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Abstract

The goal of this study was to investigate plant and soil communities along a riparian corridor at four sites in the Toms River Watershed of Ocean County, NJ. This research assessed how these communities differ between the upland and floodplain habitats by examining both biotic and abiotic factors. To accomplish this, plant communities were assessed upon tree basal area, woody shrub cover, herbaceous cover and presence/absence of “all-vegetation”. Soil microbial community composition was also measured at the same four sites. At each of the four sites, I surveyed three transects that were parallel, perpendicular and upland from the river. These transects were then classified into two habitat types; floodplain and upland. Soil samples were returned to the lab for microbial DNA fingerprinting (terminal restriction fragment length polymorphism- TRFLP). Soil chemistry samples were also taken at all four sites. Plant and fungal communities were significantly different among the four sites, however bacterial communities were not significantly different. Plant, fungal and bacterial communities were all significantly different between the floodplain and the upland habitats. Soil chemistry did not vary significantly among the sites. However, soil chemistry did vary significantly between the floodplain and upland for both soil moisture and pH. Soil moisture and pH correlated strongly with the distribution and composition of plant and microbial communities sampled in this study. Bacterial communities were unique in that they correlated with NH_4 as well as pH, but did not correlate with soil moisture. Bacterial communities also did not correlate with any of the plant groups or with fungi. Fungal communities correlated with plant communities as well as soil

moisture and pH. These results show that soil chemistry, particularly soil pH, correlates most often with plant and soil microbial community distribution in this study.

MONTCLAIR STATE UNIVERSITY

Drifting Communities: relationships between soil chemistry, plant and microbial
communities along an urban watershed.

by

Carolyn Haines-Klaube

A Master's Thesis Submitted to the Faculty of
Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

January 2016

College of Science and Mathematics
Department of Biology

Thesis Committee:

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DRIFTING COMMUNITIES: RELATIONSHIPS BETWEEN SOIL CHEMISTRY,
PLANT AND MICROBIAL COMMUNITIES ALONG AN URBAN WATERSHED.

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Master of Science

by

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Montclair State University

Montclair, NJ

2016

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EPA Disclaimer

Although the information in this document has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement CE-98212311 to Ocean County College, it has not undergone the Agency's publications review process and, therefore, may not necessarily reflect the views of the Agency and no official endorsement should be inferred.

Acknowledgements

I am grateful for valuable field assistance from Eliana Geretz and Elena Tartaglia, from Rutgers as well as Elijah Bohoroquez of Montclair State. Adam Parker and Julia Greendyk contributed critical laboratory assistance from Montclair. I thank Christopher Klaube for ArcGIS analysis and map creation. I am also grateful for valuable external quality review by James Vasslides. This work was funded by the Barnegat Bay Partnership and the United States Environmental Protection Agency.

I am thankful for my advisor, Dr. Jennifer Krumins, and co-advisor, Dr. Myla Aronson for their guidance, knowledge and patience. You both have taught me more than you know and I cannot thank you enough. I am thankful for my committee members, Dr. Dirk Vanderklein and Dr. Paul Bologna for their time and wisdom in helping me on my journey. I am grateful for my family and their never ending support, I would not have been able to do this without you.

To my son, may you always be curious.

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Introduction:

Urbanization has affected nearly one-half of the Earth's terrestrial surfaces including biogeochemical cycling and productivity on small (regional) as well as large (continental) scales (Zhang et al., 2013). Riparian areas within watersheds can create a buffer system that works as a filter that can reduce influx of pollutants into the larger water basins (Groffman et al., 2003). Communities within urban riparian areas are dynamic and unique in that they are subject to both anthropogenic disturbances as well as natural disturbances, such as flooding events. However, they are also becoming more threatened by increasing levels of urbanization, which is impacting them through increased pollution and flooding events as well as reducing their size (Burton et. al., 2009). Urbanization can impact the flow-rate and nutrient levels of streams by increasing impervious surfaces and concentrating nutrient output (Harrison et al., 2012). Changes in the rate at which the water flows will also impact the amount as well as type of organic matter that is able to settle on the soil surface. This will in turn affect how organic matter will be broken down and used by the microbial community (Yu and Ehrenfeld, 2010).

Urbanization can affect the amount of nutrients put into an ecological system (Burton et al., 2009), which in turn could have an affect on how the above ground (plant) communities and below ground (bacteria and fungi) communities interact with one another. Plants, bacteria and fungi interact with one another in a number of ways. Plants are able to provide nutrient pools to microbial communities (Lamb et al., 2011). Fungi can use their hyphae to help plants absorb nutrients and water (Hazard et al., 2014). Bacteria and fungi can have positive feedbacks with plants, as they provide nutrients that help stimulate growth and provide defenses against pathogenic soil organisms while

receiving carbon in return (Weidner et al., 2015). While these interactions are well established, it is important to see how they co-exist within the context of a riparian corridor subject to heavy anthropogenic influence. Because microbial communities play fundamental roles in nutrient cycling it is critical to understand how plants and the microbial community are being affected by their surrounding environment (Zornoza et al., 2015).

Plant communities also impact the microbiota in the soil (Bardgett and Wardle, 2010). Arbuscular mycorrhizal fungi are closely associated with plant species and live symbiotically on their root system (Fernanda et al. 2012). They not only provide the plant with access to nutrients, but also help stabilize soil structure (Fernanda et al. 2012). Soil microbiota can also be used as an indicator of soil quality and function (Vasconcellos et al., 2013). The relationship between plants and soil microbes are dynamic. These relationships can be positive or negative, as they affect both the plant and microbial communities, particularly in areas containing abiotic stressors such as contamination (Krumins et al., 2015). Some organisms may be better suited to survive in areas of high nutrient loading than others. For instance, denitrifying bacteria are able to convert NO_3 to N_2 , however they need anaerobic conditions to perform this task (Bettez and Groffman, 2012). Urban riparian zones are perfect places for high levels of denitrification to take place, due to the increased presence of NO_3 and anaerobic soil conditions. Research has shown that a combination of increased fossil fuel consumption and impervious surfaces in urban areas can increase NO_3 runoff into local water bodies by two to four times higher (Bettez and Groffman, 2012). However, there are very few

studies that examine the relationships among plant, bacterial, and fungal communities together with soil chemistry.

This study investigated plant and soil communities along a riparian corridor in the Toms River Watershed of Ocean County, NJ. This research aims to obtain initial knowledge on how plant and microbial communities are distributed between the floodplain and upland habitats and how the soil chemistry is related to the biotic community distribution. The focal point of this study is the riparian zone of Toms River, which is situated within the Barnegat Bay Watershed, a highly urbanized watershed. Soil chemistry is known to affect plant community productivity as well as fungal dominance and species diversity (Egerton-Warburton et al., 2007). The goal of this study is to investigate if the plant and microbial communities are changing along the riparian corridor as well as between the floodplain and upland habitats. Here I examined the differences in soil chemistry, microbial and plant communities in both the floodplain of the Toms River as well as the upland area adjacent to the river. I also wanted to investigate if these changes are consistent among taxa and if soil chemistry is a factor in how these communities are distributed. The majority of stream research projects have focused purely on plant communities, however an important component of a well-functioning plant community is the associated soil biotic community (Harrison et al., 2012, Yu and Ehrenfeld, 2010). This study will determine if the plant and microbial communities are changing between the floodplain and the upland and whether or not these changes are correlated with each other and soil chemistry.

Methods:

Barnegat Bay, located within New Jersey, has a 660 square mile watershed and is considered the largest estuary completely contained within the state (Conway and Lathrop, 2003). The watershed lies along the outer-coastal plain in the southeastern part of the state (Conway and Lathrop, 2005). The bay is considered to be a shallow estuary, with a maximum depth of only 6 meters and a mean depth of 1.5 meters (Kennish et al., 2011). The bay has two major freshwater inputs in the north from the Metedeconk River and Toms River (Kennish and Fertig, 2010). Most of the urban development that has occurred around Barnegat Bay is commercial strip development and low-density suburban housing built on previously forested land (Conway, 2009). Nitrogen, as well as other forms of pollutants, has been introduced into the estuary by storm runoff (surface-water discharge), atmospheric deposition, groundwater and sediments releasing nitrogen (Kennish et al., 2011).

