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Abstract

Lonicera maackii (Amur honeysuckle) invasion is extensive in forests across much of Ohio and the Midwest. Amur honeysuckle has been shown to influence headwater streams and its organisms, which depend on a certain water chemistry to survive. Little has been done to understand how honeysuckle affects water chemistry and nutrient cycling. As honeysuckle canopies prevent native organic matter from entering the streams below, while also adding its own organic matter that is high in nitrogen and phosphorus, and low in lignin, the amount and types of nutrients present in both forests and streams may be significantly altered. Over a one-year time period, five riparian stream sites were sampled and analyzed for a variety of chemical parameters. It was found that Amur honeysuckle does not have an effect on these chemical variables and does not follow a gradient of honeysuckle, as predicted. The effects from honeysuckle may be over shadowed by anthropogenic pollution and stream geology.

Dedication or Acknowledgements

This thesis is dedicated to the McEwan Lab and to everyone in the lab who has taught me so much! I would like to thank the UD Keck Environmental Fellowship, the UD Honors Department, and the National Science Foundation (DEB-1352995) for funding my project. Thank you to the Five Rivers Metroparks, Miami County Parks District, and Aullwood Farm for providing us with the field sites for this study.

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Introduction

Invasive species have harmful effects on ecosystems including outcompeting native species and altering ecosystem function (Webster *et al.* 2000; Zavaleta *et al.* 2001, Collier *et al.* 2002). *Lonicera maackii,* commonly known as Amur honeysuckle (hereafter "honeysuckle"), is a problematic invasive species in many forests across Ohio and the broader midwestern United States. Honeysuckle was introduced to North America *ca*. 1896 from Asia (Shewhart, McEwan, & Benbow 2014). Today, it has spread to 27 states in the central and eastern United States, colonizing in urban parks, riparian zones, and second-growth forests (Luken 1988, Luken and Thieret 1996, McNeish *et al.* 2012, Wilson *et al.* 2013).

Figure 1. Observed unique seasonal changes of Amur honeysuckle in southwest Ohio, USA.

The unique seasonal changes of honeysuckle can be readily observed throughout the year (Figure 1), as honeysuckle has a longer leaf duration than most Ohio native species (McEwan *et al.* 2009). Commonly in autumn, honeysuckle is the last plant to lose its leaves and appears green until the winter. In the spring, it is one of the first plants to bloom with bright green leaves and red berries. During the summer time, the invasive shrub creates dense canopies of leaves, preventing light and organic matter from reaching the environment underneath the canopy. These distinctive seasonal changes of honeysuckle may be adding organic matter to the surrounding environment at odd times of year. Additionally, honeysuckle organic matter may be different in chemical make-up.

Streams meander through many of the Ohio forests where honeysuckle has overtaken the native vegetation creating near-monocultures (Figure 1). Riparian forests and streams are interlinked as allochthonous inputs enter the stream from the terrestrial environment (Vannote *et al.* 1980; Gregory *et al.* 1991; Webster *et al.* 1995). Prior research has found that invaded riparian zones and streams experience a decrease in the amount of organic matter entering the stream, and a large portion of the organic matter is from honeysuckle (McNeish, Moore, Benbow, & McEwan 2014). Aquatic organisms and ecosystem processes are ultimately affected by these invasive allochthonous inputs from the riparian zone above (McNeish *et al.* 2014). Furthermore, invasions may modify nutrient dynamics in streams, as their organic matter is high in nitrogen, phosphorus, and low in lignin; however, there have been relatively few scientific studies of this subject. More research is needed to understand how invaded riparian zones affect the water chemistry and nutrient cycling of neighboring streams. As honeysuckle canopies alter the organic matter that enters the streams below, while also adding its own organic matter that is high in nitrogen and phosphorus and low in lignin (McNeish *et* *al.* 2014), the amount and types of nutrients present in both forests and streams may be significantly altered.

Over a one-year time period, five riparian stream sites were sampled and analyzed for a variety of chemical parameters. Two of the riparian stream sites had no honeysuckle, two sites had a moderate amount of honeysuckle, and one site had high amounts of honeysuckle with a riparian canopy that was all honeysuckle. It is hypothesized that across an invasion gradient there will be a measurable gradient in stream chemistry and nutrient concentration (H_1). Specifically, it is predicted that areas of higher honeysuckle invasion will be associated with significantly increased concentrations of different forms of nitrogen and phosphorus (H_2).

