Final Report: to the Barnegat Bay Partnership

Role of Plant and Soil Community Structure in Riparian Soil Nutrient Retention

Jennifer Adams Krumins, Myla F. Aronson and Carolyn Haines-Klaube

Introduction

Urban watersheds face unique challenges due to increased amounts of disturbance, impervious surfaces, and high levels of non-point source pollutants. They not only serve as an important habitat for both flora and fauna, but also provide essential water supplies as well as recreational activities to surrounding communities. Urbanization has affected nearly one-half of the globe's surfaces including biogeochemical cycling and productivity on small (regional) as well as large (continental) scales (Zhang et al., 2013). This can also be seen in the large increase in the amount of nitrogen being added to ecological systems (Collins et al., 2010). The capacity of landscapes to cycle nutrients vary drastically between urban and rural ecosystems, and it is important to acknowledge that it is not appropriate to apply models used in rural systems to those in urban. Further, dynamics among plants, soil organisms and their environment can be very different (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 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/flooding events as well as reducing their size (Burton et. al. 2009). Urbanization can also impact streams by increasing their

peak flows due to lack of permeable ground as well as increasing the amount of nutrients that are being transported downstream (Harrison et al., 2012). Changes in the flow rate will also impact the amount and kind of organic matter that is able to settle on the soil surface and be broken down and used by the microbial community (Yu and Ehrenfeld, 2010). Studies have shown that urban to rural gradients along riparian corridors have different impacts on forest communities. Along these gradients, forests close to urban areas had more fast growth rate plants, and rural areas had plants that grew at a slower rate (Burton et al. 2009).

Changing plant communities will 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. 2009). Soil microbiota can also be used an indicator as soil quality and function (Vasconcellos et al., 2013).

In this study, we investigated how plant and soil communities change along an urban to rural gradient in the Toms River Watershed of Ocean County. We tie our results to measures of soil chemistry along the gradient. Some species of plants and microbes may be better suited to survive in areas of high nitrogen loading than others. For instance, bacterial denitrifies 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 increase in NO_3 and the anaerobic soil conditions. Research has shown that due to the increase in fossil fuel consumption combined with increased impervious surfaces in urban area, there is an increase in NO_3

runoff into local water bodies that is anywhere between two and four times higher than what is seen in rural areas (Bettez and Groffman, 2012).

Our study is focused on a major river that discharges into the Barnegat Bay, which lies along the central east coast of NJ. One of the major contaminants of the Barnegat Bay is nitrogen (Wieben and Baker, 2009). While nitrogen is a naturally occurring element and is vital to many natural processes and organisms, in large concentrations it can be devastating, especially to aquatic environments (Library.fws.gov). This work provides a good case study to obtain initial knowledge on how anthropogenic influences along a rural to urban gradient can impact an ecosystem. The majority of stream restoration projects have focused purely on flora communities, however an important component of a well-functioning flora community is the associated soil biotic community (Harrison et al., 2012, Yu and Ehrenfeld, 2010). It is increasingly important that we understand the complex interactions between plants, bacteria and fungi and how nutrient flow and soil chemistry impact the intricate system, a system that will ultimately reduce the terrestrial effects of urbanization on downstream aquatic habitats.

Goals and Objectives of the Project

The stated goal of this research was to assess the feedbacks among non-point source pollution, plant communities, and soil community structure along a riparian corridor of the Barnegat Bay Watershed (BBW). We examined soil and plant community composition in the summer of 2013 along the Toms River. Ultimately, understanding the interactions between the plant and soil communities has allowed us here to make recommendations of restoration targets in the BBW.

The final outcome of this research is data that demonstrates the effects of urbanization on flora, bacterial and fungal communities along a riparian corridor in both floodplain and upland zones. Very few studies consider at plant, bacterial and fungal communities combined along an urban to rural transect. Because it is well established that there are interactions occurring between plants, bacteria and fungi, it is important to see how they co-exist within the context of a riparian corridor subject to heavy anthropogenic human influence. Our results have high applicability to restoration projects. Knowing the flora and microbial communities best suited for a particular remediation site with factors such as soil moisture, soil chemistry and proximity to urbanization may be the key to rebuilding functional riparian ecosystems.

Methods

Study Sites

Barnegat Bay has a 660 km^2 watershed and is considered the largest estuary completely contained within the state of New Jersey (Conway and Lathrop, 2003). The watershed lies along the out-coastal plain in the southeastern part of the sate (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 (Library.fws.gov). Barnegat Bay is separated from the Atlantic Ocean by narrow, highly populated barrier islands, Island Beach and Long Beach Island (Gao, 2002, Kennish and Fertig, 2010). The bay is fed from the north by Toms River, which results in a lower concentration of salinity in the northern

region compared to the central and southern regions (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). The area is expected to increase its population because of the expansion of nearby cities such as Atlantic City and Philadelphia (Conway, 2009). Toms River and the Metedeconk River account for more than 60% of the nitrogen load as a result of surface water discharge into the northern portion of Barnegat Bay (Wieben and Baker, 2009). Nitrogen, as well as other forms of pollutants, are introduced into the estuary by base flow or storm runoff (surface-water discharge), atmospheric deposition, direct storm-water runoff and groundwater recharge, and sediments releasing nitrogen, as well as ocean water coming into the estuary (Wieben and Baker, 2009).

