there were significant differences between treatments ($F = 6.2$, $p = 0.024$) as well as among seasons ($F = 128.6$, $p = 0.0001$), but there was no interaction effect between the two factors. The Tukey post-hoc test showed that all seasons were significantly different from one another besides Fall and Spring (Figure 15). For dissolved oxygen, there was no significant difference between treatments and among seasons, however seasons was approaching significance ($p = 0.06$, Figure 16). There was also no interaction seen between treatment and seasons. The two-way ANOVA for specific conductivity showed there to be a significant difference between treatments ($f = 13.5$, $p = 0.002$, Figure 17), but there was no difference among seasons and there was no interaction between the factors. Finally, pH was not significantly different between treatments, but there was a significant difference among seasons ($f = 4.2$, $p = 0.02$) and there was no interaction between the factors. The Tukey post-hoc test showed that summer pH was significantly more different than both winter and spring ($p = 0.04$ and $p = 0.03$ respectively, Figure 18). Average seasonal concentrations for each water quality parameter for each treatment can be seen in Tables (3 and 4).

Bacterial Assessment

Coleoptera, Ephemeroptera, Plecoptera, and Trichoptera were the orders that contained the greatest bacterial growth. Among these four orders, there was no significant difference in the numbers of organisms that exhibited bacterial presence (Figure 19). The average number of Coleoptera within each stream that had bacterial growth was 7.17 +/- 4.12; the average for Ephemeroptera was 9.8 +/- 3.1; the average for Plecoptera was 9.33 +/- 3.5; and the average for Trichoptera was 27.5 +/- 10.3.

The total percentage of macroinvertebrates with bacterial coverage was compared between the enriched and non-enriched sites, and it was found that there was no significant difference between the treatments (Figure 20). The average percentage of invertebrates with bacteria in the enriched sites was 3.33% +/- 1.2 and the average for the non-enriched sites was 1.67% +/- 0.33.

The number of EPT that had bacterial coverage was compared to the number of EPT that did not have bacterial coverage, and it was found that there was a significant difference between the two groups ($t = -2.5$, $p = 0.03$). There were more EPT without bacteria compared to EPT with bacteria (Figure 21). The average number of EPT with bacteria was 47.17 +/- 15.1 and the average number of EPT without bacteria was 1077.2 +/- 411.1.

There was no significant difference found in the number of individuals with bacterial coverage among the EPT orders. Figure 22 displays the average number of individuals with bacterial coverage within each order. Ephemeroptera had an average of 9.8 +/- 3.1; the average for Plecoptera was 9.33 +/- 3.5; and the average for Trichoptera was 27.5 +/- 10.3.

There were no seasonal differences in the presence of macroinvertebrates with bacteria within the streams (Figure 23). The average percentage of invertebrates that showed bacterial coverage in the summer was 1.67% +/- 0.64. The average in the fall was 4.9% +/- 1.6. The winter average was 3.43% +/- 1.6, and the spring average was 2.1% +/- 0.9.

Regarding percent coverage of bacteria on macroinvertebrates, I found no significant difference in coverage among seasons (Figure 24). The average percent coverage in the summer was 4.8% +/- 0.99, the average in the fall was 7% +/-1.15, the average for the winter was 5.17% +/- 0.51, and the average for the spring was 4.5% +/- 1.26. There was also no significant difference in bacterial coverage between the enriched and non-enriched sites (Figure 25). The average percentage of bacterial coverage in the enriched sites was 5.1% +/- 0.54 and the average percent bacterial coverage in the non-enriched sites was 5.8% +/- 1.3. I also found no significant difference in coverage among the EPT orders (Figure 26). The average percent coverage for Ephemeroptera was 3.23% +/- 1.04, for Plecoptera the average percent coverage was 4.5% +/- 1.2, and the average percent coverage for Trichoptera was 5.5% +/- 0.96.

Microcosm Experiment

The starting nutrient concentrations for each treatment were 4.8 µg P/L and 0.172 mg N/L for the low phosphorus treatment, 313 µg P/L and 0.171 mg N/L for the high phosphorus treatment, 0.5 µg P/L and 0.186 mg N/L for the low nitrogen treatment, and 0.17 µg P/L and 3.35 mg N/L for the high nitrogen treatment. Average nutrient concentrations for total phosphorus, orthophosphate, total nitrogen, and nitrate/nitrite are displayed in Table 5. At the end of the three-week trial, no bacterial growth was seen on the damselflies or in the glass test

tubes. The averages for the physicochemical parameters can be viewed in Table 6, and the starting and ending number of damselflies per tank can be viewed in Table 7.

Discussion

Agriculturally developed land poses a significant threat to the health of our waterways and the function and composition of aquatic freshwater macroinvertebrate communities. Here, I examined the impacts of agriculture on aquatic macroinvertebrate communities in streams within Western New York. The primary objectives of my study were to (1) determine differences in aquatic macroinvertebrate community structure (e.g., richness, diversity, community composition, biomass) between nutrient enriched streams and non-enriched streams, (2) assess water quality in both nutrient enriched and non-enriched streams, and (3) assess bacterial growth and coverage on aquatic macroinvertebrates and evaluate seasonal patterns and differences.

Macroinvertebrate Community Assessment

Intensive agricultural practices are known to have significant impacts on waterways within the United States, and the prospective intensification will contribute towards the decline of many aquatic ecosystems. The input of fertilizer and non-point source run-off in streams and tributaries has caused elevated nutrient levels such as nitrogen and phosphorus, which has induced stress on aquatic macroinvertebrates and has caused shifts in community structure and decreases in biodiversity (Tilman 1999). In this study, I observed that more macroinvertebrates were collected from the non-enriched streams (n=8,758) compared to the enriched streams (n=4,561) and the non-enriched streams had far fewer non-insect taxa present within samples. Our observation agrees with other studies showing a decrease in macroinvertebrate abundance in streams within a developed, agricultural watershed (Quist and Schultz 2014, Harding et al. 1998,

Olson et al. 2016). Row-crop agriculture is a popular planting technique and responsible for contributing large loads of sediment and nutrients to tributaries and watersheds (Baker 1993). The input of excessive nutrients into streams is a known stressor that can cause a decrease in oxygen levels and increase in the growth of biofilms, which results in a reduction in aquatic macroinvertebrate abundance and diversity (Quist and Schultz 2014, Olson et al. 2016).

The presence and survival of certain aquatic macroinvertebrate species is heavily dependent on water quality. Nutrient enrichment can modify macroinvertebrate assemblages which can often lead to an abundance of tolerant taxa (Zhang et al. 2021). Our observations also show a higher presence of non-insect taxa within the nutrient enriched streams. The enriched streams consistently had taxa belonging to Amphipoda (Gammarus spp.), Annelida (Oligochatea and Hirudinea), Isopoda (Caecidotea spp.), and Gastropoda. Nutrient enrichment in streams can enhance phytoplankton growth in water and as phytoplankton debris sinks into the sediment, it increases the organic matter content which becomes a food source for deposit feeders such as oligochaetes (Zhang et al. 2021). Oligochaetes are often abundant in polluted and degraded aquatic systems and the presence of oligochaetes is often indicative for organic pollution in streams (Lin and Yo 2008, Armendariz et al. 2012). Gastropoda and Hirudinea are also commonly identified as tolerant taxa and are known to be abundant in polluted streams and rivers (Berger et al. 2018). Our findings of more non-insect taxa present in the enriched sites matches previous studies which showed higher abundances of non-insect individuals in disturbed, agricultural sites (Gerth at el. 2017, Lorion and Kennedy 2008).

Taxa Richness, Diversity, and Community Analysis

The most notable differences seen between the enriched streams and non-enriched streams were in taxa richness and diversity. The non-enriched streams had notably higher family richness among macroinvertebrates as a whole as well as among insect families, specifically (Figures 4 and 5). The higher overall richness in the non-enriched streams could be attributed to a higher habitat quality compared to the enriched sites. Martins et al. (2017) observed a decrease in macroinvertebrate richness and an exclusion of sensitive taxa along an urbanized and developed stream gradient that resulted in high nutrient concentrations and decreased habitat quality. The non-enriched streams in our study had dense, intact riparian zones along the stream banks and were not within heavily agricultural watersheds. Both factors are known to have a significant influence on stream habitat quality and health as well as influence on the presence of taxa that are sensitive to habitat degradation (Quist and Schultz 2014). Similar to our findings, several other studies have also reported higher taxonomic richness in forested streams compared to streams near agricultural fields (Gerth et al. 2017, Zhang et al. 2018).

The contrast between the enriched streams and non-enriched streams is also evident in the differences seen among the EPT taxa (a group commonly used as bioindicators for stream quality) in terms of both average EPT abundance and average EPT density. The non-enriched streams had a higher total abundance of EPT taxa compared to the enriched sites as well as a higher average abundance of EPT and higher EPT family richness. The richness and abundance of EPT insects is known to decrease as development within a watershed catchment area increases, whereas the richness of dipterans and non-insects is known to increase (Cooper et al. 2013). Our non-enriched sites were dominated by EPT taxa which comprised 64.3% of the total

macroinvertebrate abundance while the enriched sites were dominated by non-insects and dipterans (63.2%) and only 24.9% of the total abundance was comprised of EPT taxa. Sensitive taxa such as EPT are associated with streams that are more pristine and unimpacted compared to non-insects and dipterans which are often in found in higher abundances in more degraded habitats with lower water quality (Harding et al. 1999).

Macroinvertebrate community analyses confirmed that there are distinct differences in community structure between the enriched and non-enriched sites. The non-enriched sites consisted of more sensitive and pollution intolerant taxa compared to the enriched sites, where the macroinvertebrate community was dominated by non-insects and dipterans. These results closely mirror the EPT results discussed above. There have been several other studies that have found differences in aquatic macroinvertebrate assemblages within streams that have a forested watershed and streams within a pasture/cropland dominated watershed. Danger and Robson (2004) found that aquatic macroinvertebrate communities in stream reaches within pastureland were significantly different from communities in forests that have riparian zones, where the communities within pastureland consisted of more tolerant species. The higher taxon richness and abundance of EPT in the non-enriched streams could be attributed to the presence of dense riparian zones along the stream bank which provide an influx of organic matter, decrease the amount of nutrients entering the water (by providing a buffer), and provide shade leading to lower water temperatures. These components create an optimal habitat for insects and other aquatic macroinvertebrates that require colder water temperatures and higher concentrations of dissolved oxygen. While the enriched sites in this study had some riparian zone present, the density of the vegetation was variable, and the zones were not as wide as the non-enriched sites (Edwards, personal observation). Increased nutrient levels can alter community composition and

structure that results in a decrease in sensitive taxa and an increased abundance in more tolerant taxa such as physid snails and oligochaetes (Helms et al. 2009). Upstream of the enriched sites, land use consisted primarily of crop and pastureland. The biological conditions of a site can be heavily influenced by the upstream area in an agricultural watershed. Roth et al. (1996) found that streams whose upstream catchment areas and watershed were dominated by agriculturally developed land had lower biotic and habitat indices compared to sites with a catchment area dominated by natural plant communities.

Notably, the macroinvertebrates that were most dissimilar between the enriched and non-enriched sites belonged to the non-insect taxa (*Gammarus* spp. and Oligochaeta) which were only found within the enriched streams, and the order Ephemeroptera, including *Baetis* spp., *Ephemerella* spp., *Epeorus* spp., *Leptophlebia* spp., and *Choroterpes* spp., which had much higher abundances within the non-enriched streams. Some were only found in the non-enriched sites (*Leptophlebia* spp., and *Choroterpes* spp.). Members of Ephemeroptera are often considered to be sensitive to environmental stressors, and the occurrence of certain taxa is closely related to habitat quality and pollution levels within a stream. For instance, species within the families of Baetidae and Leptophlebiidae are often used as indicators for sites that are relatively pollution-free and have good or very good water quality (Xu et al. 2013). Similar to our study, others have found an absence of Leptophlebiids from streams where the riparian vegetation had been modified for plantations (Selvakumar et al. 2014).

Macroinvertebrate biomass

Development within a watershed can influence macroinvertebrate biomass within streams (Sterling et al. 2016, Woodcock and Huryn 2007). The availability of nutrients such as nitrogen

and phosphorus in streams can regulate primary productivity (Riseng et al. 2004, Biggs 2000) and, thus, influence the presence and abundance of primary consumers such as grazing herbivores. Increased nutrient concentrations in streams are often the result of runoff due to agricultural development. Elevated nutrient concentrations in streams and rivers can lead to increased macroinvertebrate biomass through the increased production of benthic algae and other primary producers.

In this study, I found no difference in total macroinvertebrate biomass between the enriched and non-enriched streams. My hypothesis that biomass would be higher in the non-enriched sites due to higher diversity and abundance of sensitive taxa compared to the enriched sites was rejected. The average biomass for our enriched sites was 0.216 g/m^2 and the average for the non-enriched sites was 0.161 g/m^2. The lack of difference in biomass between treatments could be explained by the abundance of tolerant taxa within the enriched sites. Similar to our study, Helms et al. (2009) found that streams within urbanized watersheds had altered community structure but also had an increase in invertebrate biomass. Even though these streams were far less diverse than streams within rural watersheds, they had high biomass due to the presence and abundance of tolerant taxa (e.g., Oligochaeta, Hirudinea, and Chironomidae). Tolerant taxa do well and proliferate in more urbanized and nutrient enriched streams as there are often lower species variety and therefore low interspecific competition (Arimoro and Ikomi 2007). Higher densities of tolerant taxa in streams can also be attributed to the opportunistic tendencies of these organisms to monopolize the available space and resources created by the absence of intolerant and sensitive species. An increase in primary productivity, as a result of higher nutrient concentrations, can also lead to increased macroinvertebrate biomass in nutrient enriched streams. Cross et al. (2006) found that nutrient enrichment in streams has a bottom-up

effect and can stimulate whole-community production, thus increasing macroinvertebrate biomass.

The richness, diversity, and community composition of stream macroinvertebrate assemblages can vary seasonally (Beche et al. 2006, Linke et al. 1999). Helms et al. (2009) found that spring had the highest biomass compared to any other season. In this study, spring had higher biomass than summer and fall (but not winter). There are many abiotic factors that can drive seasonal variations in aquatic macroinvertebrate communities such as precipitation, temperature, and photoperiod (Wolda 1988, Beche et al. 2006). Most stream insects have a two-part life cycle that is dominated by the nymph/larval stage. During this stage, the organism is mainly aquatic, only to become terrestrial upon emergence as an adult. The seasonal timing of insect emergence depends primarily on internal metabolic responses that are heavily dependent on in-stream factors such as temperature (Cheney et al. 2019). Temperature is an important abiotic variable that affects aquatic invertebrates throughout their life cycles (Vannote and Sweeney 1980). In both the nutrient enriched and non-enriched streams, spring had the highest biomass compared to the other seasons, which may in part be due to sampling before the emergence of adults. Emergence is positively correlated with temperature – as temperature increases so does adult insect emergence (Huryn and Wallace 2000). In temperate streams, emergence of the insect assemblage typically peaks in early summer and sharply declines by late summer (Baxter et al. 2017). The emergence of adults in early summer may also help explain the low biomass seen in summer for both the nutrient enriched and non-enriched streams.