Methods

Field Sites

Five different first and second order headwater streams located in southwest Ohio were sampled from August 2015 until November 2016. These sites spanned a gradient of honeysuckle invasion intensity. Two of the stream sites that were used as controls had no honeysuckle: Englewood and Aullwood Reference. Two of the stream sites had a moderate amount of honeysuckle: Englewood and Charleston Falls (CF) Moderate. One site included a riparian canopy that was completely honeysuckle, known as our high invasion site: Buckeye Trail (BT) High. The streams were larger first order and smaller second order streams that were similar in terms of basic parameters including sinuosity, gradient, bedrock, and discharge. These streams were relatively uninfluenced by urban or agricultural inputs as they were surrounded by second growth forest. Within each stream a set of five permanent 6 m length in-stream plots were established along a 30 m reach following the thalweg at each stream site (Figure 2).

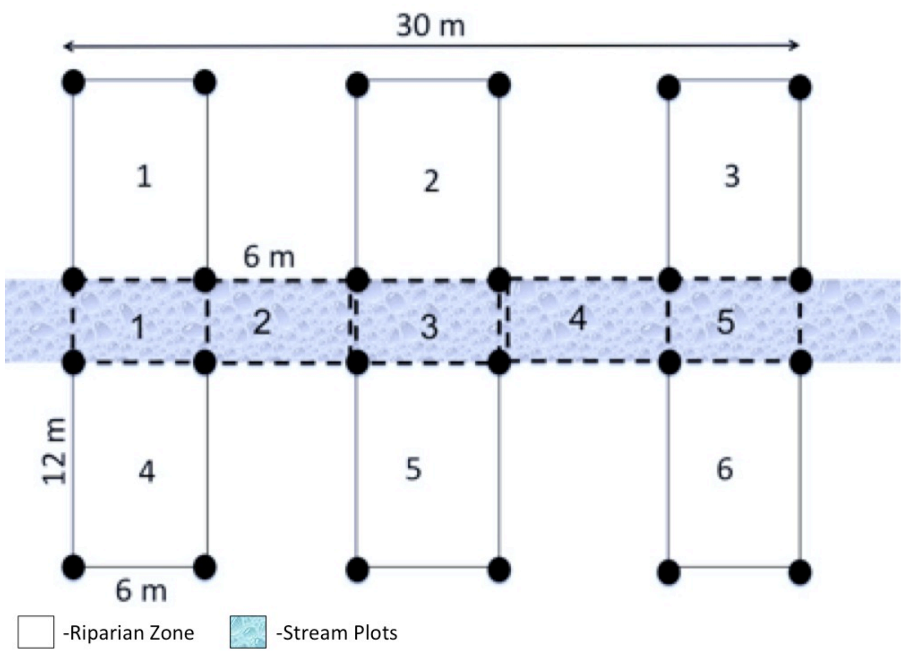

Figure 2. Illustration of the plot design

Data Collection

Streams were monitored at least monthly with an YSI Sonde Probe (Yellow Springs, OH) starting on August 4, 2015 until November 30, 2016 to record the water temperature (°C), dissolved oxygen (DO) (mg/L), pH, specific conductivity (µS/cm), and total dissolved solids (TDS) (mg/L). In addition to these *in situ* measurements, water samples were also collected and analyzed beginning October 20, 2015 and ending on September 16, 2016. These sampling events were performed monthly throughout the summer and winter, and weekly in the spring and fall, so that the influence of honeysuckle's unique phenology could be discerned.

During each sampling event, three clear plastic bottles per site, provided by Alloway Environmental Laboratory (Lima, OH), were used to collect approximately 500 mL of flowing stream water in each bottle. Two of the bottles were empty, while one held a small amount of preservative (H_2SO_4) for the appropriate analytical parameters. While in the field, one of the empty bottles was rinsed three times with the stream water and then filled completely. This water was then poured into the bottle containing the sulfuric acid preservative. Then the empty bottle was filled again. Half of the water from this bottle was then filtered in lab or in the field using a 0.45 µm glass filter into the second empty bottle.

Sample Processing and Nutrient Analysis

After each sampling event, water bottles were placed on ice, and delivered to Alloway Laboratory in Lima, OH within 48 hours. Alloway was contracted to test for the amounts of Total Kjeldahl nitrogen (TKN) (mg/L), ratio of nitrate to nitrite (mg/L), soluble reactive phosphorus (SRP) (mg/L), and dissolved organic carbon (DOC) (mg/L) in each of the water samples. The remaining water samples from each site were used to test for total suspended solids (TSS) (mg/L), hardness (mg/L as $CaCO_3$), and alkalinity (mg/L as $CaCO_3$) in lab.