Four study sites were chosen along the Toms River (See Table 1 for Latitude and Longitude for each site, as well as Fig. 1 for a map). The sites were chosen to represent an urban-rural gradient with Near Toms River (NTR) the most urban and VH (Van Hiseville) the least urban. Two of these sites were chosen in areas surrounded by urban or suburban infrastructure (NTR, DM – Dove Mill), and two sites where chosen in suburban and rural areas (BBL – Blacks Branch, VH). An estimation of impervious surfaces to indicate level of urbanization was done using a 1km and 2km radii overlay on a map of each site (Fig. 2). NTR and DM had more than 50% cover of impervious surface (as defined as roads, buildings, parking lots, etc) in the 1 km radius and were classified as suburban/urban. BBL and VH had while less than 50% impervious surface cover and were classified as suburban/rural.

Plot Design

Three transects were established at each site, representing the riparian zone, the wetland zone, and the upland zone. Each transect was 100m in length and contained 3 10x10m plots. With each plot, we established 1x1m subplots (Fig. 3). The decreasing size of the plots allowed for a more accurate assessment of the flora communities represented along each transect. The first transect that was placed was the riparian transect, which started 10m away from the river bank and ran parallel to the river. The second transect started 20m away from the riparian transect and ran perpendicular to the river and the riparian transect. The third transect, the upland transect, started 100m from the edge of the wetland transect and ran parallel to the river and the riparian transect. Each plot along the transect was numbered 1 through 3, where 1 was the most upstream plot and 3 was the most downstream plot. In the case of the wetland transect, plot 1 represented the plot that was closest to the river and 3 represented the plot that was farthest from the river. Subplots were lettered A-C and were randomly placed by using a random number generator that would pick points within the 10X10m gridded plot. If all subplots fell within one side of the plot, an inversion of the random numbers would be done on one of the plots to ensure that results were not due to an uneven sampling distribution.

Data analysis showed that there was no significant difference between riparian and wetland transects with respect to plant, bacterial, and fungal community composition or plant diversity, soil chemistry, and soil moisture. However, it did show that there was a significant difference between riparian and wetland transects grouped together and the upland transects. For this reason, the data will be referred to as “floodplain” or the

"upland" zones where 'floodplain' includes all data points from the riparian and wetland transects (Fig. 4).

Flora Sampling

Flora data was collected between May 31 and June 11, 2013. In the 10x10m plots, tree and shrub data were collected. Trees were identified to species level and their diameter at breast height (DBH) were taken and converted to basal area. Shrubs were identified to species level and their length, width and number of stems was recorded and converted to percent cover of the 10X10mplot. Herbaceous data was collected in the 1X1m plots, where plants were identified to genus (and species levels when possible). Percent cover of herbaceous species was done visually in situ and recorded. All plant species were validated in the lab using Gleason and Cronquist (1991) and species codes were obtained from current USDA data and organized to family level.

Soil Chemistry and Microbial Community Sampling

Soil samples were collected on June 20, 2013. Soil microbial data was collected at the subplot level. A small soil corer was used and 3 soil cores were taken within each subplot to account for patchy distribution of soil microorganisms. Soil samples were immediately put in a cooler with ice and transported to the lab where they were sieved through a 2mm 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 IT1 and ITS4 primers following procedures outlined in Krumins et al. (2009). PCR products were tested for consistency using gel electrophoresis and 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 *Hha1* following manufacturers instructions (New England Biolabs, Waltham, MA), and they were separated for fingperprinting using Terminal Restriction Fragment Length Polymorphism (tRFLP) using the Applied Biosystems Genetic Analyzer 3010.

Soil chemistry samples were sent to Cornell Nutrient Analysis Laboratory (CNAL) where pH, NH_4, NO_3/NO_2 and total C:N was conducted. We used CNAL because the laboratory approved in our QAPP, New Jersey Analytical Labs, failed to produce results. After several phone calls and emails by Dr. Krumins, Dr. Aronson and Carolyn Haines-Klaube, between June 2013 and January 2014, NJAL claimed they did not have enough samples to process our results. This information was given to Dr. Aronson on January 24 by Ethan Einwohner, owner of NJAL. When pressed for more information, Mr. Einwohner told Dr. Aronson they lost the samples. *In situ* soil moisture was taken within the 1 X 1 m plots using a Field Scout TDR 100 soil moisture meter on the same day as soil samples were collected. Soil chemistry samples of the riparian and wetland transects were aggregated together at the site level into the 'floodplain' as described above.

