EVALUATING THE ROLE OF BIOTIC AND ABIOTIC ECOSYSTEM COMPONENTS ON THE RETENTION AND REMOVAL OF DITCH NUTRIENTS IN DITCHES OF DIFFERENT CONSTRUCTION

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ABSTRACT

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As headwaters transporting agricultural runoff to streams and lakes, agricultural ditches may be a key component to reducing nutrient loading and harmful algal blooms. While conventional trapezoidal ditches are the most widely used, two stage ditches and self-forming streams are starting to be constructed as a means of management. Two stage ditches and self-forming streams may be useful for their wider floodplains, allowing slower movement of water and less erosion, promoting environments that retain and remove more nutrients than the more narrow conventional ditches. Here I examined multiple nutrient pools and fluxes (plants, invertebrates, sediments, water, and biofilm) of phosphorus and nitrogen in both May and July of 2018. I also tested the effects of isopods on nutrient cycling in ditch sediments in laboratory experiments. The results showed that ditch morphology did little to impact the concentration of nutrients, but did alter nutrient density and total nutrient retention, which was related to the width of the ditch. Plant and sediment pools were found to retain the most nutrients. Self-forming streams retained the most nutrients but supported a low biomass and diversity of invertebrates which can be important in nutrient cycling and multiple ecosystem functions. Bioturbation was found to be less important than excretion with invertebrates that rework surface sediments. Overall, my results suggest two stage ditches may balance increased nutrient retention while at the same time maintain aquatic habitat quality.

To my parents.

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1. INTRODUCTION

The Laurentian Great Lakes support a $6 trillion regional economy that directly provides 1.5 million jobs (Great Lakes Commission 2019). The Great Lakes are also a source of drinking water for 48 million people in the U.S. and Canada (Great Lakes Commission 2019). However, these waters are suffering from an over enrichment of nutrients leading to increasing instances of harmful algal blooms (Paerl and Huisman 2009) and causing the western basin of Lake Erie to be deemed impaired (Ohio Environmental Protection Agency 2018b). Moreover, similar phenomenon are increasingly occurring globally (Paerl and Huisman 2009).

The original concern for the eutrophication in Lake Erie came in the 1970s when conservation efforts were made to reduce pollution of the lake. In 1972 amendments were made to the Clean Water Act targeting point source pollution (e.g. wastewater effluent) (United States Congress 2002). These efforts helped to decrease nutrients and algal blooms by 1985 (Makarewicz and Bertram 1991). However, this did not address non-point source pollution (e.g. agricultural runoff) that could be the culprit of Lake Erie's re-eutrophication (Robertson and Saad 2011). In fact, much of the nitrogen and phosphorus loading in the western Lake Erie basin is likely from agricultural sources in the Maumee, Portage, and Sandusky River watersheds (Ohio Environmental Protection Agency 2018a).

Cover crops, buffer strips, conservation tillage, and other best management practices (BMPs) have been implemented across Ohio, but with these methods alone it could take decades to reach target nutrient concentrations (Muenich et al. 2016). Management of agricultural ditches may also help reduce nutrient fluxes downstream to lakes, but much is still unknown about how this

occurs. While ditches provide conduits for nutrients, delivering them from fields to rivers and lakes, ditches may also absorb nutrients before this happens (Kröger et al. 2008).

Different types of ditches can be more or less successful in removing nutrients. In Ohio, there are three ditch types, the common conventional ditches and two restoration types: two stage ditches and self-forming streams (Figure 1). Conventional ditches tend to have steep banks from the field directly into the waterway, they are manmade, and typically occur in straight lines. Two stage ditches are a management strategy of increasing the width of the ditch and adding a ledge (or second stage) to previously conventional ditches. This stage is a large flat "floodplain" surrounding the first stage, where the water flows under low-flow conditions. Self-forming streams are similar to two stage ditches in that they have a "floodplain" surrounding the flowing water and can have steep banks, but the creation of the ditch is different. To construct a self-forming stream a large area is carved out and then the water is allowed to flow and naturally meander through the created low-lying area.

With the different formation of these ditches they have the possibility of cycling nutrients differently, but all host the same general nutrient pools and fluxes (Figure 2). Below I describe how ditch geomorphology might influence each of these pools and fluxes.

1.1 Floodplain and Riparian Plants

The varying width, velocity, and sediment composition of ditches can impact their ability to absorb nutrients from the water column (e.g. Smith 2009). For instance, ditches with high slopes, typical of conventional ditches, are more likely to release fine sediments, along with their absorbed nutrients, into the water during higher flow events (Shore et al. 2015). This is due to the combination of a high velocity of channel water with the steep banks limiting the area for water

to disperse resulting in a stronger overall stream power that is more likely to erode banks and sediment. Water velocity can also impact nutrient spiraling (Newbold et al. 1983). The combination of nutrient cycling through water, sediment, and organisms, with displacement, has been termed nutrient spiraling (Webster and Patten 1979). Fisher et al. (1998) took the concept of nutrient spiraling and incorporated disturbance and succession connections to hyporheic (stream bed) and riparian (bank/floodplain) zones. Resistance to flood events is highest in the riparian zone and works its way down to being least resistant in the surface stream. Resistant systems have more structure and the ability to resist displacement, which has been shown to be related to greater storage, turnover times, and nutrient recycling (Webster et al. 1975). This is important in showing how floodplains and banks are relied upon to process nutrients, and how utilizing floodplains can help management efforts especially during spring floods.

Under anaerobic conditions, microbes can remove nitrogen from a system through denitrification, converting nitrogen ions to nitrogen gas that escapes to the atmosphere. For phosphorus, microbes will temporarily retain the nutrient, but not remove it. Plants in floodplains may also influence microbial effects on nutrients. For instance, Powell and Bouchard (2010) studied the composition of two stage ditch floodplains and conventional ditch slopes. They found that floodplain sediment had a higher percentage of organic matter than sediments of conventional ditch banks, supporting more denitrifying microbial activity and higher denitrification rates. This organic matter may come from dead ditch plants or roots and root exudates.

Floodplains in self-forming streams and two stage ditches are often excavated close to the water table resulting in soils that are saturated and can create anoxic conditions that provide an ideal environment for microbes responsible for denitrification (Gift et al. 2010, Roley et al.

2012). As a result, floodplains have the ability to remove nitrogen from influxes during flow events where slow flowing water inundates the sediment. However, anoxic soils are more likely to release phosphorus (Christophoridis and Fytianos 2006), but this possibility has been less investigated for ditch floodplains.

Floodplain retention and removal may be of particular importance in areas where tile drains are used. Some farmers may use buffer strips as a form of runoff management, but tile drains bypass this strip of vegetation and in conventional ditches the water is drained directly into the channel (Christopher et al. 2017). In two-stage ditches the water is drained into the floodplain, which would allow some retention to occur before the water reached the ditch channel.

I predict that floodplain plants will increase nutrient retention and that this will be highest in both two stage ditches and self-forming streams. However, other factors may also influence nutrient cycling (see below).

1.2 Aquatic Plants

During the growing season some channel plants, such as cut grass, can have luxury uptake in which they will store high concentrations of nitrogen and phosphorus in amounts above what is needed for growth (Kröger et al. 2007). Tyler, Moore, and Locke (2012) found that cutgrass and cattails, common plants found within channels and floodplains, retained about three times more nitrates compared to the retention in unvegetated systems. Vymazal and Březinová (2018) studied a ditch containing, cattails, phragmites, and reed manna grass, finding that cattails retained the most nitrogen and phosphorus and all plants together accounted for 16.9% - 10.2% of the phosphorus removed over two years. Unfortunately, while vegetation can increase retention of nitrogen and phosphorus during the growing season, when these plants die, the

nitrogen and phosphorus are often released (Kröger et al. 2007). This shows that plants are only a temporary mechanism of retention.

The abundance of plants, no matter their type or nutrient retention capacity, can impact other ditch components to promote retention and removal. More vegetation in a system stabilizes the sediment, reducing sedimentation and nutrient release during high flow (Lawson et al. 2012). Additionally, plants may assist in promoting nitrogen removal from the system by diffusing oxygen through their roots into to the sediment, promoting nitrification in the sediments close to roots (Reddy et al. 1989). The nitrate from this process can then make its way to the anoxic sediments away from the root zone and undergo denitrification (Reddy et al. 1989).

I predict that aquatic plant biomass will increase nutrient retention, and will be highest in self-forming streams, followed by two-stage ditches, and then conventional ditches. Not only will a higher mass of plants retain more nutrients from the ditch, a higher biomass of plants can create a higher level of habitat complexity and host more aquatic organisms, which could alter nutrients (Section 1.3).

1.3 Aquatic Macroinvertebrates and Emergent Insects

In addition to plants retaining high levels of nutrients, other living organisms such as macroinvertebrates also take part in nutrient cycling. Grazing organisms, such as snails, can change nutrient cycling by ingestion of various food items and excretion in soluble forms (Evans-White and Lamberti 2005). Burrowing macroinvertebrates can translocate nutrients from the sediment into the water column through feeding and excretion (Vanni 2002). These excretion rates of nutrients vary based on an organism's ecological stoichiometry, or the nutrient contents required per unit body mass (Vanni 2002). For example, snails have a body N:P ratio of 28,

compared to crayfish who have a smaller ratio of 18 (more P relative to N). In a laboratory study, snails have been found have a much lower N:P ratio in their excretions (excrete more P relative to N) than crayfish because snails require more nitrogen to be incorporated into their bodies (Evans-White and Lamberti 2005).

Organisms can also influence nutrient fluxes through bioturbation, which is the disruption of sediment and soils (Meysman et al. 2006). Organisms within ditches can contribute to bioturbation simply by moving over the sediment or burrowing into it. Leslie and Lamp (2017) characterized macroinvertebrates from different types of agricultural ditches in Maryland's Eastern Shore. They classified roughly 97% of all taxa found as burrowing. Macroinvertebrates with burrowing behaviors have been found to increase nutrient release from the sediment into the water (Biswas et al. 2009, Zhong et al. 2015). Although not previously tested, this sediment nutrient release throughout the summer may reduce the amount of nutrients in the sediment that will later be released during spring floods (the key time period of runoff leading to harmful algal blooms in Lake Erie). Disturbing sediments may also introduce surface water containing nitrates into anoxic sediments where denitrification can occur (Shang et al. 2013) or increase redox conditions in sediment, leading to fixation of P to metals. Thus, bioturbation could have multiple effects on nutrient cycling, leading to multiple competing hypotheses.

First, invertebrate bioturbation could potentially increase surface water nutrients and decrease sediment nutrients by releasing dissolved reactive phosphorus and dissolved nitrogen trapped deeper in the sediment by providing flow paths to the surface and by allowing labile P from deeper anoxic layers to be released into surface waters. This is because phosphorus is often trapped in sediments when surface waters are oxic because P may be fixed to metals in a layer at the top of the sediment trapping labile P in lower layers (Ahlgren et al. 2011). Conversely,

bioturbation may have the ability to decrease surface water nitrogen through movement that would introduce nitrogen rich surface water into anoxic sediments and denitrification would occur. Finally, bioturbators may cause no nutrient change in sediments due to an introduction of oxygenated water into burrows creating oxic sediments, decreasing nutrient release from sediments (Ahlgren et al. 2011).

The types of organisms in ditches can vary depending on the flow regime of a ditch. Invertebrate diversity and taxonomic richness in streams has been found to increase with flow permanence (Williams et al. 2004, Datry et al. 2007). Williams et al. (2004) compared biodiversity of rivers, ponds, streams and ditches; they found that ditches had the lowest diversity of plants as well as macroinvertebrates. While ditches had less biodiversity than the other sites with higher flow permanence, they still supported species of plants and macroinvertebrates that were unique from other water bodies. Sponseller et al. (2010) monitored macroinvertebrate populations over 16 years in Sycamore Creek in Arizona. Over the sampling period a significant difference in species composition following dry and wet years was observed, specifically a family of caddisflies completely disappeared after two droughts. Losing biodiversity in this way decreases a community's ability to tolerate disturbances (Balvanera et al. 2006).

Emergent insects, such as the caddisflies mentioned above, spend part of their lives in the water, take in nutrients from feeding on algae and organic matter, and then emerge in a new life stage, taking their nutrients with them. Sanzone et al. (2003) looked at insect emergence from a reach of Sycamore Creek in Arizona. Between May and July, they found that the total biomass of emergent insects was about 2% of the total instream biomass. The largest group to emerge from their experiment was the order Trichoptera, which includes caddisflies. With caddisflies as a

whole being an important part of the emergent nutrient flux from Sycamore Creek, losing a family from this order, may play a significant role in altering nutrient fluxes; because while most factors discussed here merely retain nutrients from the water column, emergent insects actually remove these nutrients when they emerge. Yet little is known about emergent insect's impact on water nutrient content or downstream loading.

Overall, I suggest that macroinvertebrates may play important roles in nutrient cycling, but that these roles are complex. However, these effects will likely be stronger in ditches with greater biomass and diversity of macroinvertebrates, which will be found with greater flow permanence (conventional, two-stage) and greater plant biomass and habitat complexity (two-stage, self-forming). This should increase fluxes of nutrients via emergent insects and increase bioturbation

1.4 Summary

Here I describe how differences in multiple abiotic and biotic factors in different types of agricultural ditches and natural streams work together to impact pools and fluxes of nutrients, which should ultimately influence nitrogen and phosphorus loading into Lake Erie. I expected nutrient cycling in streams and ditches to be driven by a balance between flow permanence, vegetation, and animal abundance, diversity, and composition. I compared metrics in conventional ditches to reconfiguration options, including two stage ditches and self-forming streams. I also included small streams without altered geomorphology as a reference.

2. METHODS

My research is divided into two parts. First, to examine actual pools and fluxes of nutrients in ditches of different construction I collected samples from water, sediment, plants, macroinvertebrates, and emergent insects and measured their biomass and nutrient content. I also complemented this with a laboratory investigation of the effects of a common macroinvertebrate on nutrient cycling.

2.1 Field

2.1.1 Study Sites

Samples and measurements were taken from conventional ditches, two-stage ditches, self-forming streams, and un-manipulated streams in NW Ohio. There were three replicates per site type for a total of 12 sites (Figure 3). Sampling occurred along a 50-meter section of the ditch or stream in late May and July of 2018 at each site.

2.1.2 Above Ground Riparian and Aquatic Plant Sampling

In order to measure the pool of nutrients retained in floodplains, above ground riparian plant material was collected from the bank of ditches during the May sampling. At the downstream end, middle, and upstream end of each ditch segment a 0.25 m^2 quadrat was haphazardly placed about a meter away from the channel. All plants within the quadrat were cut and bagged. To measure the flux of nutrients in the new growth of plants, the sampling area was then flagged during the May sampling so that the same area could be trimmed again during the July sampling.

A similar approach was taken to sample the pool and flux of aquatic plant nutrients. A 0.25 m^2 quadrat was haphazardly placed in the channel of the ditch. All plants within the quadrat were trimmed, placed into a paper bag and brought back to campus to be dried and analyzed.

During the first sampling in May, the quadrat area was flagged so we could return during the July sampling to collect plants to represent the flux of nutrients from new growth.

In the lab, plant samples were dried at 55 °C. The dried samples were ground into a powder using a coffee grinder. The three powdered samples for each site (upstream, middle, downstream) were then combined into one sample per site, for each sampling period. These final mixed samples were analyzed for nitrogen and phosphorus content (Section 2.3).

2.1.3 Aquatic Macroinvertebrate Sampling

To determine the aquatic macroinvertebrate nutrient pool, aquatic insects were collected by two different methods, D-net sampling, and surber samples. D-net samples were taken following EPA invertebrate sampling protocols for sampling bottom substrate and submerged macrophytes (Dorn 2013). At least three D-net samples were taken: one at downstream, middle, and upstream. When not enough organisms were found to analyze nutrient content, more samples were attempted, up to 6. Samples were collected by 3 quick 1-meter long sweeps through the ditch that were emptied into a white pan and sorted into an empty cup. These samples were then immediately placed in a glass vial and dried at 55°C once returning to the lab and then nutrient content was determined (Section 2.3).

Surber samples were taken using 0.25 m^2 quadrat and the D-net. Invertebrates were sampled from the same quadrat that the aquatic plants were removed from (Section 2.1.2) The quadrat was haphazardly placed in the channel and the D-net was place directly downstream of the quadrat. After the aquatic plants were removed, the sediment and rocks within the quadrat were stirred up so that anything in the area would flow into the D-net. This sample was then emptied into a white pan for sorting out invertebrates from debris. I assumed that D-net and surber samples both showed similar composition of aquatic insects, but the surber allowed us to

have an exact area measurement to determine biomass and diversity per square meter. In order to calculate this, any aquatic insects from the surber were placed into an ethanol filled bottle to be identified to order, dried, and weighed for total dry biomass.

Crayfish were collected separately by a timed search for 1.5 hrs with a D-net along the entire site. They were counted, and then there was an attempt to measure excretion, but excretion data was unusable due to methodological flaws. Following attempted excretion measurements, crayfish were brought back to the lab, dried at 55°C, weighed and stored in a glass container until they were crushed to prepare for nutrient analysis.

One large crayfish was not used in excretion and was weighed, and measured for total and carapace length, then placed in a field mesocosm to attempt a growth experiment. The mesocosm was formed from a five-gallon bucket with small holes drilled all around the bottom of the bucket to allow flow, and netting placed over the top. The bucket had mud, a large rock, and some plants placed in it to weigh it down and give the crayfish a more natural environment. These crayfish were measured every week throughout the experiment. If the crayfish was misplaced, a new individual was captured, weighed, and measured and the experiment started over again. Many crayfish were lost during this experiment due to flooding and predation. This resulted in only two sites having useable repeated measurements. At the end of the experiment, these crayfish were measured one final time and brought back to the lab to be dried at 55°C and analyzed for nutrients (Section 2.3).

2.1.4 Emergent Insect Sampling

Floating emergence traps (Cadmus et al. 2016) (Figure 4) were used to assess the outgoing flux of nutrients from emergent insects. These were placed at each end of the site and were collected every week between the May and July samplings. The soapy water bottles from

the traps were immediately refrigerated upon returning to the lab and identified as soon as possible to order. After identification the emergent insects were placed in glass vials and dried at 55°C. Once dried, samples were ground to a powder using a mortar and pestle, and weighed to determine if they were viable for nutrient analysis (Section 2.3). At least one sample of each site type was large enough to be tested. All three conventional sites, two natural stream sites, and only one of each the two stage and self-forming stream sites were sufficient for analysis.

2.1.5 Biofilm Sampling

To measure biofilm biomass, nutrient content, and growth, three ceramic tiles, zip-tied to scaffolding, were placed into each ditch for two intervals of time. The first interval started in May and lasted 83 days to measure biofilm biomass and nutrient content. A second trial was run in September where the tiles were in the field for 14 days to measure growth. When removed, the tiles were placed in plastic bags and frozen until analysis. To analyze biofilm biomass and nutrients, tiles were placed into a small tub and scrubbed with a toothbrush (Marshall 2019). I alternated scrubbing and rinsing with DI water until no more biofilm came off of the tiles. The slurry was then placed into petri dishes and dried at 55°C (Marshall 2019). Once dried, biofilm was carefully scraped from petri dishes, weighed, and stored until they were analyzed for nutrients (Section 2.3).

2.1.6 Abiotic Samples and Measurements

To be able to identify other pools and fluxes of nutrients, as well as other possible drivers of nutrient cycling we also took multiple abiotic measurements and collections. Water parameters were measured using a YSI multiparameter sonde (ProDDS), with a total of three sensors: pH and oxygen reduction potential, conductivity and temperature, and dissolved oxygen. YSI measurements were recorded at the top and bottom of the water column if the water was at

least 10 centimeters deep, and at each end of the site. Water depth was also recorded at each end of the sampling area. These data were recorded during the two major samplings in May and July, as well as weekly in between those dates. During each of the main samplings, wetted channel width was measured at the ends and in the middle of each site. The width was also measured at the top of the banks in those locations so I could calculate a floodplain area.

During each sampling, two samples of surface water were collected, one at the downstream and one at the upstream end of the site. These samples were collected following EPA protocols for dip sampling (Simmons 2016). Facing upstream, I rinsed then filled a labeled urine cup with ditch water and placed it into a cooler until returning to campus where samples were refrigerated. These samples were analyzed in less than a week for total nitrogen and phosphorus as well as dissolved reactive phosphorus and dissolved inorganic nitrogen (Section 2.3).

Sediment samples were collected from the center of the channel at the upstream and downstream ends, and in the middle of the 50-meter site. Sediments were collected by pushing a labeled urine cup into the sediment, sliding a spatula underneath, and removing the sample from the sediment. Samples were placed in a cooler until returning to campus where they were refrigerated if not immediately dried. To dry the samples, they were spread out in labeled aluminum pans and left out on a counter to air dry (A. Vazquez Ortega, personal communication, 2018). Once dried, the samples were crushed and run through a 2 mm sieve. The contents were then placed in two cups for greater and less than 2 mm. The less than 2 mm samples were analyzed for nutrients (Section 2.3).

2.2 Lab

2.2.1 Setup

In summer 2019, I examined the impact of bioturbation of a common ditch macroinvertebrate, isopods, on nutrient cycling in a laboratory experiment. The experiment examined the influence of isopod density on change in dissolved and total N and P, with 6 replicates of four levels of isopod density: control (0 isopods), low (6 isopods), medium (12 isopods) and high (18 isopods). These numbers were chosen because in the field, numbers of isopods collected ranged from 0-18 for the surface area of the jars. The experiment took place in twenty-four 1.89-liter (half-gallon) glass mason jars set up with collected ditch sediment and filtered ditch water. A 0.25 g sample of plant material was added to each cage for food for the isopods.

Ditch sediment was collected from a two-stage ditch site used in the field experiment. This site was the NEE Ditch (T2 on Figure 3), located in the Portage River Watershed with SnA soils, comprised of mostly sloan silty clay loam sediment which was the main composition of 4 of the 12 ditches in the field experiment. This sediment is considered very poorly drained, subject to frequent flooding, with 3-6% of the surface layer made up of organic matter (USDA and NRCS 2007). This sediment was dried and sieved at 2 mm before use. Water was collected from a nearby conventional ditch between cornfields in Bowling Green, OH, and filtered before use. Isopods and plant material for the experiment were collected at one of the conventional ditches previously used in this experiment. This site was BGE (C2 on Figure 3), located in the Portage River watershed with hoytville silty clay loam substrate. Isopods were sorted into size categories so that all individuals used in the experiment were roughly the same size, ranging from 5-8 mm in length. They were then housed in the filtered ditch water within the

environmental chamber for 3 days prior to being added to the jars. This allowed the isopods to acclimate to the water and temperature before the experiment began.

At the start of setup, a sample of dried and sieved sediment was set aside for analysis and 800 mL of sediment was added to each of twenty-four glass mason jars used for the experiment. The jars were then filled to the 1750 mL mark with filtered ditch water and allowed 5 days to settle before isopods and plant material were added. The jars were set up in an environmental chamber allowing us to control light and temperature. The temperature was set to 21.5 °C for the duration of the experiment, this air temperature allowed us to achieve a water temperature around 19-20 °C. The water temperature was selected to mimic the conditions during July of the field experiment where the average water temperature was 20 °C during the week of sampling. The light cycle was selected to have a 6:15 am sunrise and a 9:00 pm sunset, once again to mimic the day/night cycle of the July field sampling.

Mesh was used to cover the opening of the jar to prevent any possible contamination from outside insects. A hole was cut in the mesh just large enough to fit the aeration tube through it. Aeration was set up similar to the methods of Leslie and Lamp (2019), using an air pump and aquarium tubing, with the tubing inserted just under the surface of the water to add oxygen without disturbing the sediment (Figure 5a). Aeration was kept low in attempt to mimic lower oxygen levels in the field, around 60% DO in the middle of the water column.

Within the environmental chamber, the 24 jars were set up on three shelves, 8 jars per shelf. Since I used four treatments (control, low, medium, high), two jars of each treatment were randomly placed on each shelf to mitigate effects of proximity to the door or any light or aerator malfunctions on each individual treatment (Figure 5b).

2.2.2 Lab Sampling

Temperature, redox, pH and percent dissolved oxygen was measured in the surface water of each jar using a YSI, and 2 mL of water was removed to test for nitrogen and phosphorus. Additionally, to measure sediment pore water, I used a PushPoint sampler (MHE Products), rigged with tubing and a syringe to pull pore water in front of the YSI probe to get a pH and Redox reading for pore water, and collect a 2 mL sample for nitrogen and phosphorus (Figure 6). Once measurements were taken, isopods were placed in each jar at varying treatment amounts.

After the organisms were added, additional surface and pore water sampling took place every three days for 15 days following the same procedures outlined above. However, I removed and replaced (with filtered ditch water) an additional 20 mL of water in addition to sampled surface and pore water, helping to simulate downstream flow of a ditch. The replacement water was filtered ditch water that was collected at the same time as the initial water and stored in the environmental chamber to keep the same temperature. Thirty minutes after adding the new water another 2 mL sample was taken to serve as the "starting nutrient levels" for the next measurement period (Figure 7).

At the end of the experiment, the plants and isopods in each jar were removed and dried. Sediment was removed in two 4.5 cm sections of top and bottom, and air dried (A. Vazquez Ortega, personal communication, 2018). Once dried, sediment samples were crushed so that they could be analyzed. All water samples, as well as sediment samples from the beginning and end of the experiment, were tested for nitrogen and phosphorus content using the same process as those used for field samples (Section 2.3). By knowing the nutrient contents for inputs and outputs I was able to examine changes in nutrients over time.

2.3 Nutrient Analysis

All samples were analyzed for nitrogen (N) and phosphorus (P) content by the Midden lab at BGSU. First, I made sure all biotic samples were dried and ground to a powder. Once delivered to the lab these samples were digested using persulfate digestion techniques for sediments, and refined for plants and animals (Gibson et al. 2015, Metzner 2017). This technique involved creating a reagent by combining potassium persulfate, sodium hydroxide solution, and DI water. Then thoroughly mixing the reagent, sample, and more water together in culture tubes. These tubes were then autoclaved and after digestion the samples could then be analyzed using a Seal AQ2 Discrete Analyzer housed in the Midden lab.

Water samples were first filtered with using 0.2 um or 0.4 um membrane filters to remove particulate matter. Then nutrients were determined using a combination of methods from the U.S. EPA and the U.S. Geological Survey National Water Quality Laboratory (United States Environmental Protection Agency 1993, Patton and Kryskalla 2003). Total phosphorus and total nitrogen were determined using methods from Patton and Kryskalla (2003), which are similar to the methods for sediments and tissues by using an autoclave to digest water samples with the same potassium persulfate, sodium hydroxide and DI water reagent. After digestion water samples were analyzed with the Seal AQ2 Discrete Analyzer. Dissolved nutrients were determined using multiple EPA (1993) techniques outlined in Table 1 provided by the Midden lab.