Water Quality and Environmental Assessment

Site factors such as riparian vegetation, water chemistry, and in-stream habitat characteristics play a large role in the structure and composition of macroinvertebrate communities (Stone et al. 2005). The influence of nutrient enrichment on macroinvertebrate communities is evident in this study. Nitrate/nitrite (NOx) and total phosphorus (TP) were both significantly different between the enriched and non-enriched streams and NOx was approaching significance when looking at seasonal differences. Nutrient concentrations as well as the interactions nitrogen and phosphorus have with other environmental variables can often explain the variations in macroinvertebrate assemblages among streams (Wang et al. 2007). My results indicate that high concentrations of nutrients in the enriched sites could be the factor influencing the differences in the macroinvertebrate communities.

Streams within agricultural watersheds often have higher nutrient concentrations compared to streams within forested watersheds. The area of agriculturally developed land within a watershed is positively correlated with nitrogen and phosphorus concentrations within streams and negatively related to stream quality and benthic invertebrate community structure (Wang et al. 1997). Due to different pollution tolerance levels, certain aquatic macroinvertebrates may respond differently compared to other taxa. Dalu et al. (2017) found that water chemistry variables such as nitrogen and phosphorus explained a large percentage of variation in benthic macroinvertebrate community structure. Similar to my study, they found higher macroinvertebrate taxon richness in their sites that were less impacted.

Phosphorus and nitrogen are both essential nutrients for living organisms and are naturally present in freshwater streams and rivers. In undisturbed and non-impacted aquatic

ecosystems, background levels of phosphates are generally around 10 µg P/L and the natural level for nitrate is usually below 1 mg N/L; typically, these sites support a diverse ecosystem that is abundant with wildlife (Duka et al. 2017). High levels of these nutrients are usually indicative of significant agricultural runoff that contains fertilizer. In my study, TP concentrations in the enriched sites ranged from 35.4 µg P/L to 171.17 µg P/L which are well above the background TP levels present in streams. In the non-enriched streams, the TP concentrations ranged from 10.1 µg P/L to 19.6 µg P/L which are very close to the natural, background levels of total phosphorus in streams. The high levels of TP in the enriched sites may also be contributing to the differences in aquatic macroinvertebrate communities.

Nutrient effects on aquatic macroinvertebrates generally occur through indirect mechanisms such as changing the main food source (e.g., periphyton replaces detritus and leaf litter) or by causing eutrophic conditions resulting in decreased dissolved oxygen levels. However, there have been studies that report direct toxic effects from nutrients such as nitrogen, with toxicity increasing as nitrate/nitrite concentrations and exposure time increases (Camargo et al. 2005). Nitrates and nitrites are naturally present in freshwater ecosystems as they are components of the nitrogen cycle. However, anthropogenic activities such as livestock and agricultural operations have greatly contributed to an increase in nitrogen concentrations in streams and rivers through non-point source pollutants (Soucek and Dickinson 2012). High concentrations of nitrate/nitrite in freshwater aquatic systems causes a conversion of oxygen-carrying pigments, such as hemoglobin or hemocyanin, to forms that are inefficient at carrying oxygen throughout the body (Jensen 1996, Camargo et al. 2005). Camargo and Ward (1992) found that the early instars of net spinning caddisflies (*Cheumatopsyche* spp. and *Hydropsyche* spp.) were more sensitive to elevated nitrate concentrations compared to late-stage instars.

Camargo et al. (2005) proposed the maximum level of nitrate/nitrite should be 2 mg N/L to protect sensitive invertebrate taxa from nitrogen pollution. In my study, the NOx concentrations ranged from an average of 1.37 mg N/L to 2.17 mg N/L in the enriched sites compared to a range of 0.42 mg N/L to 0.68 mg N/L in the non-enriched sites. The enriched sites exceeded the proposed maximum concentration of 2 mg N/L during the winter while the non-enriched sites never exceeding 1 mg N/L. The elevated NOx concentrations in the enriched streams may have been high enough to induce stress on the sensitive macroinvertebrate species thus leading to a decreased abundance of EPT taxa.

Bacteria Assessment

The Lemly (1998) and Lemly (2000) studies both reported higher coverage of filamentous bacteria and a higher presence of bacterial colonization on aquatic insects in nutrient enriched streams. In their studies, more bacterial growth was seen in stream reaches that had significantly higher N and P concentrations due to grazing animal waste runoff; no bacteria were seen in the upstream reaches. However, in our study we did not report a significant difference in the presence of bacteria on insects between the nutrient enriched streams and the non-enriched streams. When comparing nutrient concentrations between the streams in this study and the streams used by Lemly (1998, 2000), their NOx concentrations in the enriched stream reaches were similar to the NOx concentrations in the enriched streams used in this study. Their streams had NOx ranges from 1.29 to 2.38 mg N/L, whereas I reported NOx ranges from 1.37 to 2.17 mg N/L in the streams used in this study. Although the nitrogen concentration ranges were similar, the phosphorus concentrations in this study were much lower than the concentrations reported in Lemly (1998, 2000). Lemly (1998, 2000) reported total orthophosphate concentrations that

ranged from 130 to 440 µg P/L. The total phosphorus concentrations in this study ranged from 35.4 to 171 µg P/L.

When comparing bacteria prevalence among the different insect orders, I did not find any significant differences. The four orders that showed the most colonization were immature Coleoptera, Ephemeroptera, Plecoptera, and Trichoptera. Lemly (1998, 2000) found that the prevalence and degree of colonization was highest in Ephemeroptera, specifically within the families Ephemerellidae and Heptageniidae. He also reported colonization on other insects such as Plecoptera, Diptera, and Trichoptera but not to the extent seen on Ephemeroptera. Along with the orders mentioned above, I also found bacterial colonization on immature Coenagrionidae (Odonata), Corydalidae (Neuroptera), Athericidae (Diptera), Asellidae (Isopoda), and Cambaridae (Decapoda).

In Lemly (1998, 2000), individuals from the downstream reaches were sometimes completely covered with bacterial colonies and percent coverage ranged from 12-94%. I found no significant difference between the nutrient enriched and non-enriched streams with regards to percent cover by bacteria. Compared to Lemly (1998, 2000), the insects collected in this study also had lower percent coverage of bacteria. The range of percent coverage of bacteria found at my study sites was 0.05-19%, and the majority was below 10% during each season. Seasonally, there were no significant differences in percent coverage or number of insects displaying bacterial infection, which led to a rejection of the hypothesis that bacterial growth would vary seasonally due to seasonal variation in agricultural runoff. The influx of agricultural runoff and nutrients into streams if often highest during snowmelt and periods of heavy rainfall (Komiskey et al. 2011, Good et al. 2019). Danz et al. (2010) reported greater nutrient loads in streams during

the non-growing season (November-April) with the highest amounts occurring during the spring thaw. The streams used in this study did not exhibit elevated nutrient levels during the non-growing season. The lack of seasonal differences in bacteria colonization may have been due to the lack of seasonal variation in nutrient concentrations in the water.

Microcosm Analysis

No bacterial growth was seen on the damselflies or in the individual test tubes containing treatment water. The lack of growth in both experiments could be due in part to suboptimal environmental conditions necessary to support bacterial colonization in the aquaria. In this experiment, the only variables that were manipulated were N and P concentrations. Adding only these two nutrients to the aquaria may not have been enough to create an environment that was optimal for growth and colonization by bacteria; the water may have been lacking in essential micronutrients, such as iron and manganese. In small mountain streams, the growth and presence of *Leptothrix ochracea* on stream substrate was significantly correlated to increases in iron and manganese concentrations (Sheldon and Skelly, 1990). Similarly, other neutral streams are known to see increases and blooms of sheath-forming, ferromanganese-depositing bacteria. These blooms are generally seen in areas of the stream that have iron oxide deposition zones (Wellnitz and Sheldon, 1995). Future iterations of this experiment could benefit from manipulating the concentrations of iron and manganese in the aquaria.

Summary and Conclusion

The nutrient enriched and non-enriched streams were composed of different aquatic macroinvertebrate communities in terms of both composition and diversity. The non-enriched streams were much more diverse and had higher richness than the enriched streams and

contained higher abundances of EPT taxa, indicating that the non-enriched streams were more habitable for sensitive and pollution intolerant taxa. The enriched sites had high abundances of pollution tolerant taxa such as Oligochaeta, Hirudinea, and Gastropoda which were not seen in the non-enriched streams. The agricultural presence surrounding the nutrient enriched streams has, presumably, led to elevated nutrient concentrations and decreased habitat quality which has resulted in a macroinvertebrate community mainly composed of non-insects and dipterans. The dominance of these two macroinvertebrate groups along with a low abundance of EPT indicate impairment within these reaches of the nutrient enriched streams.

While the nutrient and macroinvertebrate data show two distinct sets of streams, there were no differences within the bacteria data as there were no differences in bacterial colonization or percent coverage. Lemly (1998 and 2000) proposed the use of filamentous bacteria growth on aquatic macroinvertebrates as a rapid bioassessment protocol for nutrient enrichment as he had found the bacteria on insects in nutrient enriched stream reaches only. However, in my study I found bacterial colonization on insects in my nutrient enriched streams as well as the non-enriched streams. To determine the efficacy of using filamentous bacterial growth as a rapid bioassessment tool for nutrient enrichment, more studies should be performed on a variety of streams. Other stream parameters should be looked at such as the levels of iron and manganese since *Leptothrix* and *Sphaerotilus* use and accumulate the oxidized forms of these elements in the formation of their sheaths and the type of nutrient pollution contaminating the stream such as sewage pollution which contains high concentrations of organic matter and can support dense populations of filamentous bacteria. The use of filamentous bacterial growth may not have been an indicator of nutrient enrichment for the streams used in this study, but the previous work on this assessment method has shown its potential. Filamentous bacterial growth could be a useful

bioassessment method in regions where best management practices are not common as it is more likely that streams in those areas would have much higher nutrient levels and organic matter and in streams that have a known pollution source such as sewage effluent or wastewater drains.

Based on the results of my study, the best indication for overall stream health are macroinvertebrate communities. Macroinvertebrate community composition, the presence of sensitive species such as EPT, and diversity were the predominant differences observed between the nutrient enriched and non-enriched sites. Macroinvertebrate sampling in streams can be paired with other bioassessment methods that could provide a better, overall depiction of stream health that include a biological report as well as a physical/chemical report. Assessing the algal and periphyton communities in streams can be done in parallel to macroinvertebrate sampling and may provide a method to determine nutrient enrichment as they are also sensitive to environmental stressors, specifically pollution and high levels of nutrients. While the filamentous bacteria may not have been a useful predictor for nutrient enrichment in my study, it may still have biomonitoring implications that future studies could help determine. To definitively determine its use as a bioassessment tool, a new study could be done that includes more streams with higher levels of nutrients and pollution to see if there is more growth in those streams. We could also incorporate streams that have known pollution sources and runoff to determine if different types of pollution (i.e., sewage, wastewater, fertilizer/manure) lead to a higher presence/occurrence of the filamentous bacteria in those streams. Expanding our site selection along with including the sampling of micronutrients may help determine if filamentous bacterial growth can be used as a rapid biomonitoring method and determining the cause of stream impairment.

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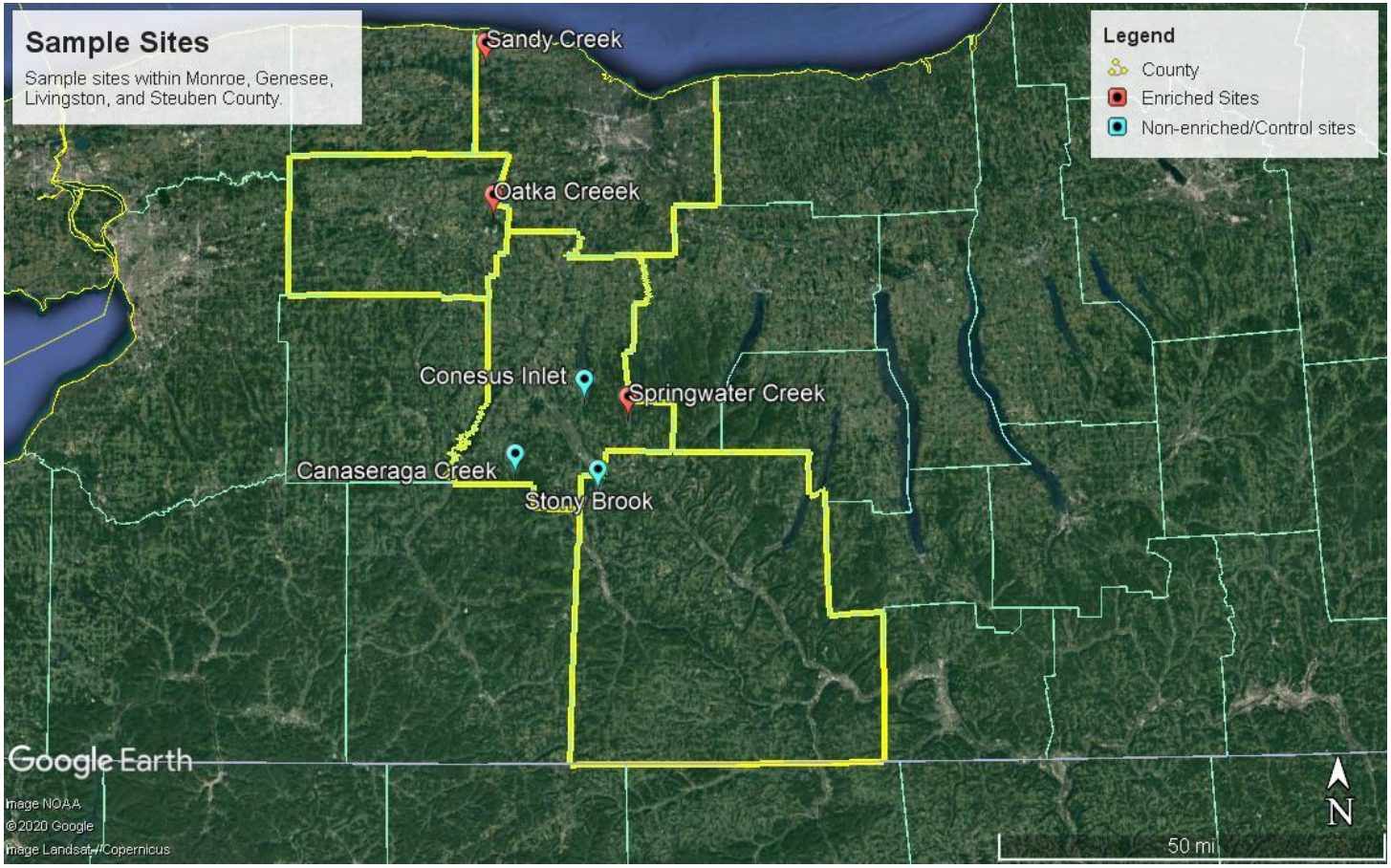

Figure 14. Map of sampling locations in Monroe, Genesee, Livingston, and Steuben Counties. The sampling coordinates for each creek are: **Sandy Creek** (43°17'47.8"N 77°57'52.5"W, 43°17'44.6"N 77°57'53.2"W, and 43°17'42.9"N 77°57'55.9"W), **Oatka Creek** (43°00'40.6"N 77°56'37.8"W, 43°00'39.2"N 77°56'35.3"W, and 43°00'38.4"N 77°56'32.6"W), **Springwater Creek** (42°38'10.6"N 77°36'07.5"W, 42°38'08.5"N 77°36'07.6"W, and 42°38'06.4"N 77°36'08.1"W), **Canaseraga Creek** (42°31'52.1"N 77°53'12.6"W, 42°31'49.2"N 77°53'12.6"W, and 42°31'44.9"N 77°53'13.6"W), **Stony Brook** (42°30'04.1"N 77°40'43.8"W, 42°30'01.8"N 77°40'41.8"W, and 42°29'58.9"N 77°40'41.3"W), **Conesus Inlet** (42°40'13.0"N 77°42'47.4"W, 42°40'08.0"N 77°42'45.8"W, and 42°40'03.4"N 77°42'44.6"W).