Results

The composition of the plant communities across the four sites overlapped heavily (Fig. 5A). When considering the trees or shrubs alone (Fig. 5 B and C), there was little

difference between the sites. However, when considering only the herbaceous cover alone (Fig. 5D), the plots at DM are notably distinct, but still not significantly different. This is likely due to the unique dominance of ferns at that site (particularly *Osmunda cinnamomea*). The sites did not differ according to the urban-rural gradient. NTR, the most urban site, was not significantly different in vegetation composition from the other sites.

The most frequently occurring plant species in the floodplain included (Table 2): *Acer rubrum, Clethra alnifolia*, various moss species, *Nyssa sylvatica, Smilax rotundifolia*, *Sphagnum* spp., and *Vaccinium corymbosum*. Other important components of these plant communities include: various *Carex* species, *Chamaecyparis thyoides, Gaylussacia* species (*G. baccata, G. dumosa*, and *G. frondosa*), *Lyonia ligustrina, Pinus rigida, Rhododendron periclymenoides,* and *Toxicodendron radicans*.

The microbial community did not vary among the four sites with one notable outlier from the DM site (Fig. 6). However, when we group all the sites together and analyze them based on their habitat type, as stated above, there were no significant differences in the plant or microbial community composition between the riparian and floodplain transects. We found significant differences between the floodplain and the upland plant communities (Fig. 7 A-D). Further, these differences were highly correlated with the microbial community composition (positive correlations are shown with green arrows overlaid on the PCA plots, Fig. 7 A-D). The key plant species associated with microbial floodplain communities were *Acer rubrum*, *Vaccinium* species (including *V. atrococcum* and *V. corymbosum*), *Sphagnum* sp., and other moss species. *Carex stricta, Carex louisianica*, and *Chamaecyparis thyoides* were also highly associated with the floodplain.

As stated above, the New Jersey Analytical Lab approved for use in our QAPP failed to provide data on soil and water chemistry, and they did not keep our samples (we have signed chain of custody forms from the lab). Therefore, we compiled remaining soil from the microbial sampling and sent it to the Cornell Nutrient Analysis Laboratory. Results showed nitrite and nitrate levels to be consistently below detection limit (bdl); NH_4 levels varied with a notable peak from the floodplain of BBL (Table 1). In fact both %N and %C were very high in the BBL floodplain. pH values were low across all sites, and consistently lowest in the upland as opposed to the floodplain for all four sites (Table 1).

Soil pH was a consistent factor with regards to community composition across all biotic communities examined within this study (Figure 8: A-F). Soil moisture was also an important factor for trees, herbs and vegetation as a whole (Figure 8: C,E & F), however it was not a factor for the shrub community (Figure 8D). This is most likely due to the large quantities of Ericaceous shrubs that were able to persist in both wet and dry soils. Interesting, NH_4 was also a contributor to the community composition of bacteria (Figure 8B), however it did not have a significant effect on fungi (Figure 8A). Fungal communities tracked with plant communities, however bacterial communities did not appear to be affected by the surrounding flora.

An important outcome of this research is a catalog of the plant taxa along this riparian transect for use as reference targets in restoration activities. For a list of the species and their frequency, please see Table 2.

Success in Achieving our Objectives

Our objectives for examining the relationships among flora and microbial communities for this study were achieved. Detailed community compositions for all four sites were gathered for flora, bacteria and fungi. This information enabled us to investigate how the communities changed between floodplain and upland areas as well as between sites.

We were unable to achieve the chemistry objectives to their fullest extent due to our issues with New Jersey Analytical labs failing to provide both the water and soil chemistry data. By utilizing soil collected for microbial analysis, we were able to obtain limited data on soil chemistry. Even though this data was limited, it still provided a general idea of the soil chemistry of the study sites and how it may be interacting with both the flora and the microbial communities. Soil pH and soil moisture are important factors in maintaining reference floodplain vegetation and soil microbial communities.

Discussion and Management Implications

The composition of the plant and microbial communities varied very little among the four sites. This is notable because our four sites were chosen based on their increasing exposure to urban influence. This result suggests that urbanization may not be the driving force affecting biotic community composition or soil chemistry. For instance, the BBL site located with moderate urban influence (Fig. 1) showed the highest values of total carbon, NH_4 and total %N (Table 1). We are not sure what the source of the nutrient loading is, but close inspection of maps reveals a possible agricultural site upstream.

Understandably, the most pronounced differences in both plant and microbial community structure were observed between the floodplain and the upland habitats. The

microbial community correlated with this difference suggesting that the plant and soil community are a cohesive unit that should be considered together to improve management and restoration. Floodplain habitats were dominated by *Acer* and *Vaccinium* species that support a very different microbial, especially mycorrhizal, rhizosphere community than the *Pinus sp.* that dominated the uplands.