Four study sites were chosen along the Toms River (See Table 1 for Latitude and Longitude for each site, as well as Figure 1 for a map). I chose to use sites that were in close proximity to USGS stream flow and water quality monitoring stations. Two of these sites (NTR - Near Toms River, DM – Dove Mill) were in areas near major roadways and residential infrastructure, while the other two sites (BBL – Blacks Branch, VH – Van Hiseville) were in more secluded areas away from major roads and houses.

The sampling design had three transects at each of the four sites along the Tom's River and its tributaries. The first transect was placed 10 meters away from the river-bank and ran parallel to the river. The second transect started 20 meters away from the 0m mark on the first transect and ran perpendicular to the river. These two transects

combined captured the floodplain habitat. The third transect (upland transect), was laid 100 meters upland from the 0m mark of the wetland transect and ran parallel to the river. Each transect was 100 meters in length and contained three 10 meter by 10 meter plots spaced every 20 meters. Within each plot, I established three 1 meter by 1 meter subplots that were distributed randomly within the 10 meter plots (Figure 2). The decreasing size of the plots allowed for a more accurate assessment of the plant communities represented along each transect and across each site. Subplots were lettered A-C and were randomly placed by using a random number generator that would pick points within the 10 × 10m gridded plot. If all subplots fell within one side of the plot, I inverted the random numbers on one of the plots to ensure that results were not due to an uneven sampling distribution. This design allows for the capture of the transition between the area immediately adjacent to the river to the areas within the floodplain that may be inundated during a flooding event. The design also allows for better sampling in an area that has a lot of variability in the topography due to erosion.

Between May 31 and June 11, 2013, trees were identified to species level and their diameters at breast height (DBH) were taken and converted to basal area in all of the 10 × 10m plots. Shrubs were identified to species level and their length, width and number of stems were recorded and converted to percent cover of the 10 x 10m plot. Herbaceous plant data were collected in the 1 × 1m plots, where plants were identified to genus (and species levels when possible) and percent cover was estimated for each species. After all tree, shrub and ground vegetation data were collected, I would briefly walk through the 10 × 10m plot and look for any other plants in the herbaceous layer that might have been missed in the 3, 1 × 1m samples. These data were combined with the

rest of the species found in the 10 × 10m plot and the 1 × 1 m plots to create a presence/absence record of all the 10 × 10m plots and was entitled “all vegetation”. All plant species were validated in the lab using Gleason and Cronquist (1991).

Soil samples were collected on June 20, 2013. Soil microbial data and soil chemistry were collected at the subplot level. A 2.54cm diameter core was used and 3 soil cores were randomly taken within each subplot and combined to create one sample to account for patchy distribution of soil microorganisms (Martiny et al., 2006). Soil samples were immediately put in a cooler with ice and transported to the lab where they were sieved through a 2mm mesh sieve and stored at -20°C.

After sieving, whole community DNA was extracted from each soil sample using a MoBio Soil DNA extraction kit (Mobio Laboratories, Carlsbad, CA) following manufacturer’s instructions. Bacterial community DNA was amplified using 16S primers and the fungal community was amplified using ITS1 and ITS4 primers following procedures outlined in Krumins et al. (2009). PCR products were tested for consistency using gel electrophoresis combined with a nano-drop nucleotide sensor, and it was determined that there was little variation in DNA concentration between samples.

Amplified whole community DNA for bacteria and fungi were digested using *HhaI* following the manufacturer’s instructions (New England Biolabs, Waltham, MA) and then were separated for fingerprinting by conducting Terminal Restriction Fragment Length Polymorphism (tRFLP) (Krumins et al., 2009) using the Applied Biosystems Genetic Analyzer 3010 (7 Kingsland Grange, Woolston, Warrington, WA 4SR, UK) to sequence the data and the Genemapper v4.0 also by Applied Biosystems to analyze the data.

Soil chemistry samples were sent to Cornell Nutrient Analysis Laboratory (CNAL) where pH, NH₄, NO₃/NO₂ concentrations and total C:N were measured. Soil chemistry samples were taken in all subplots along the transects and then aggregated together by transect for analysis. *In situ* soil moisture was taken within the 1 x 1m plots using a Field Scout TDR 100 soil moisture meter on the same day as soil samples were collected. The Field Scout TDR 100 measures the soil moisture as a percent volumetric water content in a given value of soil to a total soil volume.

I used non-metric multidimensional scaling (NMDS) to examine community composition of vegetation, fungi and soil bacteria. Tree data was analyzed by relative basal area, shrubs and ground-vegetation were analyzed as percent cover, and “all-vegetation”, fungi and bacteria were presence/absence. An arcsine transformation was done on the tree, shrubs and ground-vegetation data before performing the NMDS (Ramette, 2007). If the NMDS resulted in more than two axes, I used the R-squared values to determine which axes represented most of the variation in the data and graphed these axes. The NMDS was followed with Multi-response Permutation Procedure (MRPP) to see if there was a significant difference in communities among the four sites and also between the floodplain and upland habitats. P-values for MRPP pairwise comparisons among sites were corrected using the Bonferonni correction at $\alpha = 0.0083$. Soil chemistry data were used as a secondary layer to each biotic factor (plants and microbes) to analyze if there were any correlations between biotic community and soil chemistry. I used ANOVA to test for significant differences in the soil chemistry types (soil moisture, soil pH, NH₄, NO₃/NO₂ and total C:N) among sites and between habitats. For the correlations of plants and soil chemistry, as well microbial and soil chemistry, all

data was entered by the upland and floodplain transects at each site. A log transformation was done to normalize the soil chemistry data. The Pearson's correlation was used in PC-ORD to show if there was a correlation between the microbial and plant communities as well as soil chemistry. All statistical analyses were carried out in either SAS (Version 9.1 SAS Institute, Cary, NC) or PC-ORD (Version 6, MjM Software. Glendeden Beach, Oregon).

Results

There were 121 species of plants identified in this study, within 66 genera across 46 families. There were 125 operational taxonomic units (OTUs) of bacteria and 256 OTUs of fungi identified within this study.

MRPP showed no significant separation among sites for bacterial communities, but fungi, all-vegetation, trees, shrubs and ground cover composition was significantly different among sites (Table 4).

For tree composition, BBL was significantly different from DM and from VH (MRPP: $A = 0.213$, $T = -6.962$, $p < 0.001$) (Table 4). Upland and floodplain tree composition was also significantly different (MRPP: $A = 0.227$, $T = -13.846$, $p < 0.001$). Results from NMDS showed that Axis 1 (56.3%) and 2 (87.1%) represented 94.6% of the variation in the data. NMDS showed that *Acer rubrum* and *Pinus rigida* are driving the difference among sites (Figure 4, Table 3). *Acer rubrum* was highly correlated with the positive end of Axes 1 and 2 and *Pinus rigida* was associated with the negative end of Axis 1 and 2 (Table 3). As expected, *Acer rubrum* was associated with wetland communities while *P. rigida* was associated with upland communities (Figure 5).

Shrub community composition was significantly different among all sites except for DM and NTR (MRPP: $t = -2.160$, $A = 0.0365$, $p = 0.031$; Table 4). However, all sites showed a significant difference between the floodplain shrub community and the upland shrub community (MRPP: $A = 0.0909$, $T = -9.243$, $p < 0.001$). Results from NMDS showed that Axis 2 (53%) and 3 (71.2% %) represented 99.8% of the variation in the data. NMDS showed that *Gaylussacia baccata*, *Smilax rotundifolia* and *Vaccinium corybosum* drove the differences among and within the four sites (Figure 4, Table 3). *G. baccata* was associated with the negative end of Axes 2 and 3, while *S. rotundifolia* and *V. corybosum* were associated with the positive end of Axis 2 (Table 3). This is expected because *G. baccata* is an upland species and does not live in wet areas, while *S. rotundifolia* and *V. corybosum* can live in both upland and floodplain areas, but are more typically associated with wet soils.