2.4 Statistics

2.4.1 Summary Calculations

We determined biomass of all pools and fluxes and then determined their nutrient content per unit of mass (content), per m^2 (density), and per m length of ditch or stream (total). This last

metric multiplied the per m^2 estimated by the widths measured in the field to better understand how channel and floodplain size may impact nutrient retention. To estimate sediment nutrient pools, I used the sediment bulk density of 1.48 g/cm^3 from Calhoun et al. (2001), which measured sediments like the ones in the ditches sampled here. I also assumed a 7 cm depth for calculating the size of the sediment pool because that was the depth from which I acquired a sample (this represented a conservative estimate of the true sediment pool, which may have been deeper, but likely included the part of the sediment most active in exchanges with the surface).

2.4.2 Comparisons of Nutrient Pools Among Ditches

To examine the influence of ditch type and other potential covariates on nutrient pools and fluxes, I fit general linear models or mixed effects models, with either F, χ^2, or Kenward-Roger tests and post hoc Tukey tests, as appropriate, using the program *R* (R Core Team 2019) and the *lme4* package (Bates et al. 2015). Assumptions of equal variance and normality were assessed via plots of residuals and transformed when necessary. For data that could not meet assumptions even after transformations, a nonparametric Kruskal-Wallis test was used. Mixed effects models were used for data that were collected in both May and July, with inclusion of site as a random effect to control for repeated sampling. This included data for water nutrients and depth, invertebrate weight and nutrients, sediment weight and nutrients, and channel width. Some repeated sampling data could not be made to meet the equal variance assumption and thus was split into May and July separately, with each run as a general linear model with a Bonferroni correction of $\alpha = 0.025$. This included data for certain invertebrate nutrient measurements, surface water DIN and pH. Invertebrate richness, which also could not meet assumptions for mixed effects models, was tested with separate general linear models for May and July using a Poisson distribution for count data. Some data only had one data point for each site

(accumulation of nutrients in new plant and biofilm biomass, and emergent insect biomass) and thus I used a general linear model without a Bonferroni correction. Finally, community composition of macroinvertebrates was assessed using NMDS plots and PERMANOVA tests using the *RVAideMemoire* package (Hervé 2020).

2.4.3 Effects of Bioturbation on Nutrient Cycling

With the lab results, I used repeated measures ANOVAs with the Greenhouse Geiser correction for temporal autocorrelation to analyze changes in all water nutrients and parameters. This analysis made use of the *ezANOVA* in the *ez* package in R (Lawrence 2016). Assumptions of equal variance and normality were assessed via plots of residuals and transformed when necessary. Data for change in surface water phosphorus did not meet assumptions, even after transformation, and thus I ran a Kruskal - Wallis test on data from the first change in phosphorus measurement and the last change in phosphorus measurement, with a Bonferroni correction on each. This would allow me to test for a difference between treatments at the start and end of the experiment. Because sediment nutrients were measured only once, at the end, a general linear model was fit to test differences in treatment and an interaction with location (top or bottom) of the sediment, with a block for the jar the sample was taken from, and an F test. To determine if nutrients in the sediment were different at the end of the experiment compared to the beginning, 95% confidence intervals were calculated for the nutrients in top and in bottom sediments of each treatment at the end of the experiment and compared to the single initial homogenized sediment sample that was set aside for analysis.

3. RESULTS

3.1 Field

3.1.1 Site Characteristics and Drying

I examined differences in water parameters such as dissolved oxygen levels, conductivity, oxidation reduction potential (Redox) and pH taken with the YSI during the two sampling periods. I did not detect a significant difference in these parameters between site type. Only redox was significantly different between samplings ($F = 7.55$, $df = 1$, $p=0.032$; Table 5), generally increasing between May and July.

The depth of the water in the channel was found to be significantly lower during the July sampling than the May sampling ($F = 6.88$, $df = 1$, $p = 0.038$; Table 5; Figure 8). Wetted width of the channel was also found to significantly decrease between samplings ($F = 9.10$, $df = 1$, $p = 0.020$; Table 5; Figure 9) and differ between site types ($F = 8.29$, $df = 3$, $p = 0.009$), with natural streams being significantly wider than conventional ditches (Tukey's: $p = 0.003$) and two stage ditches (Tukey's: $p = 0.023$). While some self-forming streams had the highest number of dry weeks , overall I did not detect a statistically significant difference between ditch types ($F = 2.12$, $df = 8$, $p = 0.176$; Figure 10).

3.1.2 Riparian Plants

In May, there was significantly more bank plant biomass per meter length collected in self-forming streams than in conventional ditches (Tukey's: $p< 0.001$) and natural streams (Tukey's: $p < 0.001$) ($F = 7.72$, $df =3$, $p = 0.009$; Table 2; Figure 11). In July self-forming streams had a significantly higher biomass of new growth compared to conventional (Tukey's: $p<0.001$), two stage (Tukey's: $p = 0.046$) and natural streams (Tukey's: $p<0.011$). Two-stage

ditches also had a significantly larger amount of new growth than natural streams (Tukey's: $p = 0.019$) ($F = 10.8$, $df = 3$, $p = 0.004$; Table 2; Figure 11).

I did not detect a significant difference in the nutrient content (mg phosphorus or nitrogen per g of tissue) in bank plants, in either the May or July sampling (Table 2; Figure 12).

The density of phosphorus (per m^2) in the riparian plant pool was higher in self-forming streams than conventional ditches (Tukey's: $p<0.001$) and natural streams (Tukey's: $p = 0.0497$) ($F=5.51$, $df=3$, $p = 0.024$; Table 2). The flux of phosphorus into new plant biomass (per m^2) was also found to be higher in self-forming streams than all other site types (Tukey's: conventional $p<0.001$, two stage $p<0.001$, natural streams $p<0.001$) ($F = 56.4$, $df = 3$, $p<0.001$; Table 2). I did not detect differences in the density of nitrogen (per m^2) in the plant pool nor the flux of nitrogen (Table 2).

In May, total nutrients (per m length of ditch) were higher in riparian plants in self-forming streams than conventional ditches (Tukey's: phosphorus $p <0.001$, nitrogen $p = 0.026$) and natural streams (Tukey's: phosphorus $p = 0.001$, nitrogen $p = 0.016$) (phosphorus $F = 7.31$, $df = 3$, $p = 0.011$, nitrogen $F = 4.05$, $df = 3$, $p = 0.050$; Figure 13; Table 2). There was greater total flux of P into new plant material (per m length) in self-forming ditches than all other sites (Tukey's: conventional $p<0.001$, two stage $p = 0.007$, natural streams $p<0.001$) and two stage ditches had higher plant P accumulation than natural streams (Tukey's: $p = 0.020$) ($F = 10.8$, $df = 3$, $p = 0.002$; Table 2). No significant differences were detected in the flux of nitrogen into new riparian plant growth (Table 2).

3.1.3 Aquatic Plants

While some of the self-forming samples had a high biomass of aquatic plants per meter length compared to other site types, no significant differences between sites were found for May or July (Figure 11; Table 2).

In both the pool and flux of aquatic plants, nutrient content (per g of tissue), density (per m^2) and total (per m length) were also not found to be significantly different between site type (Figures 14 and 15; Table 2).

3.1.4 Aquatic Macroinvertebrates

I found a total of 11 different orders across sites, but any single site only had a maximum of 8 different orders. Community composition differed significantly between ditches and natural streams ($F = 2.80$, $df = 1$, $p = 0.003$). Ditch sites contained more Isopoda, Odonates (dragonflies/damselflies), Coleopterans (beetles), and Hirudineans (leeches); while natural stream sites contained more Ephemeropterans (mayflies), Trichopterans (caddisflies), Hemipterans (water striders/water boatmen) and Tricladidans (flatworms) (Figure 16). Between ditches, community composition was not significantly different ($F = 1.35$, $df = 2$, $p = 0.170$; Figure 17). However, there was a significant association between aquatic invertebrate community composition and the number of dry weeks ($F = 2.32$, $df = 1$, $p = 0.010$; Figure 18), with increases in relative abundance of Isopoda and Amphipoda (scuds) and decreases in Tricladidans and Hemiperans as drying events occurred. Wet width was also significantly associated with invertebrate community composition ($F = 2.24$, $df = 1$, $p = 0.025$; Figure 18), with increases in Diperans and Coleopterans as wet width increased. Some self-forming stream sites had the lowest order richness, but I did not detect any significant differences in order richness overall (Figure 19; Table 3).

The effect of site type on macroinvertebrate biomass changed between the May and July samplings ($F = 8.74$, $df = 3$, $p = 0.009$; Table 4; Figure 20). Self-forming streams had the lowest biomass of inverts (per m length) during both samplings, but two stage ditches had the highest biomass in May and conventional had the highest invertebrate biomass in July (Figure 20). Although there were differences in water depth and drying between stream types (see Section 3.1.1), I did not find a significant relationship between aquatic macroinvertebrate biomass and water depth ($F = 1.95$, $df = 1$, $p = 0.193$), or the number of weeks where the channel was dry ($F = 0.166$, $df = 1$, $p = 0.692$).

I did not find a significant difference in nutrient content (mg per g of tissue) between site types or samplings (Figure 21). There was also no detected significant difference between sites in nutrient density per m^2 or total nutrients per m length (Tables 3 and 4). Total nutrients did however drastically decreased from May to July in all ditch sites, but slightly increased in natural streams (Figure 22) (Phosphorus $F = 10.13$, $df = 1$, $p=0.016$; Nitrogen $F = 24.93$, $df = 1$, $p = 0.002$; Table 4).

Crayfish were analyzed separately from other invertebrates due to differences in collection. Crayfish were not caught at all sites, only natural stream sites had crayfish measurements for all three sites over both samplings. There were no significant differences detected in crayfish biomass (Table 3). Moreover, there were no significant differences found in crayfish nutrient content (mg per g tissue) or total nutrients (g per meter length) (Tables 3 and 4).

Due to predation and flooding, the samples collected for the crayfish growth experiment were insufficient to conduct a statistical test. The experiment resulted in two useable sites with three and seven weeks' worth of growth data. These crayfish averaged a biomass increase of

0.335g per week. Using the average nutrient contents this would equal an accumulation of 0.0059 g of P per week, and 0.032 g of N per week in individuals.

3.1.5 Emergent Insects

Total biomass of emergent insects collected over 12 weeks from July to September was below 20 mg per m length at each dich site. No significance was found between the biomass of emergent insects at each ditch type (Table 2). Due to such small samples collected from sites, many sites had to be combined to test nutrients resulting in insufficient data to conduct a statistical test on nutrient content. On average, emergent insects contained 2.90 mg phosphorus per gram of tissue and 42.98 mg of nitrogen per g of tissue. Density of nutrients (g per m^2) averaged in 1.80×10^{-5} g of P per day or 0.002 g of P per 123 day growing season (May-September). Nitrogen density was 2.23×10^{-4} g of N per day or 0.027 g of N per growing season. Total nutrients removed through emergent insects in one meter length of ditch averaged 3.61×10^{-5} g of P per day or 0.004 g per growing season, and 4.81×10^{-4} g of nitrogen per day or 0.059 g per growing season (Figure 23). To provide context at a broader scale, Wood County, OH contains 4800 km of ditches (Duane Abke, Wood County engineers office, personal communication, 2020); based on my measurements, fluxes of emergent insects from this county could potentially remove 19.2 kg of P and 283.2 kg of N per growing season. To my knowledge, the total length of all ditches in the entire Western Basin of Lake Erie has not been previously calculated, so scaling to a larger area is not possible at this time.

3.1.6 Biofilm

I did not detect differences in the standing biomass of biofilms (accumulation over 83 days; Figure 24) or the growth of new biofilm (accumulation over 14 days) across site types

(Table 2). There were also no significant differences detected between site types in nutrient content (mg per g of tissue), density (g per m^2; Figure 25) or total nutrients (g per m length).

Flux measurements are missing for conventional ditches due to one site being dry during the time of flux and not generating biofilm, one site was dredged before flux measurement and was therefore unusable, and at the last conventional site's biofilm tiles were lost after flooding. For all other site types nutrient concentrations were similar resulting in no significant differences detected between them for nutrient content (mg per g of tissue), density (g per m^2; Figure 25) or total nutrients (g per m length) (Table 2).

3.1.7 Surface Water

I did not find significant differences between sites or over time for average dissolved reactive phosphorus (DRP) in surface water (Figure 26; Table 5). I observed a smaller average concentration of dissolved inorganic nitrogen (DIN) in the surface water in July than in May across all site types (Figure 26). This effect of nutrient change over time was determined to be significant for both the content, mg N per L ($F = 8.76$, $df = 1$, $p = 0.024$; Table 5) and density of N in one square meter of water ($F = 13.2$, $df = 1$, $p = 0.010$), but not per meter length of the waterway (Table 3).

3.1.8 Sediment

From May to July, the amount of water in the channels decreased, therefore decreasing the channel width and wetted sediments. This resulted in a significant change in our calculations of wetted channel sediment between samplings ($F = 19.6$, $df = 1$, $p = 0.002$). Since all sediment was estimated using the same bulk density measurement (Section 2.4.1) declines in weight over one meter length occurred due to the declines in site wetted channel width. However, the differences in weight between site types were consistent over time. The wider wetted channel of

natural streams had a statistically higher mass of sediment, based on our calculations, than conventional ditches (Tukey's: $p<0.001$) and two-stage ditches (Tukey's: $p = 0.042$) ($F = 5.06$, $df = 3$ $p = 0.030$; Table 6).

Phosphorus content (mg per g) in sediment of natural streams was significantly lower than in conventional ditches (Tukey's: $p=0.014$) and self-forming streams (Tukey's $p=0.0498$) ($F = 4.75$, $df = 3$, $p = 0.035$; Table 6). Sediment nitrogen content (mg per g) was significantly higher in conventional ditches than both two stage ditches (Tukey's: $p=0.039$) and natural streams (Tukey's: $p=0.006$)($F = 4.88$, $df = 3$, $p = 0.032$; Table 6).

Densities (g per m^2) of nutrients were significantly lower in natural stream sediments than in conventional ditch (Tukey's: phosphorus $p= 0.014$; nitrogen $p<0.001$) and self-forming stream sediments (Tukey's: phosphorus $p = 0.0498$; nitrogen $p = 0.001$) (phosphorus: $F = 4.75$, $df = 3$, $p = 0.035$; nitrogen: $F=8.75$, $df = 3$, $p = 0.007$; Figure 27; Table 6). Nitrogen density was also found significantly different between samplings ($F = 9.01$, $df = 1$, $p = 0.017$; Figure 27; Table 6). Nitrogen per m^2 increased between May and July for all sites except self-forming streams. I did not detect a significant difference in total nutrients (per m length) between sites or samplings (Table 6).

3.2 Lab

3.2.1 Surface Water

I did not detect any significant difference in phosphorus over time or by treatment (Figure 28). However, surface water nitrogen significantly decreased over time ($p[GG] = 0.001$; Table 7; Figure 29), but less so with increasing isopod density, resulting in a marginally significant effect of treatment and time ($p[GG] = 0.050$; Table 7; Figure 30). Redox in the surface water also

significantly decreased over time but not differently between isopod densities (p[GG]<0.001; Table 7; Figure 31).

3.2.2 Pore Water

In the pore water, redox significantly decreased over time, but was not different between treatments (p[GG]<0.001; Figure 28). Pore water P was found to have a significant treatment plus time effect (p[GG] = 0.006; Table 7; Figure 28). All treatments experienced an average high at the third sampling, decreased to a low at the fifth sampling, then spiked again at the last sampling. But high and medium densities of isopods experienced higher peak in P concentrations at the third sampling compared to the control and low density treatment. Pore water N significantly changed over time but not between treatments (p[GG]<0.001; Figure 29; Table 7). Pore water N increased by the second or third sampling and then decreased over time.

3.2.3 Sediment

At the end of the experiment, sediment nutrients were similar across treatments and were not significantly different between treatments or between top and bottom samples (Figure 32; Table 8). Nitrogen significantly increased over time in the bottom half of the sediment in the control treatment as well as the bottom and top sediment in the high isopod treatment (Table 9). Phosphorus significantly declined over time in the top half of the sediment in the low treatment as well as both the top and bottom sediment in the medium treatment (Table 9).

4. DISCUSSION

4.1 Field

4.1.1 Summary

Overall, I found that plants and sediment are the largest pools of nutrients in streams and ditches of NW Ohio. Differences in morphology/construction of these waterways has large effects on the storage of nutrients in the plant pool and thus overall. In general, wider ditches harbor more plant biomass within larger floodplains. The higher plant biomass at self-forming streams followed predictions. However, the fact that higher plant density (per m^2) at self-forming streams also contributed to this effect was unexpected. There are many potential explanations for this finding and it needs further study, but I suggest it is possible that these wider floodplains may allow for greater plant diversity, leading to greater biomass, or a different assemblage that includes species less tolerant of inundation and capable of achieving high biomass.

Moreover, I discovered that nutrient storage and cycling was controlled more by the biomass of each pool and magnitude of fluxes than by differences in nutrient content (mg/g) (Figure 33). Very few pools were found to have differences in nutrient content between sites and the most significant differences between sites were found among total nutrients (per m length), which takes into account the width of the channel and floodplain, maximizing potential biomass differences (Figures 34 and 35).

In addition to biomass effects, the width of the floodplain has been shown to play an important role in denitrification through increased bioreactive surface area (Roley et al. 2014, Mahl et al. 2015). Gift et al. (2010) found positive relationships between plant root biomass and organic matter, and organic matter and denitrification enzyme activities in riparian zones. This could suggest that a greater area to accumulate plant biomass would also increase root biomass

leading to more organic matter, and consequently greater denitrification in large floodplains. These findings are important in that the area of the floodplain is one of the key differences that sets two stage ditches and self-forming streams apart from the widely employed conventional ditch (and self-forming also tend to be wider than two-stage).

While width of ditches has been found to play an important role in retention of nutrients, channel width may also play a role in phosphorus release. Wider channels allow the water to spread out over a larger area resulting in lower flows and could result in drying events like those we observed in self-forming streams. Inundation after these drying events has been found to lead to a release of P from sediments (Kinsman-Costello et al. 2014, 2016). Kinsman-Costello et al. (2016) examined nutrient release in a variety of sediments collected in Michigan and found higher rates of P release after drying events than P release under constant inundation. These release rates varied with sediment properties making it hard to estimate how the ditches in this study may release P without further analyzing sediment properties.

4.1.2 Surface Water

Although other studies have found that floodplains can help reduce the amount of nutrients making their way into the ditches, I did not discover a difference in surface water nutrients linked to floodplain presence (Roley et al. 2012, Hodaj et al. 2017). Per meter length of stream, self-forming ditches appear to have lower nutrients (Figures 34 and 35), but this is due to self-forming streams having less surface water than other site types and not due to any differences in concentrations. There are several possible explanations for the lack of observed difference in surface water nutrient concentration in this study. First, I sampled a limited number of sites for a limited amount of time. Roley et al (2012) and Hodaj et al (2017) were both able to monitor nutrients in two stage ditches for 2 to 3 years yielding much more data than I was able to

gather here. Second, due to an increase in surface area for denitrification, floodplains have the most ability to remove nutrients when inundated during high flow (Roley et al. 2014). Mahl et al. (2015) found that two stage ditches with the shallowest channel/lowest benches, which may have been more often inundated, consistently decreased nitrate concentration in surface water. I did not measure surface water nutrients during conditions of inundation. A final possible reason for a lack of observed treatment effect on reduction in nutrients is that surface water nutrients may have been more controlled by local or watershed-level field management and fertilizer application than by local floodplain conditions during the period of this study.

4.1.3 Sediment

Sediment nutrient pools were also significantly different between sites; but were different based on nutrient content (mg per g sediment) and density (g per m^2) and not total nutrients per meter length which takes into account the width of the channel (Figures 27. 36 and 37). Conventional ditches and self-forming streams were found to have the highest nutrient contents and two stage and natural streams had the lowest. Conventional ditches likely had the highest sediment nutrients due to a lack of other pools to retain nutrients since conventional ditches do not have a floodplain and had a low biomass of channel plants compared to other ditch sites. A possible reason for self-forming streams to have high sediment nutrients may be linked to the high plant biomass, due to the larger root systems present diffusing oxygen into sediments creating aerobic sediments around them, therefore promoting nitrification in those zones (Reddy et al. 1989, 2000). Conversely root biomass has been linked to an increase in organic matter which has been found to promote denitrification (Gift et al. 2010, Powell and Bouchard 2010). From this we would think that self-forming streams with high plant biomass would also have high organic matter from roots and decaying plants from the previous year, which would

increase denitrification rates resulting in less sediment nitrogen compared to other sites. A possible reason for self-forming streams not experiencing this expected decline in N in sediments could be because of the more frequent drying of self-forming streams. While organic matter is supposed to increase denitrification, this can only occur in anaerobic conditions and constant inundation of sediments helps achieve anaerobic conditions required for denitrification to occur (Powell and Bouchard 2010).

Smith (2009) found that ditches with a lower velocity of water flowing through them had more organic matter, finer particle sediments, and increased P uptake rate. This may explain why self-forming streams specifically had higher P content in sediments, since they had wide channels with low flow, silty clay sediments, and lots of plants creating organic matter. Another possible reason for self-forming streams and conventional ditches having higher sediment phosphorus contents could be particle sizes of the sediment. Conventional ditches and self-forming streams had the highest nutrients and were both found to be comprised of the most silt/clay substrate compared to two stage ditches that had more sand and pebbles in addition to clay, and natural streams contained more sand, pebble, and cobble. It has been found that finer sediments adsorb more P (Smith 2009, Xiao et al. 2015, Zhu et al. 2015), which is consistent with our findings. As a previously mentioned possibility, more frequent drying of self-forming sediments and aerobic zones created around plant roots could also be a cause of increased P in self-forming streams since P binds to sediments under aerobic conditions (Reddy et al. 1989, 2000, Kinsman-Costello et al. 2016).

Finally, there are other properties of sediments that we did not measure that may be impacting sediment nutrient cycling such as metals and microbes. Metals like iron can influence nutrients by binding to phosphorus removing dissolved P from surface and pore waters (Reddy et

al. 2000, Miao et al. 2006). Microbial communities are responsible for facilitating denitrification and can uptake P from the system and retain it. Both microbial processes and metal binding ability are linked to redox potential (Reddy et al. 2000, Miao et al. 2006, Seo and DeLaune 2010, Hunting and van der Geest 2011). Redox potential was measured in sediment pore water but was not found significantly different between site types. Microbial processes can also be influenced by invertebrates present; studies have found invertebrates can increase bacterial activity in sediments (Mermillod-Blondin et al. 2002, Hunting and van der Geest 2011, Hunting et al. 2012). With macroinvertebrate weight being significantly different between sites this may have contributed to the differences found in sediments.

The increased nutrient retention in self-forming stream sediments may be helpful in lessening surface water nutrients before drying events but may become problematic during a period of reflooding later on. Inundation of previously dried sediments has been found to release a flux of P larger than the release of P from constantly inundated sediments (Kinsman-Costello et al. 2014, 2016). This means self-forming streams with a larger pool of sediment phosphorus and drying events could experience the largest flux of nutrients compared to other ditches in the fall or spring when sediments become inundated again. The magnitude of P release relies on a variety of sediment properties that were not measured here such as previously mentioned metals.

4.1.4 Invertebrates

Aquatic invertebrates and emergent insects had the highest content of nutrients of all pools, but their low biomass meant that they were a tiny part of the total nutrient budget per m length of waterway (Figures 34 and 35). Even though invertebrates do not represent a large sink of nutrients, emergent invertebrates are one of the few paths by which nutrients can be permanently removed from the system and not just retained (Sanzone et al. 2003, Metzner 2017).

However, aquatic invertebrates may also be a source of nutrients through excretion and bioturbation (Section 4.2).

Although macroinvertebrates may not strongly control nutrient cycling in these systems, their biodiversity is still important because higher species richness has been found to result in positive effects to ecosystem processes and a higher resistance to external factors such as nutrient pulses (Balvanera et al. 2006, Caliman et al. 2007, Perkins et al. 2015). Macroinvertebrate biodiversity and biomass in ditches has been linked to flow and flow permanence (Williams et al. 2004, Datry et al. 2007, Leslie et al. 2012). As predicted, some self-forming sites experienced the most drying events through the season and had the lowest biomass and richness of invertebrates, but other self-forming sites did not show this pattern and the relationship was not significant overall. Shallow ditches are generally more susceptible to drying events than other types of ditches. These drying events can lead to loss of key taxa, and decreased food chain length (Sponseller et al. 2010, Sabo et al. 2010) which can potentially have broad indirect effects on nutrient cycling and the surrounding ecosystem (Covich et al. 1999, Raitif et al. 2019). Similar to other research, I found a shift in community composition with drying events, with more Isopoda and Amphipoda orders with more drying events and fewer Tricladida and Hemiptera (Figure 18). Changes in wetted width, which is partly associated the loss of water in channels, also resulted in community composition differences. Narrower wetted channels lost two key emergent orders of Diptera and Ephemeroptera, but then gained more Odonates (Figure 8). Therefore, management goals related to nutrient retention should also consider potential effects on aquatic biodiversity. Two-stage ditches may balance both management goals.

4.2 Lab

4.2.1 Summary

Results of the bioturbation experiment showed an effect of decreasing nutrients in most parts of the system, but the exact mechanisms behind these changes are still unclear; although one possible explanation is that excretion had a stronger impact than bioturbation and assisted in creating nutrient pumps in the system (Sections 4.2.2 and 4.2.3; Figure 38). Future testing of the isopods N:P ratio may further help explain this story of excretion. If isopods are higher in P relative to N they may have been sequestering more P and excreting higher levels of N that could explain the insignificant decrease of P and the differences in N decrease we saw related to density.

Many previous studies that found effects from bioturbation on nutrient cycling have used higher densities of invertebrates than the natural densities I used here. However, in the field other species would also be present which could result in a stronger bioturbation effect. Caliman et al. (2007) ran bioturbation tests on chironomid larvae, snails, and worms independently as well as combined. They found that the mixed treatment resulted in a higher phosphorus flux than the highest performing single species treatment. This suggests that while surface reworking may not be a very impactful form of bioturbation, in combination with other kinds of bioturbation, results can be more pronounced.

4.2.2 Phosphorus

Phosphorus in surface water followed the same general trend across treatments with, on average, a slight non-significant decrease between samplings. A study by Caliman et al. (2007) found something similar on a silt/clay substrate, increasing densities of the sediment reworking species *Heleobia australis* did not significantly increase the release of total dissolved

phosphorus. While I also saw no significant changes in surface water phosphorus, the general trend in my experiment was a decrease in surface water dissolved phosphorus. This difference could be related to redox, with phosphorus binding to suspended particles in the surface water under oxic surface water conditions. Despite the lack of change in surface water P, pore water P initially increased, peaking around samples two to three, and then decreased until sample five (Figure 28).

A possible explanation of my results would be that excretion of P by isopods initially increased phosphorus in the system, which was mostly taken up by pore water in the beginning. Over time oxygen may have become depleted in the sediment while the surface water remained oxic and phosphorus in the surface water bound to suspended particles. Through this process a pump could have been produced diffusing more phosphorus from the pore water into more P depleted and oxygenated surface water to bind to suspended particles, decreasing dissolved reactive phosphorus in both surface and pore water (Figure 38).