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. Regular flooding will have a strong effect on the nature of the soil community and its capacity to process excess nutrients. For instance, the oxidized form of nitrogen (NO_2 and NO_3) was below detection at all sites (Table 1). This may be due to anaerobic soil conditions caused by inundation. These conditions will also select for plants and soil microorganisms that thrive in the variable environment. The distinct decrease in pH from the floodplain to the upland likely interacted with the plant and soil microbial communities. Pines are noted for decreasing soil pH (Foreman 2001), and soil pH is a critical driver of microbial community composition (Fierrer et al. 2004).

All four sites offer good examples of restoration reference sites. All sites have relatively few non-native invasive species and those that are present are not dominant components of the vegetation (Table 2). In using these sites as reference sites, new restorations should focus on planting and maintaining healthy populations of *Acer rubrum*, *Vaccinium* species (including *V. atrococcum* and *V. corymbosum*), *Sphagnum* sp. and other moss species as these are the species most closely associated with the microbial communities. Additional species that should be planted include *Gaylussacia* and *Carex* species.

Acknowledgements and EPA Disclaimer

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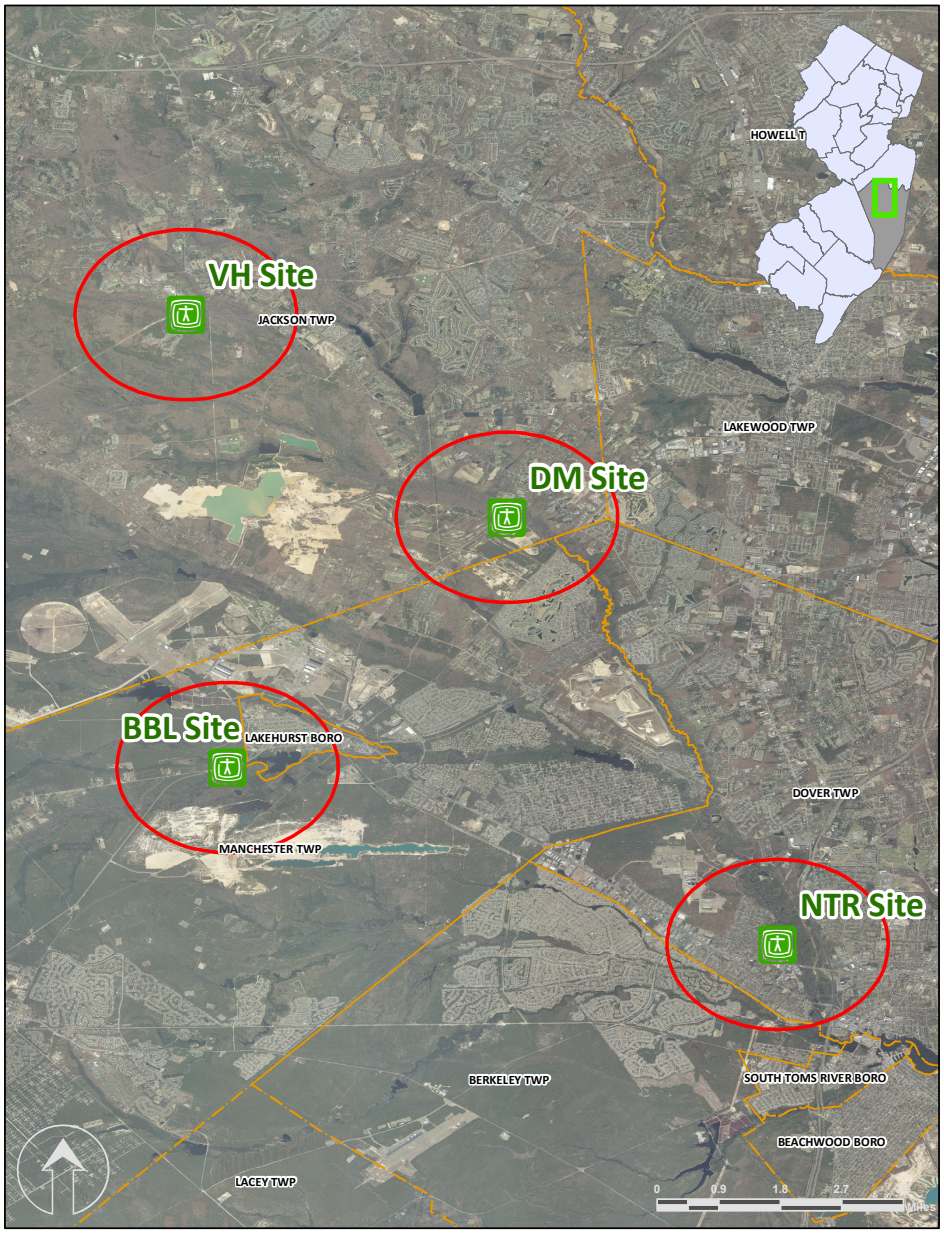


Figure 1. Site locations in the Barnegat Bay Watershed, New Jersey. Mapped using ArcGIS.