The ground cover community composition was significantly different between DM and BBL, NTR, and VH (MRPP: $A = 0.101$, $T = -7.775$, $p < 0.001$; Table 4). The floodplain and upland shrub communities were significantly different from each other (MRPP: $A = 0.091$, $T = -12.437$, $P < 0.001$). Results from NMDS showed that Axis 1 (14.7%) and 3 (82.5%) represented 100 % of the variation in the data. *Carex lousianica*, *Carex stricta*, *Clethra alnifolia*, *Nyssa sylvatica*, *Smilax glauca*, *Smilax rotundifolia*, *Gaylussacia baccata* and *Gaylussacia frondosa* drove the differences in the ground vegetation communities among the four sites and between the two habitat types (Table 3). *Carex lousianica* and *C. stricta* were associated with the positive end of Axis 1 and 3, while *C. alnifolia*, *N. sylvatica* and *S. rotundifolia* were associated with the negative end of Axis 1 and *G. frondosa*, *G. baccata* and *S. glauca* were associated with the negative

end of Axis 3. *C. lousianica* and *C. stricta* are wetland species, *G. baccata* is an upland species, and *G. frondosa*, *N. sylvatica*, *S. rontundifolia*, and *S. glauca* are able to tolerate both wetland and upland soils.

For the “All-vegetation” community composition, NTR was not significantly different from BBL or VH (MRPP: $A = 0.099$, $T = -8.701$, $p < 0.001$; Table 4). “All-vegetation” was significantly different between the floodplain and the upland habitats among all four sites (MRPP: $A = 0.059$, $T = -9.216$, $p < 0.001$). Results from NMDS showed that Axis 1 (63.6%) and 2 (78%) represented 90.1% of the variation in the data. *Acer rubrum*, *Lindera benzoin* and *Sphagnum* spp. were the species driving the changes among and between the four sites and the two habitats (Table 3). *A. rubrum* and *Sphagnum* spp. were associated with the positive end of Axis 1, while *L. benzoin* was associated with the negative end of Axis 2 (Table 3). *A. rubrum* and *Sphagnum* spp. are wetland plants and *L. benzoin* is an upland species.

Bacterial communities were not significantly different among sites (MRPP: $A = 0.018$, $T = -1.66$, $p = 0.063$; Table 4), however they were significantly different between the floodplain and the upland habitats (MRPP: $A = 0.0307$, $T = -4.87$, $p = 0.011$). Results from NMDS showed that Axis 1 (40.3%) and 2 (71.8%) represented 94.4% of the variation in the data. Fungal communities were significantly different between BBL and DM and NTR (MRPP: $A = 0.031$, $T = -4.53$, $p = 0.001$; Table 4). The fungal communities were also significantly different between the floodplain and upland habitats (MRPP: $A = 0.0378$, $T = -9.649$, $p < 0.001$) (Table 4). Results from the NMDS showed that Axis 1 (38.3%) and 2 (75.8%) represented 99.9% of the variation in the data. Further, the differences seen among sites and between habitats were highly correlated with the

fungal community composition (correlations are shown with green arrows overlaid on the NMDS plots (Figure 6).

Results from the soil chemistry data showed nitrite and nitrate levels to be consistently below detection limit (BDL) and were not included in the analysis. The soil chemistry for percent moisture, pH, NH_4 , %C and %N were not significantly different among sites ($F = 0.28$, P-value: 0.8407, $df = 3$; $F = 0.32$, P-value = 0.813, $df = 3$; $F = 0.41$, P-value = 0.752, $df = 3$; $F = 1.28$, P-value = 0.395, $df = 3$; $F = 0.66$, P-value = 0.619, $df = 3$, respectively, Table 1). Chemistry data are shown as an overlay against plant and microbial communities in Figure 7 A-F. Percent moisture was significantly different between the floodplain and the uplands, with the floodplains having a higher percent soil moisture than the uplands ($F = 22.85$, P-value = 0.0031, $df = 1$, Table 1). pH values were low across all sites, and consistently lowest in the upland for all four sites (Table 1) and were significantly different between the floodplain and upland ($F = 16.62$, P-value = 0.0065, $df = 1$, Table 1). NH_4 levels varied with a notable peak from the floodplain of BBL, but were not significant between the floodplain and upland ($F = 5.24$, P-value = 0.0621, $df = 1$, Table 1). The %C and %N were not significantly different between the floodplain and upland ($F = 0.38$, P-value = 0.559, $df = 1$; $F = 1.63$, P-value = 0.248, $df = 1$, respectively, Table 1).

Results from the Pearson correlations showed that trees and fungi had a correlation along Axis 1 and Axis 2 (Axis 1: $r = -0.506$, Axis 2: $r = 0.749$), Shrubs and fungi had a correlation along Axis 3 ($r = -0.587$). Ground cover and fungi had a correlation along Axis 3 ($r = 0.666$). "All-vegetation" and fungi had a correlation along Axis 1 ($r = -0.563$). (Figure 6 A-D). The chemistry data showed that there is a correlation

between “all vegetation” and pH along Axis 2 ($r = 0.855$), and soil moisture along Axis 1 ($r = 0.654$). There was a correlation between shrubs and pH along axes 1 and 2 ($r = -0.584$, $r = -0.515$, respectively). Ground cover showed a correlation with pH and soil moisture along Axis 1 ($r = -0.789$, $r = -0.682$, respectively) and pH along Axis 2 ($r = 0.663$). There was a correlation between trees and soil moisture along Axis 1 and 2 ($r = 0.582$, $r = 0.663$) and a strong correlation between trees and pH along Axis 1 ($r = 0.807$). Fungi had a correlation with pH along Axis 1 ($r = 0.481$). Bacteria had a correlation with pH and NH_4 along Axis 1 ($r = 0.527$, $r = 0.524$, respectively).

Table 3 demonstrates which species have the strongest correlation across axes scores. The two species with the highest and lowest r-values were chosen, unless there were fewer than two species that had r-values greater than 0.5. These results show that *Acer rubrum* and *Pinus rigida* are the species that show the strongest correlation across Axis 1 and Axis 2 (Table 3). In the shrub layer, *Gaylussacia baccata* (Axis 1), *Smilax rotundifolia* (Axis 2) and *Vaccinium corybosom* (Axis 2) are the key species (Table 3). The ground cover layer was greatly influenced by *Carex* and *Gaylussacia* species (*C. bullata*, *C. stricta* on Axis 2, *G. baccata*, *G. frondosa* on Axis 1) (Table 3). Bacteria and Fungi had a

Discussion:

Other studies have shown changes in plant communities in both diversity and presence of invasive species across urban/suburban gradients (Groffman et al., 2003; Vakhlamova et al., 2014; White et al., 2014). It has also been established that distance from an urban area as well as patch size has an effect on species richness (Knapp et al.,

2008; Matthies et al., 2015). Land use cover and its impacts on flooding potential and erosion were not considered in this study, but are also known factors that can affect the biotic communities (White et al., 2014). The site closest to two major roadways (The Garden State Parkway and Route 9), was in a municipal park containing a large floodplain area between the river and my sampling sites. Floodplains can act as a buffer zone (Rassam et al., 2006), reducing the amount of pollutants/nutrients entering into the stream and ultimately my sampling area.

Plant community composition (analyzed NMDS and MRPP) was significantly different among the four research sites. In the field, it appeared that some of these sites were very distinct, particularly in the floodplains. For example, DM was dominated by *Osmundastrum cinnamomea* (Cinnamon fern) and BBL was dominated by *Chamaecyparis thyoides* (Atlantic white cedar), however this did not make any difference on soil chemistry or soil bacterial communities. In contrast, at the community level, there was a difference among the sites. The tree community at BBL was significantly different from the communities at VH and DM, however it was not different from NTR (Table 4). This is likely due to the upland habitats at BBL and NTR being almost exclusively populated by *Pinus rigida*, while VH and DM had more tree species present. For the shrub species, all the sites were significantly different from each other, except for DM and NTR (Table 4). Both DM and NTR were dominated by Ericaceous shrubs and had very few shrubs not in that family and may explain why those two sites are more similar to each other than the other sites. DM was significantly different from the rest of the sites with respect to the ground cover (Table 4). This is most likely due to DM being dominated by *Osmunda cinnamomea*, where none of the other sites had such a drastic

difference in the ground cover. For “All-vegetation”, NTR was not significantly different from BBL or VH, even though BBL and VH were significantly different from each other (Table 4). Fungal communities were significantly different between BBL and DM, and BBL and VH (Table 4). This is very interesting because both trees and shrubs were also significantly different between those two site pairs, suggesting that there may be a relationship between the trees, shrubs and fungi.