Redox values lower than 300 mV is where we start to see facultative (300-0 mV) and anaerobic (<0 mV) processes take place (Reddy et al. 2000, Søndergaard 2009). Iron will bind P at redox potentials around >200 mV, and release P at lower values as iron is reduced (Miao et al. 2006, Søndergaard 2009). While I did not test for iron in my system, the redox results provide evidence that iron binding P could be a strong mechanism behind our results. In surface water, redox potentials were consistently above 200 mV (with ability to bind P) between samplings 1 and 5. In pore water, redox potential was more variable among treatments, but all treatments fell below 200 mV (releasing P) during samplings 5 and 6. Overall, although there were not strong patterns in surface water P, and other explanations are possible such as P uptake based on N:P

ratios, I suggest that the results are consistent with the idea of a P "pump," with P binding in surface water and release from sediment pore water.

4.2.3 Nitrogen

The change in surface water nitrogen was variable in the beginning of the experiment but over time experienced more constant declines (Figure 29). With a significant treatment effect over time, it appeared that the increasing density of isopods decreased the magnitude of N decline. It is also worth noting that higher densities appear to create a lag in peak N removal. While control and low treatments experienced a more consistent N loss across replicates between samplings three and four, for medium and high treatments this occurred between samplings four and five (Figure 30). Nitrogen in pore water also saw a variable start but then started to decline over time, but without a significant difference between treatments. A possible reason for these declines could be due to excretion and a denitrification pump occurring. Higher densities of isopods would excrete more N, which could have led to the lag in maximum decline since there would have been more nitrogen to remove from the system. Over time as the sediment became depleted of oxygen denitrification would have started removing N from the pore water. This could have triggered a pump of nitrogen from the nitrogen rich surface water into the depleted pore water, to also be denitrified.

Denitrification has been found to occur at relatively higher average redox potential in sediments than what is considered anaerobic (Reddy et al. 2000, Seo and DeLaune 2010, Hunting and van der Geest 2011). With approximately 300 mV being classified as the start of reduction, where nitrate can reduce to nitrite (Reddy et al. 2000) we can estimate that 300 mV is the highest potential where denitrification would start. Redox in pore water reached this threshold between the first and second samplings, with continued declines in redox and likely

increases in denitrification until the end of the experiment. Redox in surface water also hit this threshold but later in the experiment at the third sampling. The surface water redox was on average higher than pore water redox so it would still be possible that nitrogen from surface water was being pumped into sediments to denitrify as more denitrification was occurring there.

4.2.4 Effects of Invertebrates on Redox

Redox may play an important role in nutrient cycling of this experiment, but it is unclear if redox was impacted by invertebrate bioturbation. Redox in both surface and pore water changed over time but did not change based on treatment. Hunting et al. (2012) compared how redox potential was impacted over time with different movement strategies using isopods and scuds as "biodiffusors" compared with burrowing species. They found that the isopods and scuds increased redox over time within the first few millimeters of sediment. The results from my experiment showed a decrease in redox in sediments over time, but I was not able to carefully sample from only the top few millimeters of sediment which may have been more impacted considering the mobility of the isopods used. In future experiments, detailed profiles of redox and anoxia could greatly help explain nutrient cycling.

5. CONCLUSION

This study was the first of its kind in comparing multiple pools and fluxes of nutrients among multiple ditch morphologies. Specifically, there is a knowledge gap for self-forming stream ditches, with restoration studies focused mostly on two stage ditches. Thus, the data collected for this experiment can be important pilot data for future studies comparing pools or morphologies.

While I expected to find one type of ditch standing out from the rest as the best management strategy for retaining and removing nutrients, these findings suggest either type of the wider ditch morphologies of two stage ditches or self-forming streams can effectively retain nutrients. Both widened ditches provide a larger plant biomass compared to conventional ditches, which I found to be one of the most important pools and fluxes. Focusing on promoting this plant pool helps to promote plants retaining nutrients, provides organic matter to improve sediment denitrification, stabilizes sediments, and provides invertebrate habitat (Kröger et al. 2007, Powell and Bouchard 2010, Lawson et al. 2012, Tyler et al. 2012). Each type of widened ditch presents its own benefits such as the largest plant biomass retaining nutrients in self-forming streams, but an improved invertebrate community performing multiple ecosystem functions in two stage ditches. Thus, there is a tradeoff between maximizing nutrient retention and maintaining aquatic biodiversity. There is also a tradeoff between high sediment retention capacity in self-forming streams, and the possibility of high phosphorus release from sediments after reflooding of previously dry ditches. Additionally, the bioturbation experiment results show that macroinvertebrates could play a role in nutrient cycling. Thus, two-staged ditches could be best at simultaneously meeting nutrient management and biodiversity goals. By employing a widened

ditch management to current conventional agricultural ditches, we can start to mitigate agricultural nutrient effects at the headwaters and help to prevent downstream eutrophication.

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APPENDIX A. FIGURES

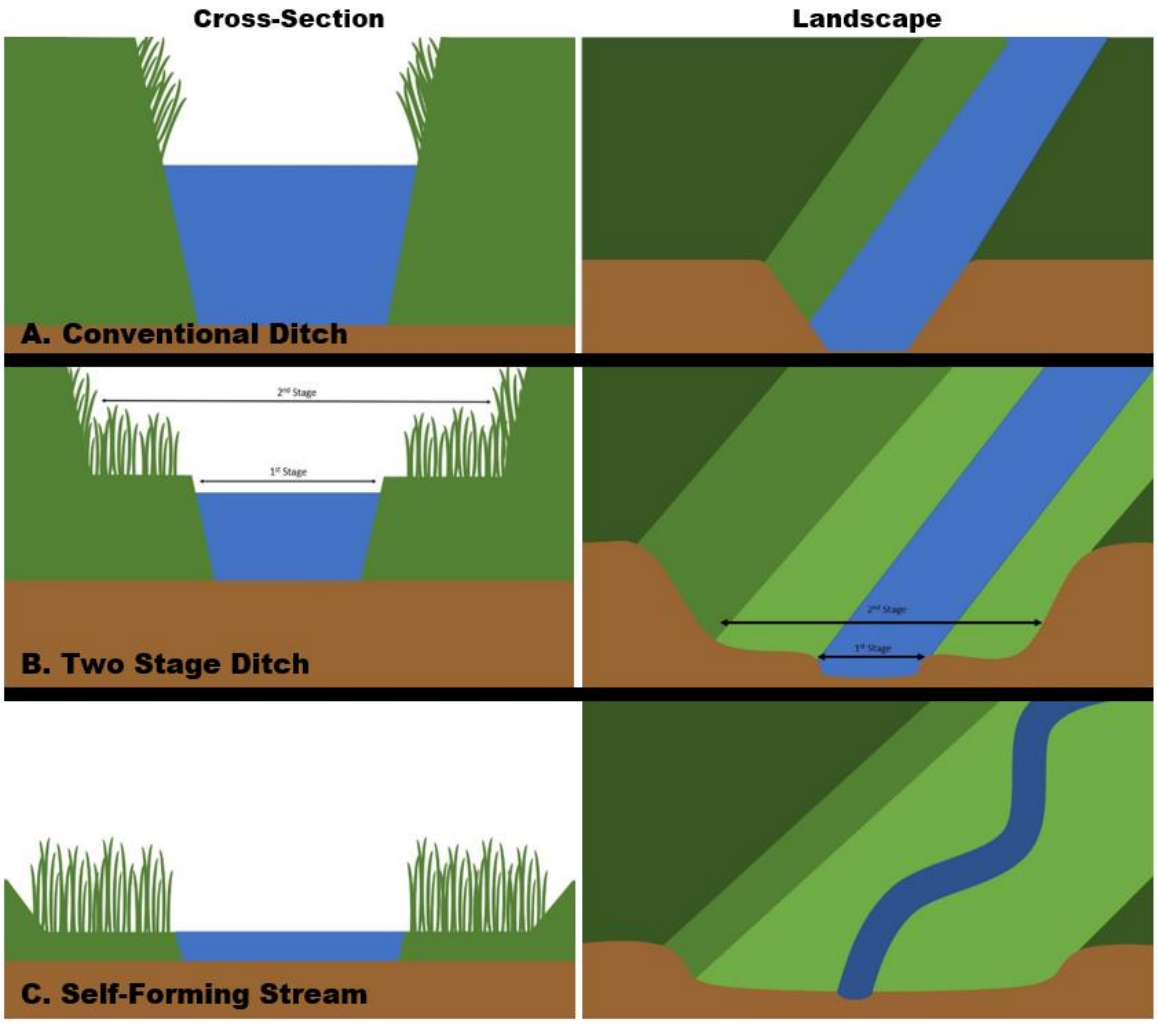


Figure 1. Types of ditches sampled. A) Two stage ditch B) Self-forming stream C) Conventional ditch.

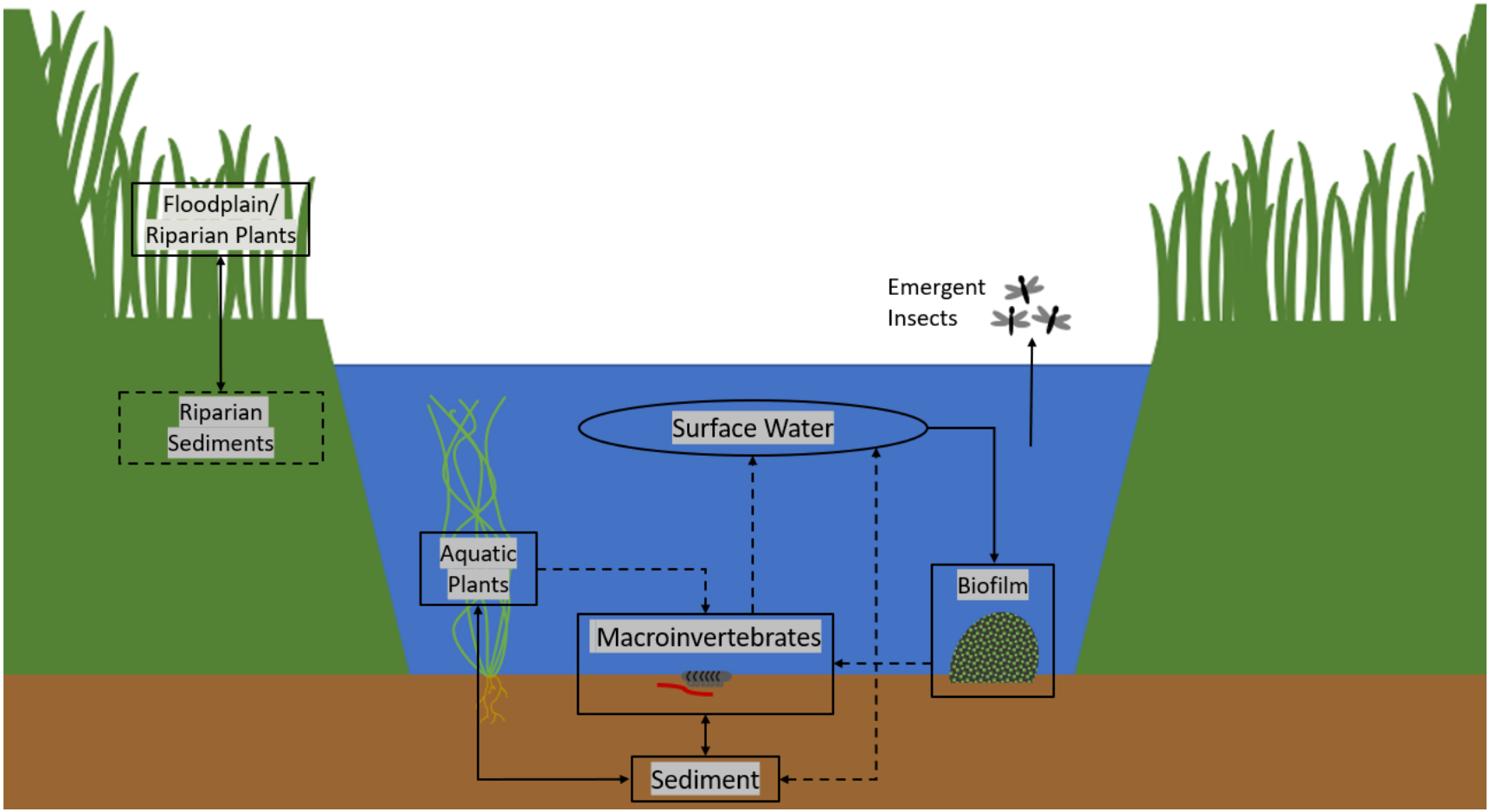


Figure 2. Pools and fluxes of agricultural ditches, solid boxes and arrows are pools and fluxes I have studied. Dashed boxes and arrows are not quantified in this study.

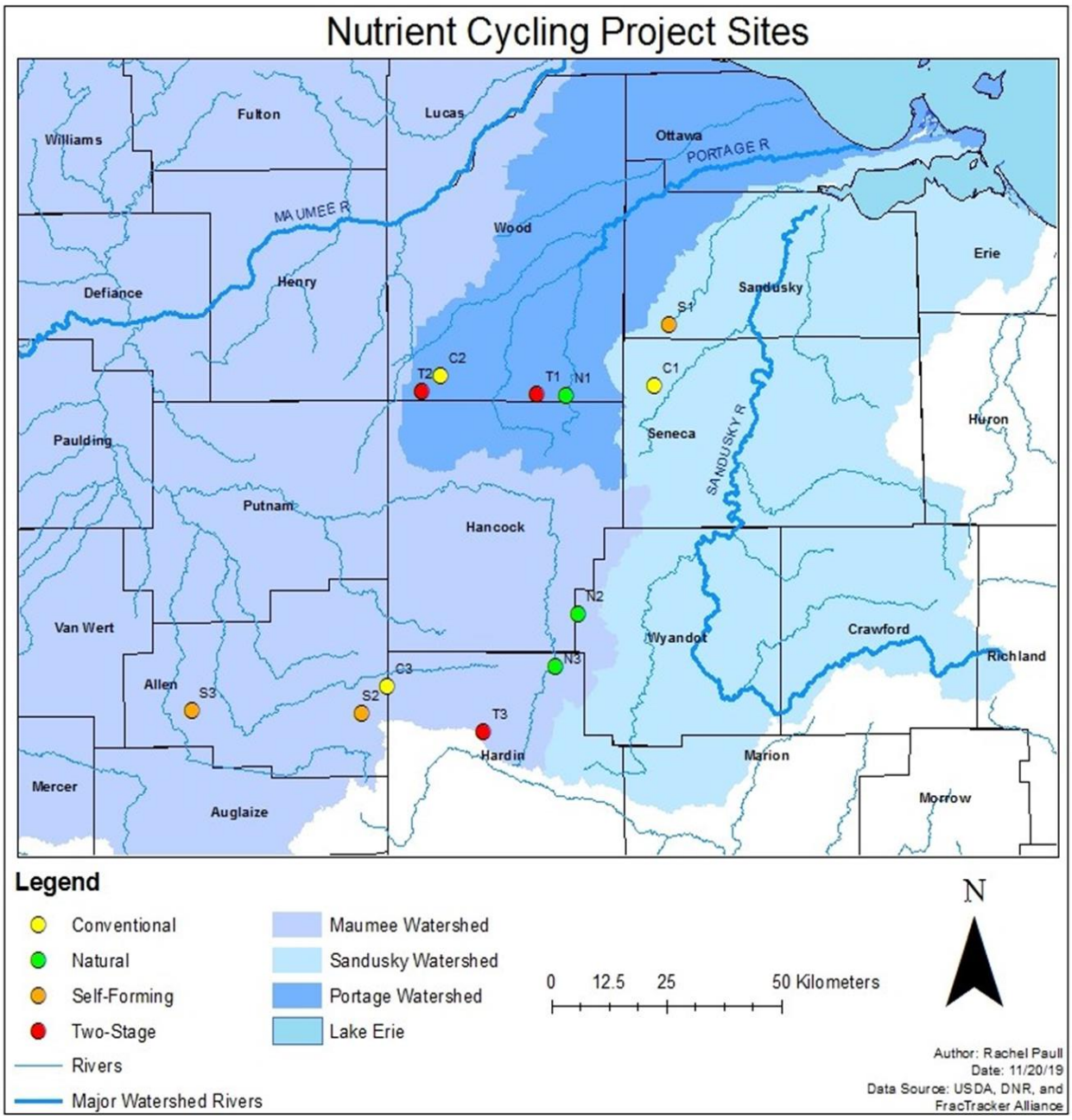


Figure 3. Locations of twelve sites used for sampling throughout Northwest Ohio. Conventional ditches 1-3 named ALL, BGE, and MEL respectively. Natural streams 1-3 named BLO, POR, and OUT. Self-forming Streams 1-3 named HEC, GAB, and HOC. Two-stage ditches 1-3 named BUC, NEE, and KEN respectively.

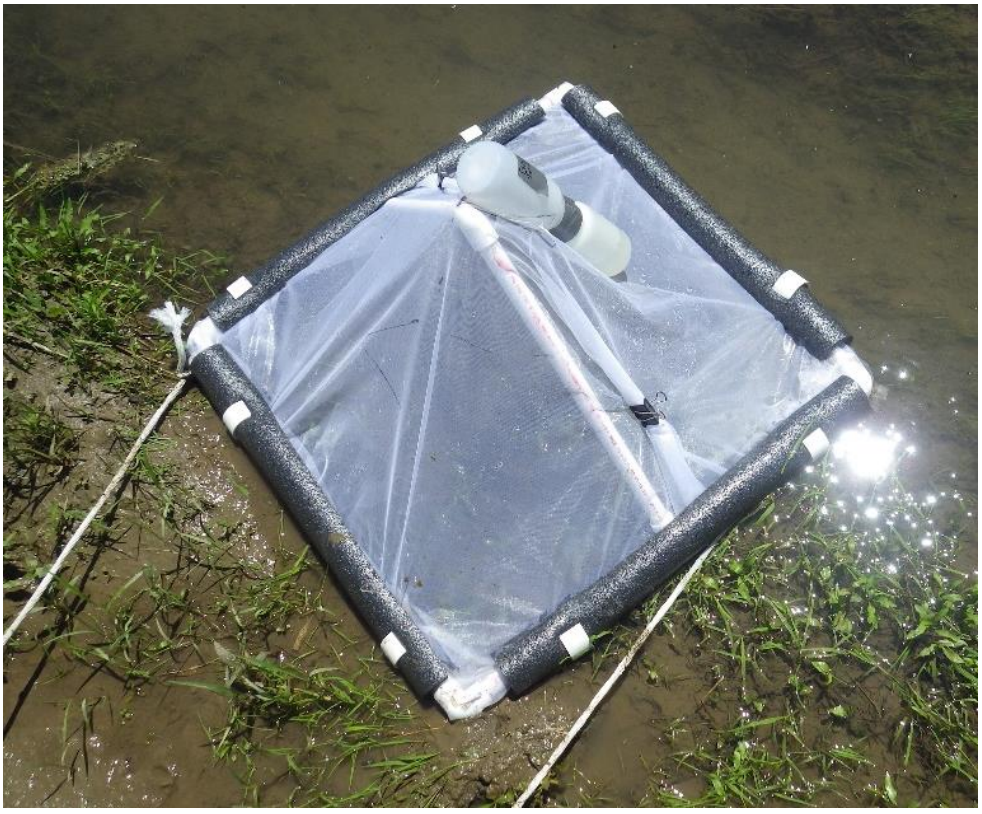


Figure 4. Emergence trap deployed in a ditch.

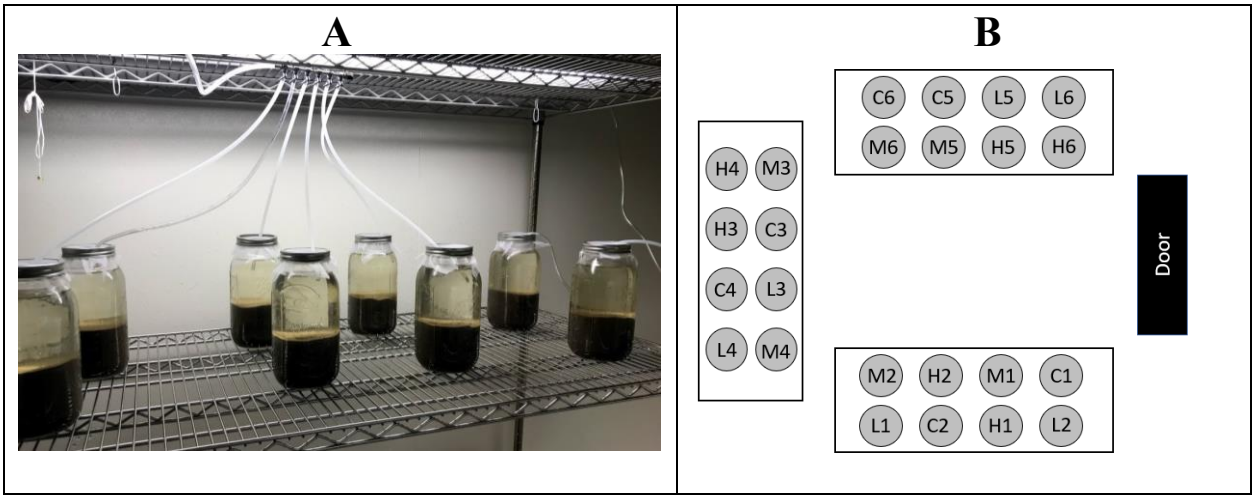


Figure 5. A)Bioturbation experimental setup, shelf 1 of 3. B) Randomized placement of jars on three different shelves. Letter represents the density treatments, control (0), low (6), medium (12), high (18), and the number represents the replicate.

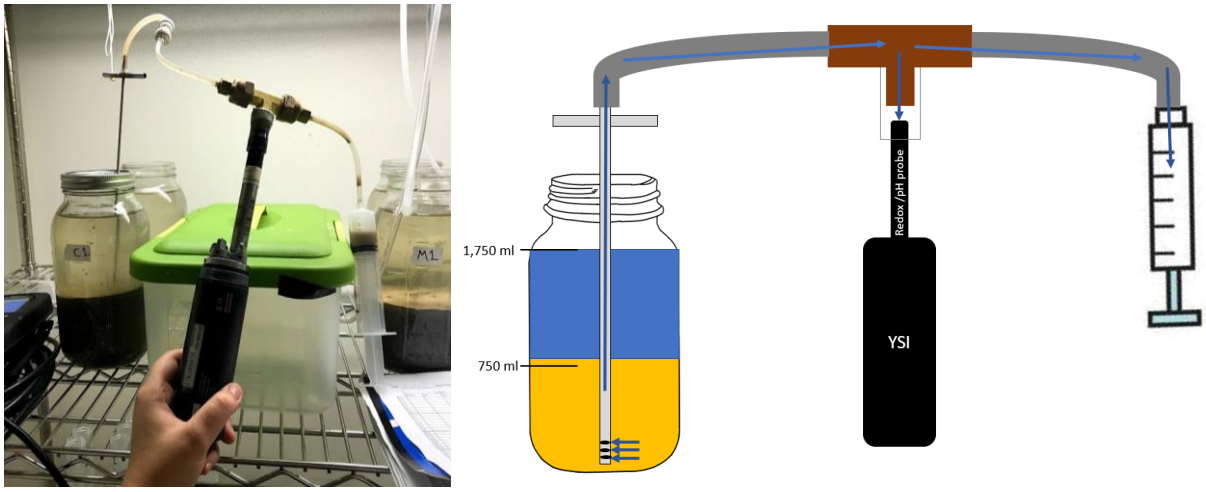


Figure 6. Pore water sampling setup in bioturbation experiment.

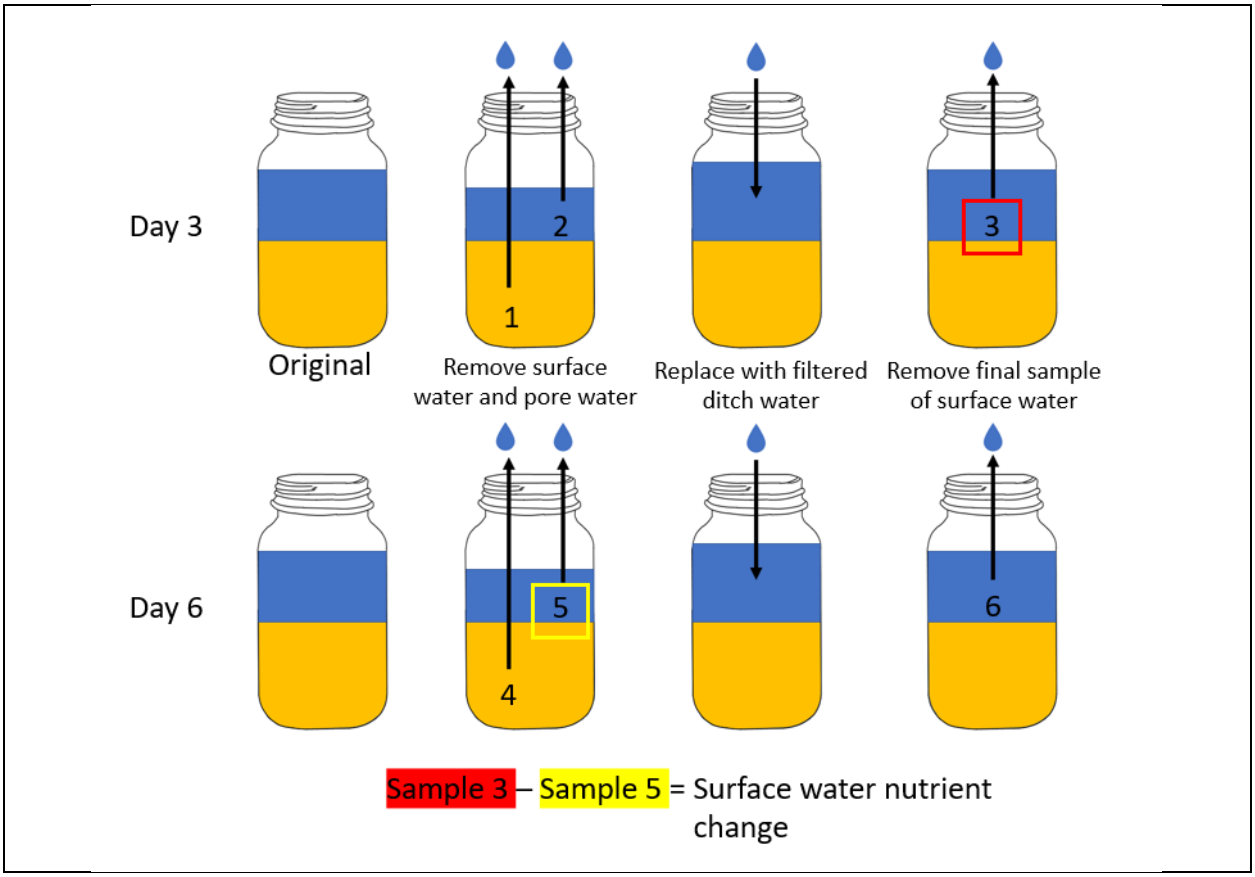


Figure 7. Water sampling and surface water calculation methods for bioturbation experiment.