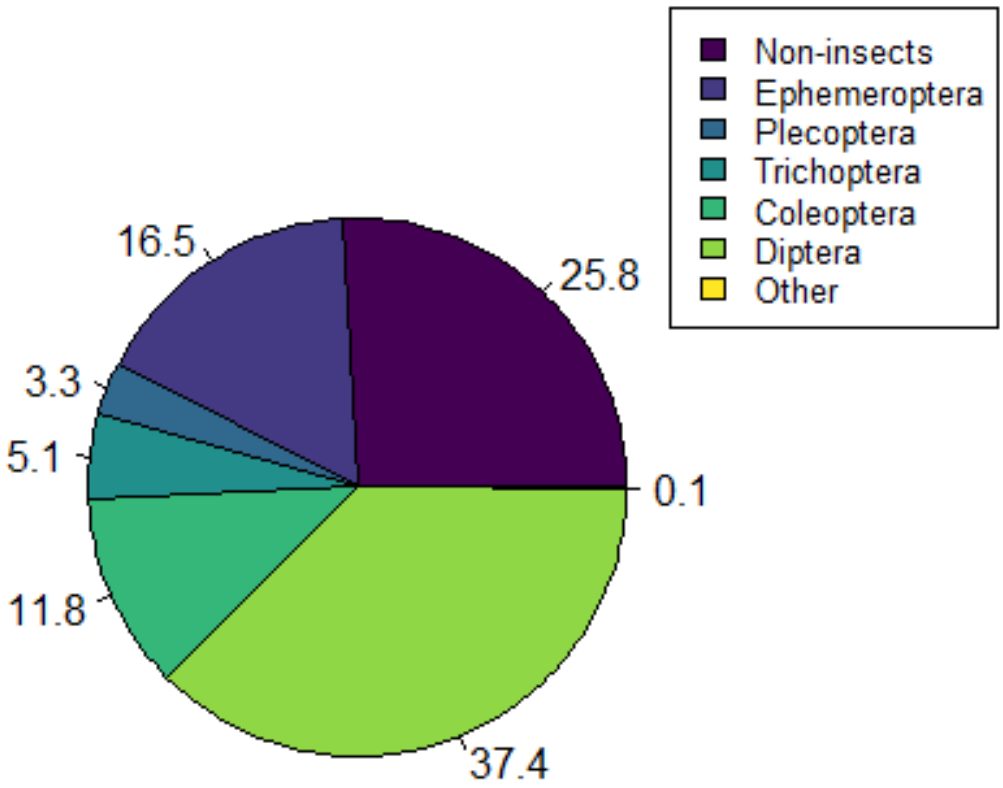

Figure 15. Percent composition of macroinvertebrate taxa in the nutrient enriched sampling sites.

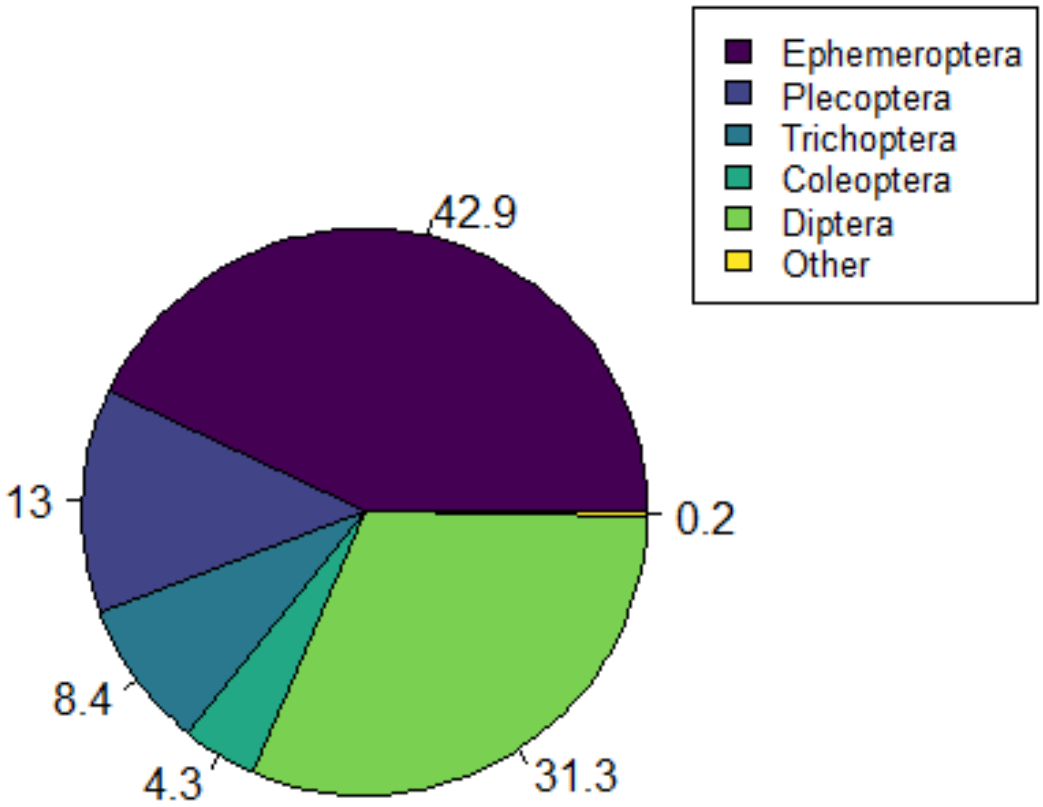

Figure 16. Percent composition of macroinvertebrate taxa in the non-nutrient-enriched sampling sites.

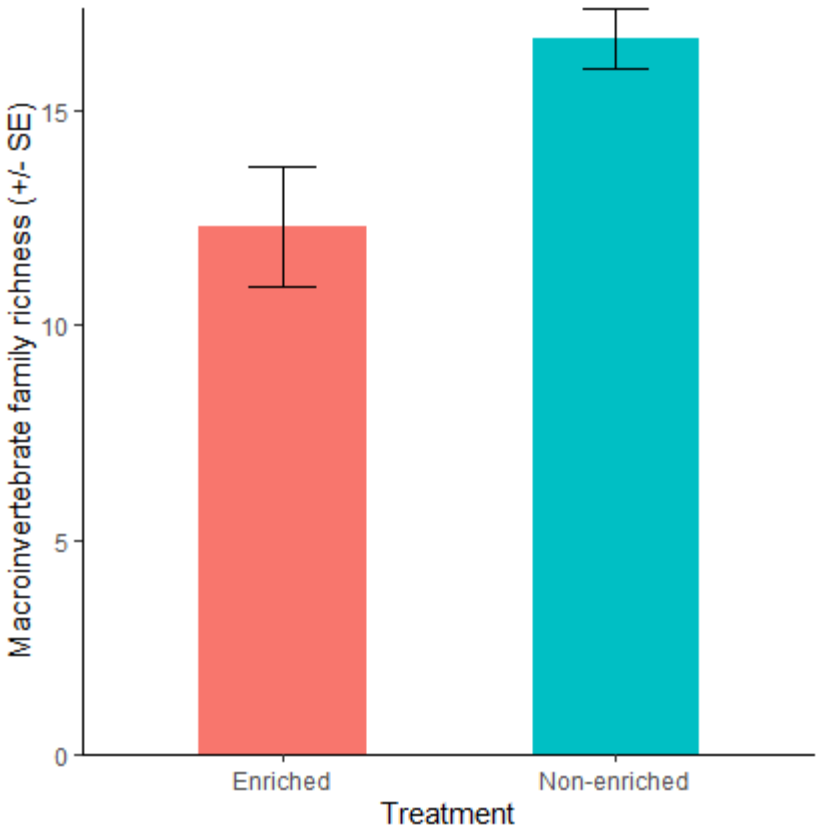

Figure 17. Average macroinvertebrate family richness in the nutrient enriched and non-enriched streams.

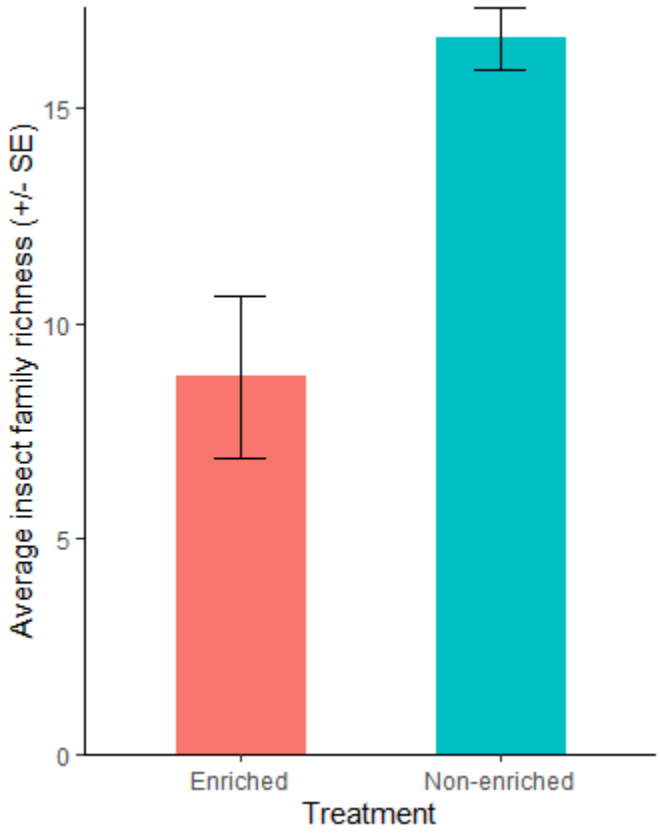

Figure 18. Average insect family richness in the nutrient enriched and non-enriched streams.

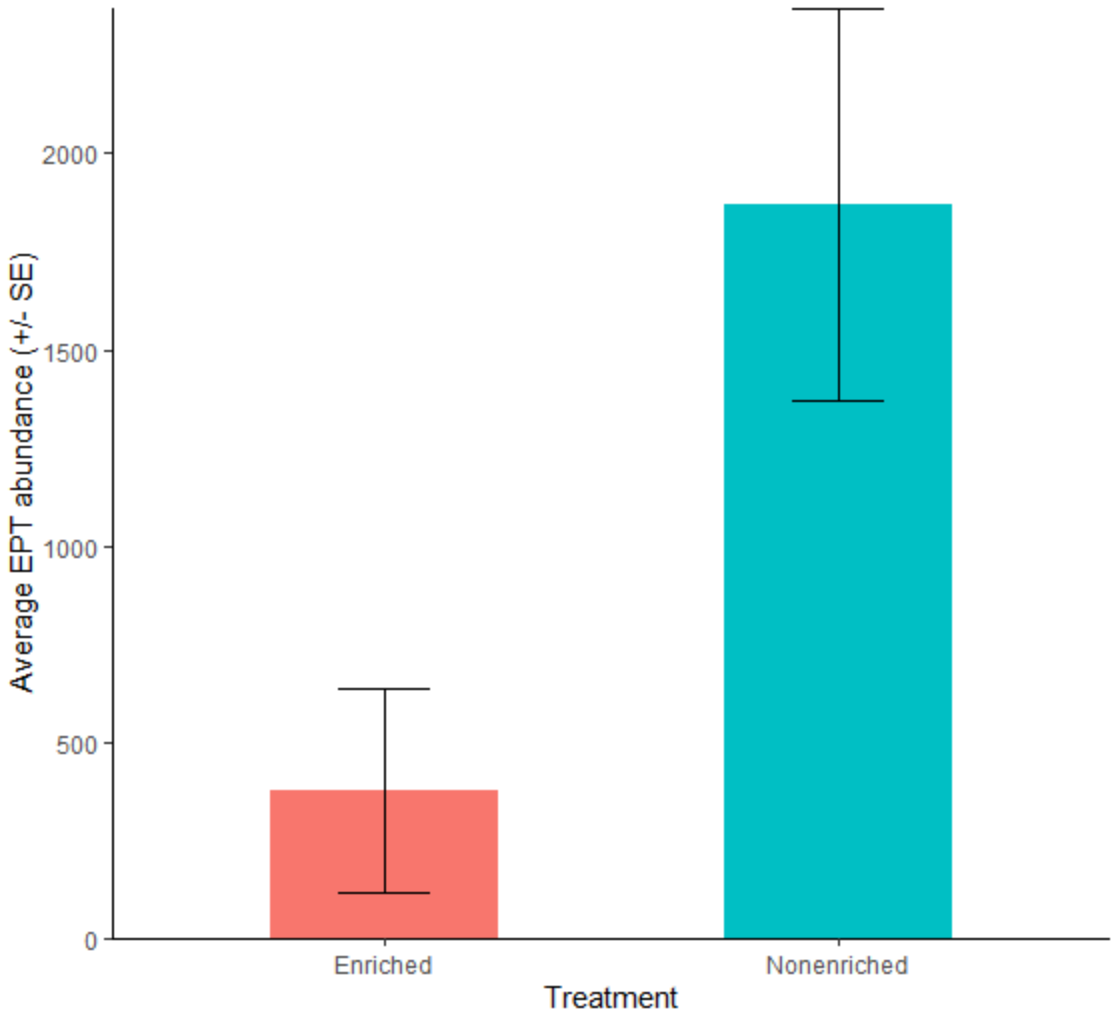

Figure 19. Average abundance of taxa belonging to EPT (Ephemeroptera, Plecoptera, and Trichoptera) in the nutrient enriched and non-nutrient enriched streams.