To measure the amount of TSS in each sample, aluminum weigh pans were dried at 105^{o}C for 24 h to standardize each pan. After 24 h, each pan was given a number, weighed (Mettler Toldeo (Toledo, OH) balance), and recorded. Each pan received a Whatman glass microfiber filter disc, 47 mm, and was re-weighed. A Buchner funnel, side arm flask, hose, and vacuum were set up for filtration. While the technician was wearing sterile gloves, the glass microfiber filter was placed with forceps onto the Buchner funnel. The glass microfiber filter was rinsed three times with a total of 50-60 mL of DI water. Each water sample was shaken thoroughly and then carefully measured to 50 mL with a graduated cylinder. Next, the sample was quickly poured directly on to the glass filter. The graduated cylinder was rinsed with DI water onto the filter. After filtration, each glass filer was placed back into its respective pan with forceps and all pans were placed into the 105 ^{o}C drying oven for one hour. After the first hour, each pan was re-weighed and then the pans went back into the drying oven for another hour to repeat the process. To calculate TSS, the average weight of the combined pans, filter, and dried suspended solids was calculated. The weight of the tin and the filter was then subtracted from this average and the resulting weight was converted to milligrams per liter.

Water hardness was measured using titration. A 25 mL sample was poured into a 50 mL Erlenmeyer flask with a small metallic stirring rod. While sitting on a stir plate, four drops of nitrate buffer and a pinch of Eriochrome Black-T reagent were added to the sample. The mixture was then titrated with Ethylenediaminetetraacetic acid (EDTA) until a color change. The amount of EDTA used was multiplied by its corresponding correction factor, divided by 25 mL, and multiplied by 1,000 to calculate hardness.

Similarly, the alkalinity of the water was found using a titration. A 25 mL sample was poured into a 50 mL Erlenmeyer flask with a small metallic stirring rod. Three drops of phenolphthalein and three drops of bromcresol green-methyl red indicator were added to the sample. The mix then sat on a stir plate and was titrated with 0.02 N H_2SO_4 until a color change. The amount of 0.02 N H_2SO_4 used was multiplied by its corresponding correction factor, divided by 25 mL and multiplied by 1,000 to calculate alkalinity.

Data Preparation and Analysis

If samples were found to be under their detection limits, then the data was adjusted using United States Geological Survey methodology (Helsel & Hirsch 2002). Corresponding blanks were subtracted from each sample, if greater than zero. Point and line plots were created for each parameter across all sites and dates.

Results

Solids

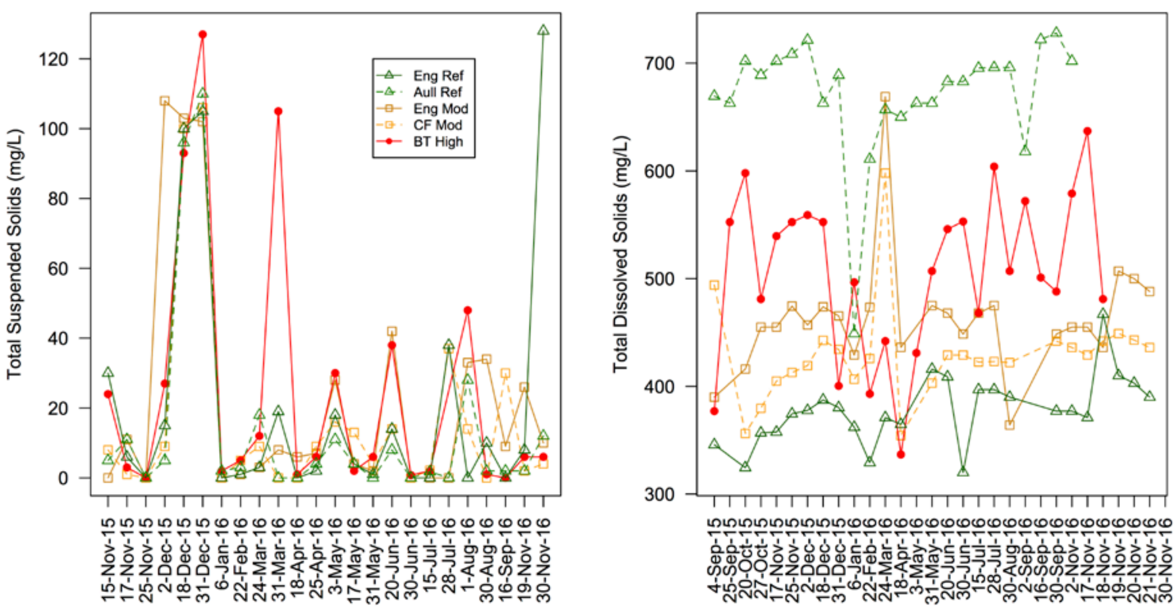

Figure 3. Total suspended solids (left) and total dissolved solids (right) at each stream site over time.