NTR = Near Toms River, DM = Dove Mill, BBL = Blacks Branch, VH = Van Hiseville.

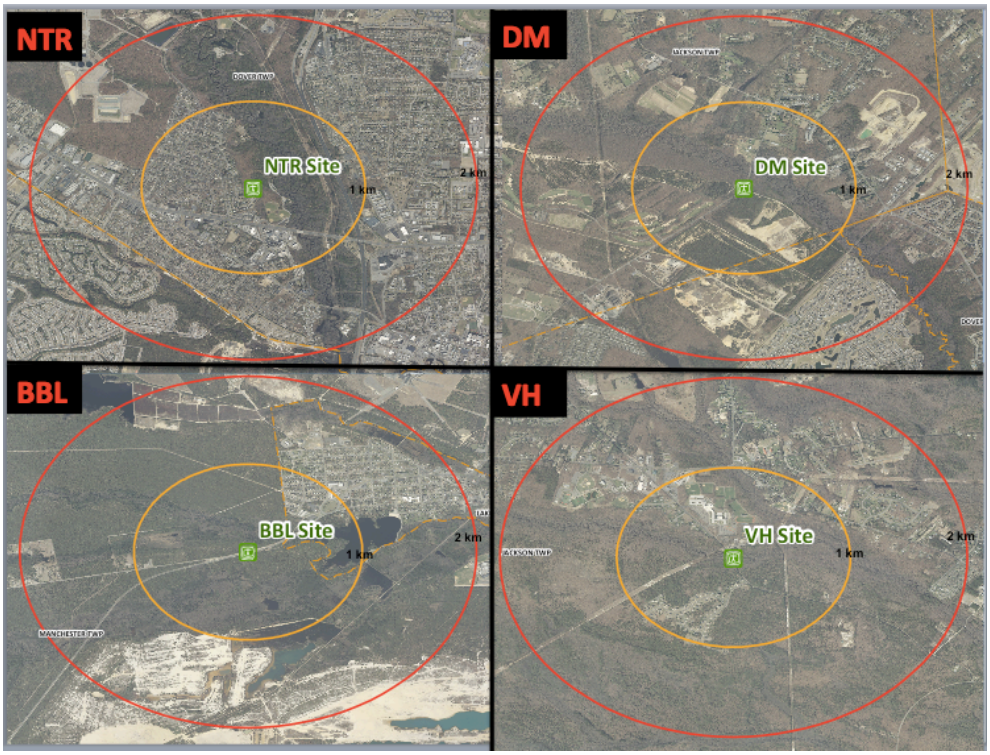


Figure 2. All sites with designated 1km and 2km radius, Barnegat Bay Watershed, New Jersey. Mapped using ArcGIS. NTR = Near Toms River, DM = Dove Mill, BBL = Blacks Branch, VH = Van Hiseville.

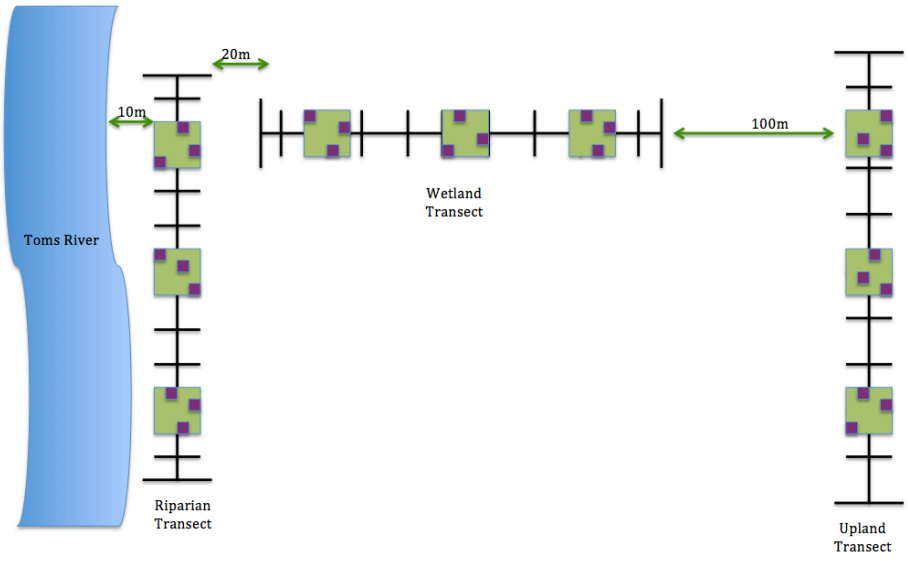


Figure 3. Site layout and plot design, including riparian, wetland and upland transects.