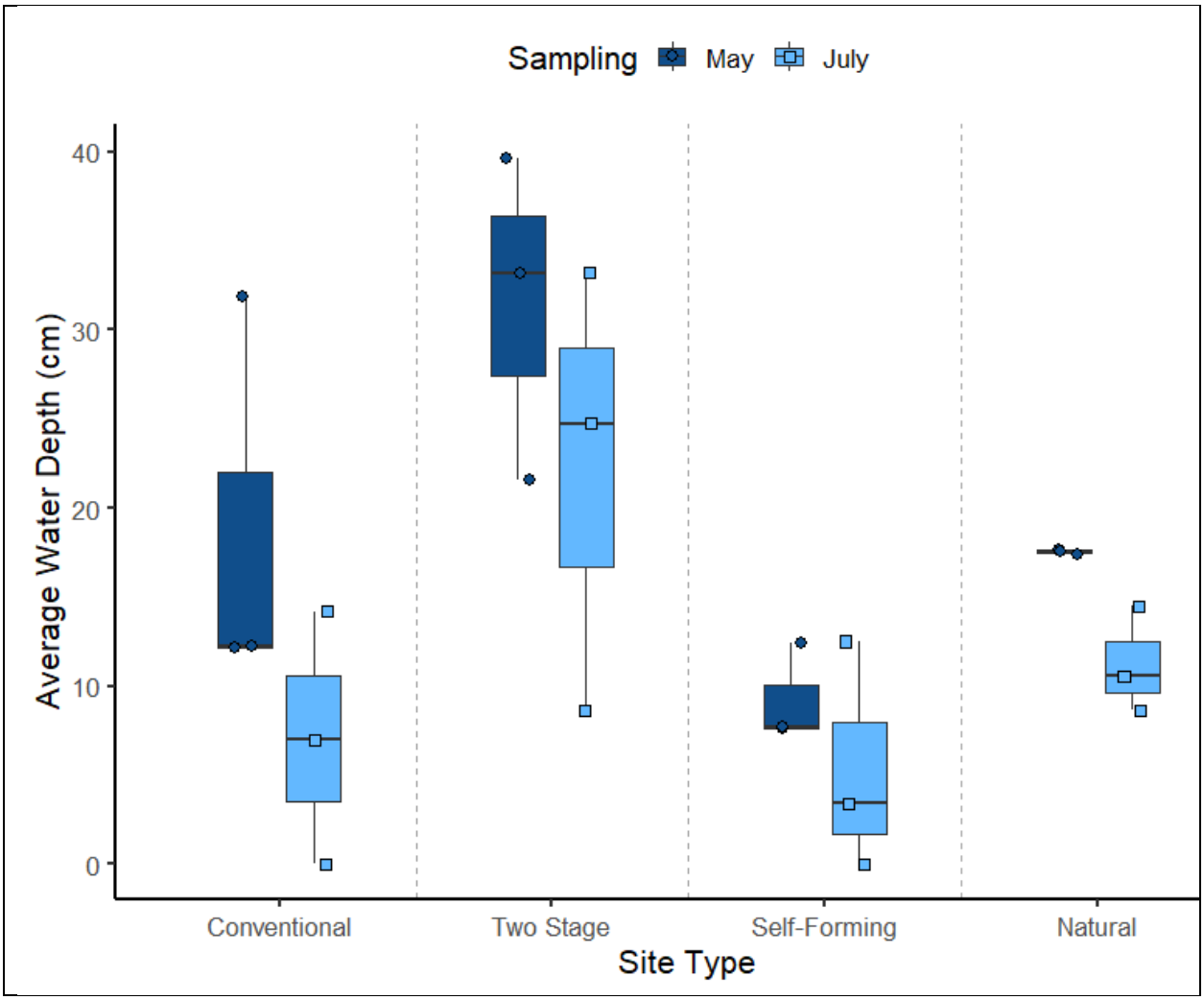


Figure 8. Average depth of channel water in centimeters in May and July for all sites

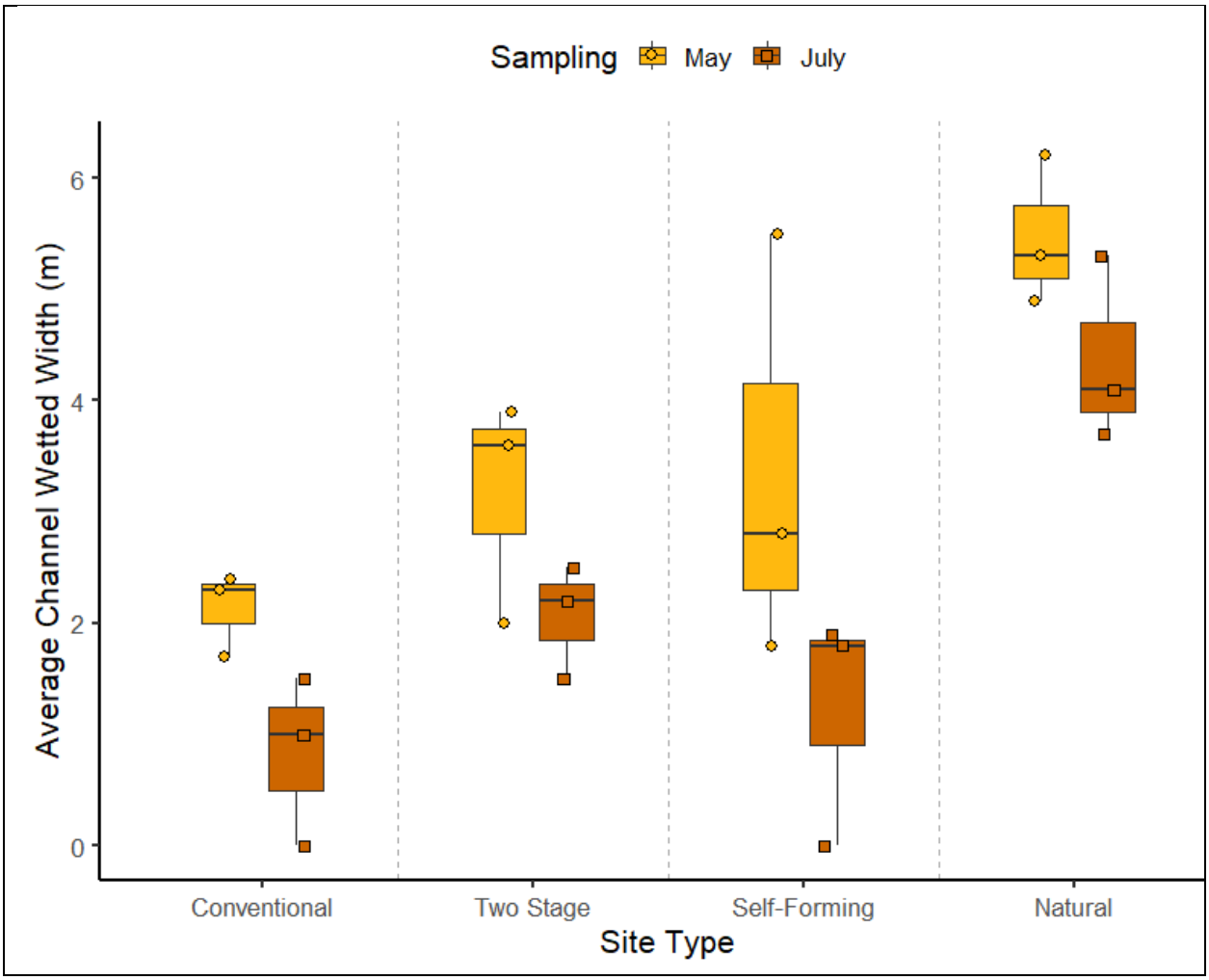


Figure 9. Average wetted width of the channel of each site type in May and July.

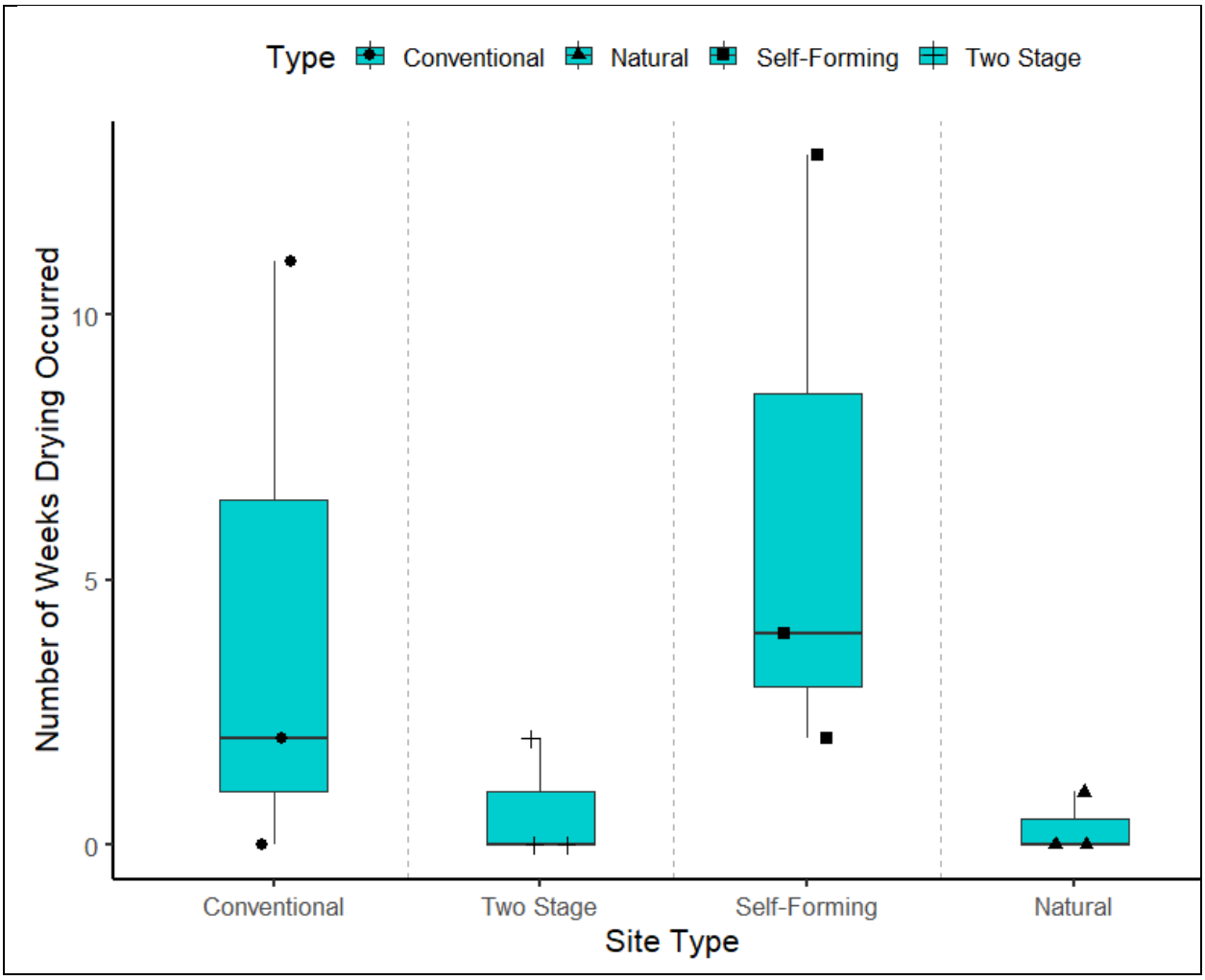


Figure 10. Number of weeks between May and September where a drying even occurred at each site type.

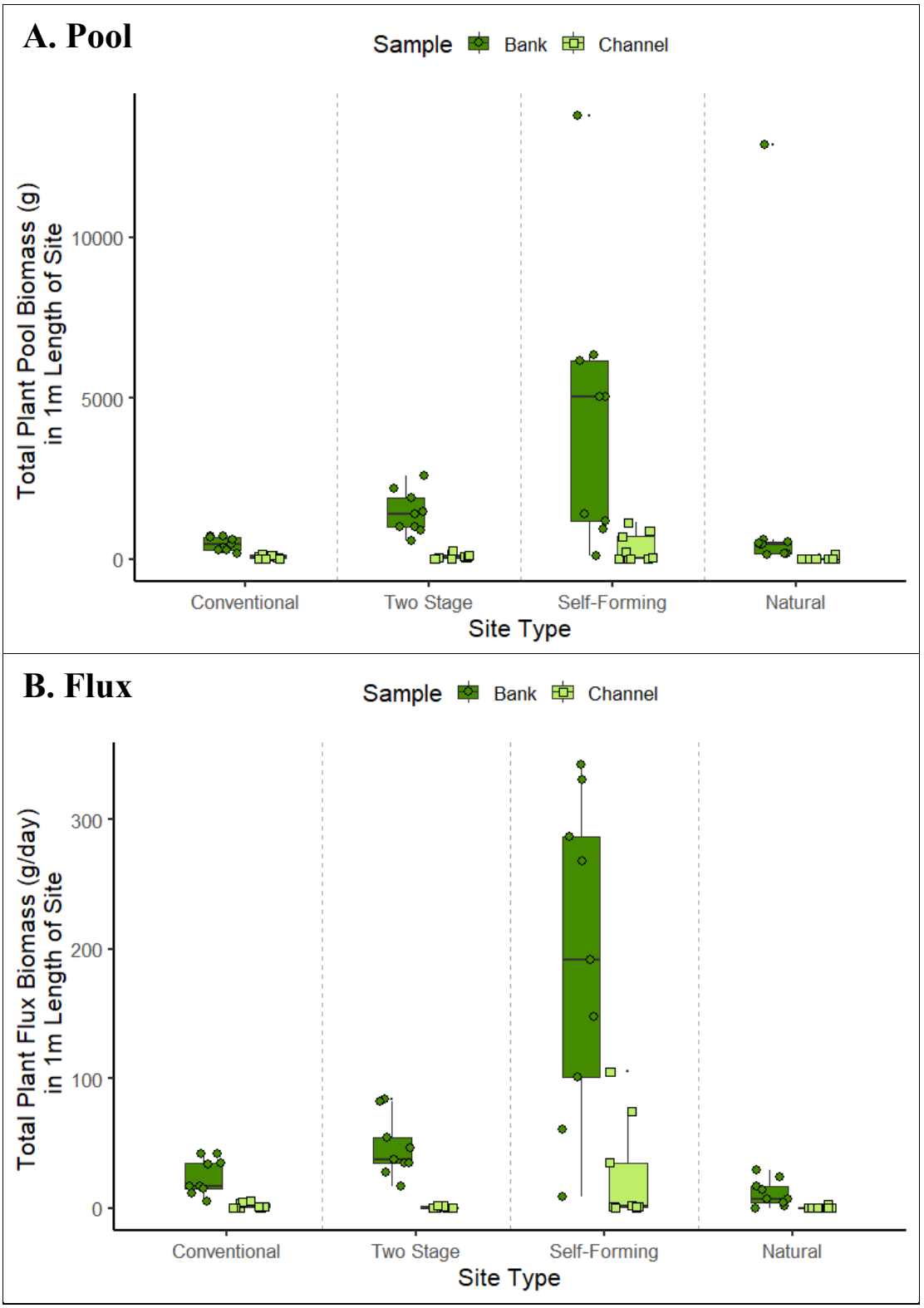


Figure 11. Biomass of plants collected. The biomass flux was collected in July and calculated to biomass gained per day from the first sampling.

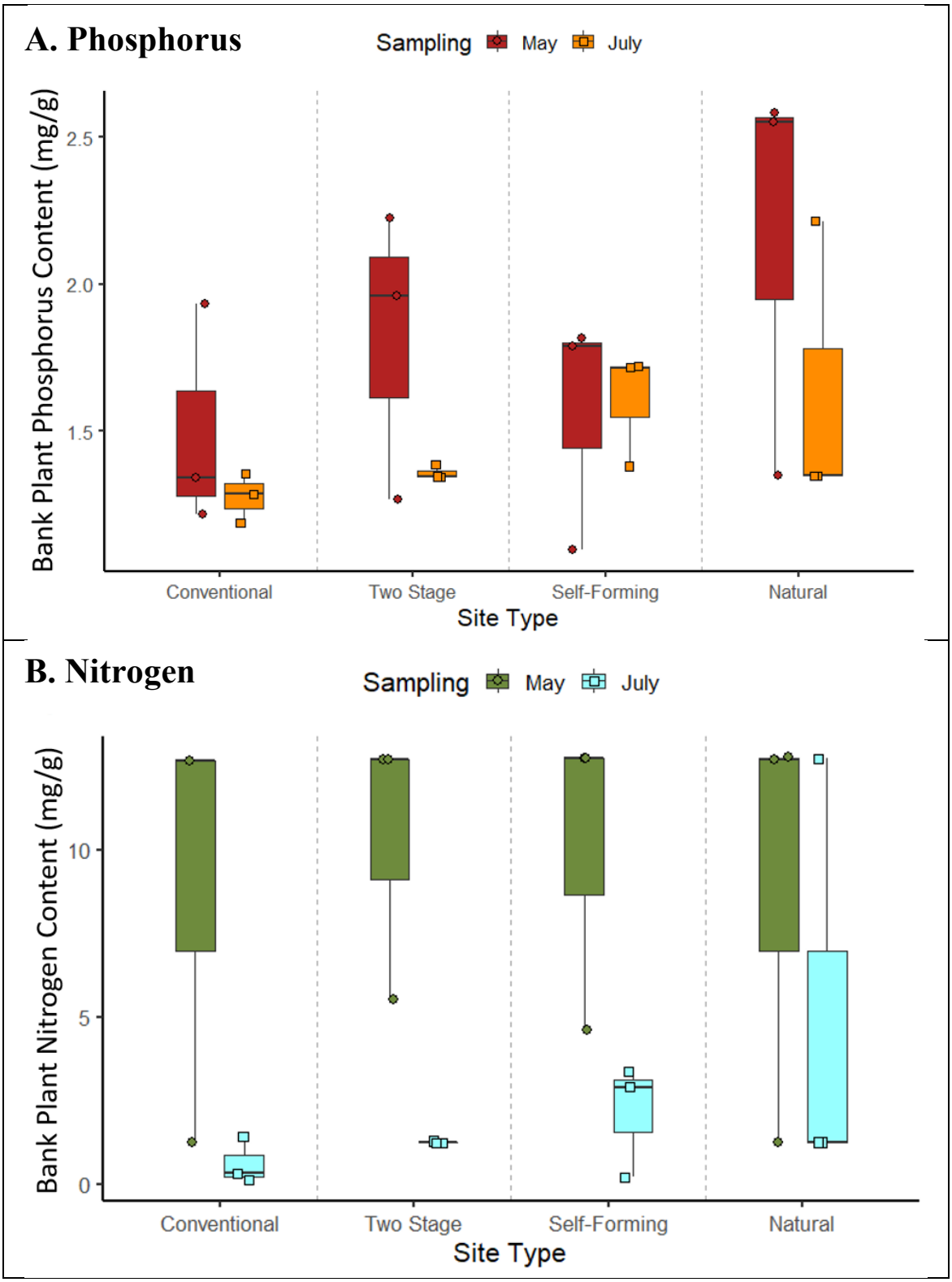


Figure 12. Bank plant phosphorus and nitrogen content in milligrams of nutrient per gram of tissue in May and July.

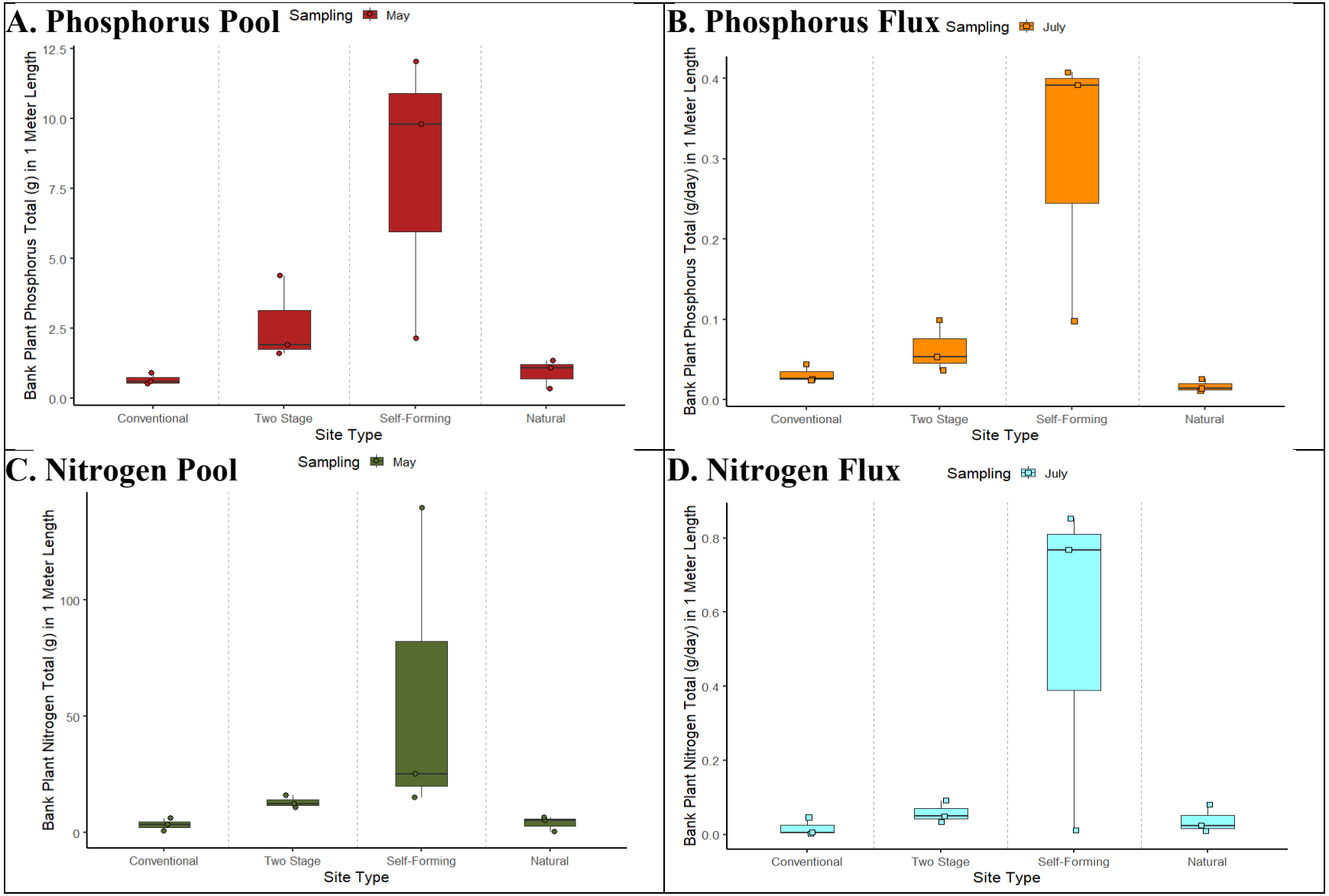


Figure 13. Phosphorus and nitrogen totals (g/meter length) of plants collected from banks.

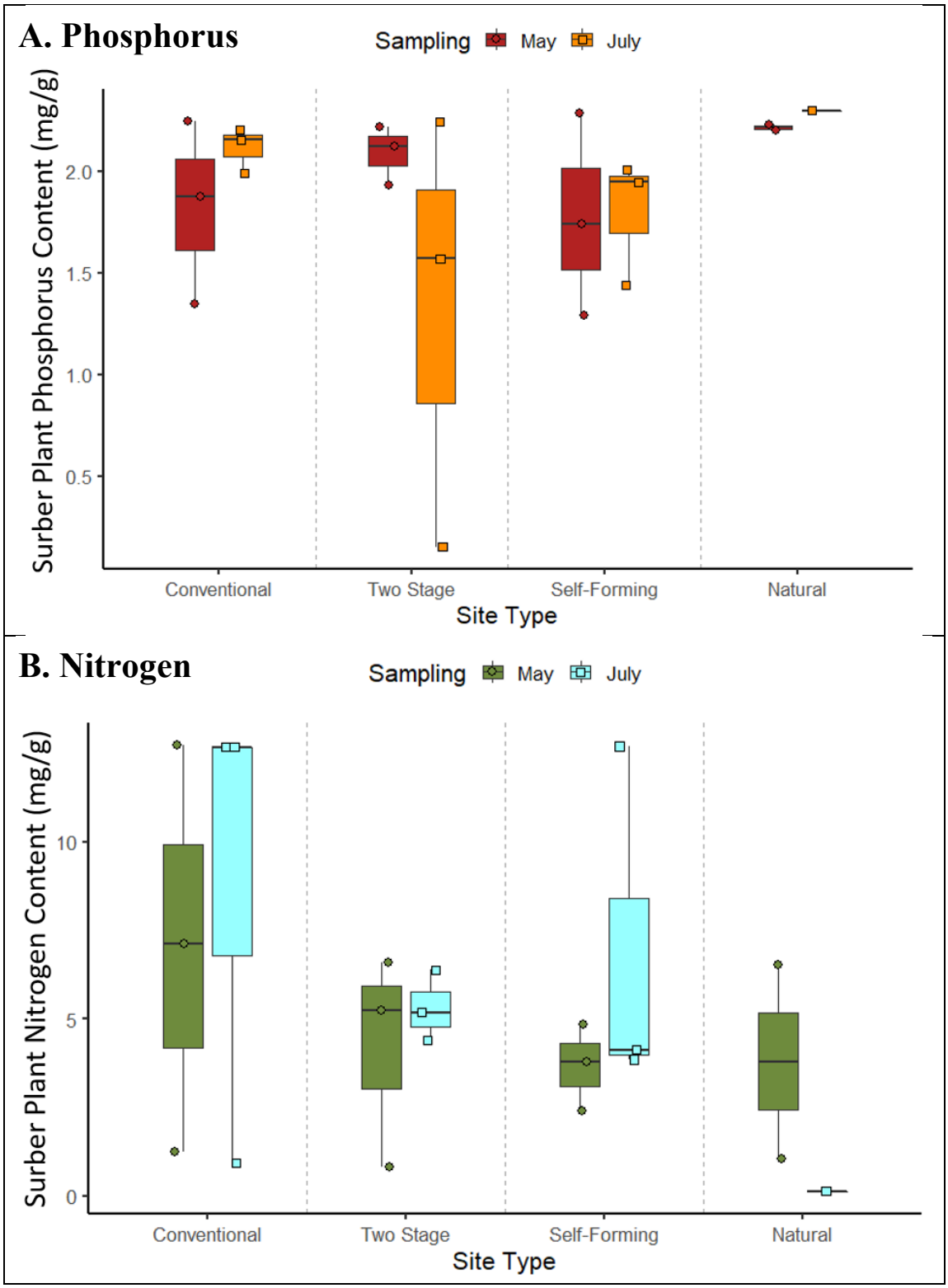


Figure 14. Bank plant phosphorus and nitrogen content in milligrams of nutrient per gram of tissue in May and July.