Recent research has shown that plant species identity is one of the most important factors in determining soil microbial community composition (Burns et al., 2015). However, this did not appear to only be important for fungal communities in this current study.

Trees and “all vegetation and ground cover were all correlated with soil moisture, while shrubs were not. This may be due to the dominance of Ericaceous species in both the floodplain and upland habitats. Both *Vaccinium corybosum* and *Gaylussacia frondosa* were consistently found in both habitat types. However, in the trees and ground-vegetation it was very uncommon to see the same species between the floodplain and upland. The prevalence of *V. corybosum* and *G. frondosa* in both the floodplain and upland sites may have been a contributing factor in why these were the only plants that did not differentiate between the floodplain and upland. There were also very few exotic species found in the sites (Table 2) which is contradictory to what we had thought we would find..

One issue that could have confounded my results is that on the day of soil microbial and chemistry sampling, all of the floodplain sites were inundated due to a recent rain event. There was no standing water when these sites were initially selected

and sampled for plant community composition. The inundation could have affected the soil chemistry (Unger, Muzika and Motavalli, 2010) as well as the soil microbial communities (Unger, Kennedy and Muzika, 2009). When soils become flooded, oxygen is depleted the environment becomes anaerobic (Wagner et al., 2015). The microorganisms that were previously living in this aerobic environment are then replaced by anaerobic organisms, mainly gram-positive bacteria (Wagner et al., 2015). Many fungal communities also decline in anaerobic conditions (Wagner et al., 2015), which could have impacted our results greatly. Previous research has shown that gram-positive bacteria are associated with plant rhizospheres (Wagner et al., 2015), the replacement of gram-positive for gram-negative bacteria in the floodplain could also have impacted our results.

All plant groups were correlated with soil chemistry differences between floodplain and upland sites (Figure 7). Interestingly, shrubs were correlated with pH but not soil moisture (Figure 7). The majority of the shrub species in this study belonged to the Ericaceae family, which is known to have close associations with mycorrhizal fungi, referred to as ericoid fungi (Perotto et al., 2002; Bougoure et al., 2007). Species identification for the microbial communities was not done in this study. Ericoid fungi enable their hosts to live in a variety of stressful habitats, which may explain why they were the most abundant plant family across both habitats (Hazard et al., 2014). Soil moisture was not an important factor for both fungi and shrubs, although soil pH was important in my study. These findings were not consistent with a study conducted by Rousk et al. (2010) who found that soil pH was not a strong driver for fungal communities. Fungi and pH were correlated in this study, but not strongly so pH might

not have been a strong driver for the fungal community, but did have the strongest correlation of all of the soil chemistry done in this study. Another study conducted by McHugh and Schwartz (2015) found that fungi were greatly influenced by soil moisture. These discrepancies may be due to the close association of the Ericaceous shrubs and their ericoid fungi (Perotto et al., 2002). It is also important to note that while ericoid fungi are known to have close associations with Ericaceous species (Perotto et al., 2002), ericaceous species are also able to have associations with other types of fungi (Bougoure et al., 2007).

Bacterial communities did not correlate with any of the plant groups in this study. This is also interesting because the bacterial communities were the only community in this study that was also not significantly different among the four sites. Bacterial communities were only distinct between the floodplain and upland. Previous studies have shown close associations between plants and bacteria (Marschner et al., 2001), particularly due to plants influencing available nutrient pools via litter and root exudates (Lamb et al., 2011). Due to the nature of the microbial community sampling techniques, it is possible that I may have not captured all of the functional groups that were in the soil (Burns et al., 2015). Alternatively, there was a very large outlier in the microbial community at the DM sight in the upland transect. It is the same point for both bacteria and fungi, which indicates that it may be due to an error that occurred when soil sampling or initial soil DNA extraction occurred. I decided to keep the outlier because it is not possible to collect another soil sample from the exact location as well as I would be comparing samples taken in different years. However this outlier may have affected the results, particularly across the second Axis. Soil pH can be directly affected by flooding

events, where a flooding event can lower the pH of the soil (Tsheboeng et al., 2014; Saint-Laurent et al., 2014). This is consistent with my data, where the floodplain sites consistently and significantly have a lower pH than the upland sites (Table 1). This may in part be due to an increase in leaf litter accumulation in the upland, which would increase the pH of the soil (Saint-Laurent et al., 2014). The anoxic conditions in the soil that were created by flooding (Grunth et al., 2008) are important habitat for denitrifying bacteria (Revsbech et al., 2005). Since the bacteria were not correlating with any plant groups, I hypothesized that the bacteria in this study are more greatly correlated with the soil chemistry than any other factor. Even though NO_3 concentration was below detection limit for this study, I believe that this may be due to the fact that the day that the soil chemistry almost all of the floodplain sites were inundated. This could lead to a dilution of the NO_3 and NO_2 , making them below detection limit for sample that I had, however if the sample was taken when the site was not inundated, I might have received different results. Due to the bacterial communities being different between the floodplain and the upland, I hypothesize that the anoxic conditions of the floodplain host denitrifying bacteria, while the upland habitats do not. This may be due to anoxic conditions created within the floodplain during flooding (Revsbech et al., 2005).

Soil pH was the only abiotic factor that correlated with both microbial and plant communities (Figure 7 A-F) which is consistent with current literature (Rousk et al., 2010; Eskelinen et al., 2009). Soil moisture was the second most important factor when determining community composition for both plants and bacteria, however it was not a factor in shrubs (Figure 7 B) or fungal communities (Figure 7 F). The shrub community was dominated by species that belonged to the *Ericaceae* family (Table 2). Fungi

correlated with plant species across all groups (Figure 6). pH was the only soil chemistry factor that correlated with fungal community composition (Figure 7 F). Soil pH is an important factor in nutrient availability for soil fungi and bacteria (Rousk et al., 2009) and would explain why my data show that soil pH and the microbial communities are correlated.

NO₂ and NO₃ were considered below detection limit (BDL) by the Cornell Nutrient Analysis lab (Table 1). The detectable levels for this lab were measured in mg/kg of soil. Due to the levels of NO₂ and NO₃ being BDL, it is impossible to determine whether it impacted plant and microbial communities in this study. Soil nitrogen levels can be decreased during flooding events (Unger, Muzika and Motavalli, 2010), which could have been the reason why the NO₂ and NO₃ levels were below the detection limit. However, the primary difference between these two habitats would be the intensity and frequency of flooding events. The floodplain sites were almost all inundated on the day of microbial and chemistry sampling. Flooding is known to have an affect not only on soil moisture, but also on pH. Regular flooding will have a strong effect on the nature of the soil community and its capacity to process excess nutrients. This may be due to anaerobic soil conditions caused by inundation (Grunth et al., 2008). These conditions will also select for plants and soil microorganisms that thrive in the variable environment. Influxes of N are known to decrease both microbial as well as plant diversity (Krumins et al., 2009; Baer et al., 2004).

The %C and %N did not appear to have any correlation with the plant and microbial communities in this study. These results are not consistent with other studies (Marschner et al., 2001; Baer et al., 2004; Krumins et al., 2009), but the results of the soil

chemistry may have been skewed due to the floodplain being inundated on the day of sampling. Other studies have shown that N is an important factor in plant community diversity, where an increasing amount of soil N reduces overall plant diversity (Baer et al., 2004). Interestingly, studies have shown that C:N ratios, Total N and Total C are not affected by flooding events (Gelsomino et al., 2006; Unger et al., 2009). A study conducted by Krumins et al. (2009) showed that changes in N concentrations in the soil did not affect fungal communities, but did have an effect on bacterial communities.

Conclusion:

Understandably, the most pronounced differences in both plant and microbial community structure were observed between the floodplain and the upland habitats. Soil moisture as well as pH varied greatly between these two habitats and are known to affect plant and microbial diversity (Rousk et al., 2010; Eskelinen et al., 2009; McHugh and Schwartz, 2015). These results suggest that soil moisture and pH are driving the shifts in plant and microbial communities between the two habitats. More research needs to be done in order to determine whether the microbial communities are also being affected seasonally and if their relationships to plant and soil chemistry change throughout the year. Due to the short nature of this study as well as the design, I was unable to determine cause and effect of the relationships observed. This case study provides important information on plant-soil interactions in a suburban riparian zone and how these interactions change with respect to distance from the river and changes in soil chemistry.