No distinction in the amounts of total suspended solids (TSS) was found along the gradient of honeysuckle over time. TSS consistently spiked at each of the stream sites at least once a month. Each of the stream sites followed approximately the same changes in TSS no matter the amount of honeysuckle present (Figure 3).

Conversely, total dissolved solids (TDS) demonstrated a distinction among stream sites over time. The Aullwood Reference site had the highest amount of TDS throughout the study at all but two sampling dates. Interestingly, the Englewood Reference site usually exhibited the lowest amount of TDS throughout the study. The high honeysuckle site usually demonstrated the second highest amount of TDS followed by the moderate honeysuckle sites in the middle. When ignoring the Aullwood Reference site, the TDS does follow the gradient of honeysuckle from low to high (Figure 3).

Nitrogen

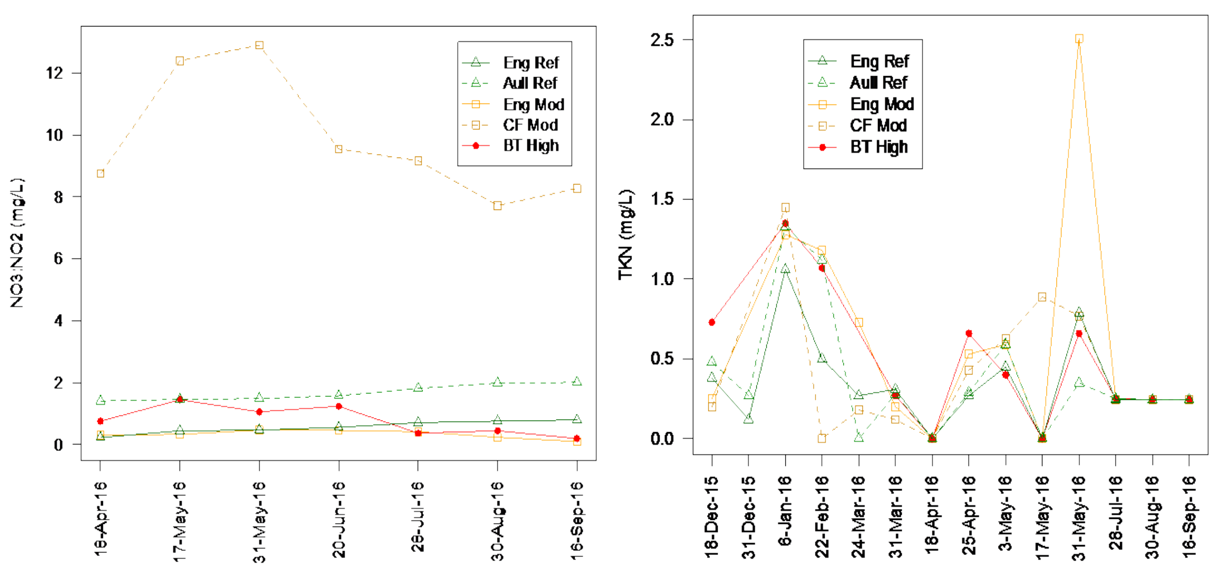

Figure 4. Total Kjeldahl Nitrogen (right) and the ratio of nitrate to nitrite (left) at each stream site over time.

The ratio of nitrate to nitrite was measured from the spring 2016 to fall 2016 of the study. The CF Moderate site consistently demonstrated the highest ratio of nitrate to nitrite over time. The highest ratio of nitrate to nitrite at the CF Moderate site was followed by the Aullwood Reference site, the BT High site, and finally an approximate tie between the Englewood Reference and Moderate sites (Figure 4).