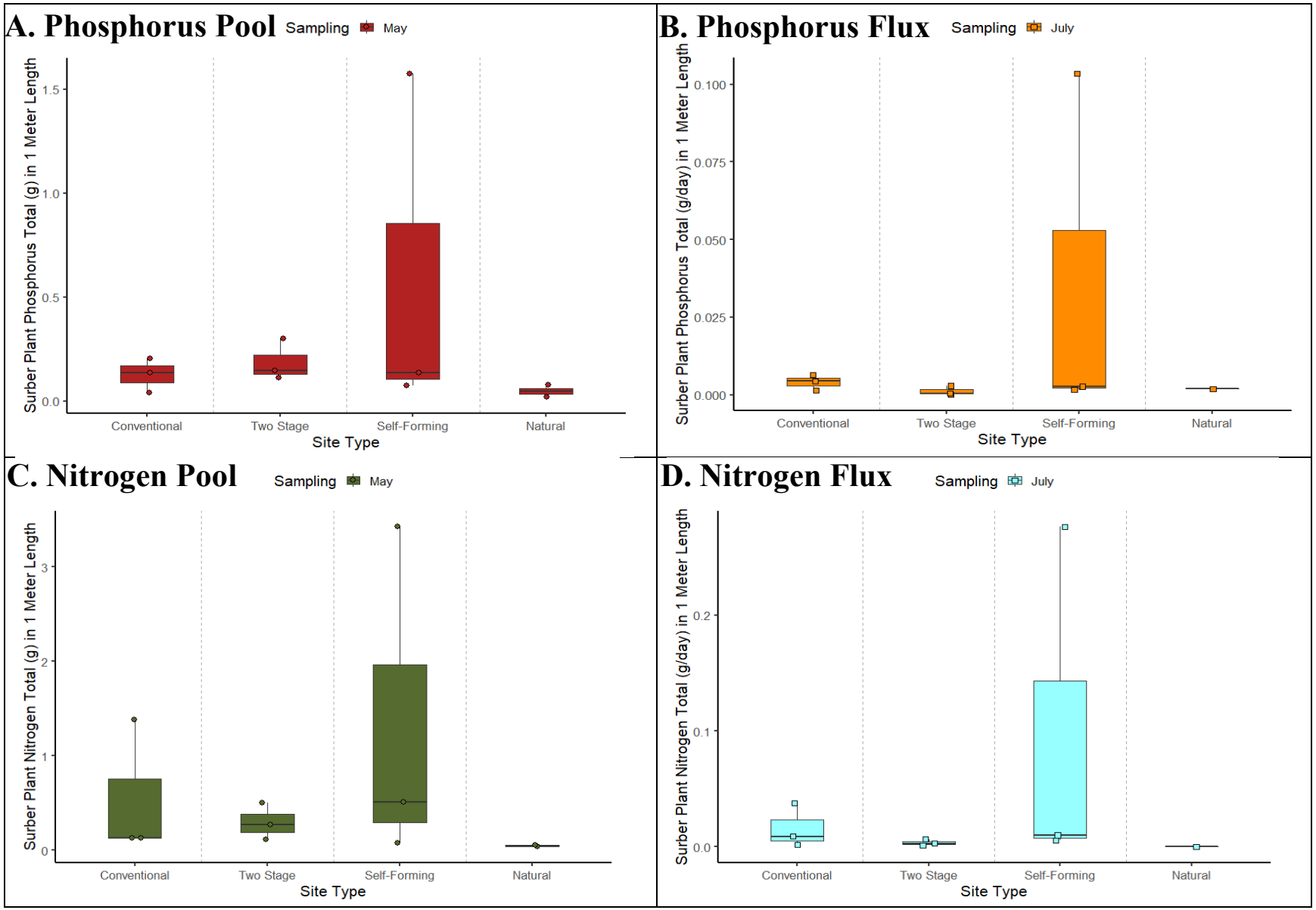


Figure 15. Phosphorus and nitrogen totals (g/meter length) of plants collected from channels.

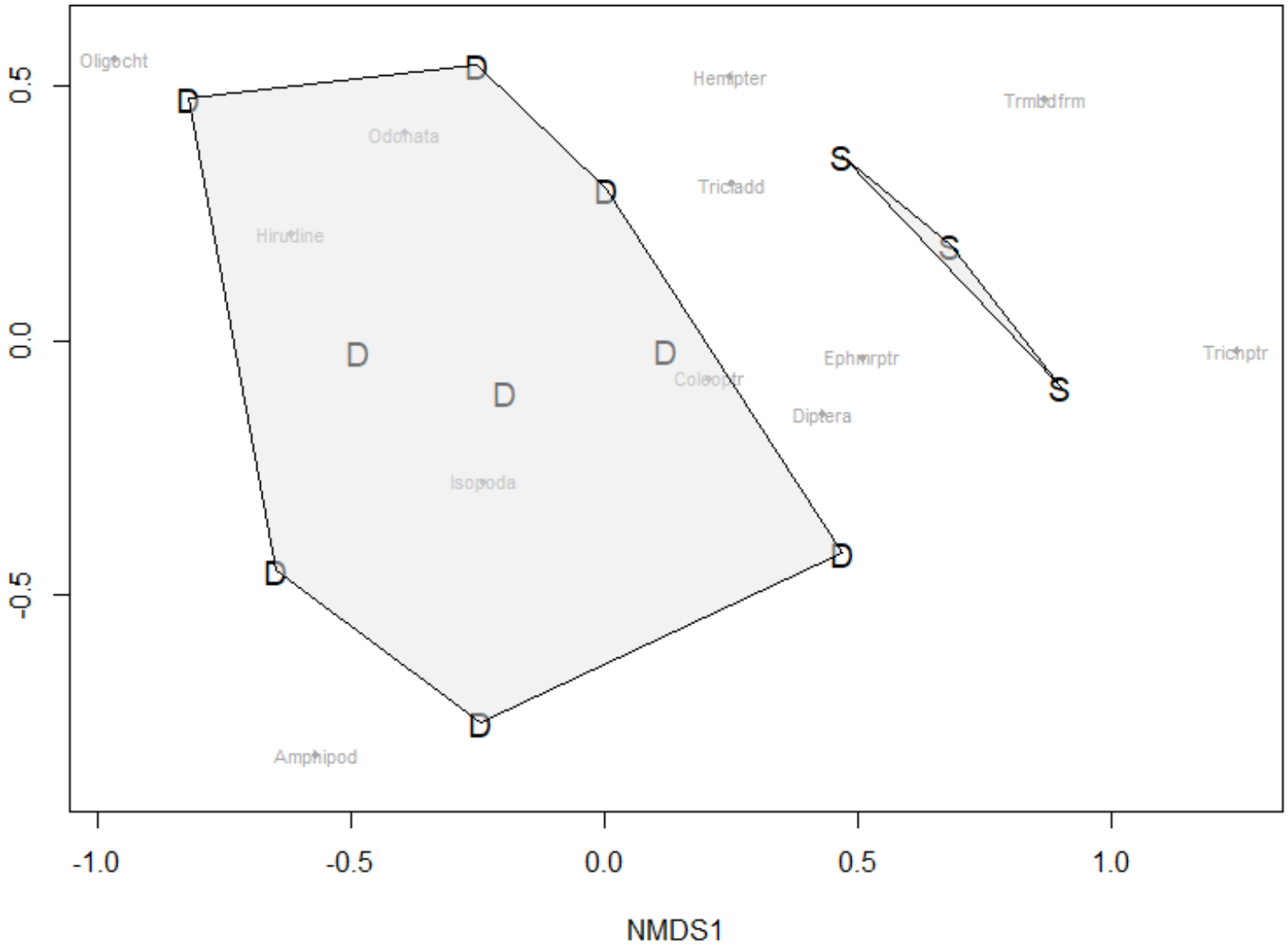


Figure 16. Community composition differences between ditch sites (D) and natural stream sites (S).

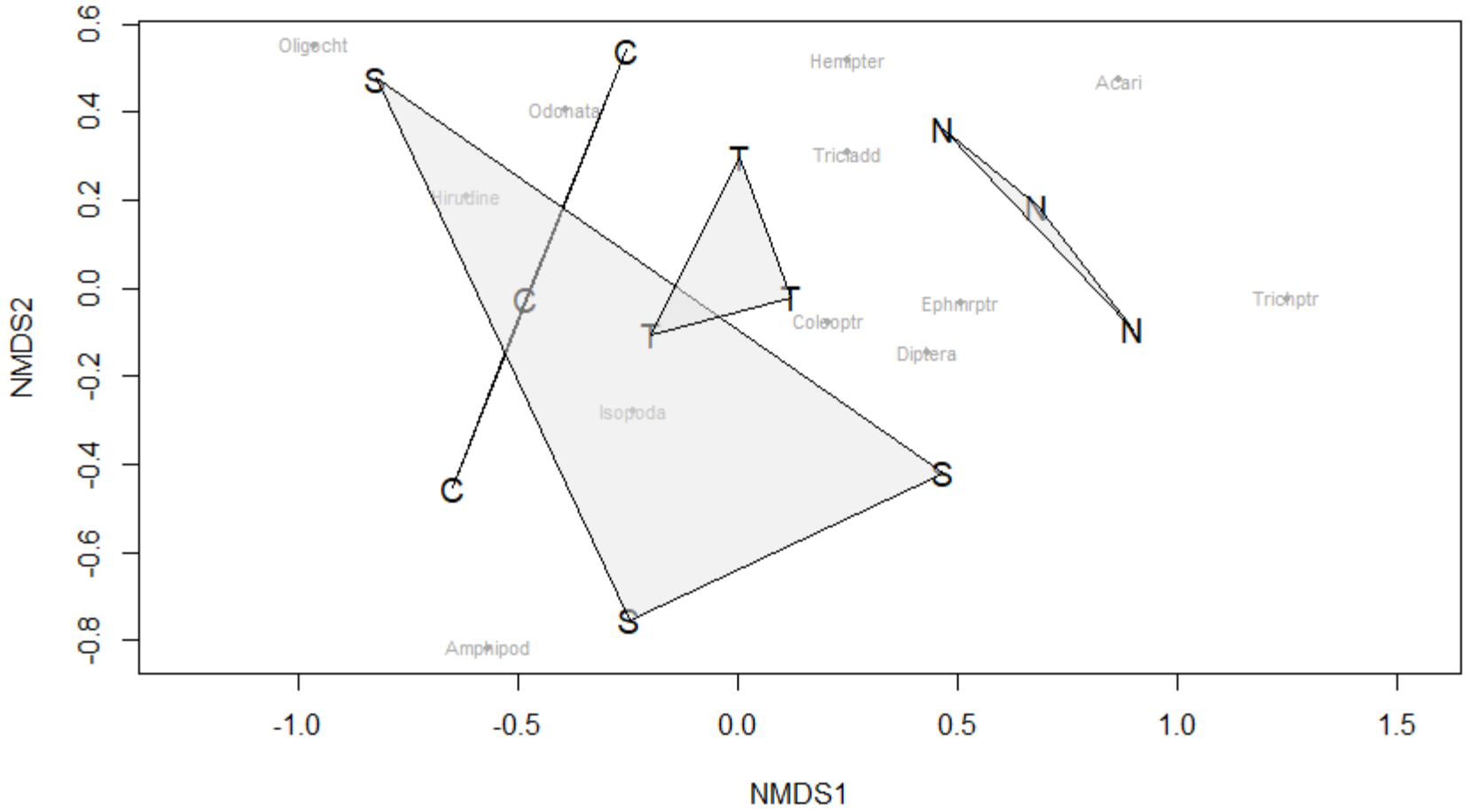


Figure 17. Community composition differences between conventional ditches (C), two stage ditches (T), self-forming streams (S) and natural streams (N).

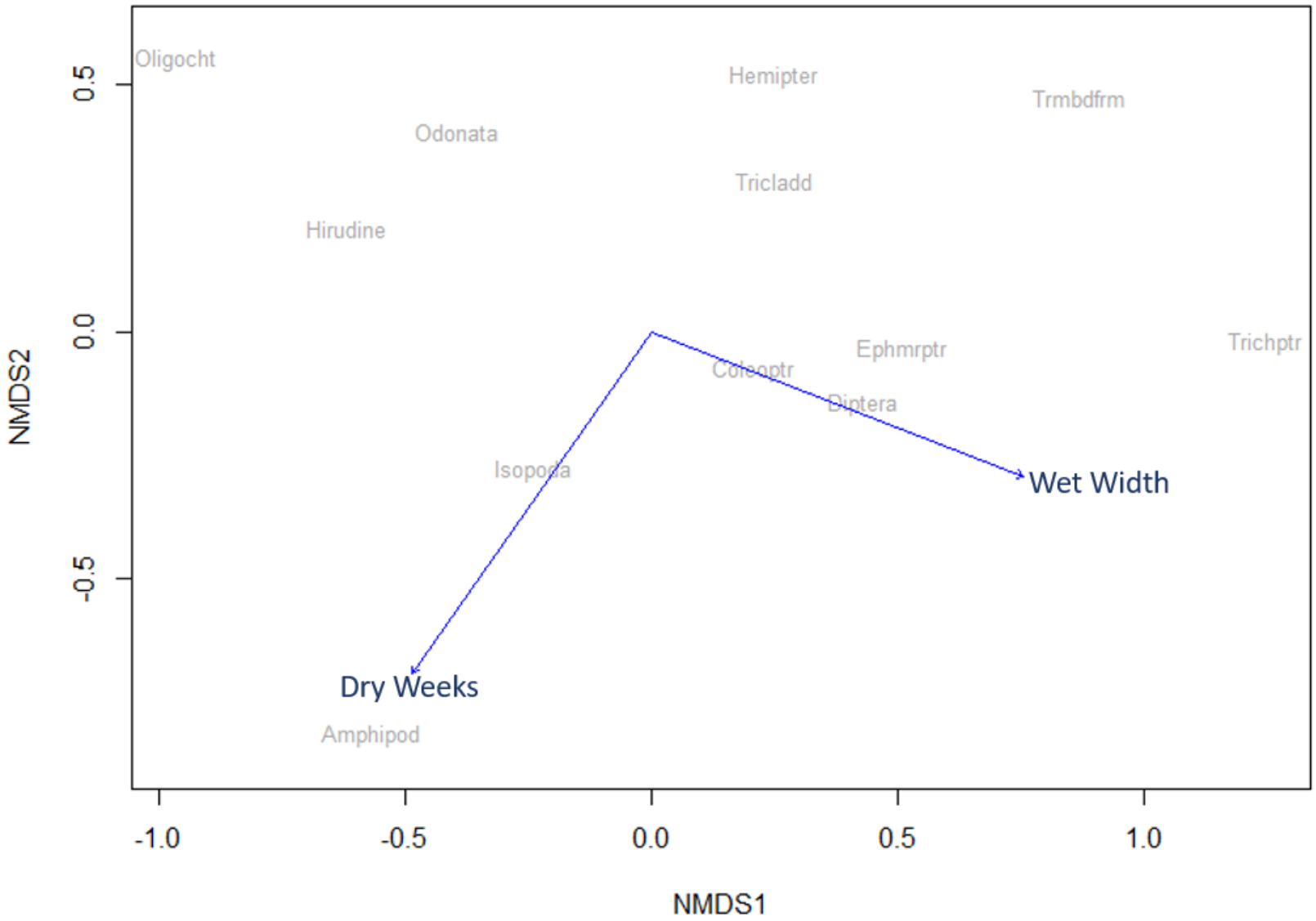


Figure 18. Aquatic invertebrate community composition with significant associations of dry weeks and wet width

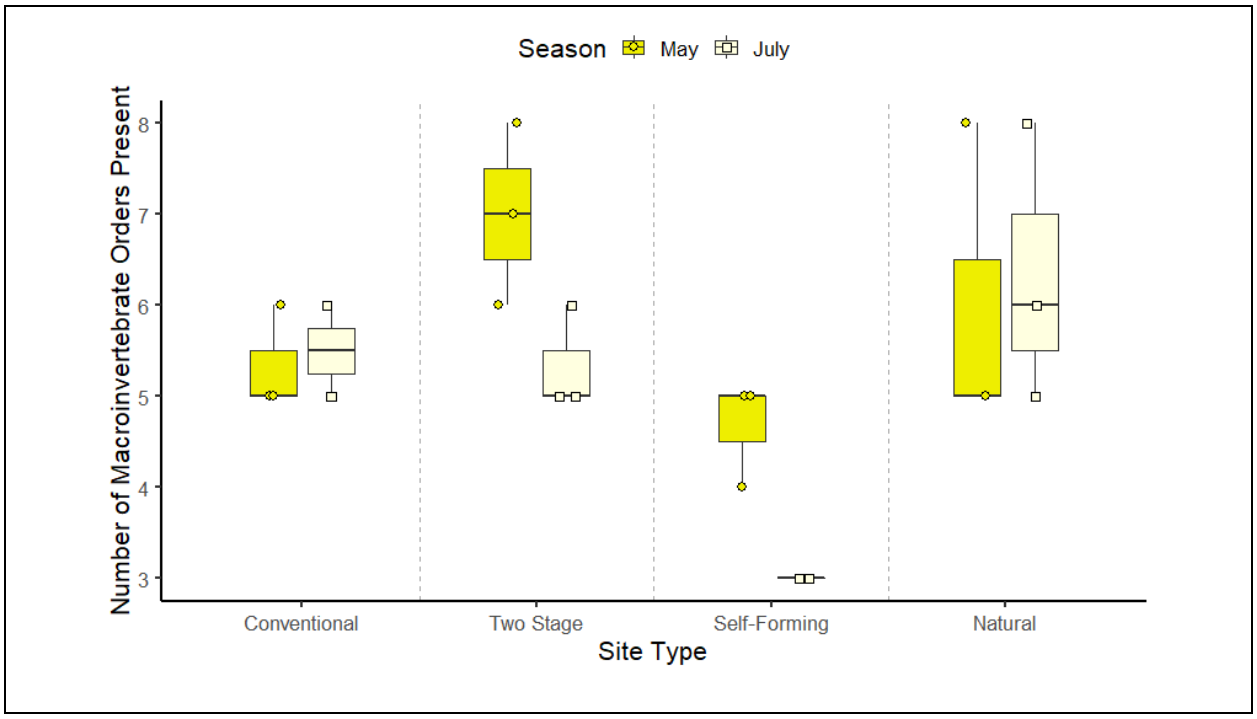


Figure 19. The total number of macroinvertebrate orders, excluding mollusks, found in surber samples in May and July.

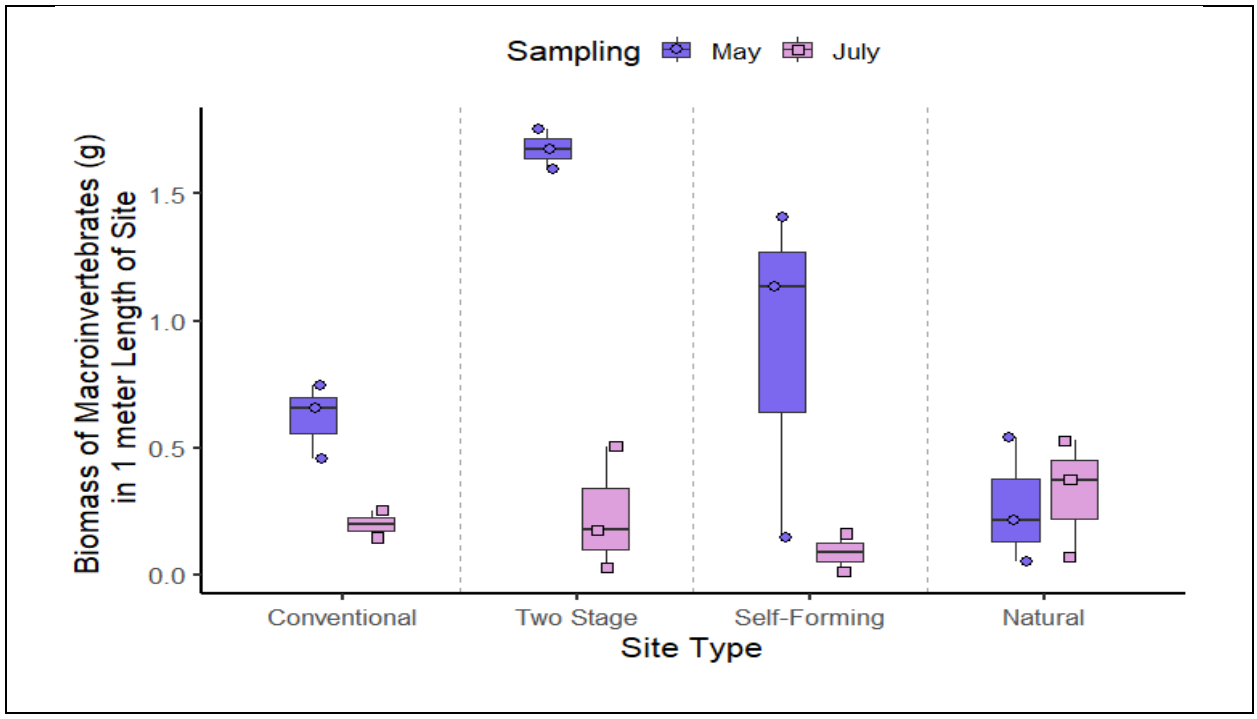


Figure 20. Total biomass of macroinvertebrates, excluding mollusks and crayfish, found in surber samples in May and July.

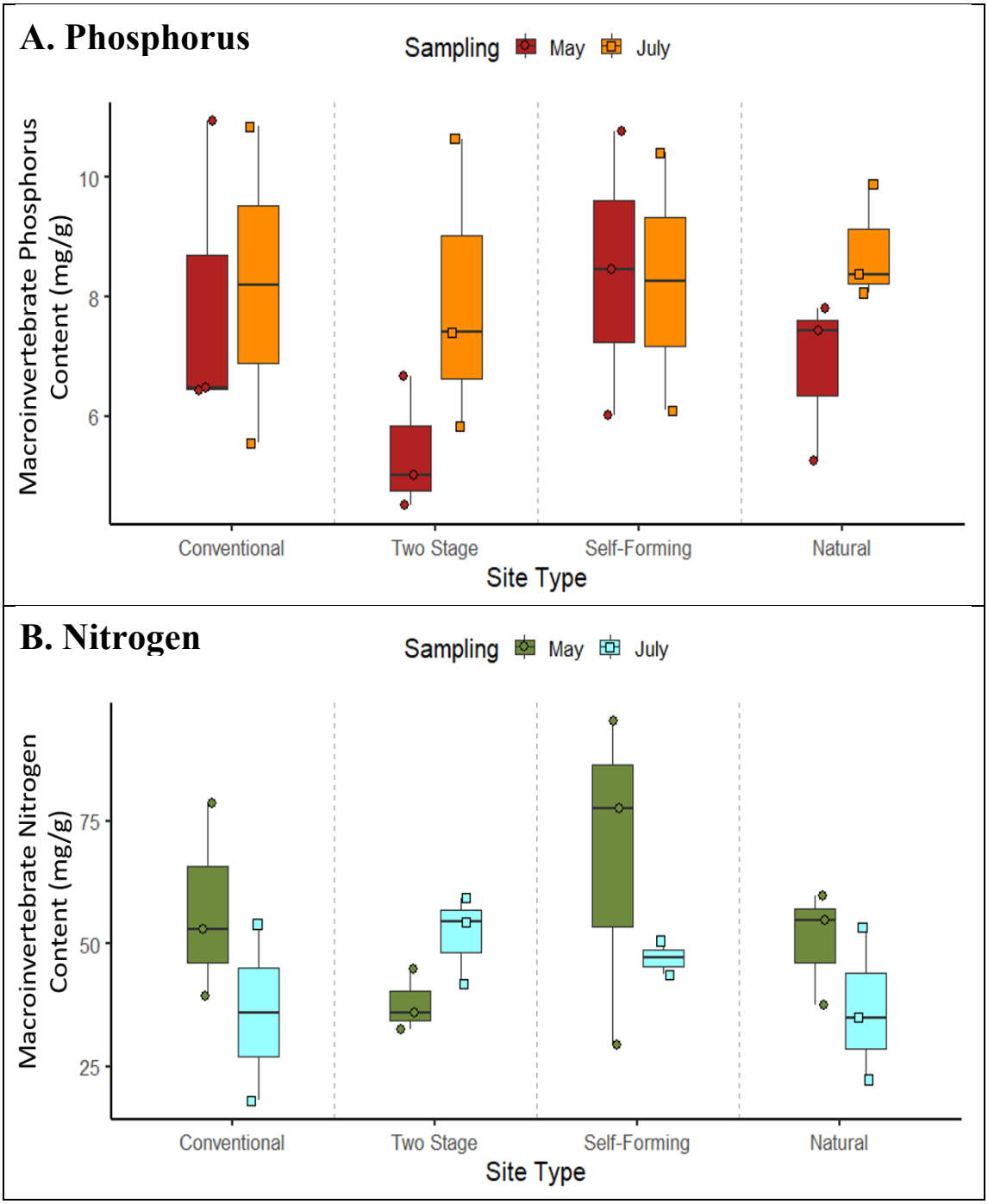


Figure 21. Macroinvertebrate phosphorus and nitrogen content in milligrams of nutrients per gram of tissue in May and July.

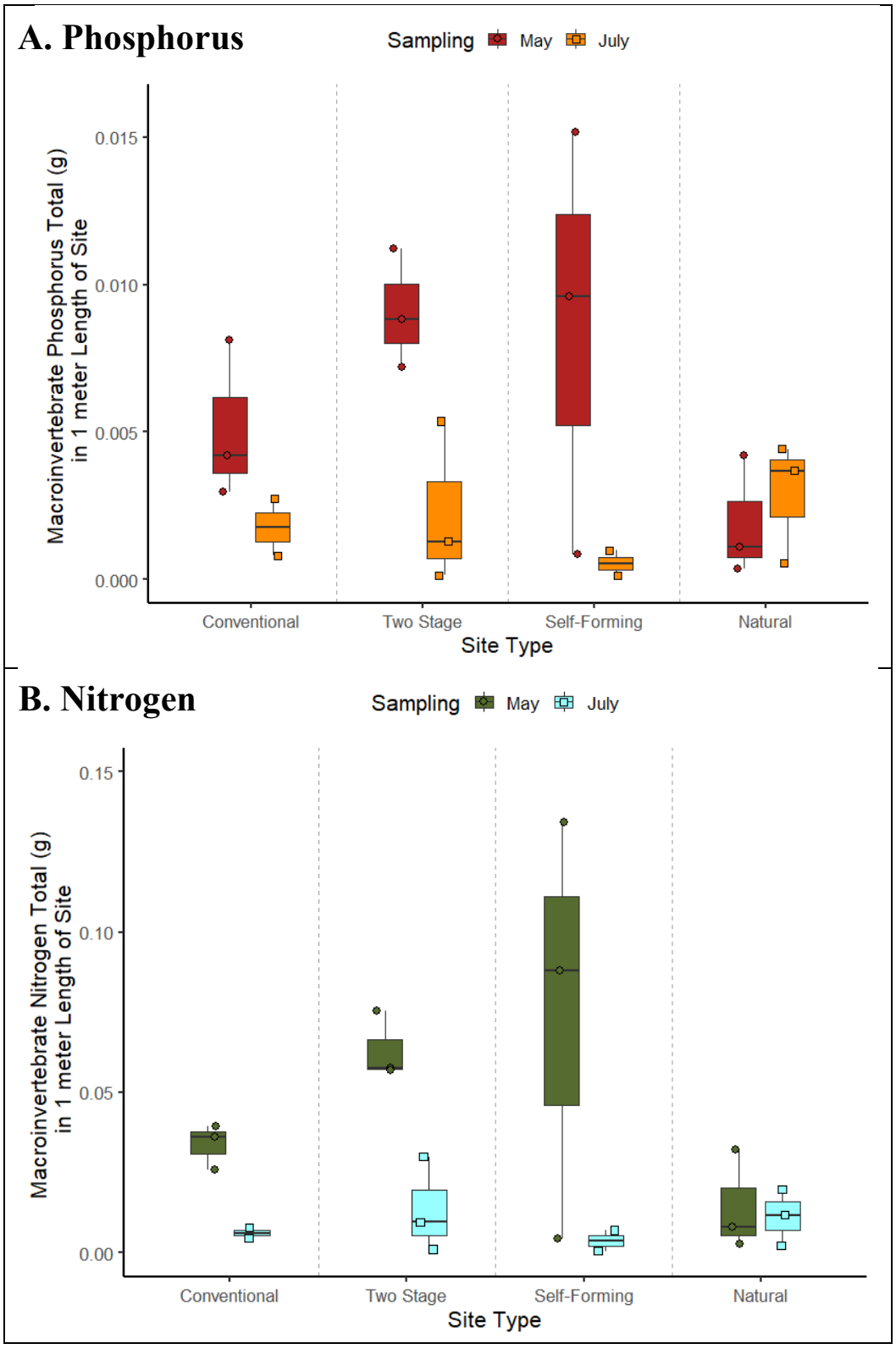


Figure 22. D-Net Macroinvertebrate nutrient content within 1 meter of ditch length.