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Figures:

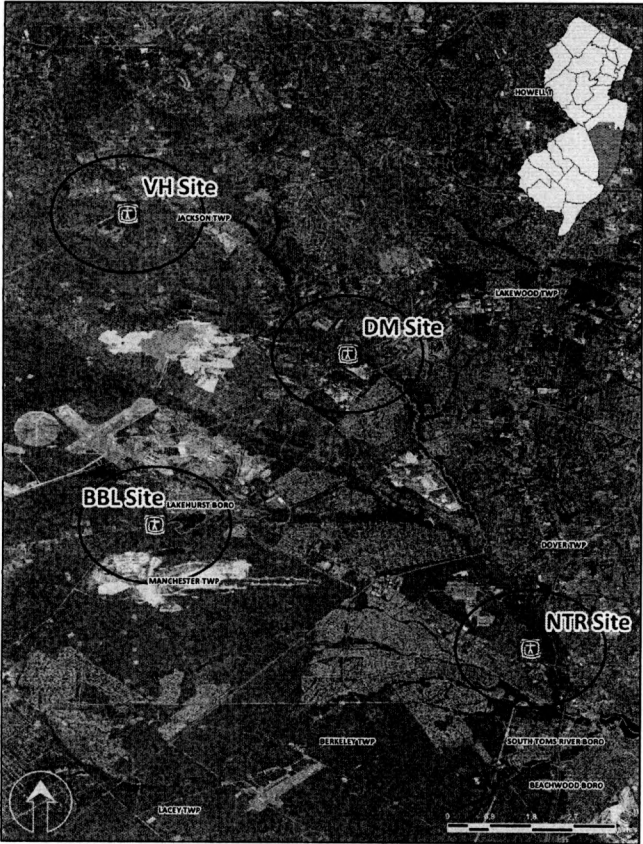


Figure 1: Aerial image of the 4 research sites within this study



Figure 2: In-situ images of the four research sites

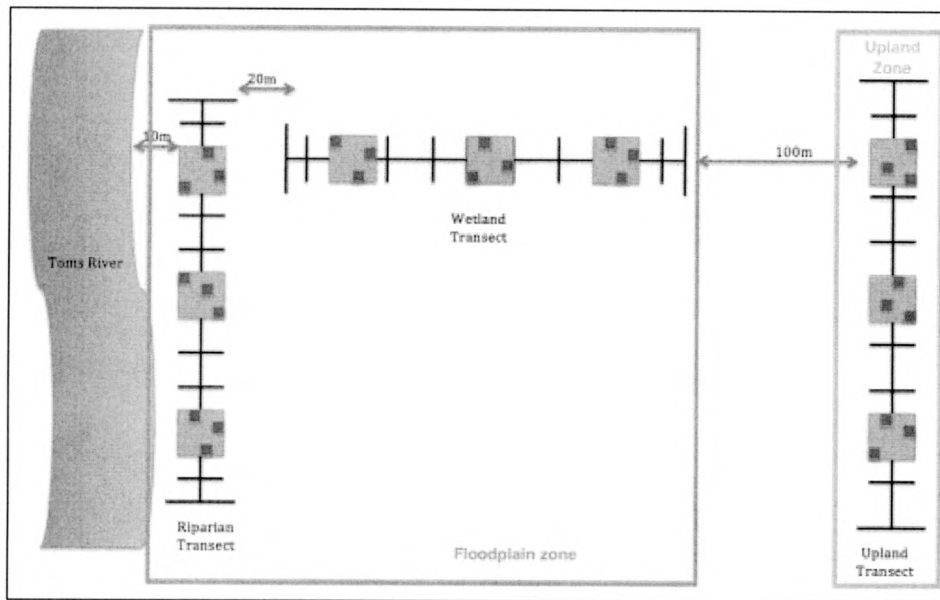


Figure 3: Diagram illustrating the plot design

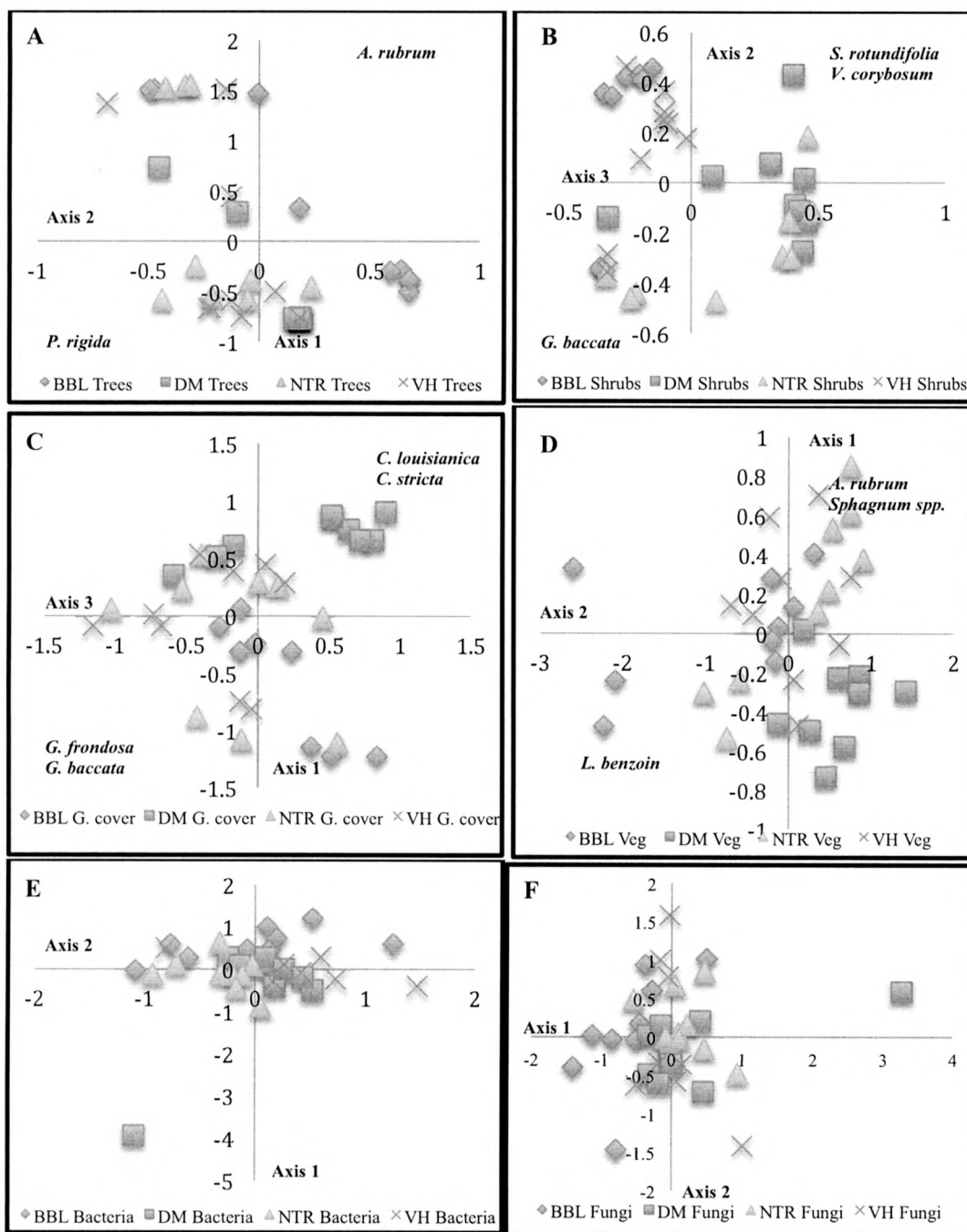


Figure 4: NMDS ordination of plant and microbial community distribution among the four sites. For significance values refer to Table 4.