TKN was measured at every stream site throughout the winter of 2015 to the fall of 2016. TKN levels ranged from 0 to about 1.5 mg/L over time. Conversely of the previous nitrogen findings, all of the sites were found to have approximately the same amount of TKN over time. The Englewood Moderate site exhibited one spike in TKN of about 2.5 mg/L in the summer of 2016, but other than this, all sites exhibited about the same amount of TKN over time (Figure 4).

Phosphorus

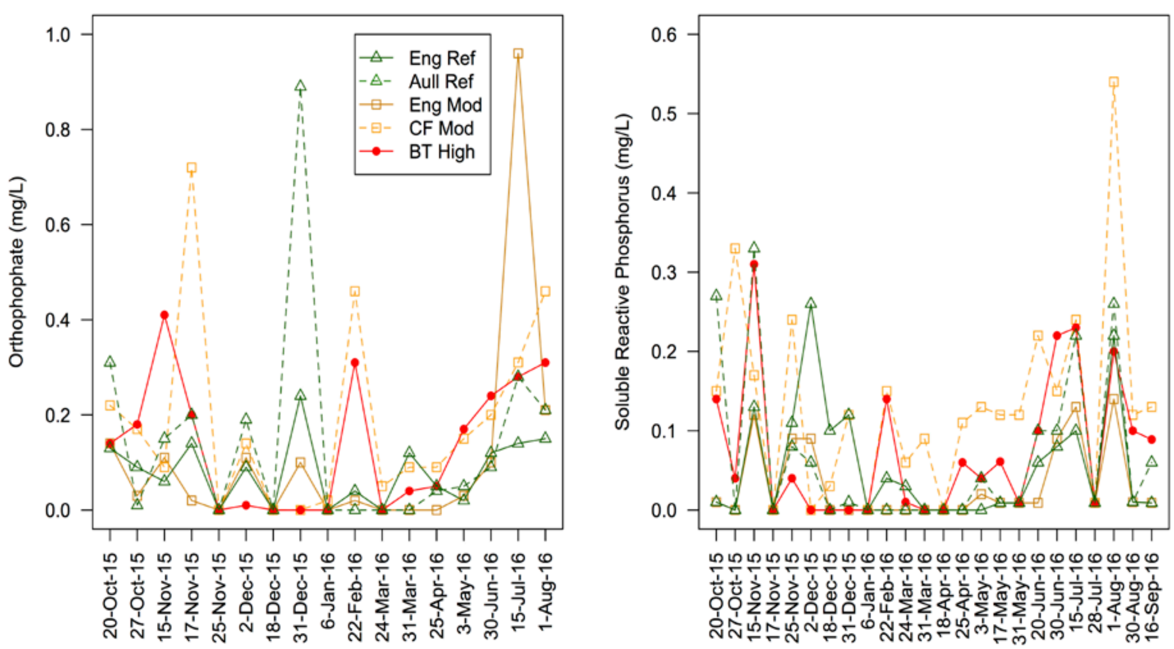

Figure 5. Orthophosphate (left) and soluble reactive phosphorus (right) at each stream site over time.

Orthophosphate levels did not demonstrate a consistent pattern across the honeysuckle gradient over time. Throughout the study, the stream sites exhibited five spikes in orthophosphate levels over time, but four of those spikes were seen across most of the stream sites. The Aullwood Reference site and the CF Moderate site demonstrated the largest spikes in orthophosphate with levels ranging from approximately 0.75 to 0.90 mg/L; however, the largest amount of orthophosphate was found at the Englewood Moderate site at about 1.0 mg/L in July of 2016 (Figure 5). Overall, orthophosphate levels followed the same pattern across sites over time.

Similarly, SRP levels across each stream site followed the same pattern over time. In the spring of 2016, the CF Moderate site demonstrated the highest levels of SRP of 0.54 mg/L. Additionally, the reference sites usually exhibited the lowest amounts of SRP over time; however, the BT High site frequently displayed middle amount of SRP or was also the lowest amounts of SRP with 0 mg/L (Figure 5).