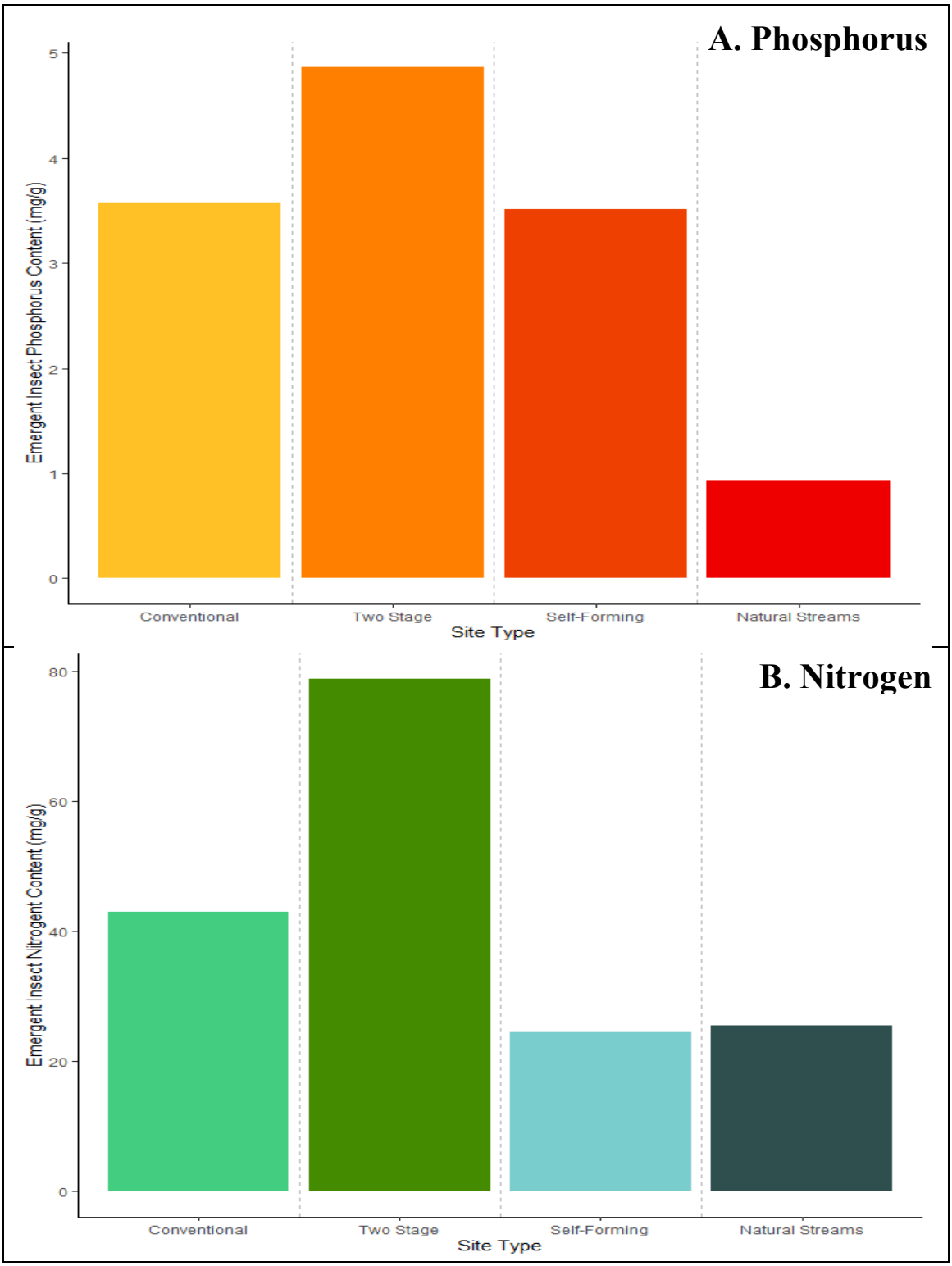


Figure 23. Average emergent insect phosphorus and nitrogen content milligrams of nutrient per gram of tissue over the sampling period

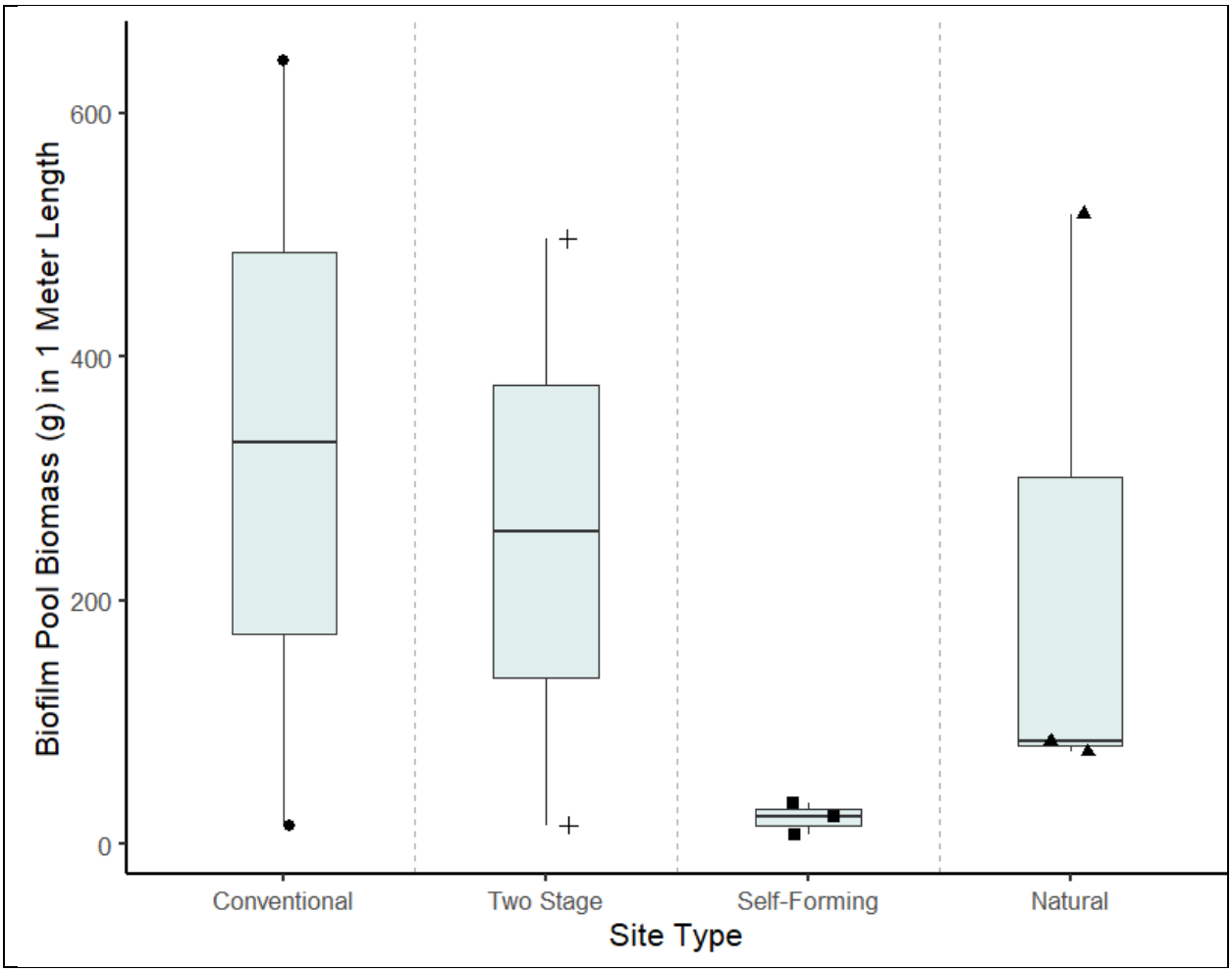


Figure 24. Average biomass of biofilm accumulated 1-meter length of ditch at each site type. This is assuming all sediment surface is covered in biofilm.

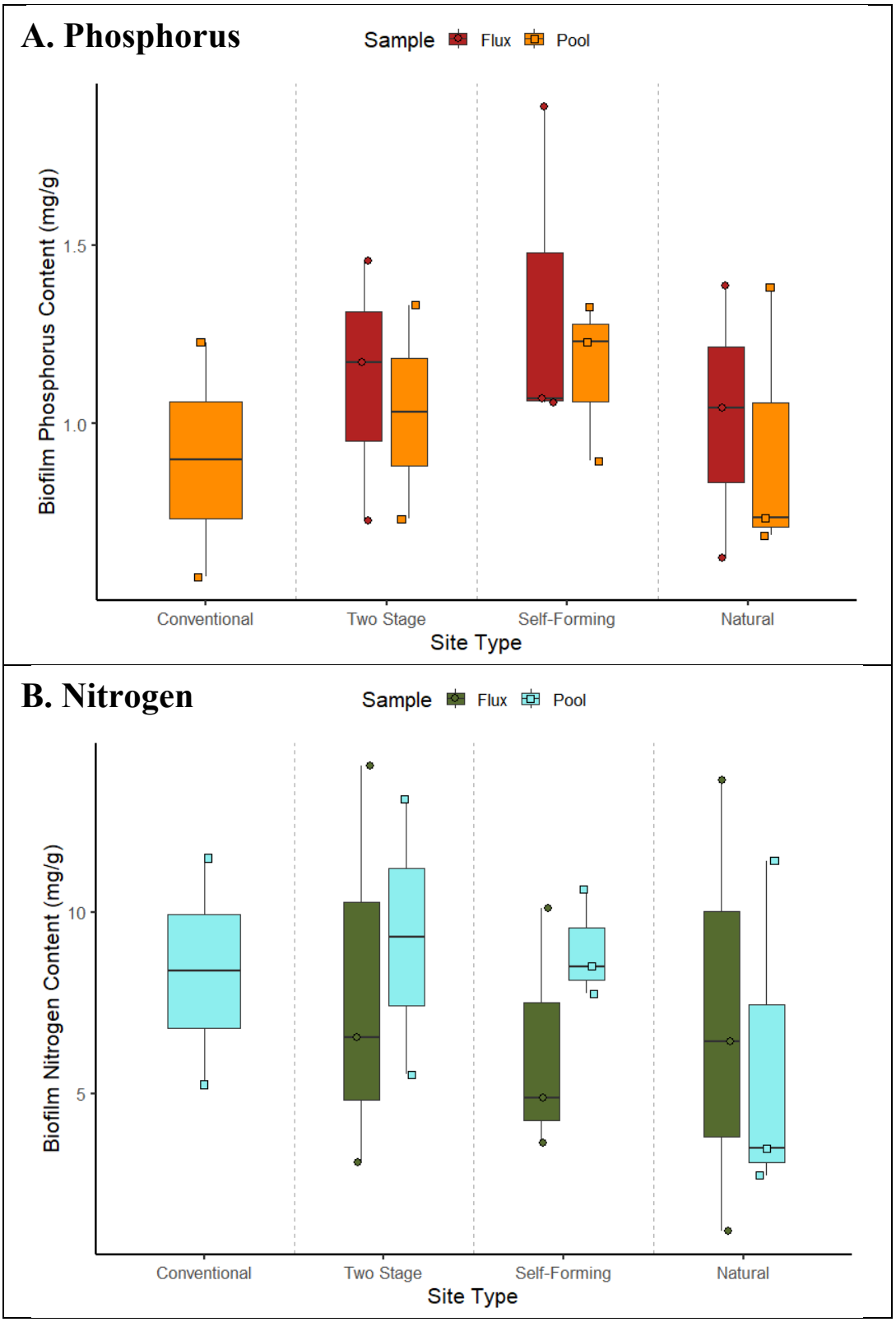


Figure 25. Biofilm phosphorus and nitrogen content in milligrams of nutrients per gram of tissue in May and July.

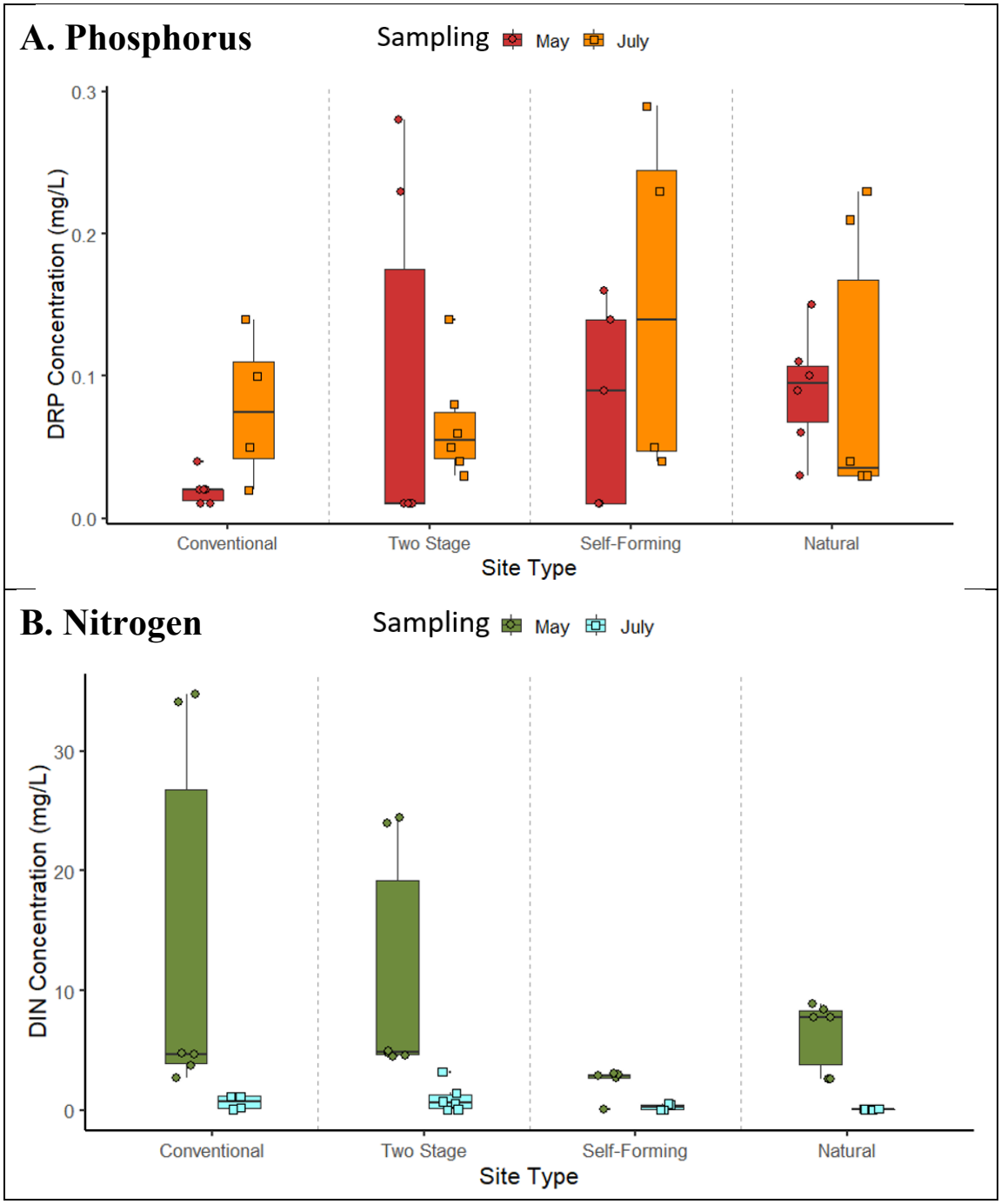


Figure 26. The average nutrient concentrations in surface water in May and July of conventional ditches, two stage ditches, self-forming streams, and natural streams.

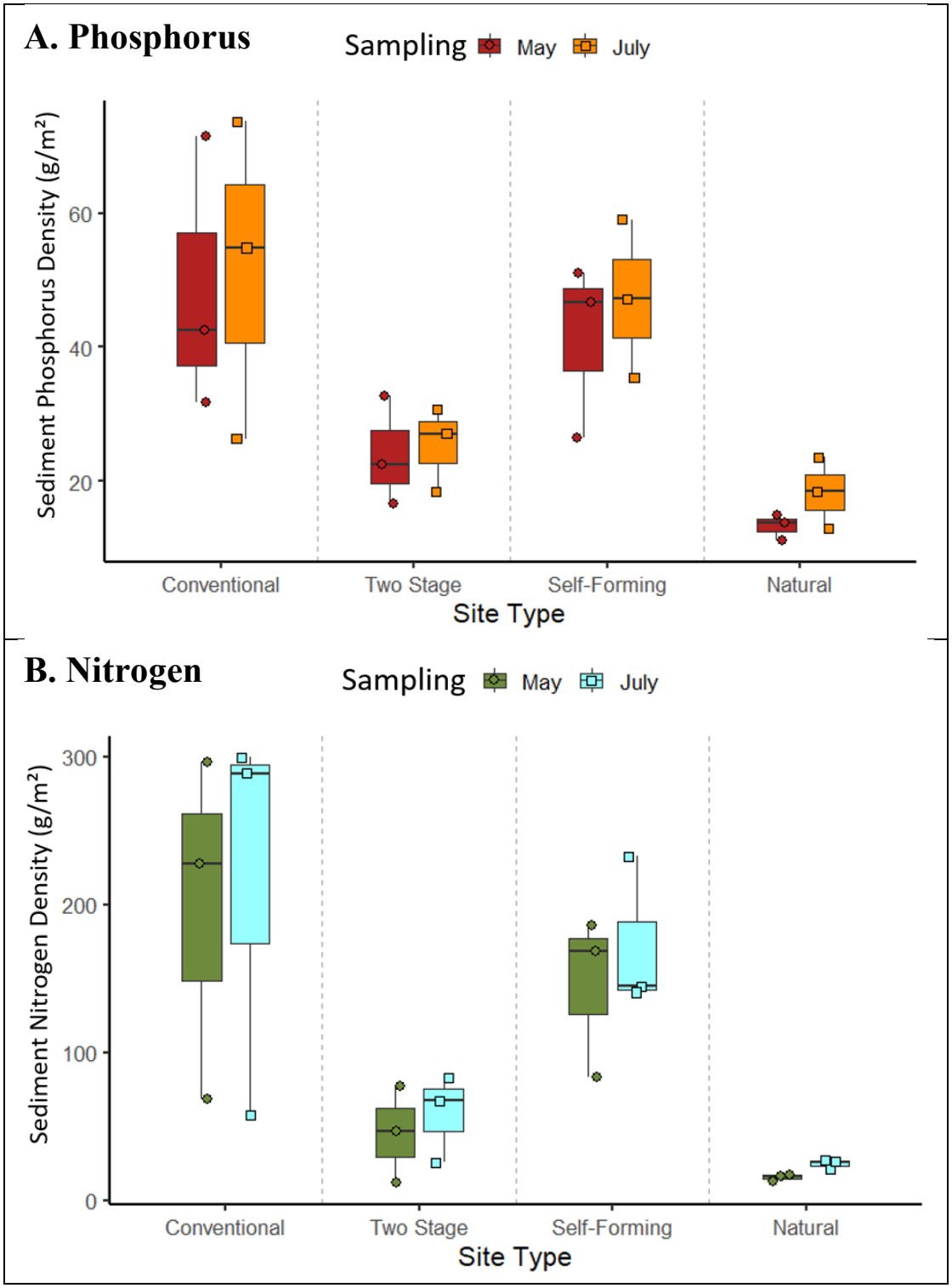


Figure 27. Sediment nutrient densities (g/m²) at each ditch type in May and July.

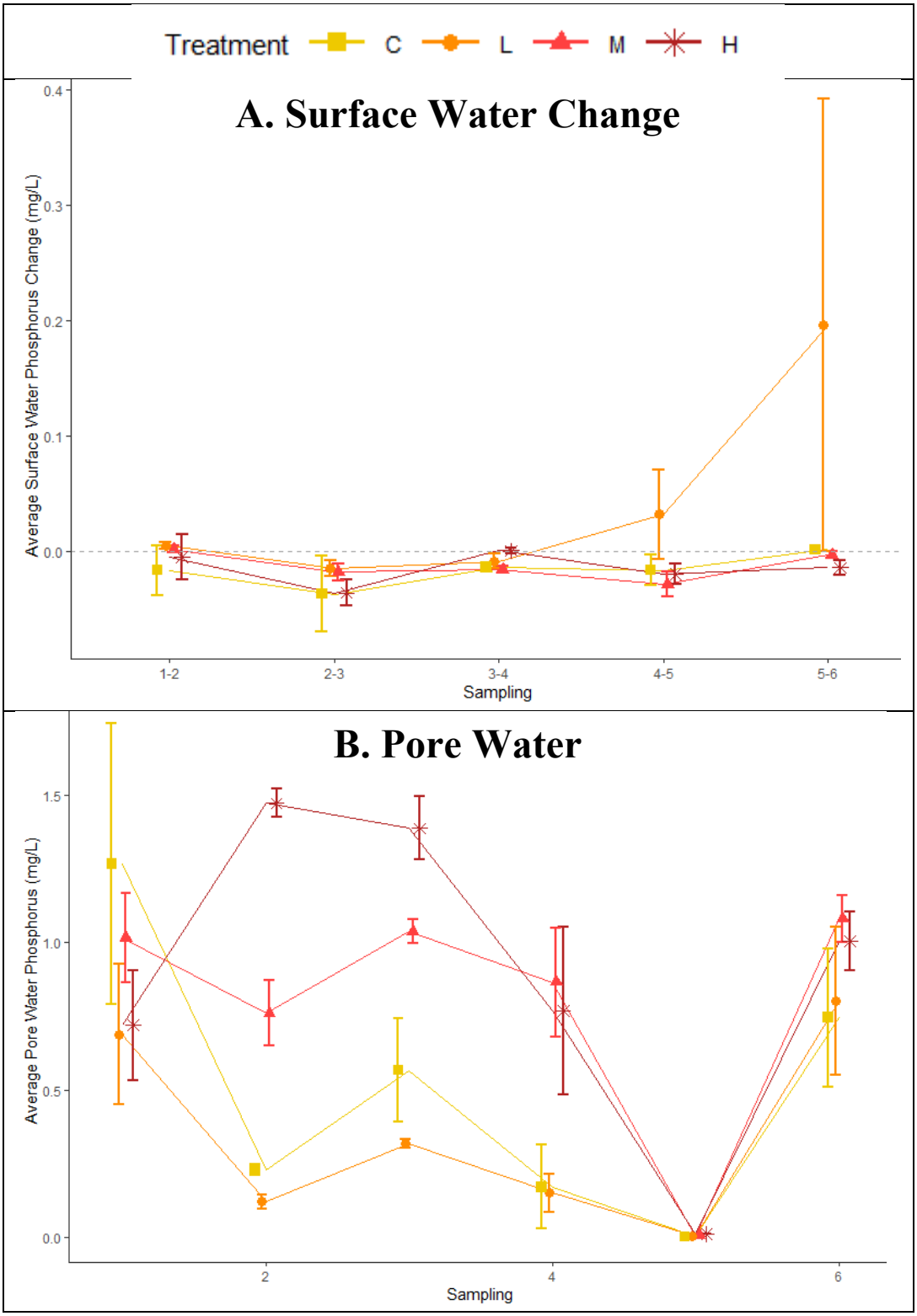


Figure 28. Phosphorus change (mg/L) in the surface water between sampling and phosphorus content at each sampling of the pore water of the bioturbation experiment.

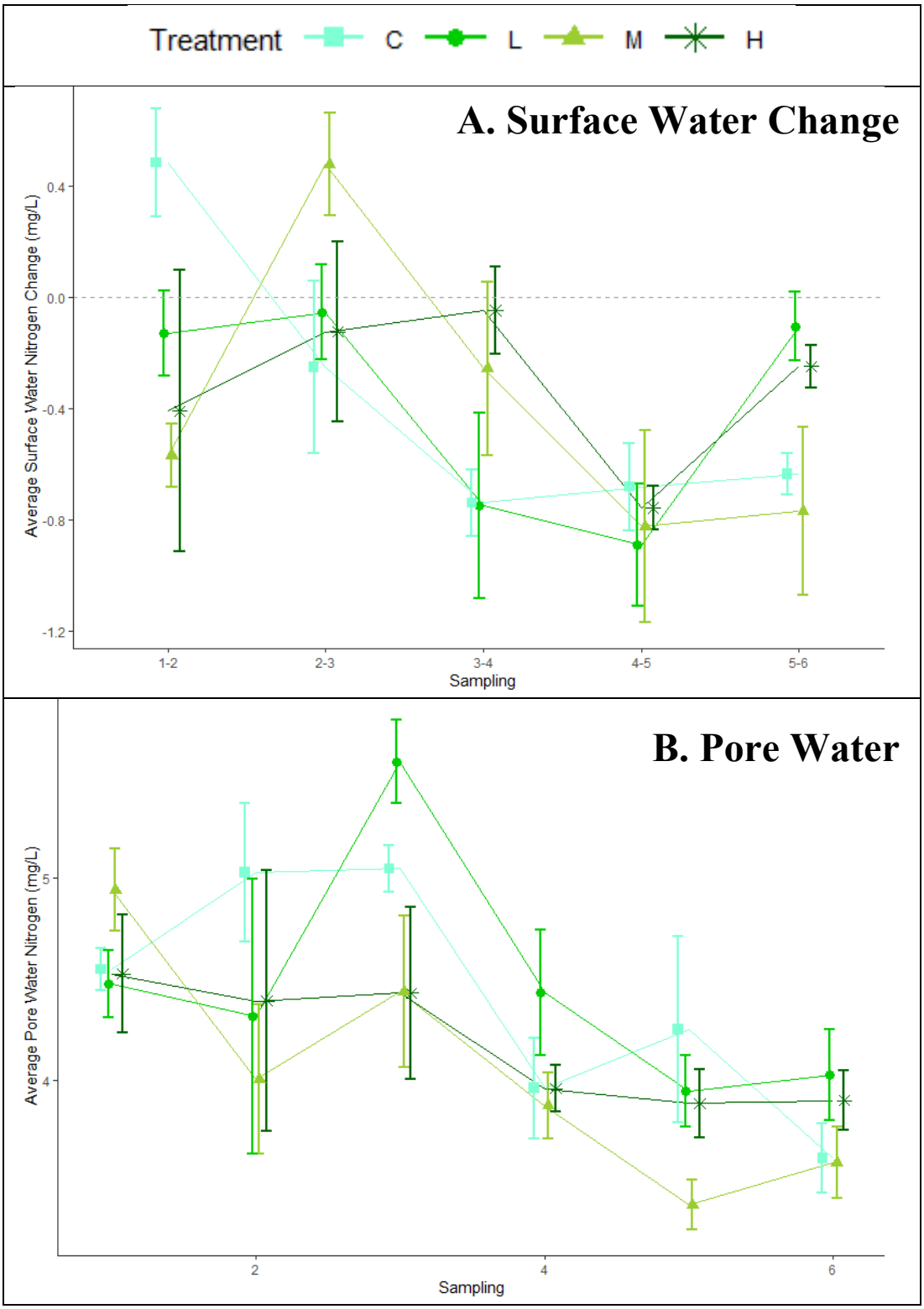


Figure 29. Nitrogen change (mg/L) in the surface water between sampling and nitrogen content at each sampling of the pore water of the bioturbation experiment.