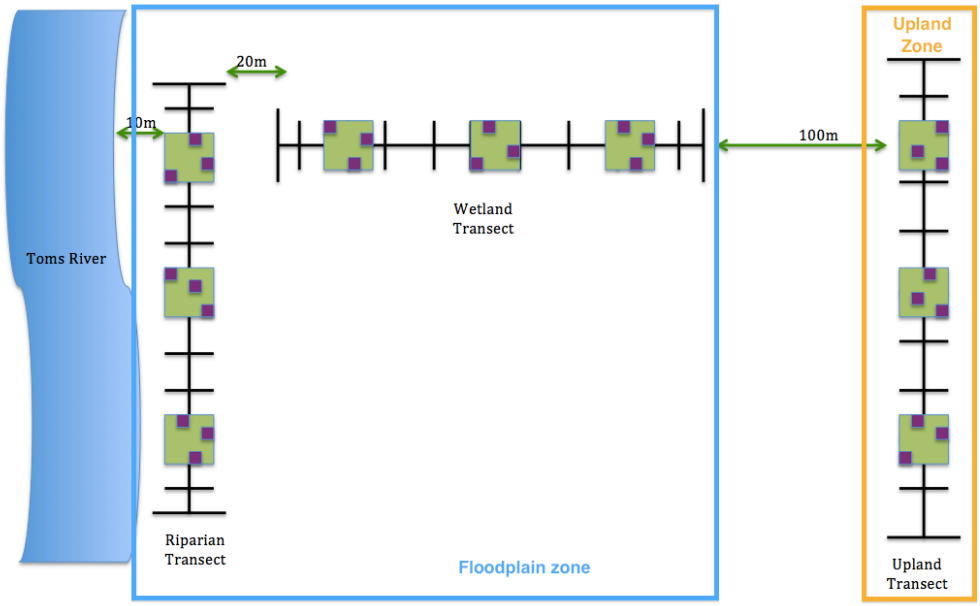


Figure 4. Site layout and analysis design after consolidation of riparian and wetland transects into the floodplain.