Dissolved Oxygen and Organic Carbon

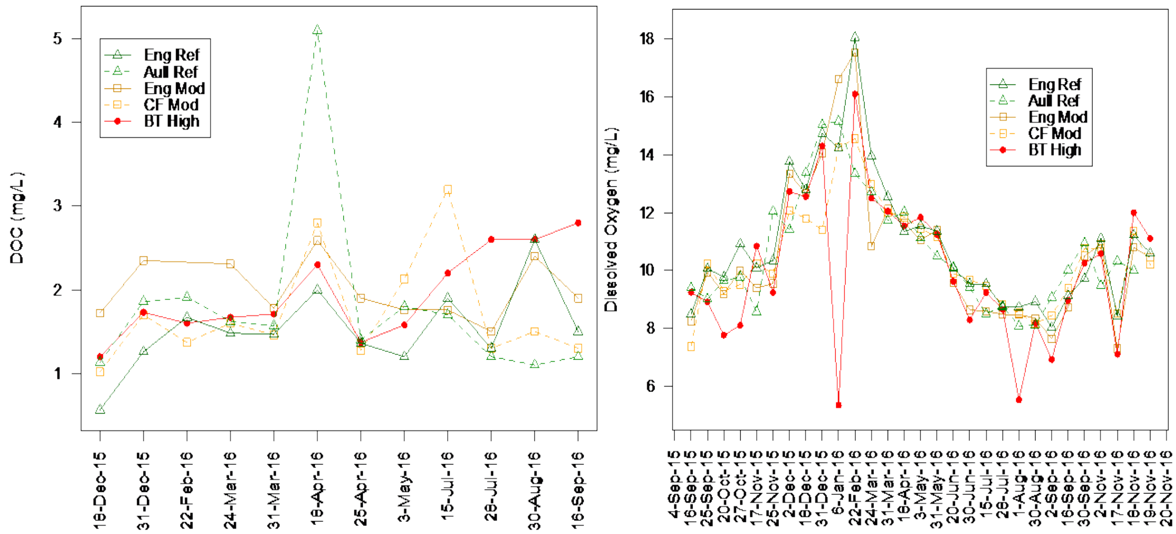

Figure 6. Dissolved organic carbon (left) and dissolved oxygen (right) at each stream site over time.

Dissolved oxygen (DO) was measured at all stream sites throughout the entire study. DO levels typically followed the same trends across all field sites throughout the study. BT High demonstrated downward spikes in DO throughout the year, decreasing to less than 6 mg/L at some dates. Generally, the same amounts of DO were found at each stream site throughout the year, with the greatest amount of DO in the winter and decreasing in the warmer months (Figure 6).

Dissolved organic carbon (DOC) was measured from the winter of 2015 to the fall of 2016. The moderate sites frequently displayed the largest amounts of DOC throughout the study, expect for a spike of 5.1 mg/L at the Aullwood Reference site. BT High also presented the largest amount of DOC from 28-Jul-16 to 16-Sept-16. Reference sites typically displayed the lowest levels of DOC (Figure 6).