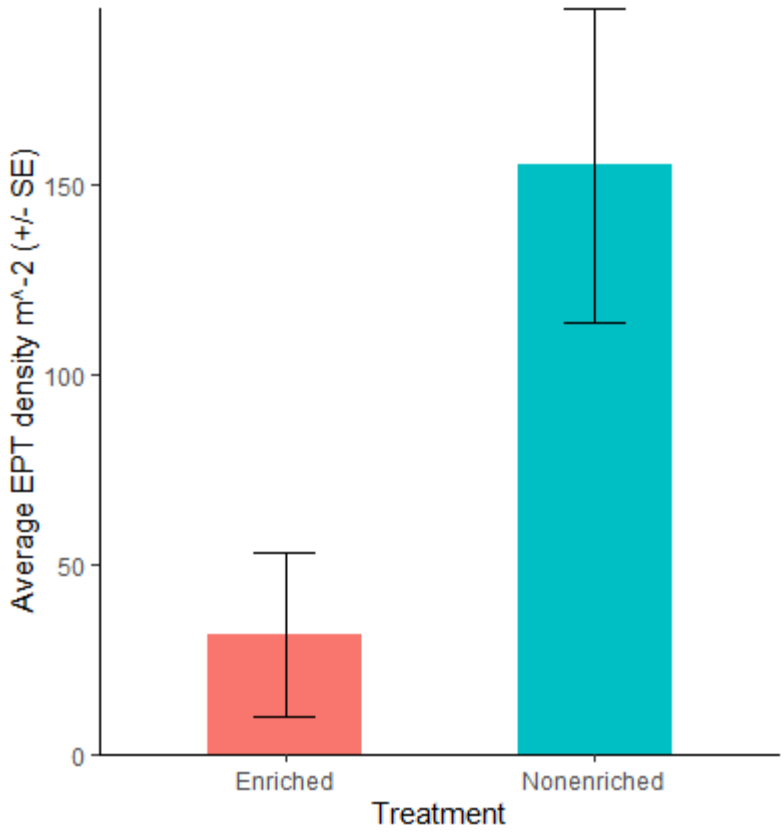

Figure 20. Average density of EPT taxa per m^{-2} in the nutrient enriched and non-enriched streams.

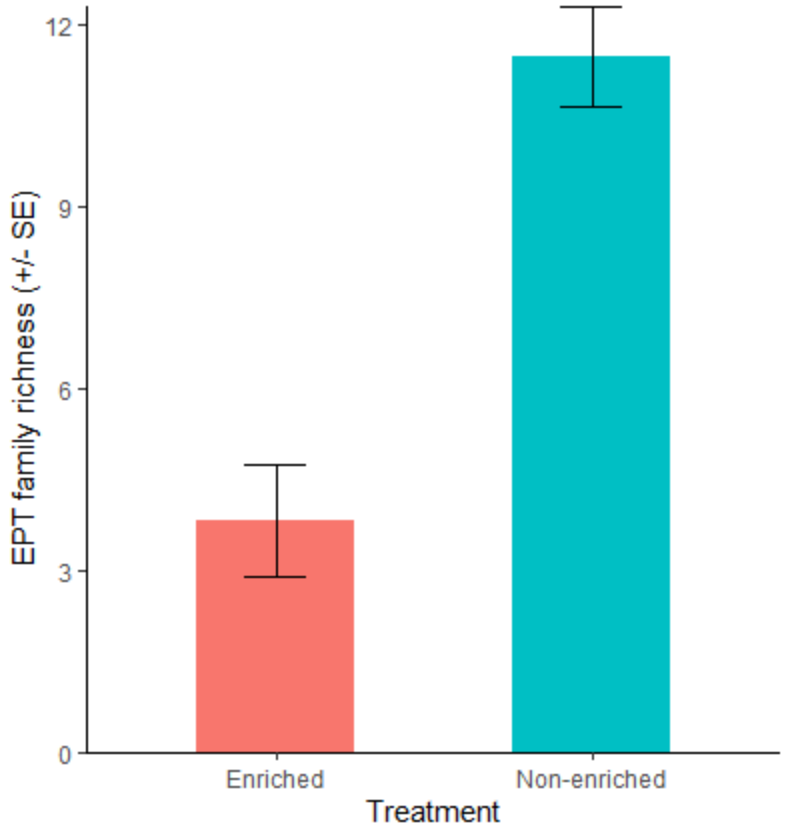

Figure 21. Average EPT family richness in the nutrient enriched and non-enriched sites.

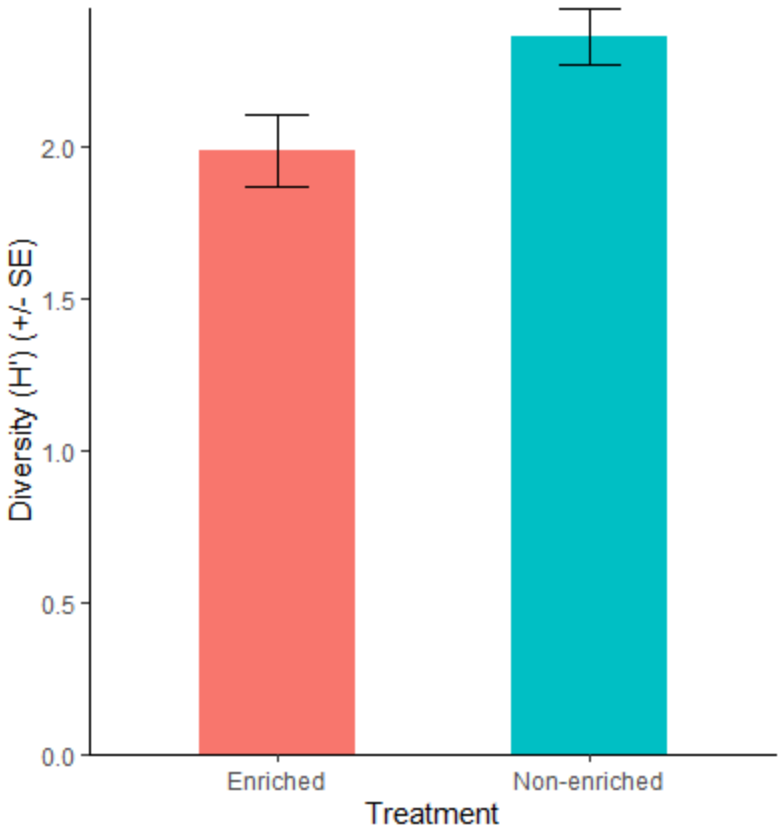

Figure 22. Average diversity (H') between the nutrient enriched and non-enriched sites.

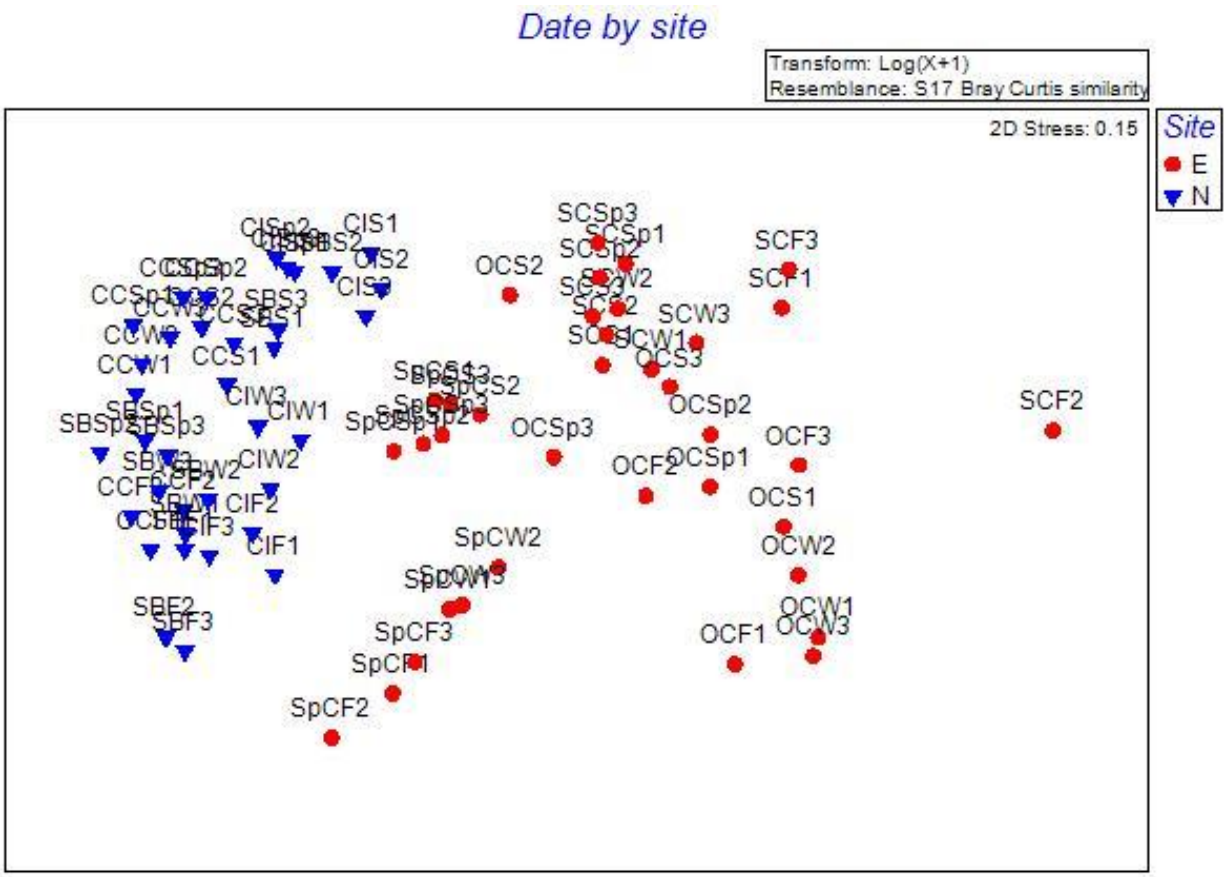

Figure 23. Nonmetric multi-dimensional scaling (nMDS) of the macroinvertebrate communities, Summer 2019 – Spring 2020, with each sample kept separate as a single point in the ordination space. Sites labels include whether they are in the nutrient enriched (E) or non-enriched (N) treatment (see key).

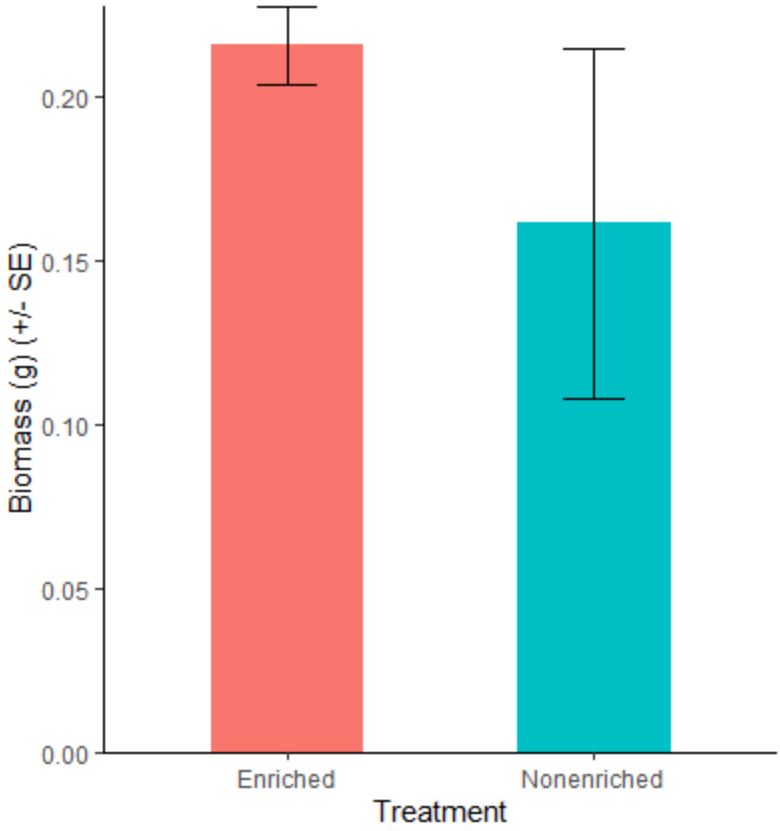

Figure 24. Average biomass between the nutrient enriched and non-enriched sites.

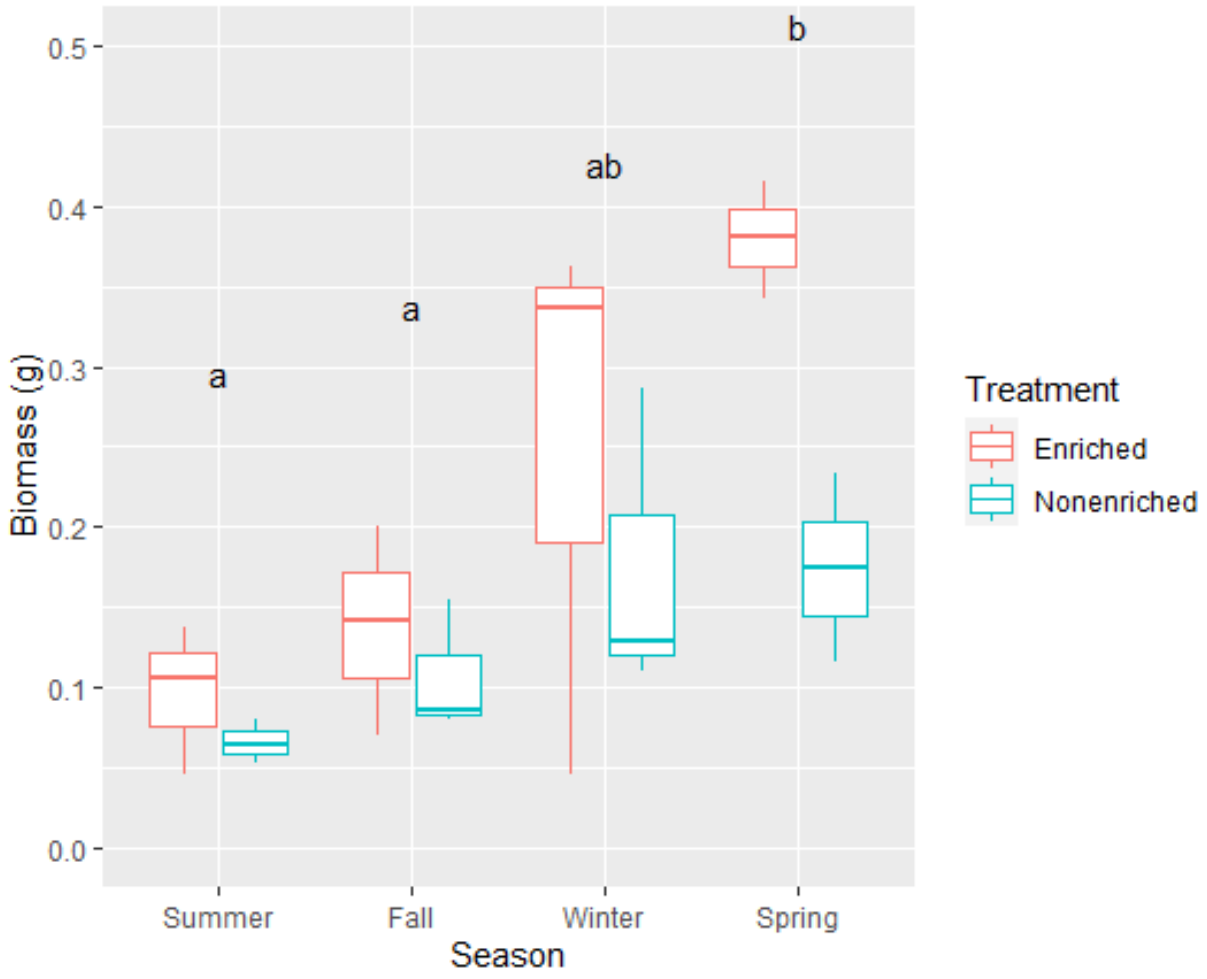

Figure 25. Average biomass per treatment during the summer, fall, winter, and spring. Different letters indicate significant differences in biomass among seasons.