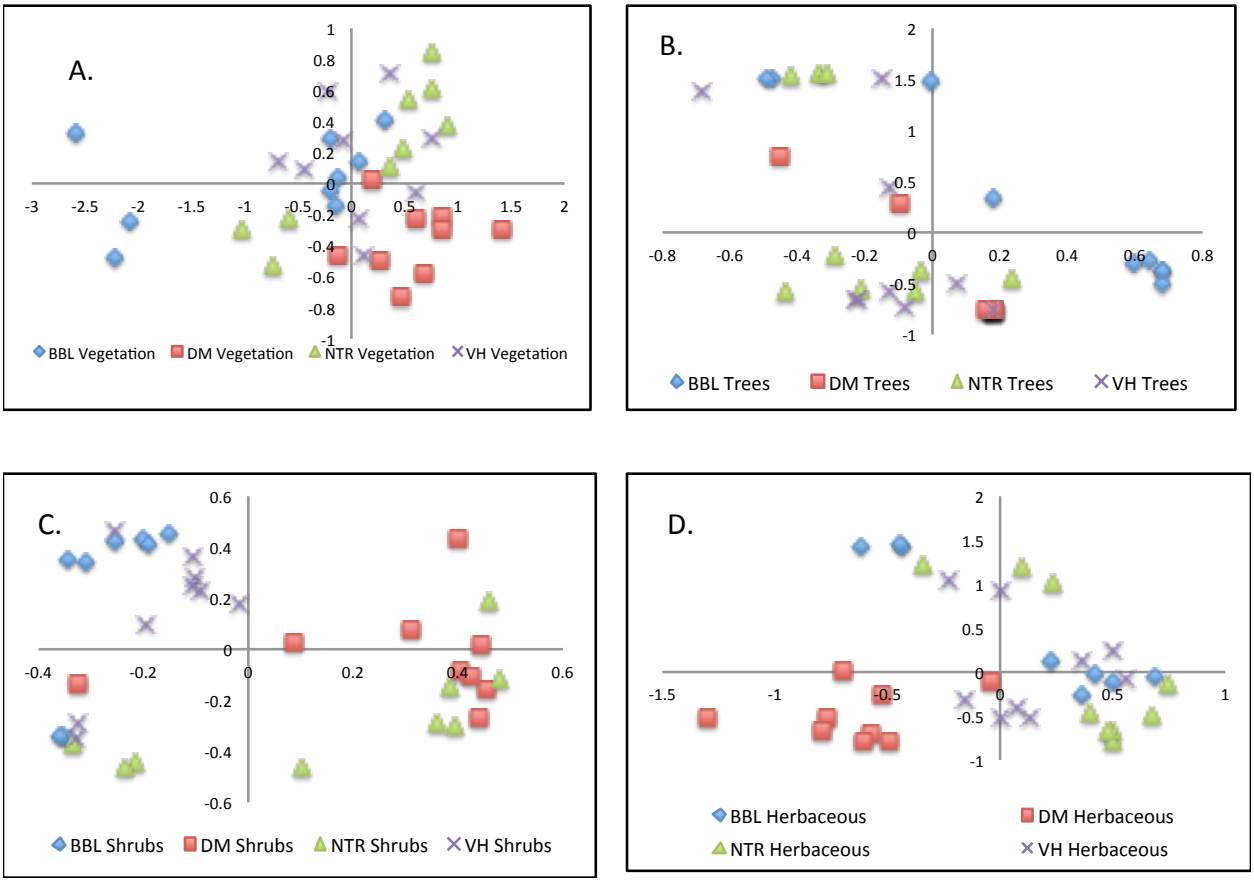


Figure 5. NMDS separation of plant community composition according to site along the rural to urban transect including: A. all vegetation together, B. trees, C. shrubs and D. herbaceous plants. NTR = Near Toms River, DM = Dove Mill, BBL = Blacks Branch, VH = Van Hiseville.

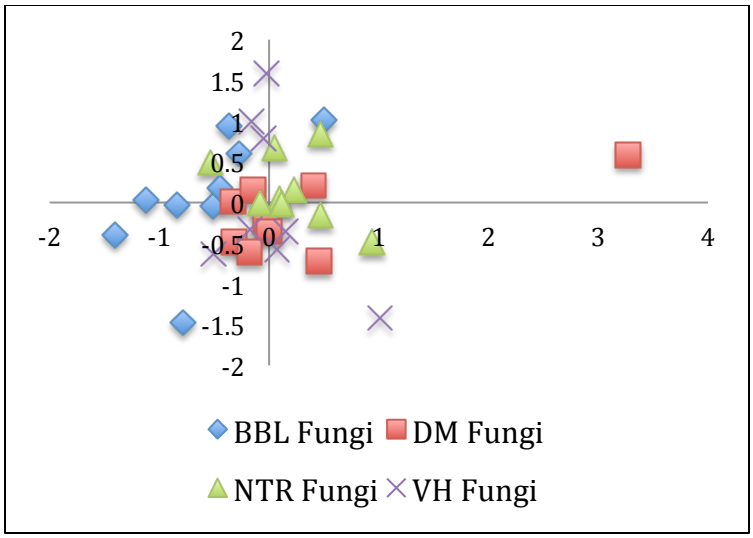


Figure 6. NMDS ordination of soil fungi community composition according to site along the rural to urban transect. NTR = Near Toms River, DM = Dove Mill, BBL = Blacks Branch, VH = Van Hiseville.