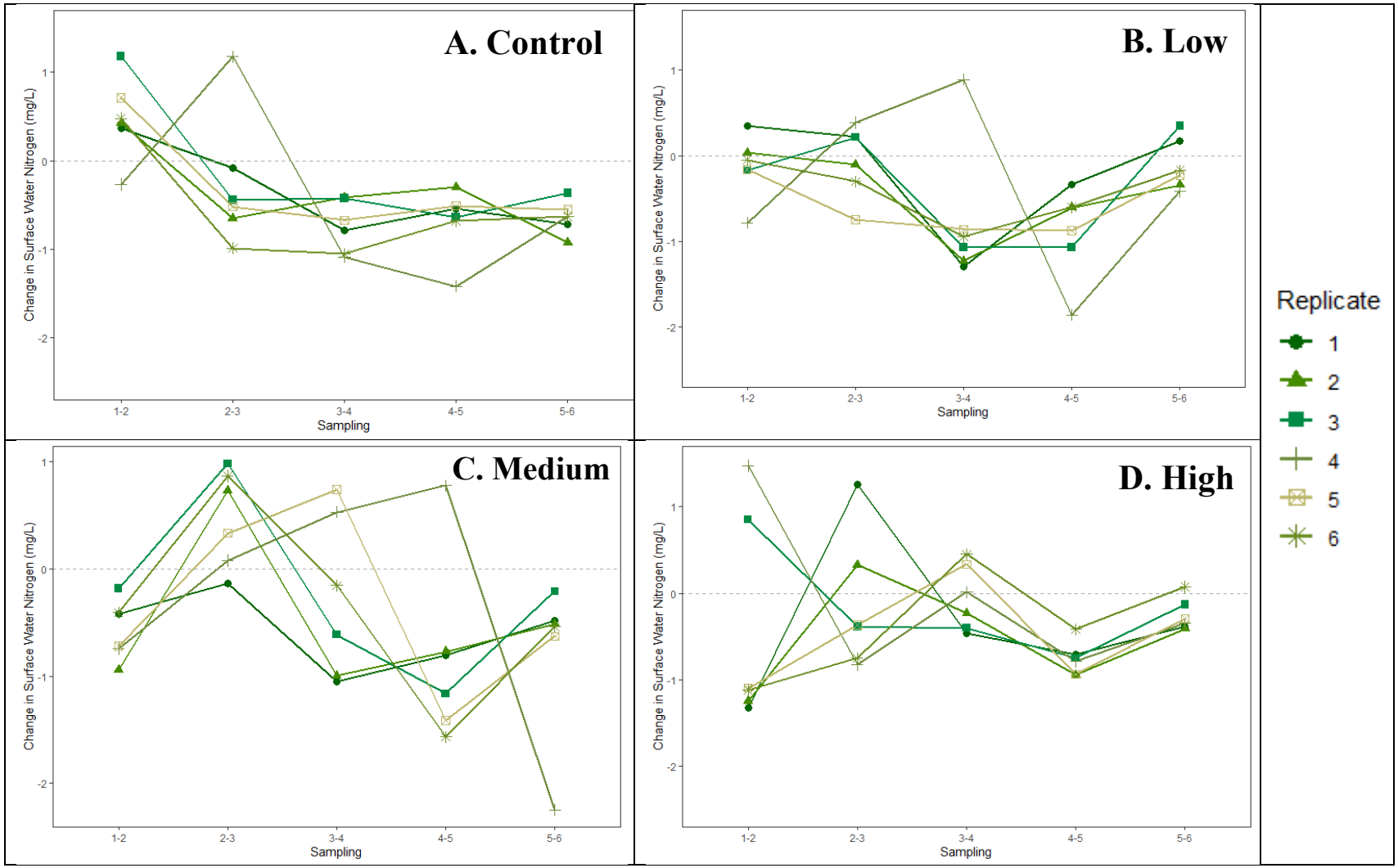


Figure 30. Change in surface water nitrogen (mg/L) between each sampling throughout the bioturbation experiment.

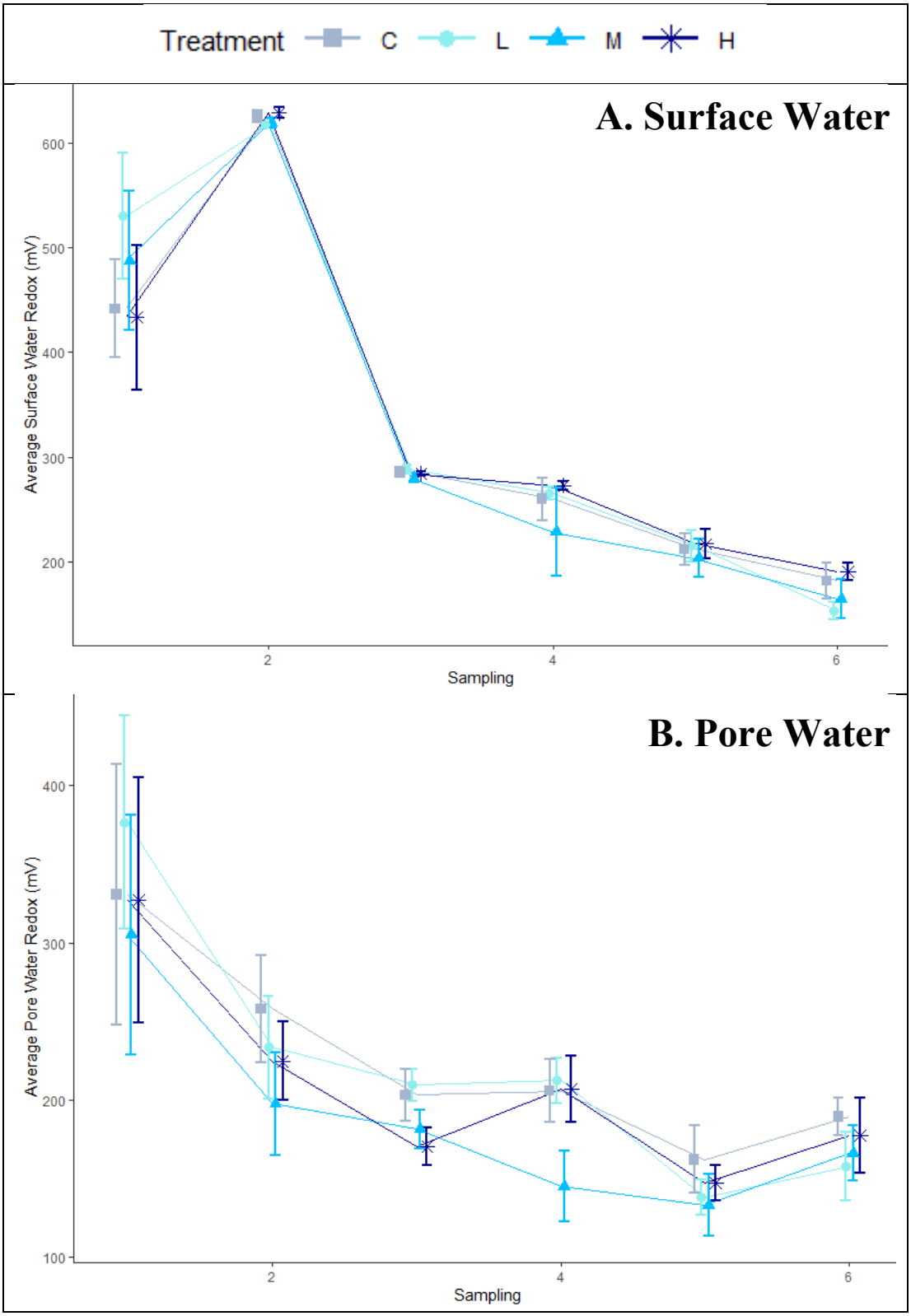


Figure 31. Redox in the surface and pore water at each sampling of the bioturbation experiment.

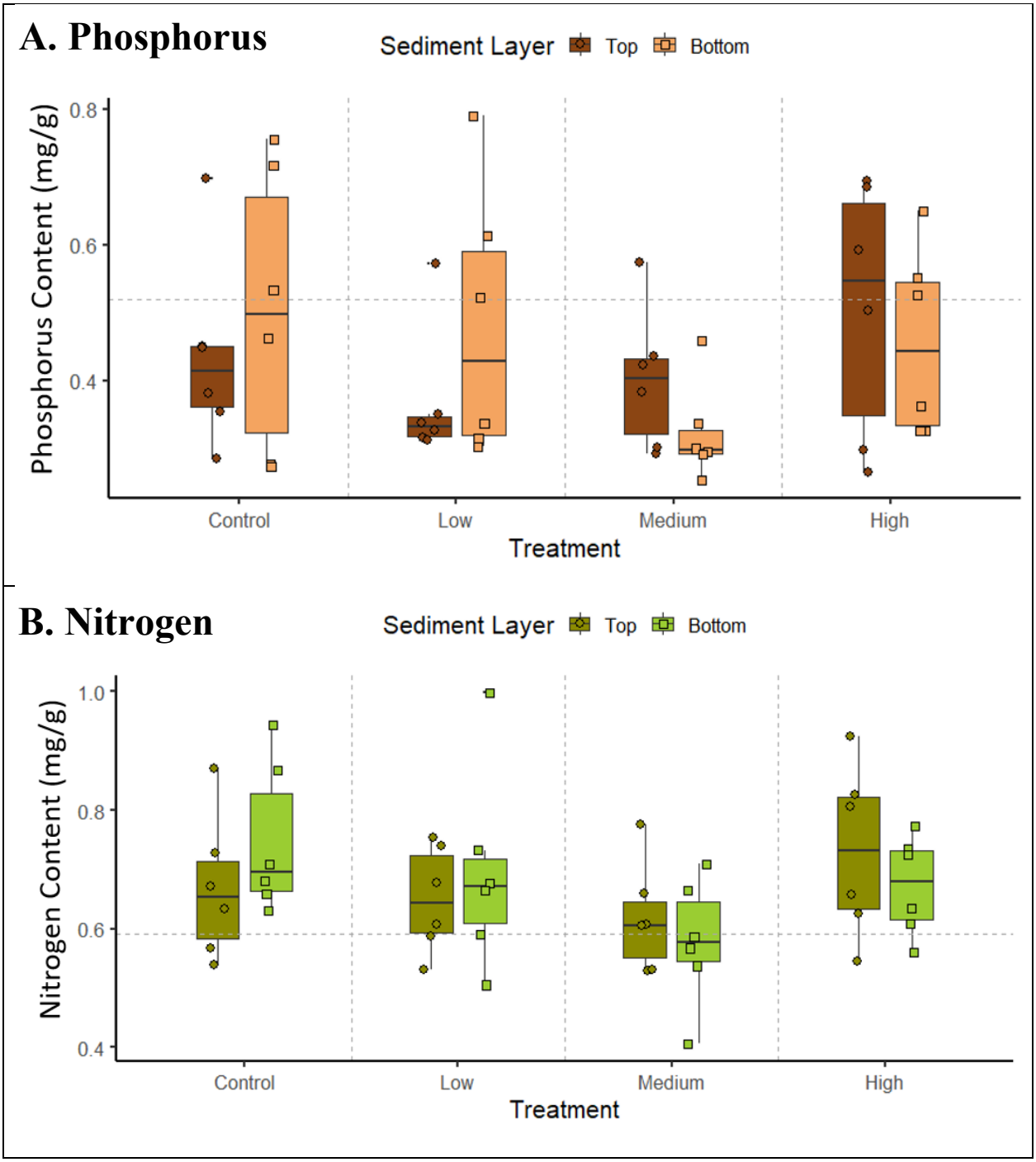
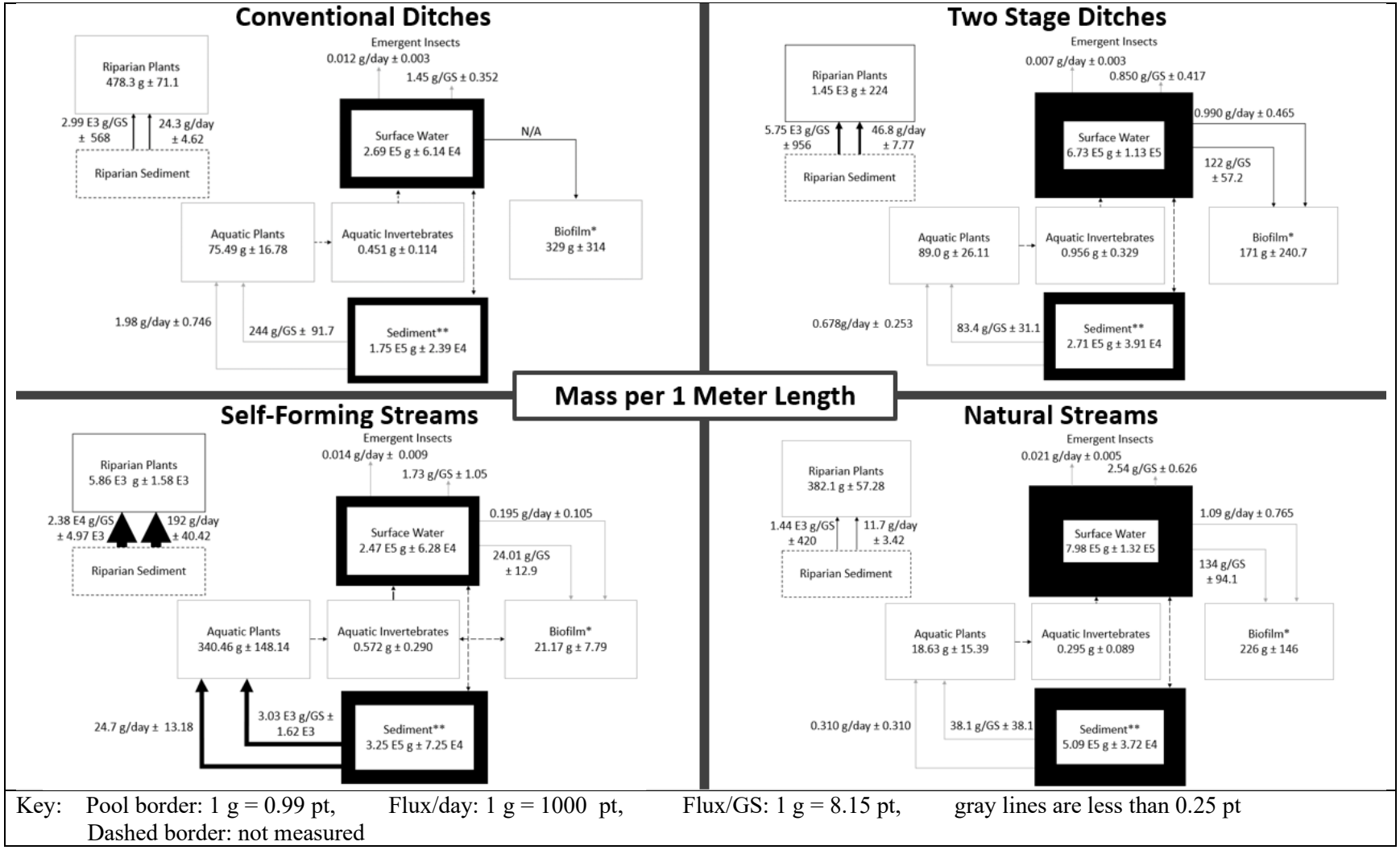
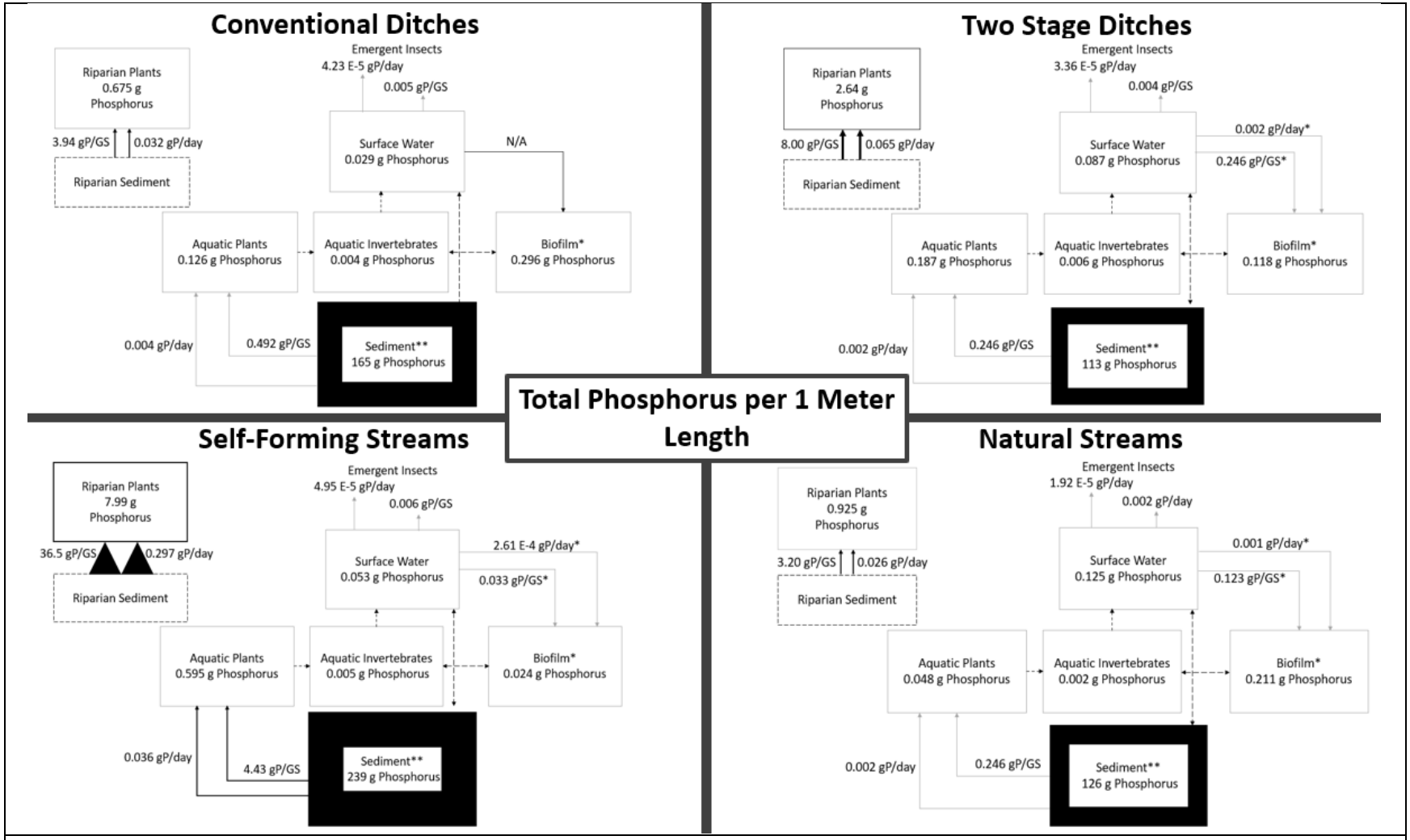


Figure 32. Bioturbation sediment nutrients in mg per g in the top and bottom halves of sediment separately. The horizonal dashed line represents the nutrient content of the tested initial sample.



Key: Pool border: 1 g = 0.99 pt, Flux/day: 1 g = 1000 pt, Flux/GS: 1 g = 8.15 pt, gray lines are less than 0.25 pt
Dashed border: not measured

Figure 33. Mass averages in grams per meter length of ditch, averaged over May and July for all site types. Arrows labeled with units g/GS are fluxes over a 123 day growing season from May through August.
*Assuming all sediment surface is covered in biofilm
**Mass of the top 7cm depth of sediment, assuming a 1.48g/cm^3 bulk density.



Key: Pool border: 1 g = 0.209 pt, Flux/day: 1 g = 50.5 pt, Flux/GS: 1 g = 0.411 pt, gray lines are less than 0.25 pt
Dashed border: not measured

Figure 34. Total phosphorus in pools and fluxes in 1 meter of ditch length, averaged over May and July, for all site types. Arrows labeled with units gP/GS are fluxes over a 123 day growing season from May through August.
*Assuming all sediment surface is covered in biofilm
**Nutrients contained in the top 7cm depth of sediment, assuming a 1.48g/cm^3 bulk density.

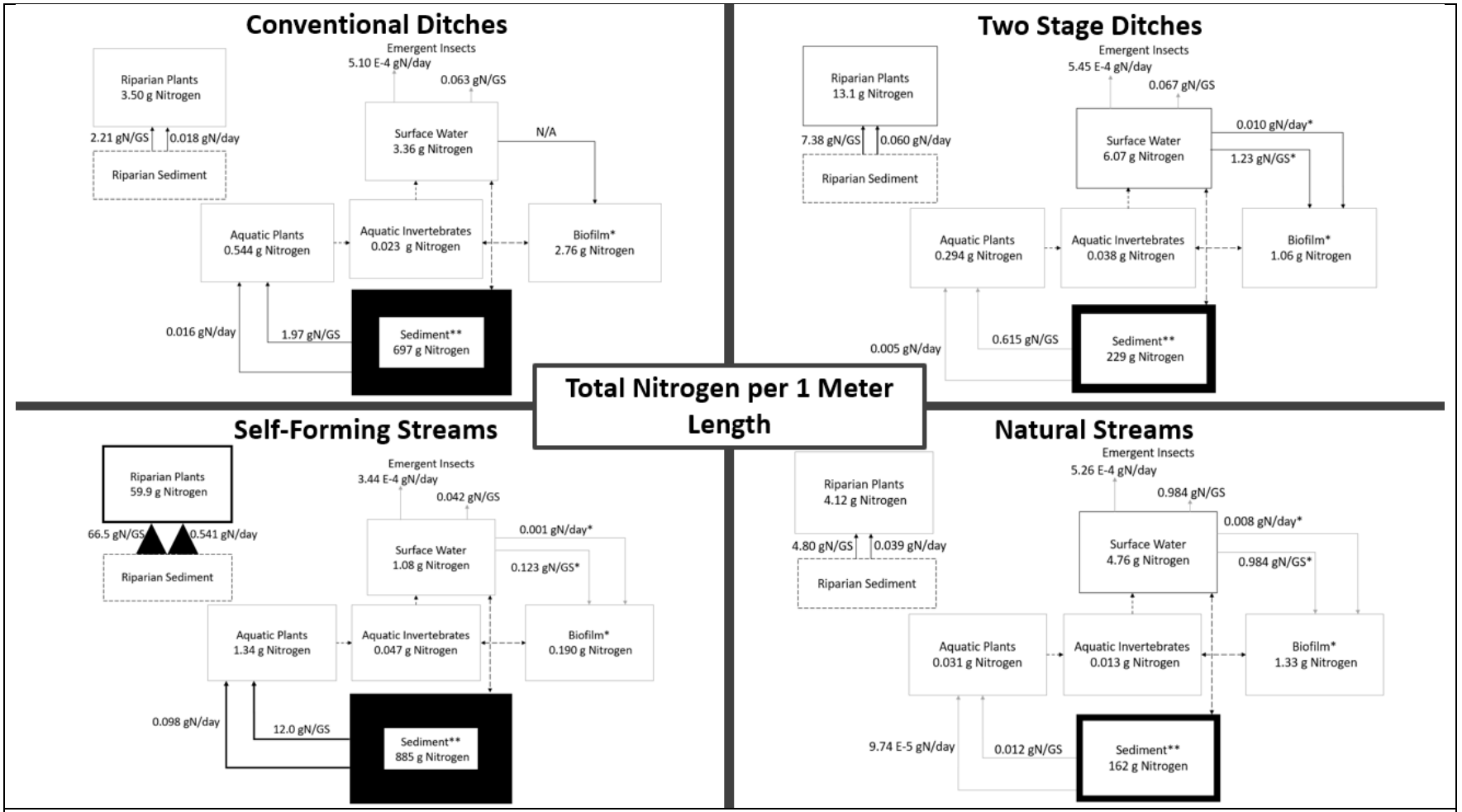
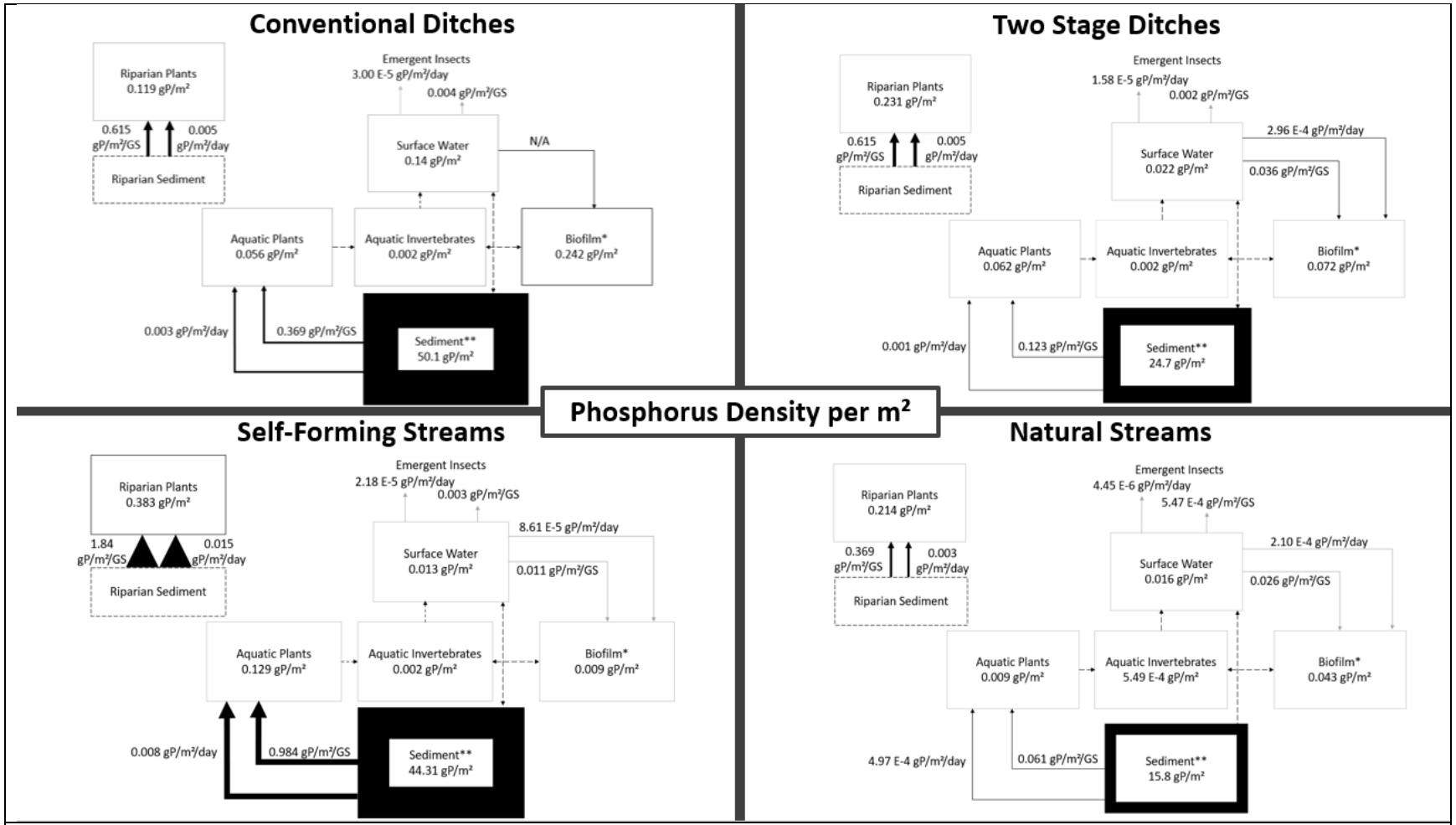


Figure 35. Total nitrogen in pools and fluxes in 1 meter of ditch length, averaged over May and July, for all site types. Arrows labeled with units gN/GS are fluxes over a 123 day growing season from May through August.