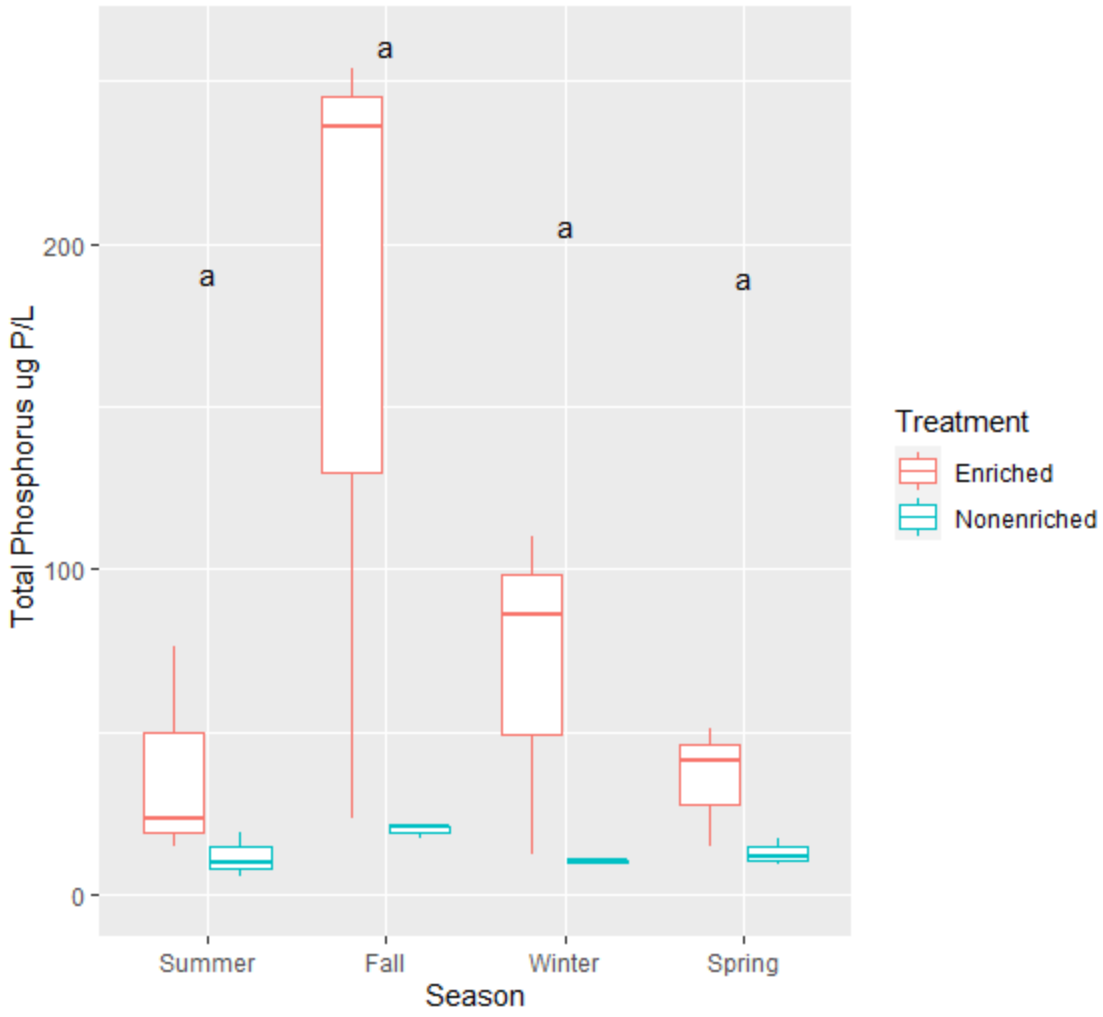

Figure 13. Average total phosphorus (µg P/L) concentrations per treatment during the summer, fall, winter, and spring.

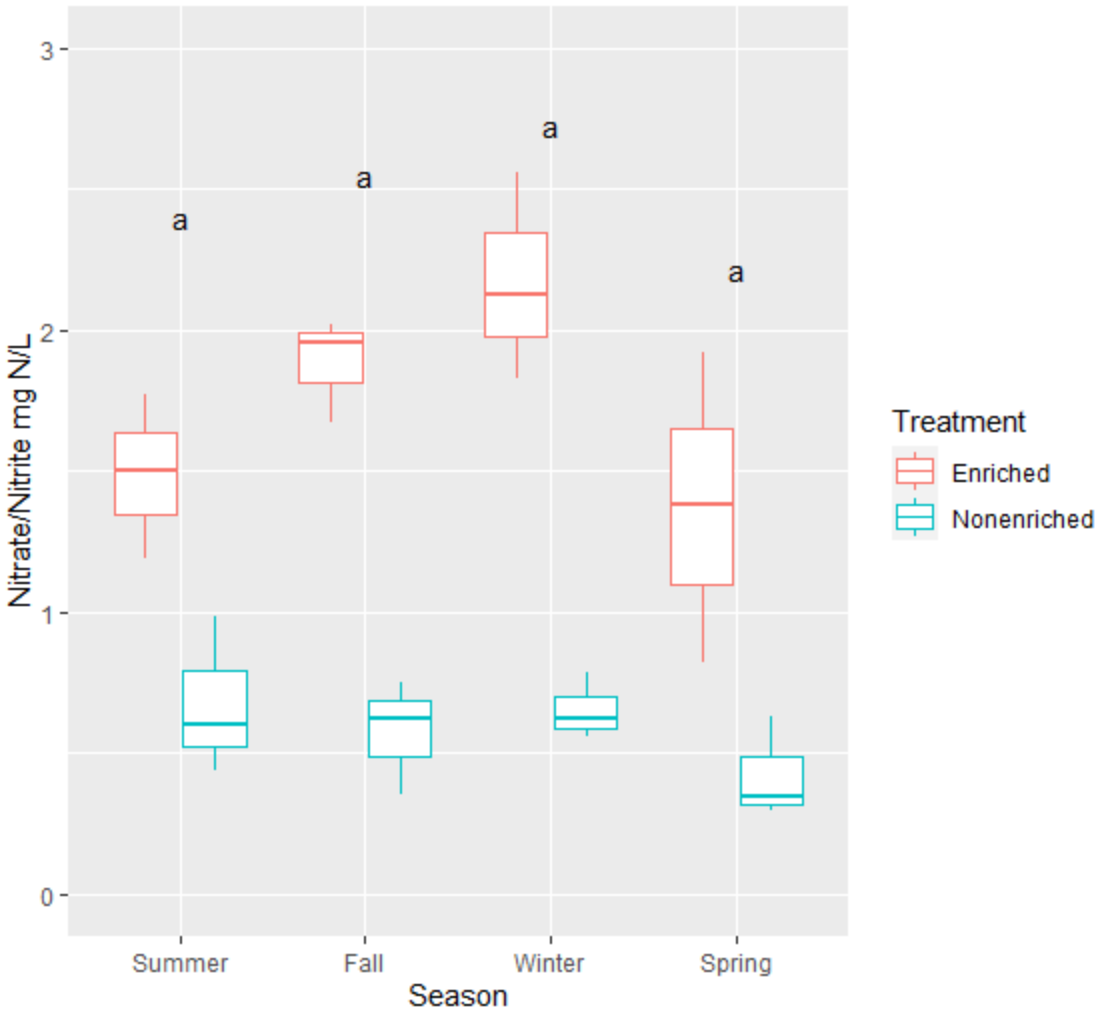

Figure 14. Average nitrate/nitrite (mg N/L) concentrations per treatment during the summer, fall, winter, and spring.

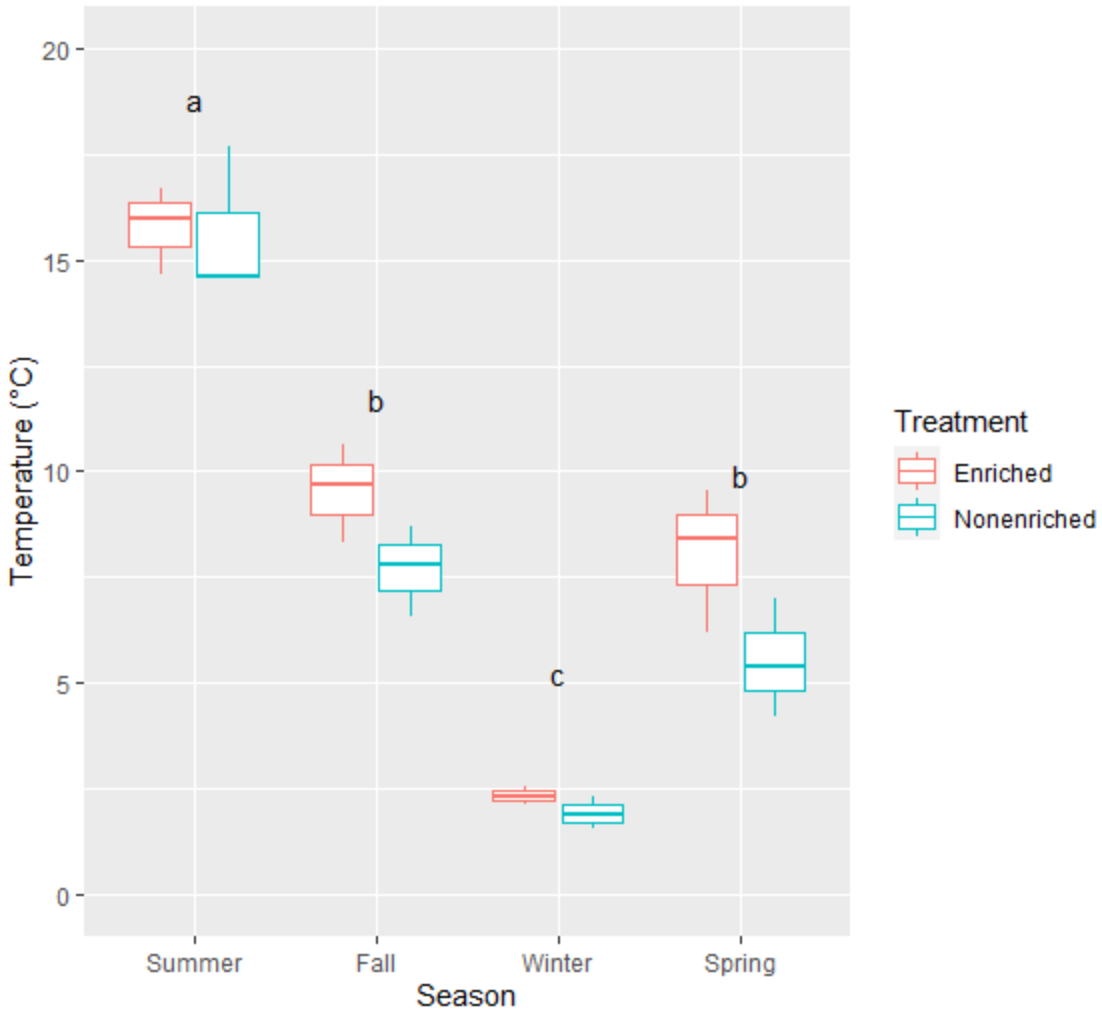

Figure 15. Average temperature (°C) per treatment during the summer, fall, winter, and spring. Different letters indicate significant differences among seasons.

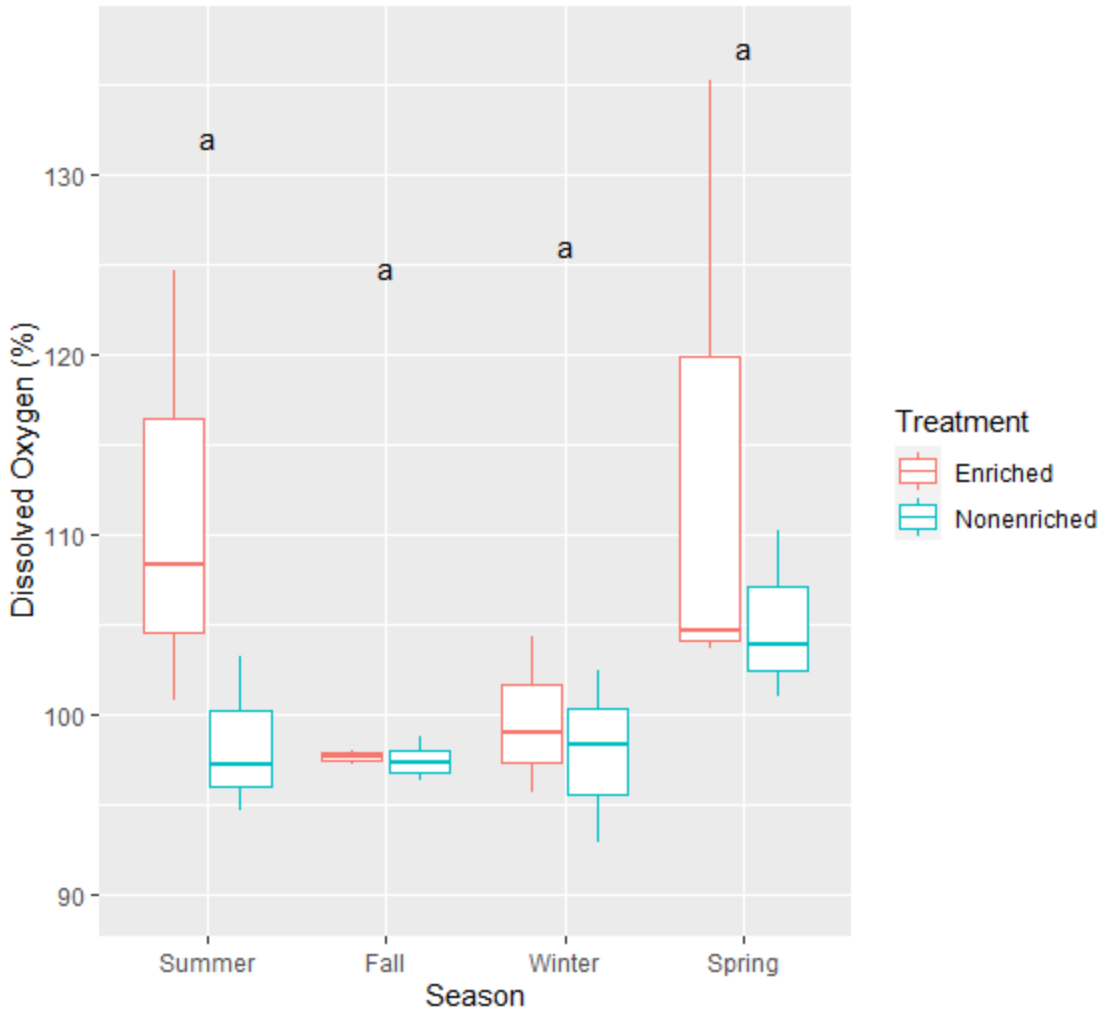

Figure 16. Average percentage of dissolved oxygen (%) per treatment during the summer, fall, winter, and spring.