Physiochemical Prosperities

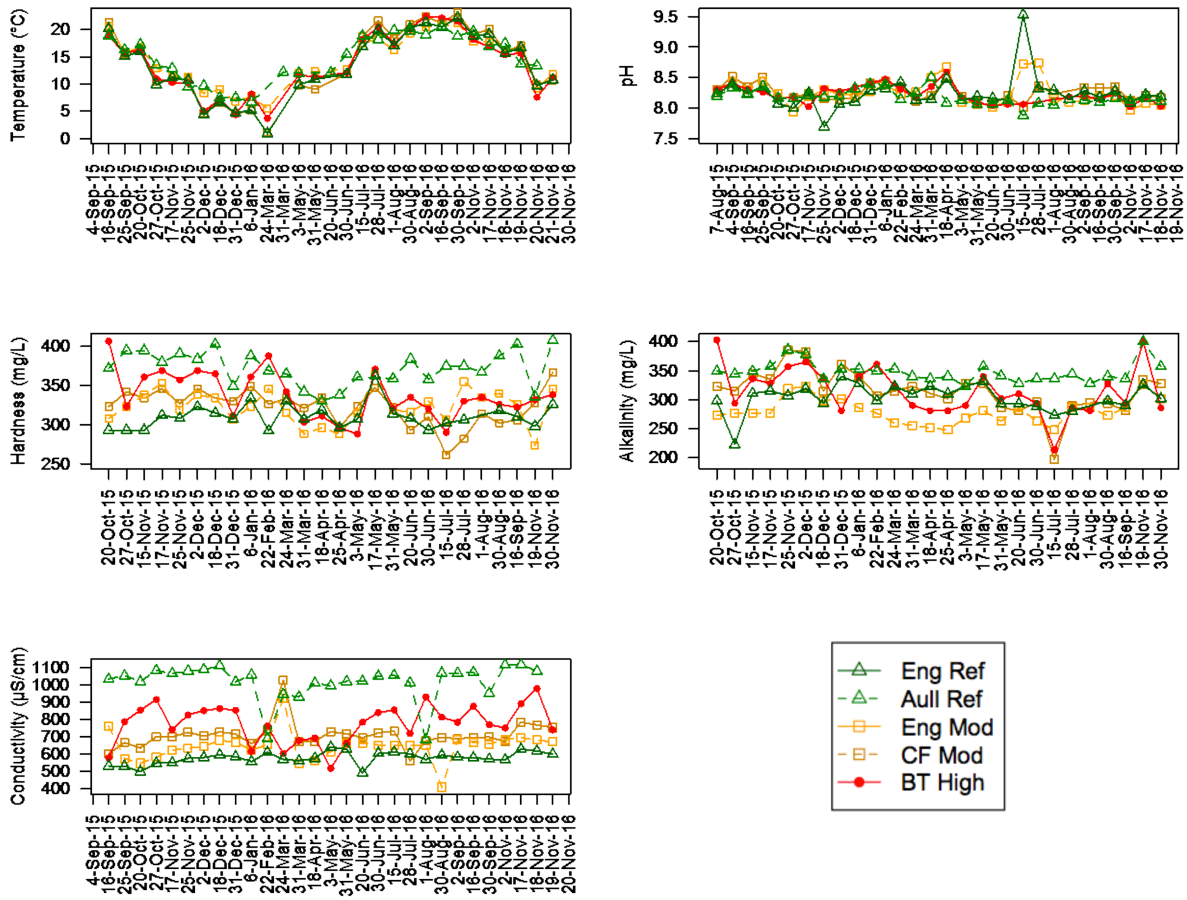

Figure 7. Stream temperature (top left), stream pH (top right), water hardness (middle left), alkalinity (middle right), and conductivity (bottom left) at each stream site over time.

Over time, the stream sites were consistently found to have relatively the same temperature. Similarly, the pH at each stream site was found to be approximately the same throughout the year. Englewood Reference displayed a decrease in pH of 7.69 on 25-Nov-15 and a sudden increase in pH of 9.53 on 15-Jul-16 (Figure 7).

The water hardness varied among each site. The Aullwood Reference site usually had the highest water hardness and Englewood Reference usually had the lowest. The BT High site varied from having the highest or second highest water hardness to having the second lowest water hardness. The moderate sites generally revealed water hardness somewhere in the middle of the other sites. When the Aullwood Reference site is removed, the water hardness generally follows the gradient of honeysuckle (Figure 7).

The alkalinity of the water also varied among the sites throughout the study. The Aullwood Reference site typically exhibited the highest alkalinity and Englewood Reference site generally exhibited the second lowest to lowest in water alkalinity. Frequently, the Englewood Moderate site displayed the second highest alkalinity and the CF Moderate displayed the lowest. The alkalinity of the BT High site greatly varied over time (Figure 7).

The conductivity of the water displayed a more prominent pattern among the sites. The Aullwood Reference site frequently demonstrated the highest conductivity and Englewood Reference usually demonstrated the lowest. The moderated sites typically ranged between 600 and 800 μS/cm, placing them towards the lower range among all of the sites. BT high varied the most among all of the sites, ranging from lowest to the second highest (Figure 7).

Discussion

Overall, there were no significant differences among the stream sites that followed the gradient of honeysuckle, refuting the hypothesis (H_1). Stream sites either demonstrated the same pattern for the studied parameter, or a pattern was found that did not follow the gradient of honeysuckle. When starting this study, physiochemical properties were thought to be similar among each stream site, but this was not always true. Each of the five streams had approximately the same water temperature and pH, which would be expected among streams in the same region (Figure 7). However, the stream sites exhibited differences in hardness, conductivity, and alkalinity throughout the study that did not follow the gradient of honeysuckle, suggesting that these differences are due to another source. The Aullwood Reference site demonstrated large amounts of alkalinity, hardness, and conductivity, possibly due to the limestone bedrock of the stream (Figure 7). Additionally, the gradient of honeysuckle demonstrates no effect on TDS. Reference sites exhibited both the highest and lowest levels of TDS, with the high and moderate sites varying between the two reference sites (Figure 3). When the Aullwood Reference data is removed from each of these aforementioned parameters, the parameter generally does follow the gradient of honeysuckle; however, further exploration into the geology of the Aullwood Reference site would need to be conducted to account for these measurements.