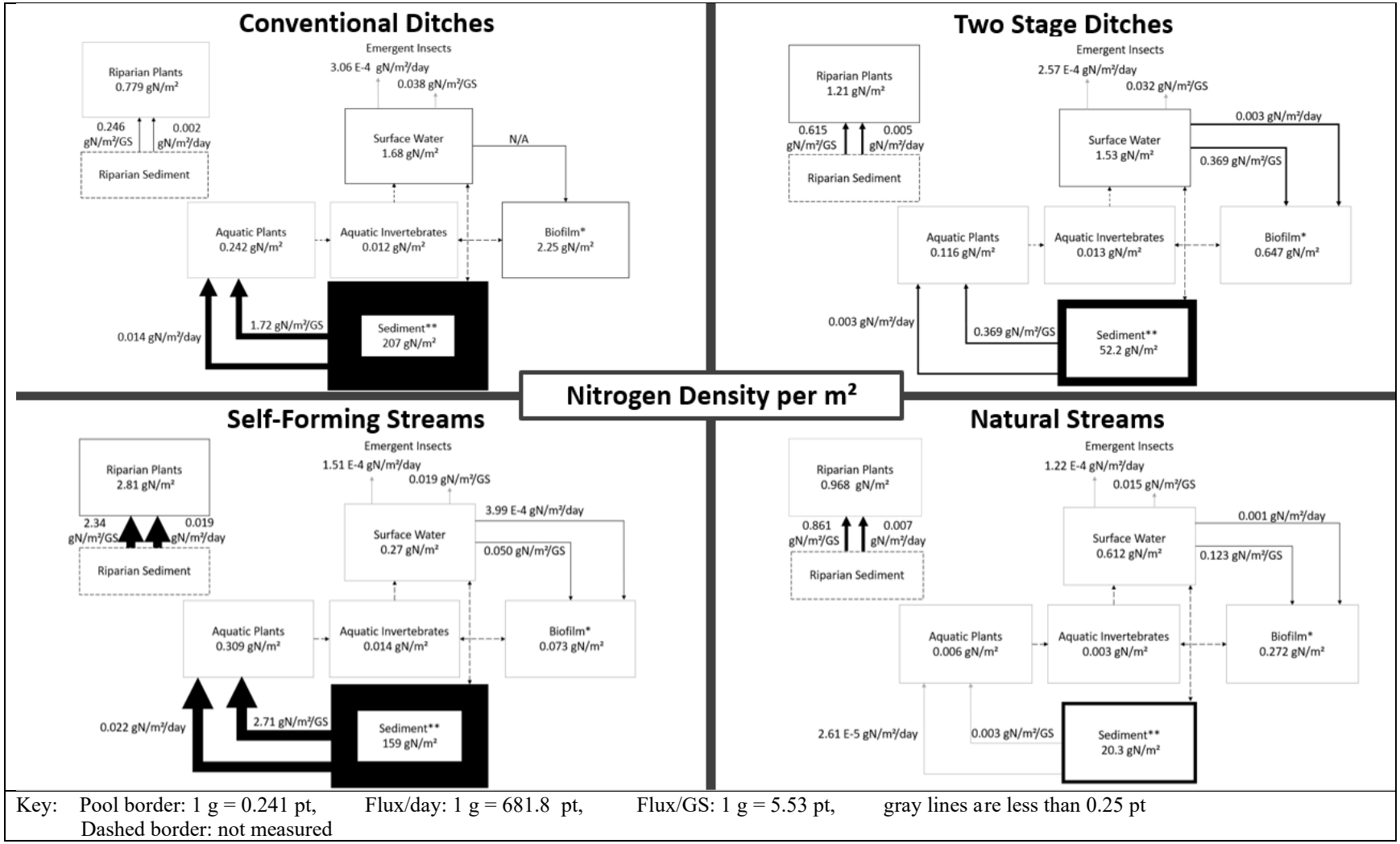
*Assuming all sediment surface is covered in biofilm

**Nutrients contained in the top 7cm depth of sediment, assuming a 1.48g/cm^3 bulk density.



Key: Pool border: 1 g = 0.99 pt, Flux/day: 1 g = 1000 pt, Flux/GS: 1 g = 8.15 pt, gray lines are less than 0.25 pt
Dashed border: not measured

Figure 36. Phosphorus density of pools and fluxes in 1 m^2 area, averaged over May and July, for all site types. Arrows labeled with units gP/GS are fluxes over a 123 day growing season from May through August.
*Assuming all sediment surface is covered in biofilm
**Nutrients contained in the top 7cm depth of sediment, assuming a 1.48g/cm^3 bulk density.



Key: Pool border: 1 g = 0.241 pt, Flux/day: 1 g = 681.8 pt, Flux/GS: 1 g = 5.53 pt, gray lines are less than 0.25 pt
Dashed border: not measured

Figure 37. Nitrogen density of pools and fluxes in 1 m^2 area, averaged over May and July, for all site types. Arrows labeled with units gN/GS are fluxes over a 123 day growing season from May through August.
*Assuming all sediment surface is covered in biofilm
**Nutrients contained in the top 7cm depth of sediment, assuming a 1.48g/cm^3 bulk density.

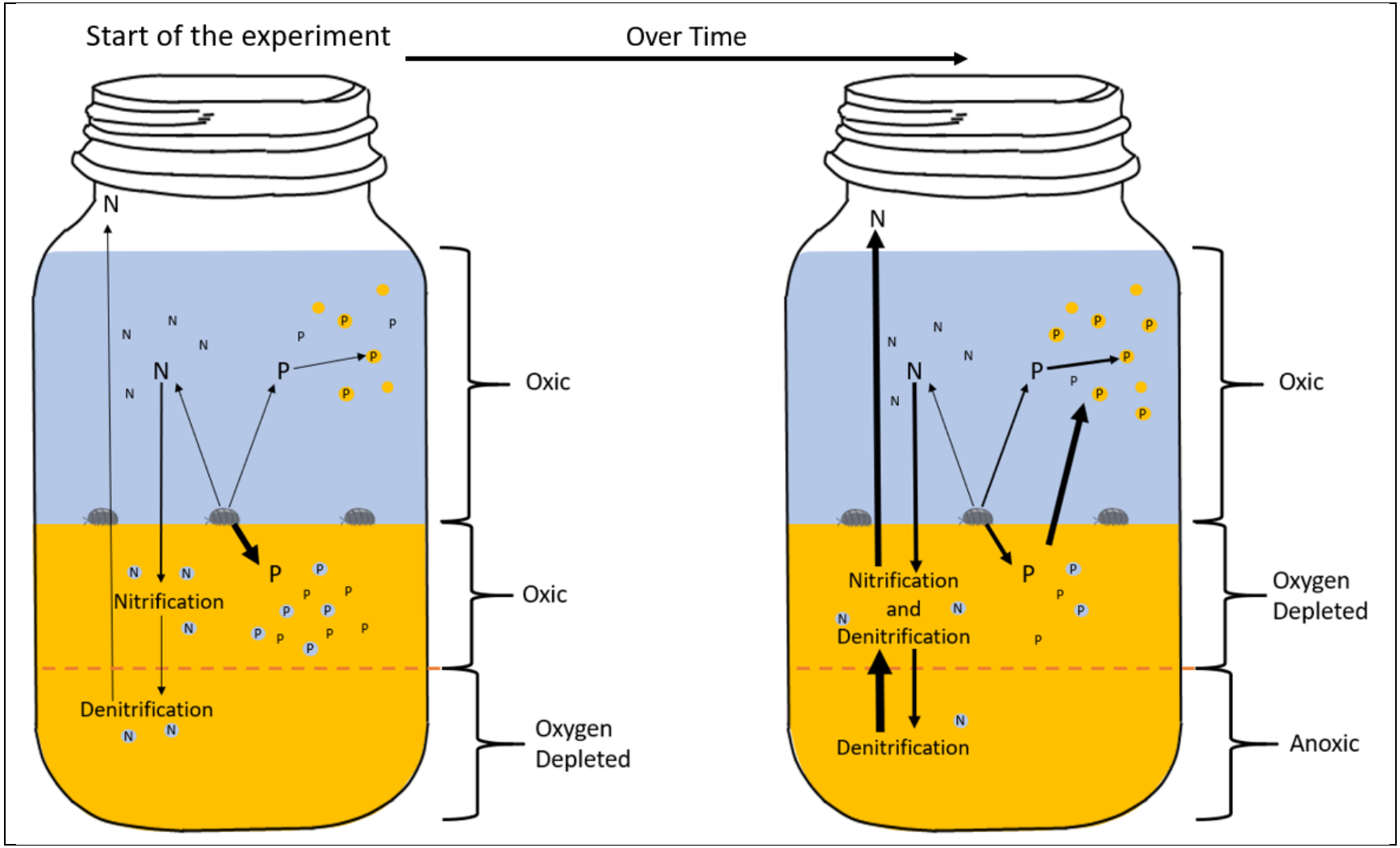


Figure 38. Predicted story of how nitrogen and phosphorus decreased over time during the lab experiment through denitrification and phosphorus binding to suspended particles in the surface water.

APPENDIX B. TABLES

Table 1. Methods used by the Midden Lab to analyze water samples for dissolved and total nutrients. Brackets indicate the source of the methods from either the U.S. Environmental Protection Agency [EPA] or Patton and Kryskalla [USGS]**.**

Constituent	NWIS parameter code	Method and NWIS method code	Reporting limit, mg/L
Dissolved ammonia, as nitrogen (NH_3)	00608	Colorimetry, alkaline phenol and hypochlorite (auto phenate), EPA-103-A, EPA 350.1 [EPA], CL015	0.05
Dissolved nitrite plus nitrate nitrogen (NOx)	00631	Colorimetry, cadmium reduction, EPA-114-A, EPA 353.2 [EPA], CDR06	0.31
Dissolved reactive phosphorus (DRP)	00671	Colorimetry, acidic molybdate, EPA-118-A, EPA 365.3, [EPA], 00119	0.013
Total nitrogen (TN)	62855	Colorimetry, after alkaline persulfate digestion [USGS, EPA], AKPO1	0.31
Total phosphorus (TP)	00665	Colorimetry by discrete analyzer, after alkaline-persulfate digestion, [USGS, EPA], PSF03	0.01

Table 2. ANOVA and Kruskal-Wallis (K-W) test results for data tested individual for May and July samplings. Significant relationships ($p<0.05$) are bolded.

	Response	Sample	Test	p value
3.1.2 Riparian Plants	Riparian Plant Pool Weight (g/meter length)	May	ANOVA	**0.009**
	Riparian Plant Pool Phosphorus (mg/g of tissue)	May	ANOVA	0.430
	Riparian Plant Pool Phosphorus (g/m^2)	May	ANOVA	**0.024**
	Riparian Plant Pool Phosphorus (g/meter length)	May	ANOVA	**0.011**
	Riparian Plant Pool Nitrogen (mg/g of tissue)	May	K-W	0.577
	Riparian Plant Pool Nitrogen (g/m^2)	May	ANOVA	0.252
	Riparian Plant Pool Nitrogen (g/m length)	May	ANOVA	**0.050**
3.1.3 Aquatic Plants	Aquatic Plant Pool Weight (g/meter length)	May	ANOVA	0.102
	Aquatic Plant Pool Phosphorus (mg/g of tissue)	May	K-W	0.765
	Aquatic Plant Pool Phosphorus (g/m^2)	May	K-W	0.192
	Aquatic Plant Pool Phosphorus (g/m length)	May	ANOVA	0.342
	Aquatic Plant Pool Nitrogen (mg/g tissue)	May	ANOVA	0.697
	Aquatic Plant Pool Nitrogen (g/m^2)	May	K-W	0.177
	Aquatic Plant Pool Nitrogen (g/m length)	May	K-W	0.192
3.1.2 Riparian Plants	Riparian Plant Flux Weight (g/meter length)	July	ANOVA	**0.004**
	Riparian Plant Flux Phosphorus (mg/g of tissue)	July	K-W	0.154
	Riparian Plant Flux Phosphorus (g/m^2)	July	AOV	**<0.001**
	Riparian Plant Flux Phosphorus (g/m length)	July	ANOVA	**0.002**
	Riparian Plant Flux Nitrogen (mg/g of tissue)	July	ANOVA	0.387
	Riparian Plant Flux Nitrogen (g/m^2)	July	K-W	0.374
	Riparian Plant Flux Nitrogen(g/m length)	July	ANOVA	0.184
3.1.3 Aquatic Plants	Aquatic Plant Flux Weight (g/meter length)	July	K-W	0.156
	Aquatic Plant Flux Phosphorus (mg/g of tissue)	July	K-W	0.280
	Aquatic Plant Flux Phosphorus (g/m^2)	July	K-W	0.263
	Aquatic Plant Flux Phosphorus (g/meter length)	July	ANOVA	0.548
	Aquatic Plant Flux Nitrogen (mg/g of tissue)	July	K-W	0.233
	Aquatic Plant Flux Nitrogen (g/m^2)	July	K-W	0.213
	Aquatic Plant Flux Nitrogen (g/m length)	July	K-W	0.470
3.1.5	Emergent Insect Weight (g/meter length)	NA	ANOVA	0.407
3.1.6 Biofilm	Biofilm Pool Weight (g/meter length)	NA	ANOVA	0.488
	Biofilm Pool Phosphorus (mg/g of tissue)	NA	ANOVA	0.853
	Biofilm Pool Phosphorus (g/m^2)	NA	K-W	0.161
	Biofilm Pool Phosphorus (g/m length)	NA	ANOVA	0.830
	Biofilm Pool Nitrogen (mg/g of tissue)	NA	ANOVA	0.755
	Biofilm Pool Nitrogen (g/m^2)	NA	K-W	0.232
	Biofilm Pool Nitrogen (g/m length)	NA	ANOVA	0.454
	Biofilm Flux Weight (g/meter length)	NA	K-W	0.288
	Biofilm Flux Phosphorus (mg/g of tissue)	NA	ANOVA	0.640
	Biofilm Flux Phosphorus (g/m^2)	NA	K-W	0.561
	Biofilm Flux Phosphorus (g/m length)	NA	K-W	0.430
	Biofilm Flux Nitrogen (mg/g of tissue)	NA	ANOVA	0.812
	Biofilm Flux Nitrogen (g/m^2)	NA	K-W	0.561
	Biofilm Flux Nitrogen (g/m length)	NA	ANOVA	0.651

Table 3. Data tested individually for May and July through ANOVA or Kruskal Wallis (K-W) with Bonferroni correction because the combined dataset did not fit a mixed effects model. For this table $\alpha = 0.025$

	Response	**Sample**	**Test**	**p value**
3.1.4 Aquatic Macroinvertebrates	Invertebrate Richness	May	ANOVA	0.673
	Invertebrate Phosphorus (mg/g of tissue)	May	ANOVA	0.315
	Invertebrate Phosphorus (g/m^2)	May	ANOVA	0.176
	Invertebrate Nitrogen (g/m^2)	May	K-W	0.033
	Invertebrate Richness	July	ANOVA	0.402
	Invertebrate Phosphorus (mg/g of tissue)	July	ANOVA	0.981
	Invertebrate Phosphorus (g/m^2)	July	ANOVA	0.592
	Invertebrate Nitrogen (g/m^2)	July	K-W	0.693
	Crayfish Weight (g/meter length)	May	ANOVA	0.333
	Crayfish Phosphorus (g/m length)	May	K-W	0.654
	Crayfish Nitrogen (g/m length)	May	ANOVA	0.869
	Crayfish Weight (g/meter length)	July	K-W	0.721
	Crayfish Phosphorus (g/m length)	July	K-W	0.550
	Crayfish Nitrogen (g/m length)	July	K-W	0.375
3.1.7	DIN (g/m length)	May	ANOVA	0.465
	DIN (g/m length)	July	K-W	0.693
	pH	May	ANOVA	0.620
	pH	July	ANOVA	0.367

Table 4. Mixed effects model results for aquatic macroinvertebrate data containing May and July data in one dataset. Invertebrates were all collected in surber samples excluding mussels, clams, and snails. Crayfish were those separately collected from surber samples. Significant relationships ($p<0.05$) are bolded.

3.1.4 Aquatic Macroinvertebrates

Response	Predictor	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Invertebrate Weight (g/m length)	Type	0.855	0.285	3	7.45	4.22	**0.0497**
	Sampling	2.14	2.14	1	6.79	31.6	**<0.001**
	Type:Sampling	1.77	0.591	3	6.86	8.74	**0.009**
Invertebrate Phosphorus (g/m length)	Type	20.71	6.90	3	7.46	0.859	0.503
	Sampling	81.38	81.38	1	6.77	10.13	**0.016**
	Type:Sampling	66.02	22.00	3	6.83	2.74	0.125
Invertebrate Nitrogen (mg/g tissue)	Type	390.9	130.3	3	7.51	0.588	0.641
	Sampling	395.2	395.2	1	6.71	1.78	0.225
	Type:Sampling	1089	363.1	3	3.77	1.64	0.268
Invertebrate Nitrogen (g/m length)	Type	0.484	0.161	3	7.77	0.420	0.744
	Sampling	9.58	9.58	1	6.37	24.93	**0.002**
	Type:Sampling	3.78	1.26	3	3.40	3.28	0.095
Crayfish Phosphorus (mg/g tissue)	Type	0.177	0.059	3	5.37	3.012	0.126
	Sampling	0.074	0.074	1	6.56	3.78	0.096
	Type:Sampling	0.292	0.097	3	5.49	4.66	0.058
Crayfish Nitrogen (mg/g tissue)	Type	0.175	0.058	3	5.31	0.834	0.527
	Sampling	0.366	0.366	1	6.99	5.24	0.056
	Type:Sampling	0.741	0.247	3	5.42	3.29	0.109

Table 5. Mixed effects model results for surface water data containing May and July data in one dataset. Significant relationships ($p<0.05$) are bolded.

3.1.7 Surface Water

Response	Predictor	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
DRP (mg/L)	Type	0.007	0.002	3	7.43	0.424	0.742
	Sampling	0.002	0.002	1	6.81	0.395	0.550
	Type:Sampling	0.006	0.002	3	6.88	0.403	0.756
DRP (g/m length)	Type	5.96	1.99	3	7.44	2.85	0.110
	Sampling	0.956	0.956	1	6.79	1.37	0.281
	Type:Sampling	1.29	0.430	3	6.86	0.626	0.626
DIN (mg/L)	Type	51.4	17.1	3	7.84	0.789	0.534
	Sampling	190.4	190.4	1	3.27	8.76	**0.024**
	Type:Sampling	47.7	15.9	3	6.30	0.731	0.569
DIN (g/m^2)	Type	0.103	0.134	3	7.87	1.50	0.287
	Sampling	1.18	1.18	1	6.22	13.2	**0.010**
	Type:Sampling	1.08	0.361	3	6.24	4.03	0.066
Dissolved Oxygen (Percent)	Type	1531.5	510.5	3	7.60	0.497	0.695
	Sampling	2177.4	2177.4	1	6.59	2.12	0.191
	Type:Sampling	7596.7	2532.2	3	6.64	2.46	0.151
Conductivity (µS/cm)	Type	268228	89409	3	7.65	3.15	0.089
	Sampling	14287	14287	1	6.53	0.503	0.503
	Type:Sampling	121278	40426	3	6.57	1.42	0.319
Redox (mV)	Type	4846.4	1615.5	3	6.73	0.547	0.666
	Sampling	22282	22282	1	6.34	7.55	**0.032**
	Type:Sampling	12522	4174.0	3	6.35	1.413	0.324
Depth (cm)	Type	174.5	58.2	3	7.81	3.05	0.094
	Sampling	131.3	131.3	1	6.30	6.88	**0.038**
	Type:Sampling	54.7	18.3	3	6.33	0.957	0.469
Wet Channel Width (m)	Type	15.3	5.09	3	7.51	8.29	**0.009**
	Sampling	5.60	5.60	1	6.70	9.10	**0.020**
	Type:Sampling	0.064	0.021	3	6.77	0.035	0.990

Table 6. Mixed effects model results for sediment data, containing May and July data in one dataset. Significant relationships ($p<0.05$) are bolded.

3.1.8 Sediment

Response	Predictor	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Sediment Weight (g/m length)	Type	3.92 e 10	1.31 e 10	3	8	5.06	**0.030**
	Sampling	5.07 e 10	5.07 e 10	1	8	19.6	**0.002**
	Type:Sampling	4.22 e 10	1.41 e 10	3	8	0.544	0.666
Sediment Phosphorus (mg/g)	Type	0.087	0.029	3	8	4.75	**0.035**
	Sampling	0.018	0.018	1	8	2.98	0.123
	Type:Sampling	0.003	0.001	3	8	0.207	0.889
Sediment Phosphorus (g/m^2)	Type	428.8	142.9	3	8	4.75	**0.035**
	Sampling	89.0	89.0	1	8	2.96	0.124
	Type:Sampling	18.6	6.21	3	8	0.206	0.889
Sediment Phosphorus (g/m length)	Type	6585.2	2195.1	3	8	1.18	0.377
	Sampling	3408.4	3408.4	1	8	1.83	0.213
	Type:Sampling	6097.1	2032.4	3	8	1.09	0.406
Sediment Nitrogen (mg/g)	Type	1.47	0.491	3	8	4.89	**0.032**
	Sampling	0.347	0.347	1	8	3.45	0.100
	Type:Sampling	0.052	0.017	3	8	0.171	0.913
Sediment Nitrogen (g/m^2)	Type	1.22	0.406	3	8	8.75	**0.007**
	Sampling	0.418	0.418	1	8	9.01	**0.017**
	Type:Sampling	0.164	0.055	3	8	1.18	0.376
Sediment Nitrogen (g/m length)	Type	127500	42500	3	8	1.97	0.196
	Sampling	28116	28116	1	8	1.31	0.286
	Type:Sampling	110617	36890	3	8	1.71	0.241

Table 7. Results of ezANOVAs run with bioturbation data. Significant relationships (p<0 .05) are bolded.

	Response	Effect	GGe	p[GG]	Hfe	p[HF]
3.2.1	Surface Water Nitrogen Change	Sampling	0.805	**0.001**	0.978	**<0.001**
		Treatment: Sampling	0.805	0.050	0.978	0.037
	Surface Water Redox	Sampling	0.404	**<0.001**	0.449	**<0.001**
		Treatment: Sampling	0.404	0.502	0.449	0.809
3.2.2	Pore Water Phosphorus	Sampling	0.494	**<0.001**	0.569	**<0.001**
		Treatment: Sampling	0.494	**0.006**	0.569	**0.003**
	Pore Water Nitrogen	Sampling	0.506	**<0.001**	0.585	**<0.001**
		Treatment: Sampling	0.506	0.292	0.585	0.285
	Pore Water Redox	Sampling	0.531	**<0.001**	0.620	**<0.001**
		Treatment: Sampling	0.531	0.809	0.620	0.833

Table 8. ANOVA results for bioturbation sediments, sample refers to the top or bottom half sample of sediment sampled. Results are Bonferroni corrected, α = 0.025.

3.2.3 Bioturbation Sediment

Response	Effect	DFn	Deviance	Resid. DF	Resid. Dev.	F	p value
Phosphorus	Treatment	3	0.105	44	0.929	1.41	0.269
	Sample	1	0.002	43	0.987	0.073	0.790
	Treatment: Sample	3	0.074	20	0.499	0.996	0.415
Nitrogen	Treatment	3	0.092	44	0.610	2.32	0.106
	Sample	1	0.0005	43	0.610	0.041	0.841
	Treatment: Sample	3	0.039	20	0.266	0.992	0.416

Table 9. Results from calculating 95% confidence intervals of nutrients in bioturbation sediments, compared to the initial nutrient content. Bolded rows show factors where the initial content fell outside the final confidence interval.

3.2.3 Bioturbation Sediment

Treatment	Sample	Initial Content (mg/g)	Upper Limit	Lower Limit
Control	Top mgP/g	0.519	0.550	0.322
	Bottom mgP/g	0.519	0.670	0.337
	Top mgN/g	0.592	0.765	0.572
	Bottom mgN/g	**0.592**	**0.850**	**0.647**
Low	**Top mgP/g**	**0.519**	**0.450**	**0.289**
	Bottom mgP/g	0.519	0.638	0.322
	Top mgN/g	0.592	0.721	0.579
	Bottom mgN/g	0.592	0.829	0.560
Medium	**Top mgP/g**	**0.519**	**0.485**	**0.319**
	Bottom mgP/g	**0.519**	**0.380**	**0.265**
	Top mgN/g	0.592	0.691	0.544
	Bottom mgN/g	0.592	0.663	0.493
High	Top mgP/g	0.519	0.656	0.356
	Bottom mgP/g	0.519	0.567	0.347
	Top mgN/g	**0.592**	**0.846**	**0.616**
	Bottom mgN/g	**0.592**	**0.740**	**0.606**