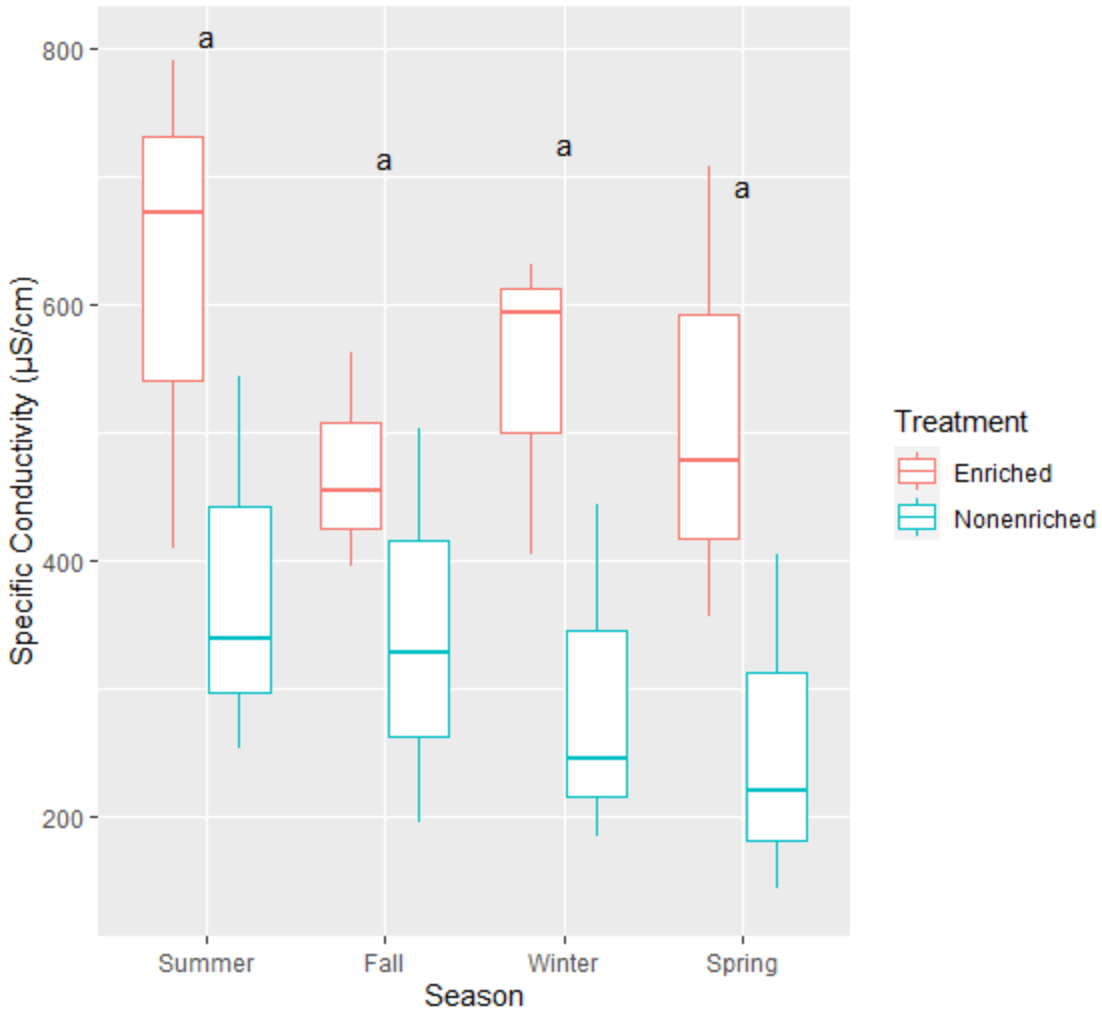

Figure 17. Average specific conductivity (µS/cm) per treatment during the summer, fall, winter, and spring.

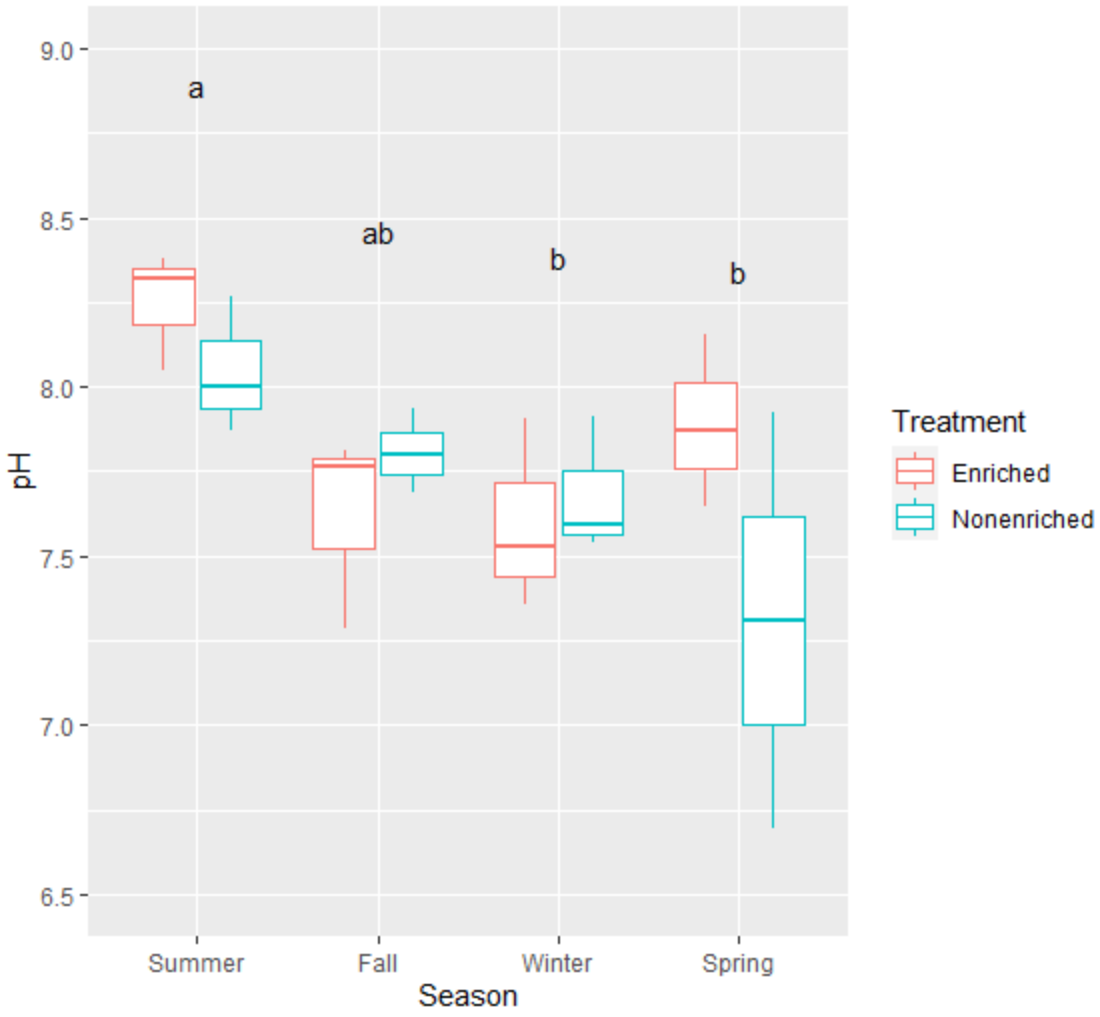

Figure 18. Average pH concentration per treatment during the summer, fall, winter, and spring.

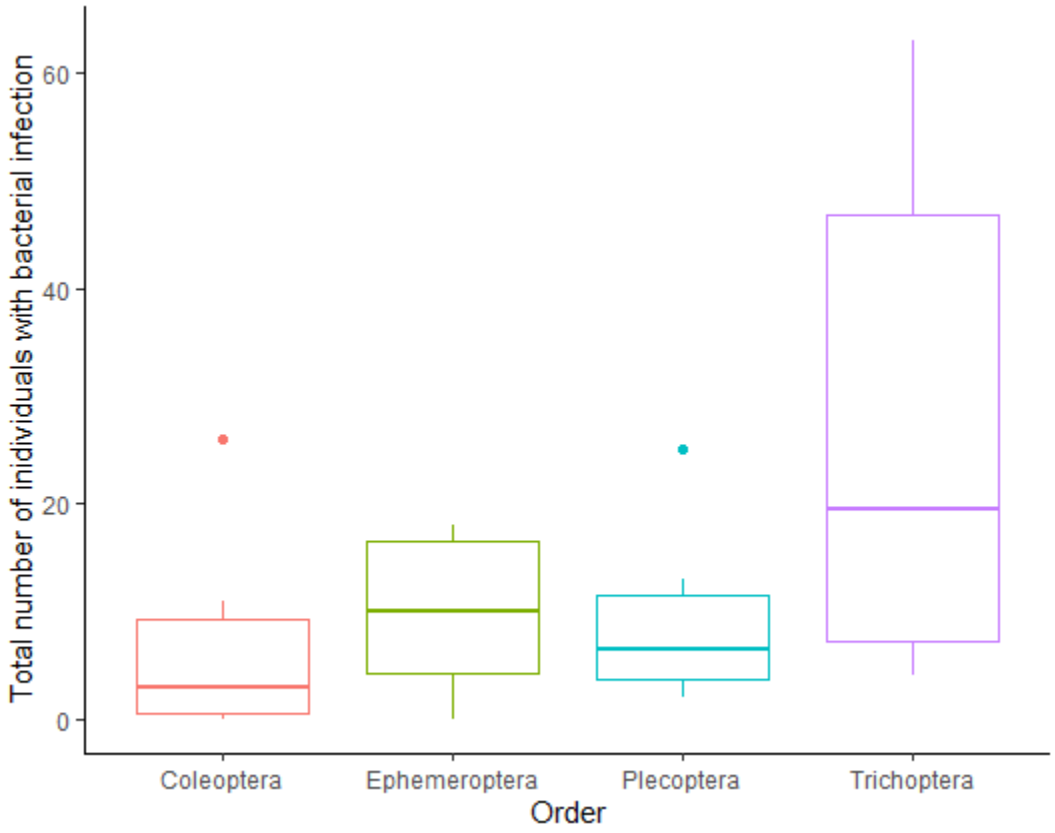

Figure 19. The total number of individuals in Coleoptera, Ephemeroptera, Plecoptera, and Trichoptera that showed bacterial colonization. Seasons and treatments were combined and the dots above Coleoptera and Plecoptera represent outliers.

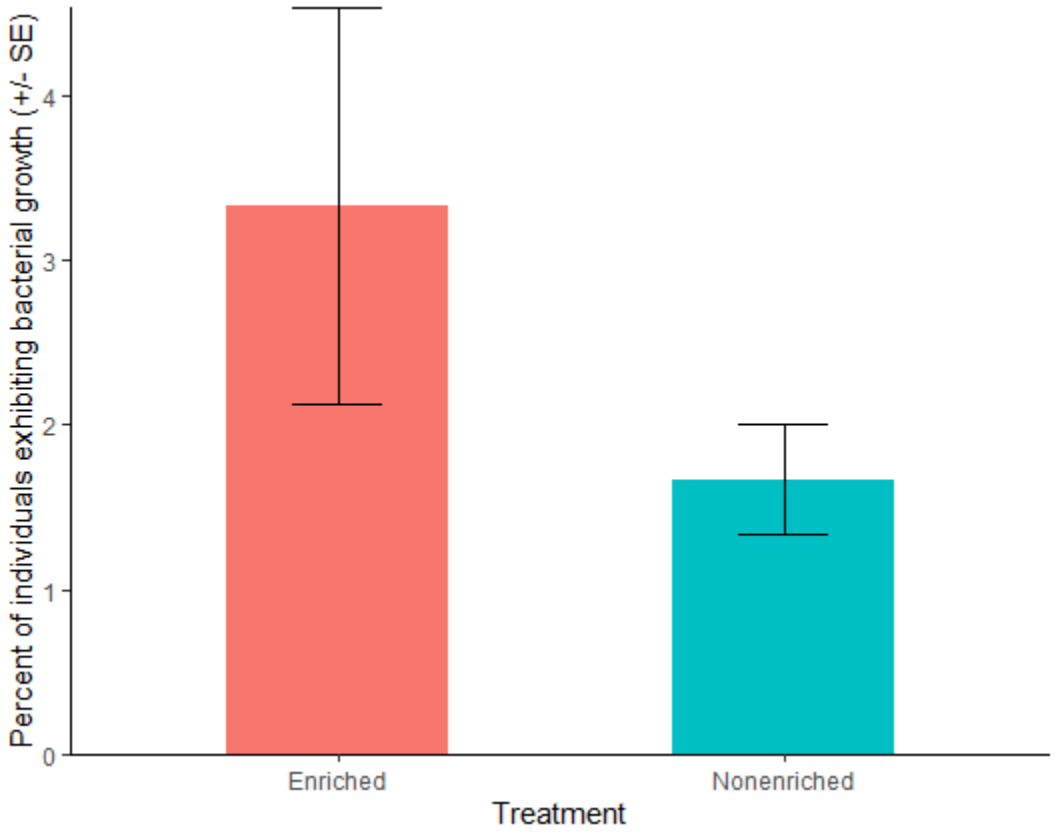

Figure 20. Percentage of all individual macroinvertebrates in the nutrient enriched and non-nutrient enriched streams with bacterial coverage.