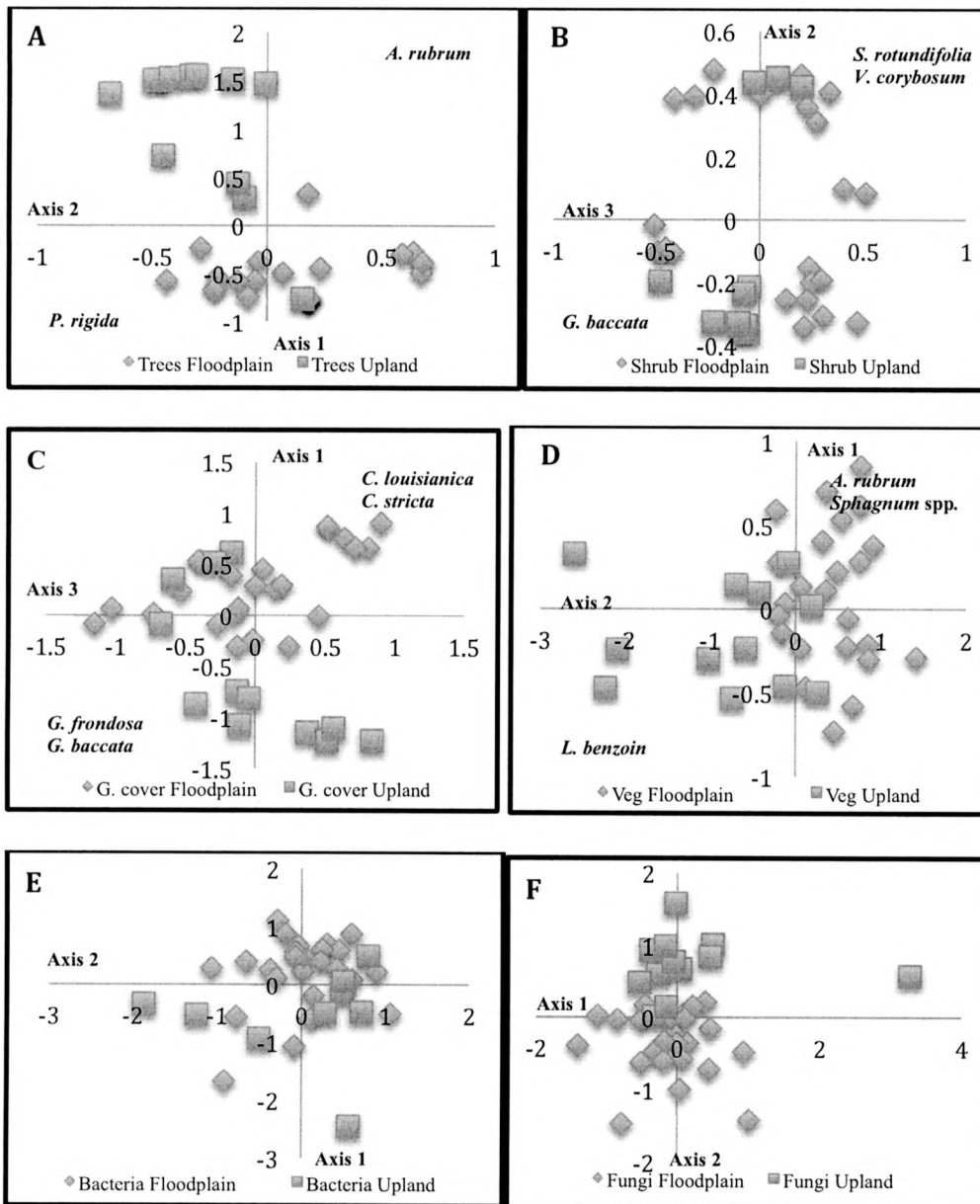


Figure 5: NMDS ordination of plant and microbial community distribution between floodplains and uplands. (A) Trees ($A = 0.227$, $p < 0.001$), (B) Shrubs: ($A = 0.0909$, $p < 0.001$), (C) Ground cover (G. cover) ($A = 0.0915$, $p < 0.001$), (D) “All-Vegetation”: ($A = 0.059$, $p < 0.001$), (E) Bacteria: ($A = 0.0378$, $p = 0.011$), (F) Fungi ($A = 0.0378$, $p < 0.001$)

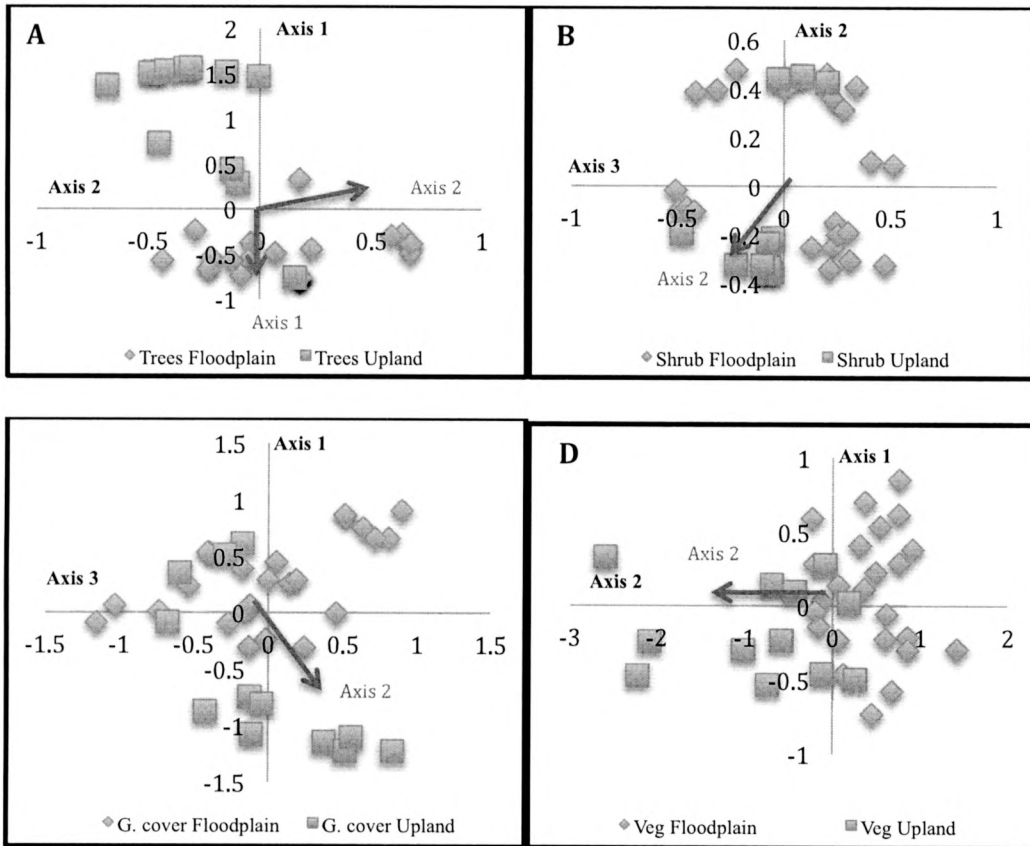


Figure 6: NMDS ordination with (A) Trees (Axis 1: $r = -0.506$, Axis 2: $r = 0.749$), (B) Shrubs (Axis 3: $r = -0.587$), (C) Ground cover (G. cover) (Axis 3: $r = 0.666$), (D) “All-Vegetation” (Axis 1: $r = -0.563$) as the primary layer and fungi as the second layer.