Levels of nitrogen and phosphorus also did not follow the gradient of honeysuckle, refuting the original hypothesis (H_2). Other allochthonous inputs like non-point source pollution may have overshadowed any affect that invasive honeysuckle may have had on the water chemistry. In particular, the ratio of nitrate to nitrite levels were always found to be considerably higher at the CF Moderate site than the other stream sites, suggesting that some other process besides honeysuckle invasion was driving nitrogen dynamics in this stream (Figure 4). CF Moderate site was downstream of a residential neighborhood, leading one to the possibility that there was nitrogen pollution from the residents living near the stream. Furthermore, for both measures of phosphorus, levels spiked on certain dates amongst all five sites no matter the amount of honeysuckle, suggesting other processes besides honeysuckle invasion was driving phosphorus dynamics in the study sites (Figure 5).

In summary, this study found (H_1) no measurable gradient in stream chemistry and nutrient concentrations that could be linked to honeysuckle and (H_2) no significantly increased concentrations of nitrogen and/ or phosphorus where honeysuckle invasions were high in the riparian zone. To further assess how invasive honeysuckle may be affecting stream chemistry and nutrient cycling, steps would have to be taken to discern anthropogenic influences from the effects of honeysuckle. In this study, point and non-point source pollution, such as road salt or agriculture run-off, may have had too great of effects on streams, leaving the honeysuckle effects unrecognizable. Invasive plants are found to have negative effects on stream ecosystems and other biota (McNeish *et al.* 2014); however, anthropogenic influences may be too strong and more of a concern than invasive plants, such as Amur honeysuckle.

References

Collier, M. H., J. L. Vankat, & M.R. Hughes. (2002). Diminished plant richness and abundance below *Lonicera maackii*, and invasive shrub. American Midland Naturalist 147:60-71.

Gregory, S. V., F. J. Swanson, W. McKee W, & K. W.Cummins. (1991). An ecosystem perspective of riparian zones. *BioScience* 41:540-551.

Helsel, D. R., & Hirsch, R. M. (2002). Methods for Data Below the Reporting Limit. In *Statistical Methods in Water Resources Techniques of Water Resources Investigations* (pp. 357-376). United States Geological Survey.

Luken, J.O., & Goessling, N. (1995). Seedling distribution and potential persistence of the exotic shrub *Lonicera maackii* in fragmented forests. *Am. Midl. Nat.* 133: 124–130.

Luken, J.O., & Thieret J.W. (1996). Amur honeysuckle, its fall from grace. *Bioscience* . 46: 18–24.

McEwan RW, Birchfield MK, Schoergendorfer A, & Arthur MA. (2009). Leaf phenology and freeze tolerance of the invasive shrub Amur honeysuckle and potential native competitors. *Journal of the Torrey Botanical Society*. 136: 212–220.

McNeish, R.E., Benbow, M.E., & McEwan, R.W. (2012). Riparian forest invasion by a terrestrial shrub (*Lonicera maackii*) impacts aquatic biota and organic matter processing in headwater streams. *Biol. Invasions.* 14: 1881–1893.

McNeish, R., Moore, E., Benbow, M., & McEwan, R. (2014). Removal of the Invasive Shrub, *Lonicera Maackii* , from Riparian Forests Influences Headwater Stream Biota and Ecosystem Function. *River Res. Applic*. River Research and Applications, 1131-1139. doi:10.1002/rra

Shewhart, Lauren, McEwan, Ryan W., & Benbow, M. Eric. (2014). Evidence for Facilitation of *Culex pipiens* (Diptera: Culicidae) Life History Traits by the Nonnative Invasive Shrub Amur Honeysuckle (*Lonicera maackii*). *Environ Entomology* 2014; 43 (6): 1584-1593. doi: 10.1603/EN14183

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, & C. E. Cushing. (1980). The river continuum concept. Canadian Journal of Fisheries and Aquatic Science **37**:130-137.

Webster, J. R., J. B. Wallace, & E. F. Benfield. (1995). Organic processes in streams of the eastern United States. Pages 117-187 in E. E. Cushing, K. W. Cummins and G. W. Minshall, editors. River and stream ecosystems. Elsevier, Amsterdam.

Wilson, H.N., Arthur, M.A., Schörgendorfer, A., Paratley, R.D., Lee, B.D., & McEwan, R.W. (2013). Site characteristics as predictors of Lonicera maackii in second-growth forests of central Kentucky, USA. *Nat. Area J.* 33: 189–198.

Zavaleta, E. S., R. J. Hobbs, & H. A. Mooney. (2001). Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology and Evolution*. 16:454-459.