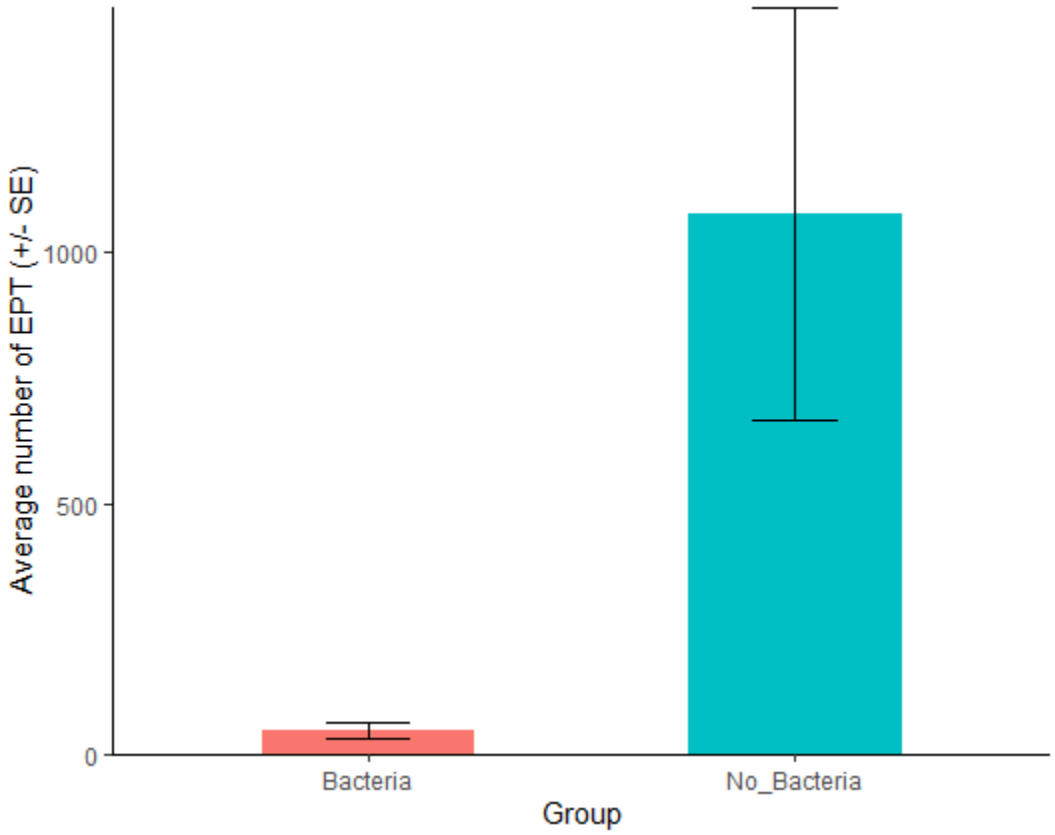

Figure 21. Average number of individuals in EPT that had bacterial coverage compared to the number of EPT individuals that exhibited no bacterial colonization.

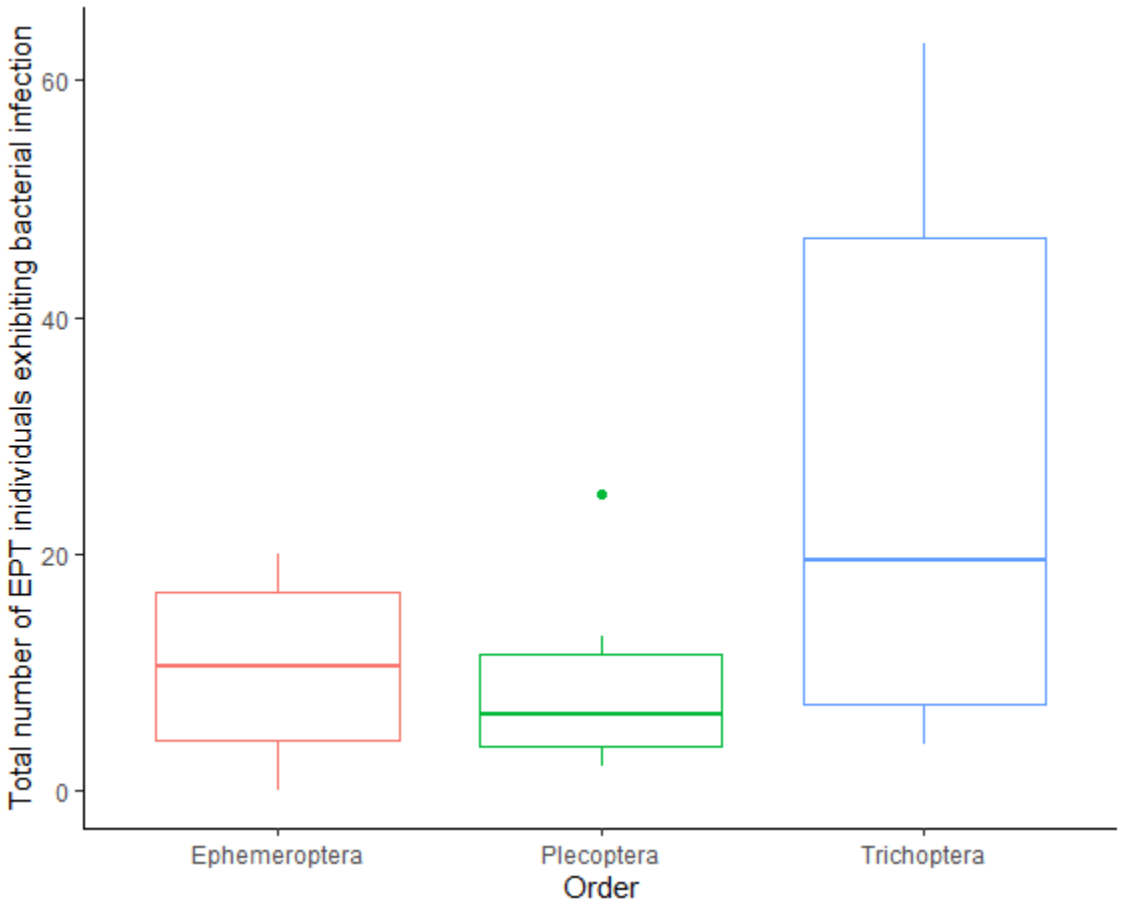

Figure 22. Total number of EPT taxa with bacterial coverage, separated by order. Seasons and treatments were combined and the dot above Plecoptera represents an outlier.

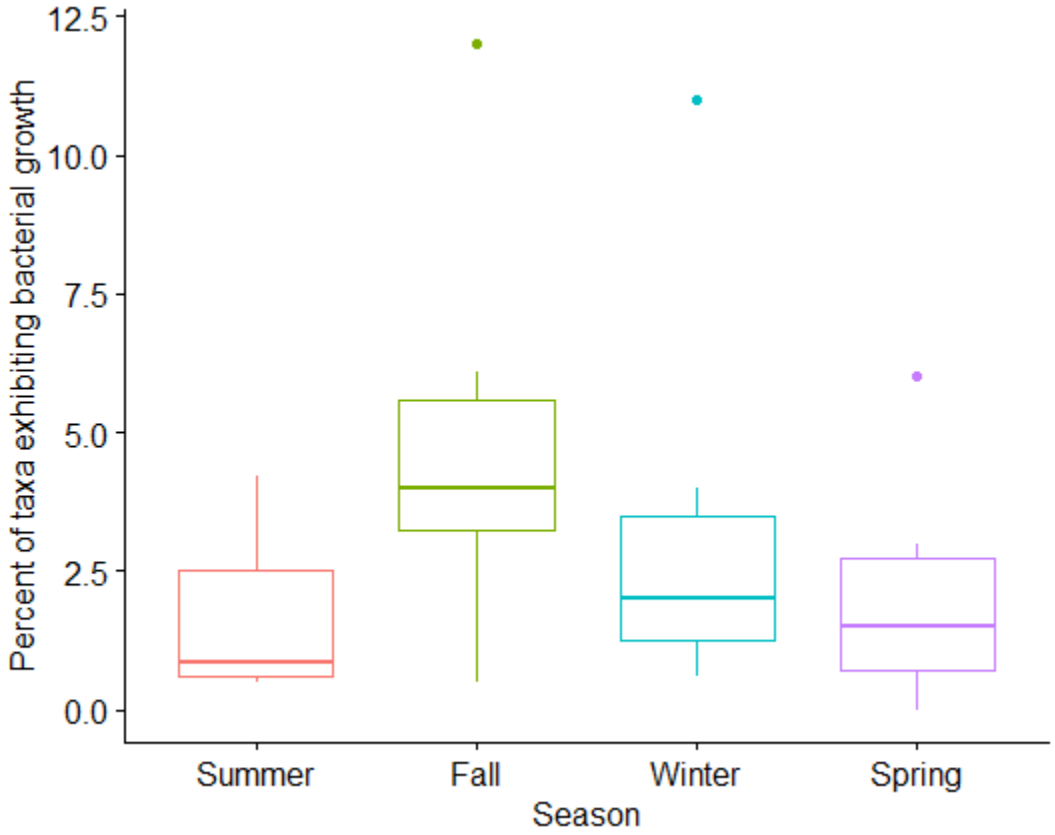

Figure 23. Percentage of taxa with bacterial coverage during the summer, fall, winter, and spring. Treatments were combined and the dots above fall, winter, and spring represent outliers.

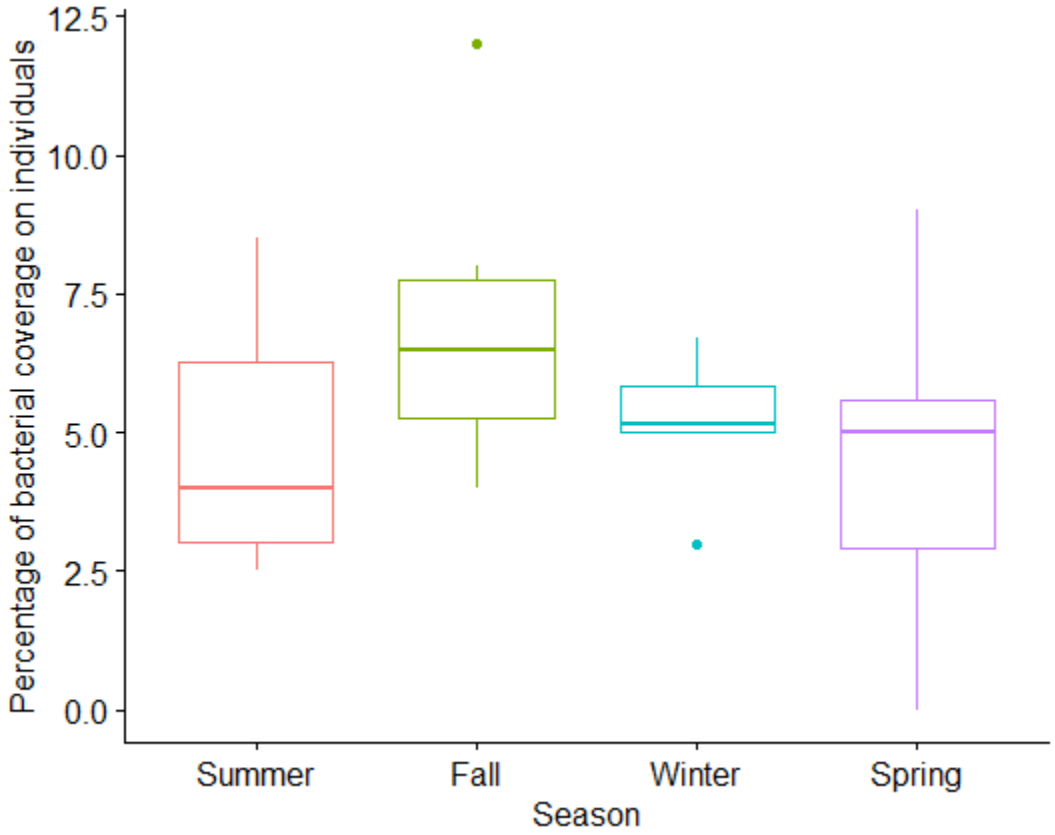

Figure 26. Percent coverage of bacterial colonization on macroinvertebrates across all sites during the summer, fall, winter, and spring.

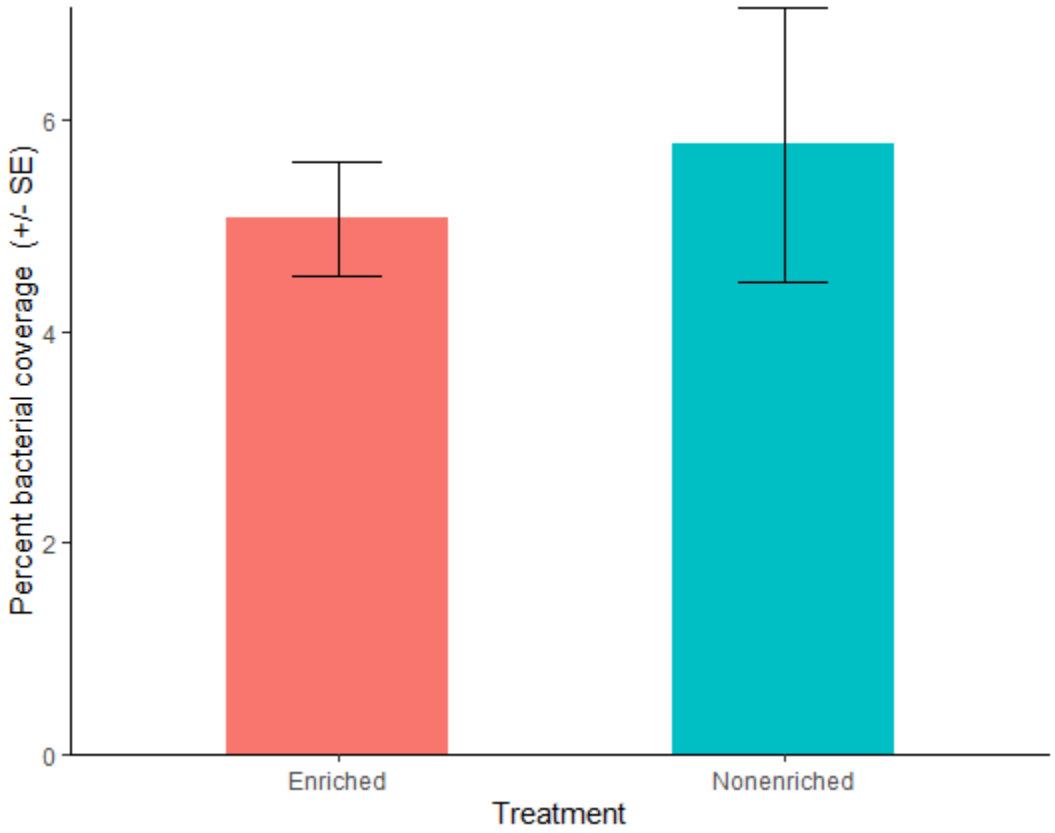

Figure 25. Average percentage of bacterial coverage on macroinvertebrates in the nutrient enriched and non-enriched streams.