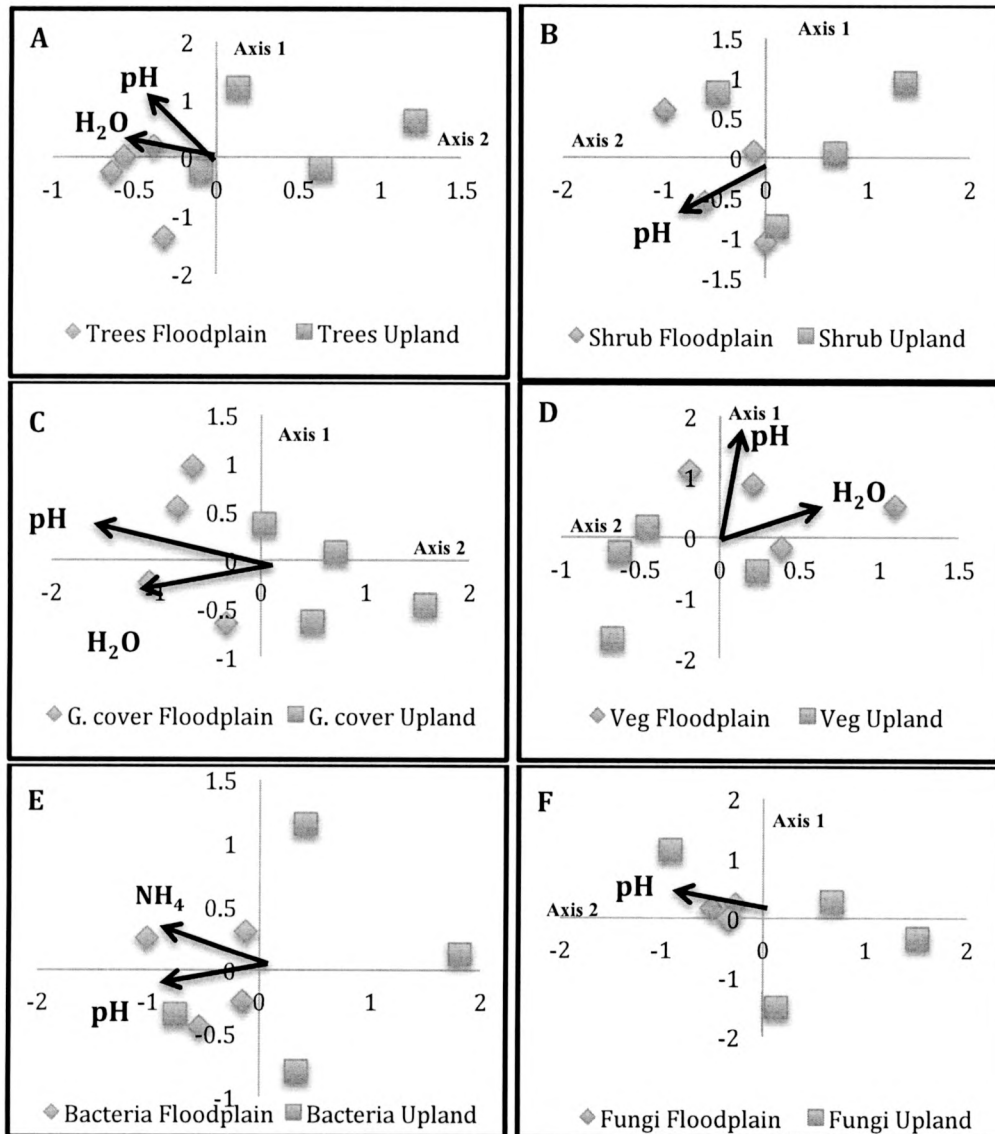


Figure 7: NMDS ordination with trees (pH Axis 1 and 2: $r = 0.582$, $r = 0.663$, respectively; H₂O Axis 1: $r = 0.897$) (A), shrubs (pH Axis 1 and 2: $r = -0.584$, $r = -0.515$, respectively) (B), ground cover (pH and H₂O Axis 1: $r = -0.789$, $r = -0.682$, respectively, pH Axis 2: $r = 0.663$) (C), “all-vegetation” (pH Axis 2: $r = 0.855$, H₂O Axis 1: $r = 0.654$) (D), bacteria (pH and NH₄ Axis 1: $r = 0.527$, $r = 0.524$, respectively) (E) and fungi (pH Axis 1: $r = 0.481$) (F) communities as the first layer and soil chemistry as the second layer.

Table 1: Soil Chemistry for habitat type within each site. Results from ANOVAs are comparing the floodplain and upland. Soil moisture (F = 16.62, P-value = 0.0065, df = 1), pH (F = 16.62, P-value = 0.0065, df = 1), NH₄ (F = 5.24, P-value = 0.0621, df = 1), Total %N (F = 1.63, P-value = 0.248, df = 1), Total %C (F = 0.38, P-value = 0.559, df = 1).

Site and Zone	Soil % Moisture	pH	NH ₄ mg/kg	NO ₃ +NO ₂ mg/kg	Total %N	Total %C
NTR Floodplain	43.08	4.17	38.61	BDL	0.48	10.61
NTR Upland	6.05	3.81	10.33	BDL	0.15	4.77
DM Floodplain	36.73	4.61	36.56	BDL	0.31	5.41
DM Upland	4.35	3.7	24.12	BDL	0.59	13.55
VH Floodplain	41.92	4.14	12.25	BDL	0.21	3.97
VH Upland	8.6	3.55	9.01	BDL	0.17	4.52
BBL Floodplain	66.83	3.99	107	BDL	1.42	39.89
BBL Upland	20.56	3.5	7.65	BDL	0.21	7.57

Table 2: List of all plant species identified within this study. * Indicates a non-native species designated according to the USDA database.

<i>Aronia arbutifolia</i>	<i>Lindera benzoin</i>	<i>Quercus bicolor</i>
<i>Betula nigra</i>	<i>Lobelia</i> spp.	<i>Quercus ilicifolia</i>
<i>Carex atlantica</i>	<i>Lycopus virginicus</i>	<i>Quercus marilandica</i>
<i>Carex bullata</i>	<i>Lyonia ligustrina</i>	<i>Quercus montana</i>
<i>Carex collinsii</i>	<i>Lyonia mariana</i>	<i>Quercus palustris</i>
<i>Carex formosa</i>	<i>Lysimachia nummularia</i> *	<i>Quercus phellos</i>
<i>Carex intumescens</i>	<i>Magnolia virginiana</i>	<i>Quercus rubra</i>
<i>Carex louisianica</i>	<i>Maianthemum canadense</i>	<i>Quercus</i> spp.
<i>Carex nigromarginata</i>	<i>Maianthemum racemosum</i>	<i>Quercus veluntina</i>
<i>Carex seorsa</i>	<i>Melampyrum lineare</i>	<i>Photinia pyrifolia</i>
<i>Carex</i> spp.	<i>Microstegium vimineum</i> *	<i>Rhododendron periclymenoides</i>
<i>Carex stricta</i>	<i>Mitchella repens</i>	<i>Rhododendron viscosum</i>
<i>Carex trisperma</i>	<i>Moss</i> spp.	<i>Rubus hispidus</i>
<i>Chamaecyparis thuyoides</i>	<i>Myrica pennsylvanica</i>	<i>Rubus</i> spp.
<i>Chimaphila maculata</i>	<i>Nyssa sylvatica</i>	<i>Sassafras albidum</i>
<i>Cinna latifolia</i>	<i>Oenothera</i> spp.	<i>Smilax glauca</i>
<i>Clethra alnifolia</i>	<i>Onoclea sensibilis</i>	<i>Smilax laurifolia</i>
<i>Cornus</i> spp.	<i>Osmunda cinnamomea</i>	<i>Smilax rotundifolia</i>
<i>Crataegus</i> spp.	<i>Osmunda regalis</i>	<i>Solidago</i> spp.
<i>Cyperus</i> spp.	<i>Oxalis</i> spp.	<i>Sphagnum</i> spp.
<i>Dichanthelium clandestinum</i>	<i>Oxydendrum arboretum</i> *	<i>Symplocarpus foetidus</i>
<i>Dioscorea villosa</i>	<i>Panicum</i> spp.	<i>Toxicodendron radicans</i>
<i>Galium asprellum</i>	<i>Parthenocissus quinquefolia</i>	<i>Trientalis borealis</i>
<i>Gaultheria procumbens</i>	<i>Peltandra virginica</i>	<i>Ulmus</i> spp.
<i>Gaylussacia baccata</i>	<i>Pinus rigida</i>	<i>Vaccinium atrococcum</i>
<i>Gaylussacia dumosa</i>	<i>Polygonum</i> spp.	<i>Vaccinium corymbosum</i>
<i>Gaylussacia frondosa</i>	<i>Potentilla</i> spp.	<i>Vaccinium</i> spp.
<i>Ilex glabra</i>	<i>Prunus avium</i> *	<i>Verbena</i> spp.
<i>Ilex opaca</i>	<i>Prunus serotina</i>	<i>Viburnum dentatum</i>
<i>Iris</i> spp.	<i>Prunus virginiana</i>	<i>Viola lanceolata</i>
<i>Leersia oryzoides</i>	<i>Pteridium aquilinum</i>	<i>Viola</i> spp.
<i>Lilium</i> spp.	<i>Quercus alba</i>	