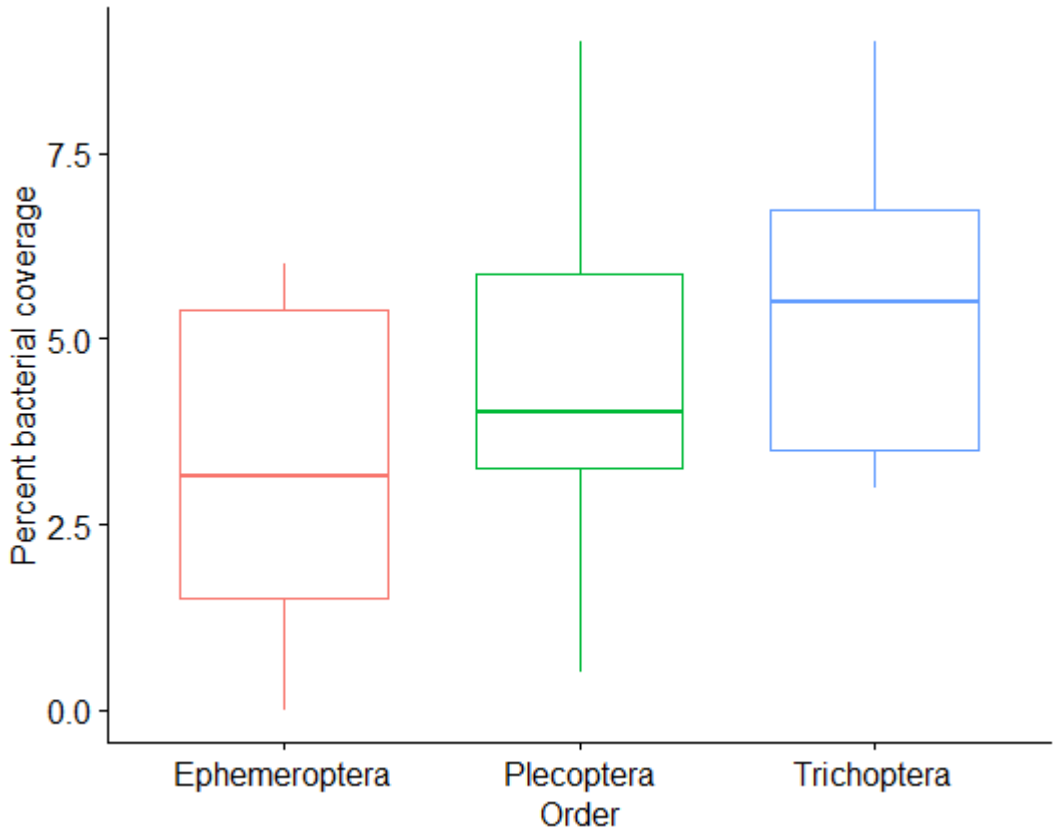

Figure 26. Comparison of average percent bacterial coverage among EPT taxa with seasons and treatment combined.

Table 2. Similarity percentages (SIMPER) of species contributing towards 70% of the dissimilarity between the nutrient enriched non-enriched sites.

	Enriched	Non-enriched			
Taxa	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib%
Gammarus	2.58	0.00	3.97	2.63	5.61
Baetis	0.98	3.22	3.77	1.78	5.33
Oligochaeta	2.02	0.00	3.14	2.29	4.43
Ephemerella	1.21	2.24	3.06	1.40	4.32
Epeorus	0.43	2.00	2.69	1.57	3.80
Leptophlebia	0.00	2.00	2.69	1.57	3.79
Orthocladiinae	2.65	2.81	2.58	1.26	3.65
Chironominae	1.45	2.56	2.53	1.32	3.58
Leuctra	0.55	1.27	1.98	1.05	2.79
Tanypodinae	0.97	1.64	1.91	1.32	2.70
Dolophilodes	0.28	1.24	1.90	1.01	2.69
Dicranota	0.63	1.26	1.88	1.28	2.66
Stenelmis	2.03	1.79	1.78	1.35	2.52
Agnetina	0.36	1.21	1.67	1.42	2.35
Psephenus	0.87	1.10	1.66	1.23	2.35
Simulium	0.92	0.66	1.60	1.04	2.27
Cheumatopsyche	0.48	1.08	1.58	1.15	2.24
Macrostemum	0.47	0.91	1.46	1.11	2.06
Macdunnoa	0.03	0.87	1.33	1.07	1.88
Choroterpes	0.00	0.86	1.20	0.59	1.69
Allocapnia	0.02	0.73	1.19	0.65	1.68
Hydropsyche	0.16	0.75	1.16	0.80	1.64
Heptagenia	0.15	0.75	1.16	0.70	1.64
Capnia	0.00	0.76	1.14	0.89	1.61
Haploperla	0.00	0.70	1.10	0.83	1.56

Table 2. Average seasonal biomass (g/m^2) per treatment (site).

	Enriched	Non-enriched
Season	*Avg. (g/m^2) +/- SE*	*Avg. (g/m^2) +/- SE*
Summer	0.096 +/- 0.027	0.065 +/- 0.01
Fall	0.14 +/- 0.038	0.11 +/- 0.024
Winter	0.25 +/- 0.1	0.18 +/- 0.056
Spring	0.38 +/- 0.02	0.3 +/- 0.13

Table 3. Average water quality parameters (+/- standard error) for the nutrient enriched treatment during the summer, fall, winter, and spring.

Parameter	Summer	Fall	Winter	Spring
TP (µg P/L)	38 +/- 19.2	171.17 +/- 74.01	69.37 +/- 29.5	35.4 +/- 10.8
NOx (mg N/L)	1.5 +/- 0.17	1.88 +/- 0.11	2.17 +/- 0.21	1.37 +/- 0.32
Temp (˚C)	15.8 +/- 0.59	9.54 +/- 0.69	2.32 +/- 0.14	8.06 +/- 0.99
DO (%)	111.2 +/- 7.07	94.7 +/- 2.93	99.64 +/- 2.5	114.5 +/- 10.35
SPC (µS/cm)	624.23 +/- 112.3	471.2 +/- 48.7	543.72 +/- 70.28	514.2 +/- 102.81
pH	8.25 +/- 0.1	7.62 +/- 0.17	7.6 +/- 0.16	7.9 +/- 0.15

Table 4. Average water quality parameters (+/- standard error) for the non-nutrient enriched treatment during the summer, fall, winter, and spring.

Parameter	Summer	Fall	Winter	Spring
TP (µg P/L)	11.6 +/- 3.9	19.6 +/- 1.32	10.1 +/- 0.4	12.69 +/- 2.45
NOx (mg N/L)	0.68 +/- 0.16	0.54 +/- 0.15	0.65 +/- 0.07	0.42 +/- 0.1
Temp (˚C)	15.6 +/- 1.03	7.7 +/- 0.62	1.91 +/- 0.22	5.53 +/- 0.81
DO (%)	98.34 +/- 2.51	97.43 +/- 0.71	97.8 +/- 2.78	105.03 +/- 2.73
SPC (µS/cm)	379.4 +/- 86	342.6 +/- 88.8	291.5 +/- 78.16	256.14 +/- 77.3
pH	8.05 +/- 0.12	7.8 +/- 0.07	7.68 +/- 0.12	7.31 +/- 0.36

Table 5. Average nutrient concentrations (+/- standard error) per treatment tank in the microcosm experiment.

Tank	Total Phosphorus (µg P/L)	Orthophosphate (µg P/L)	Total Nitrogen (mg N/L)	Nitrate/Nitrite (mg N/L)
Low N 1	10.5 +/- 4.2	1.02 +/- 0.33	0.53 +/- 0.06	0.19 +/- 0.03
Low N 2	4.5 +/- 0.6	0.55 +/- 0.11	0.42 +/- 0.03	0.19 +/- 0.04
Low N 3	4.0 +/- 0.8	0.6 +/- 0.07	0.47 +/- 0.04	0.2 +/- 0.05
High N 1	2.9 +/- 0.5	0.5 +/- 0.1	3.61 +/- 0.06	3.23 +/- 0.02
High N 2	3.07 +/- 0.9	0.47 +/- 0.7	3.22 +/- 0.4	3.28 +/- 0.04
High N 3	5.7 +/- 1.2	0.52 +/- 0.06	3.62 +/- 0.09	3.33 +/- 0.03
Low P 1	12.9 +/- 3.13	3.01 +/- 1.24	0.434 +/- 0.1	0.17 +/- 0.01
Low P 2	18.4 +/- 6.6	3.94 +/- 0.45	0.48 +/- 0.05	0.17 +/- 0.03
Low P 3	7.9 +/- 1.2	2.92 +/- 1.03	0.51 +/- 0.06	0.17 +/- 0.04
High P 1	308 +/- 4.8	302.7 +/- 7.13	0.79 +/- 0.15	0.18 +/- 0.02
High P 2	310 +/- 6.7	303 +/- 5.8	0.52 +/- 0.01	0.18 +/- 0.01
High P 3	310 +/- 3.5	303 +/- 9.5	0.66 +/- 0.06	0.18 +/- 0.06

Table 6. Average physicochemical measurements (+/- standard error) for each treatment tank in the microcosm experiment.

Tank	Temperature (°C)	Dissolved O_2 (%)	Dissolved O_2 (mg/L)	Specific Conductivity (µS/cm)	pH	Irradiance (W/m^2)
Low N 1	22.5 +/- 0.7	99.5 +/- 0.1	8.51 +/- 0.1	309.0 +/- 5.8	8.21 +/- 0.0	27.5 +/- 0.1
Low N 2	22.5 +/- 0.7	99.5 +/- 0.7	8.48 +/- 0.1	302.0 +/- 3.2	8.23 +/- 0.0	13.6 +/- 0.1
Low N 3	22.3 +/- 0.6	100.2 +/- 0.1	8.58 +/- 0.1	304.5 +/- 3.2	8.25 +/- 0.0	15.7 +/- 0.1
High N 1	22.0 +/- 0.5	100.3+/- 0.2	8.63 +/- 0.1	329.2 +/- 4.1	8.24 +/- 0.0	24.5 +/- 0.3
High N 2	22.2 +/- 0.6	100.1 +/- 0.2	8.58 +/- 0.1	323.6 +/- 4.1	8.21 +/- 0.0	19.3 +/- 0.2
High N 3	22.1 +/- 0.6	100.3 +/- 0.2	8.61 +/- 0.1	329.6 +/- 5.0	8.26 +/- 0.0	29.5 +/- 0.2
Low P 1	22.3 +/- 0.7	100.2 +/- 0.2	8.58 +/- 0.1	307.8 +/- 4.4	8.24 +/- 0.0	31.7 +/- 0.1
Low P 2	22.2 +/- 0.6	100.0 +/- 0.1	8.59 +/- 0.1	304.4 +/- 2.8	8.24 +/- 0.0	16.9 +/- 0.1
Low P 3	22.1 +/- 0.6	100.2 +/- 0.1	8.61 +/- 0.1	303.1 +/- 2.1	8.21 +/- 0.0	13.9 +/- 0.1
High P 1	22.1 +/- 0.6	100.2 +/- 0.1	8.61 +/- 0.1	310.5 +/- 6.2	8.25 +/- 0.0	11.2 +/- 0.2
High P 2	22.0 +/- 0.7	100.2 +/- 0.1	8.63 +/- 0.1	310.0 +/- 4.2	8.24 +/- 0.0	16.7 +/- 0.1
High P 3	22.1 +/- 0.6	100.2 +/- 0.1	8.61 +/- 0.1	311.0 +/- 5.6	8.25 +/- 0.0	17.6 +/- 0.1

Table 7. Starting and ending number of damselflies for each treatment tank in the microcosm experiment. The damselflies were identified as either *Ischnura* or *Enallagma.*

	Start			End		
Tank	*Ischnura*	*Enallagma*	**Total**	*Ischnura*	*Enallagma*	**Total**
Low N 1	5	2	7	3	0	3
Low N 2	4	3	7	1	2	3
Low N 3	6	1	7	2	1	3
High N 1	6	1	7	4	0	4
High N 2	3	4	7	2	3	5
High N 3	4	3	7	0	2	2
Low P 1	7	0	7	3	0	3
Low P 2	5	2	7	2	1	3
Low P 3	3	4	7	2	2	4
High P 1	1	6	7	0	4	4
High P 2	4	3	7	3	1	4
High P 3	4	3	7	0	3	3

Appendix 1. Aquatic macroinvertebrate taxa list. All organisms were collected between summer 2019 and spring 2020. Abundances are divided between the nutrient enriched and non-enriched sampling sites.

Taxon	Enriched	Non-enriched
Non-insect taxa		
Amphipoda		
Gammaridae		
Gammarus	727	0
Isopoda		
Asellidae		
Caecidotea	50	0
Decapoda		
Cambaridae		
Orconectes	3	2
Collembola		
Isotomidae	1	0
Acari	5	0
Anellida		
Oligochaeta	317	0
Glossophoniidae	2	0
Helobdella	1	0
Placobdella	17	0
Gastropoda	12	0
Physidae		
Physa	17	0
Hydrobiidae	2	0
Pleuroceridae	9	0
Lymnaeidae	2	0
Bivalvia		
Sphaeriidae		
Musculium	14	0
Ephemeroptera		
Baetidae		
Baetis	191	1145
Centroptilum	0	3
Ephemerellidae		
Ephemerella	421	944
Drunella	9	9
Serratella	0	30

Ephemeridae		
Ephemera	0	6
Heptageniidae	1	
Heptagenia	10	173
Epeorus	54	439
Macdunnoa	2	92
Stenacron	9	31
Rhithrogena	36	14
Stenonema	20	1
Leptophlebiidae		
Leptophlebia	0	514
Choroterpes	0	319
Caenidae		
Caenis	0	30
Isonychiidae		
Isonychia	0	5
Odonata		
Aeshnidae	0	4
Aeshna	0	2
Gomphidae	1	1
Coenagrionidae	1	0
Enallagma	1	0
Plecoptera		
Perlidae	10	8
Agnetina	33	114
Paragnetina	2	0
Eccoptura	4	5
Acroneuria	1	3
Beloneuria	0	2
Perlodidae		
Isoperla	1	32
Remenus	0	27
Diploperla	0	1
Leuctridae		
Leuctra	61	377
Capniidae		
Capnia	0	97
Allocapnia	1	126
Peltoperlidae		
Peltoperla	0	9

Nemouridae		
Amphinemura	8	125
Ostrocerca	0	121
Taeniopterygidae		
Taeniopteryx	29	15
Chloroperlidae		
Haploperla	0	70
Alloperla	0	3
Hemiptera		
Belostomatidae		
Belostoma	2	0
Trichoptera		
Hydropsychidae	11	13
Macrostemum	55	105
Cheumatopsyche	43	141
Hydropsyche	11	71
Arctopsyche	0	1
Hydroptilidae		
Hydroptila	22	49
Limnephilidae		
Apatania	0	2
Limnephilus	36	2
Hydatophylax	4	0
Hesperophylax	1	0
Pycnopsyche	1	0
Philopotamidae		
Dolophilodes	25	226
Polycentropodidae		
Cyrnellus		17
Polycentropus	2	26
Helicopsychidae		
Helicopsyche	13	3
Lepidostomadtidae		1
Lepidostoma	0	4
Theliopsyche	0	1
Glossosomatidae		
Glossosoma	0	4
Rhyacophilidae		
Rhyacophila	3	48
Uenoidae		

Neophylax	3	20
Odontoceridae		
Psilotreta	0	3
Leptoceridae		2
Molannidae		
Molanna	2	0
Lepidoptera		
Pyralidae		
Parapoynx	1	0
Coleoptera		
Elmidae		
Stenelmis	448	239
Psephenidae		
Psephenus	85	133
Dytiscidae		
Hydroporus	2	0
Hydrphilidae		
Hydrobius	1	1
Neuroptera		
Corydalidae		
Neohermes	0	10
Diptera		
Chironomidae		
Orthocladiinae	1056	1286
Chironominae	234	761
Tanypodinae	109	226
Simuliidae		6
Simulium	142	67
Prosimulium	10	115
Tipulidae		
Tipula	8	16
Limnophila	2	5
Dicranota	85	146
Hexatoma	0	48
Antocha	5	13
Dolichopodidae	14	0
Tabanidae	1	0
Stratiomyidae		
Oxycera	2	0
Stratiomys	2	0

Ceratopogonidae		
Bezzia	16	26
Athericidae		
Atherix	2	4
Empididae	17	13
Chelifera	0	5