Table 3: List of plants and microbial OTUs that are the key players in community composition between the floodplain and the upland habitats

Species/O.T.U.	r	Axis	Species/O.T.U.	r	Axis	Species/O.T.U.	r	Axis
Trees			Fungi			Fungi continued		
<i>Acer rubrum</i>	0.566, 0.746	1, 2	F87	-0.8	2	F162	-0.58, -0.836	1,2
<i>Pinus rigida</i>	-0.876, -0.889	1, 2	F80	-0.882	2	F191	-0.58, -0.836	1,2
Shrubs			F76	-0.715	2	F226	-0.58, -0.836	1,2
<i>Gaylussacia baccata</i>	-0.523, -0.606	2, 3	F58	-0.836	2	F244	0.574, 0.6	1,2
<i>Smilax rotundifolia</i>	0.512	2	F46	-0.821	1	F251	0.574, 0.6	1,2
<i>Vaccinium corybosum</i>	0.635	2	F45	-0.821	1	F254	0.513, 0.543	1,2
Ground Cover			F40	-0.8	2	F85	0.546, 0.562	1,2
<i>Carex louisianica</i>	0.564, 519	1, 3	F38	-0.654	1	F97	0.536	2
<i>Carex stricta</i>	0.565, 565	1, 3	F37	-0.628	1	F94	0.531	1
<i>Clethra alnifolia</i>	-0.625	1	F33	-0.821	1	F9	0.625	1
<i>Gaylussacia baccata</i>	-0.801	3	F31	-0.628	1	F81	0.555	1
<i>Gaylussacia frondosa</i>	-0.632	3	F28	-0.821	1	F79	0.625	1
<i>Nyssa sylvatica</i>	-0.636	1	F26	-0.574	1	F77	0.625	1
<i>Smilax glauca</i>	-0.531	3	F24	-0.675	1	F70	0.625	1
<i>Smilax rotundifolia</i>	-0.726	1	F239	-0.502	2	F62	0.625	1
All Vegetation			F238	-0.589	1	F61	0.591	1
<i>Acer rubrum</i>	0.799	1	F235	-0.692	2	F253	0.8	2
Moss spp.	0.596	1	F233	-0.62	1	F249	-0.574	1
<i>Gaylussacia frondosa</i>	-0.529	1	F23	-0.821	1	F248	0.559	1
<i>Lindera benzoin</i>	-0.507	2	F221	-0.715	2	F236	0.625	1
<i>Sphagnum</i> spp.	0.608	1	F220	-0.762	1	F232	0.715	2
Bacteria			F209	-0.8	2	F229	0.625	1
B13	-0.607	2	F208	-0.8	2	F227	0.591	1
B19	-0.717	1	F200	-0.882	2	F222	0.625	1
B42	-0.613	1	F197	-0.821	1	F22	0.625	1
B59	-0.775	2	F196	-0.514	1	F213	0.677	2
B64	-0.775	2	F190	-0.8	2	F202	0.625	1
B71	-0.723	2	F18	-0.821	1	F201	0.558	2
B72	-0.717	1	F170	-0.502	2	F19	0.625	1
B74	-0.581	1	F17	-0.8	2	F151	0.501	1
B81	-0.775	2	F163	-0.615	2	F146	0.654	1
B84	-0.707	2	F16	-0.8	2	F14	0.715	2
B99	-0.59	2	F159	-0.821	1	F134	0.574	1
B115	-0.821	2	F157	-0.675	1	F124	0.625	1
B124	-0.59	1	F156	-0.821	1	F11	0.625	1
B51	0.777, -0.669	1, 2	F15	-0.821	1			
B35	0.777, -0.669	1, 2	F149	-0.628	1			
B88	0.777, -0.669	1, 2	F147	-0.666	2			
B101	0.777, -0.669	1, 2	F145	-0.715	2			
B8	0.607		F138	-0.558	2			
B96	0.717	1	F131	-0.677	2			
B93	0.59	1	F128	-0.589	1			
B87	0.615	1	F127	-0.558	2			
B85	0.538	1	F122	-0.514	1			
B78	0.707	2	F12	-0.628	1			
B77	0.538	1	F117	-0.821	1			
B76	0.641	2	F116	-0.821	1			
B70	0.538	1	F115	-0.675	1			
B69	0.8	1	F109	-0.715	2			
B68	0.649	1	F108	-0.821	1			
B67	0.717	1	F106	-0.8	2			
B55	0.59	1	F104	-0.882	2			
B52	0.59	1	F102	-0.821	1			
B49	0.59	2	F164	-0.58, -0.836	1, 2			
B43	0.613	1	F175	-0.762, -0.663	1, 2			
B39	0.538	1	F203	-0.762, -0.663	1, 2			
B32	0.723	2	F215	-0.58, -0.836	1, 2			
B28	0.75	2	F148	-0.556, -0.701	1, 2			
B123	0.828	1	F55	-0.58, -0.836	1, 2			
B120	0.828	1	F241	-0.562, -0.69	1, 2			
B12	0.828	1	F113	-0.762, -0.663	1, 2			
B117	0.738	1	F114	-0.762, -0.663	1, 2			
B116	0.8	1	F118	-0.58, -0.836	1, 2			
B108	0.75	1	F119	-0.58, -0.836	1, 2			
B107	0.75	1	F120	-0.503, -0.634	1, 2			

Linked

Table 4: MRPP on plant and microbial communities among the four sites, corrected using the Bonferonni correction at $\alpha = 0.0083$. Significant differences are bolded and starred.

Community	T	A	P
Trees			
* BBL vs. DM	-6.7509	0.3560	0.0002
BBL vs. NTR	-3.5116	0.1393	0.0095
* BBL vs. VH	-5.5725	0.2048	0.0007
DM vs. NTR	-3.1535	0.1383	0.0157
DM vs. VH	-1.9554	0.0815	0.0520
NTR vs. VH	-0.4085	0.0128	0.2469
Shrubs			
* BBL vs. DM	-6.6814	0.1635	0.0001
* BBL vs. NTR	-3.8950	0.1132	0.0052
* BBL vs. VH	-4.0103	0.1323	0.0044
DM vs. NTR	-2.1602	0.0366	0.0315
* DM vs. VH	-7.2387	0.1435	0.0001
* NTR vs. VH	-4.7135	0.1065	0.0014
Ground Cover			
* BBL vs. DM	-6.5428	0.1304	0.0001
BBL vs. NTR	-1.5523	0.0331	0.0785
BBL vs. VH	-2.0799	0.0394	0.0446
* DM vs. NTR	-6.0408	0.1049	0.0001
* DM vs. VH	-5.2989	0.0863	0.0007
NTR vs. VH	-2.1437	0.0336	0.0372
All Vegetation			
* BBL vs. DM	-6.3567	0.1131	0.0001
BBL vs. NTR	-3.2508	0.0581	0.0093
* BBL vs. VH	-3.9884	0.0567	0.0018
* DM vs. NTR	-4.7273	0.0798	0.0008
* DM vs. VH	-4.4970	0.0666	0.0013
NTR vs. VH	-3.2419	0.0470	0.0100
Bacteria			
BBL vs. DM	-1.5497	0.0205	0.0783
BBL vs. NTR	-2.2397	0.0247	0.0281
BBL vs. VH	-0.9740	0.0142	0.1504
DM vs. NTR	-0.5242	0.0067	0.2616
DM vs. VH	0.0873	-0.0014	0.4503
NTR vs. VH	-0.9284	0.0127	0.1651
Fungi			
* BBL vs. DM	-5.0619	0.0382	0.0001
* BBL vs. NTR	-3.4261	0.0304	0.0039
BBL vs. VH	-2.8436	0.0276	0.0133
DM vs. NTR	-2.4596	0.0179	0.0149
DM vs. VH	-0.2771	0.0024	0.3335
NTR vs. VH	-1.4252	0.0138	0.0900