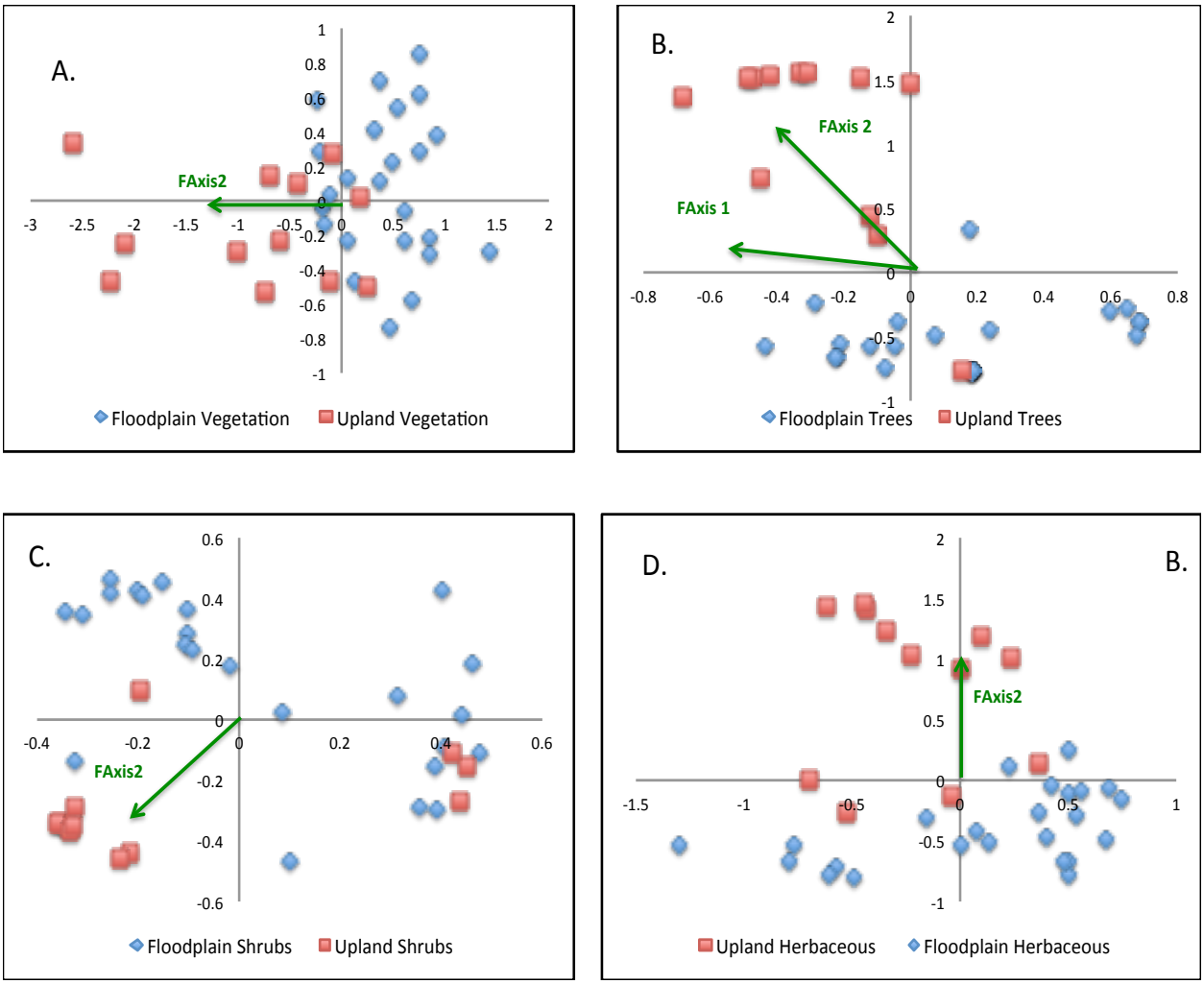
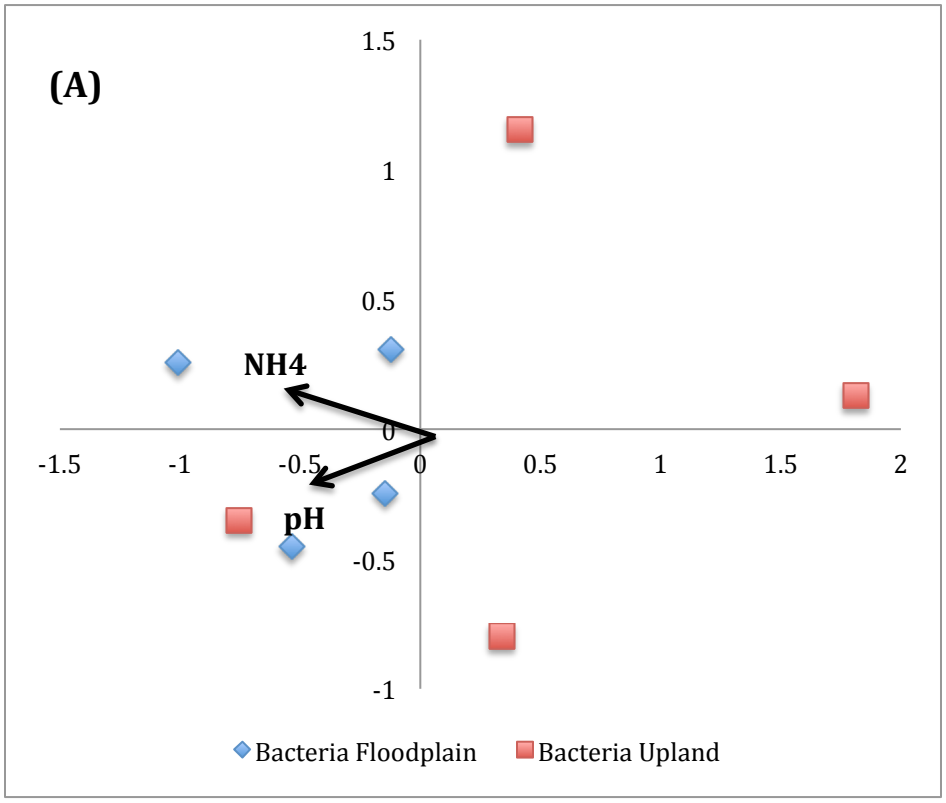


Figure 7. NMDS separation of plant community composition as associated with either upland (red squares) or floodplain (blue diamonds) sites. Correlation with microbial community composition is overlaid as arrows. Panels represent: A. all vegetation together, B. trees, C. shrubs and D. herbaceous plants.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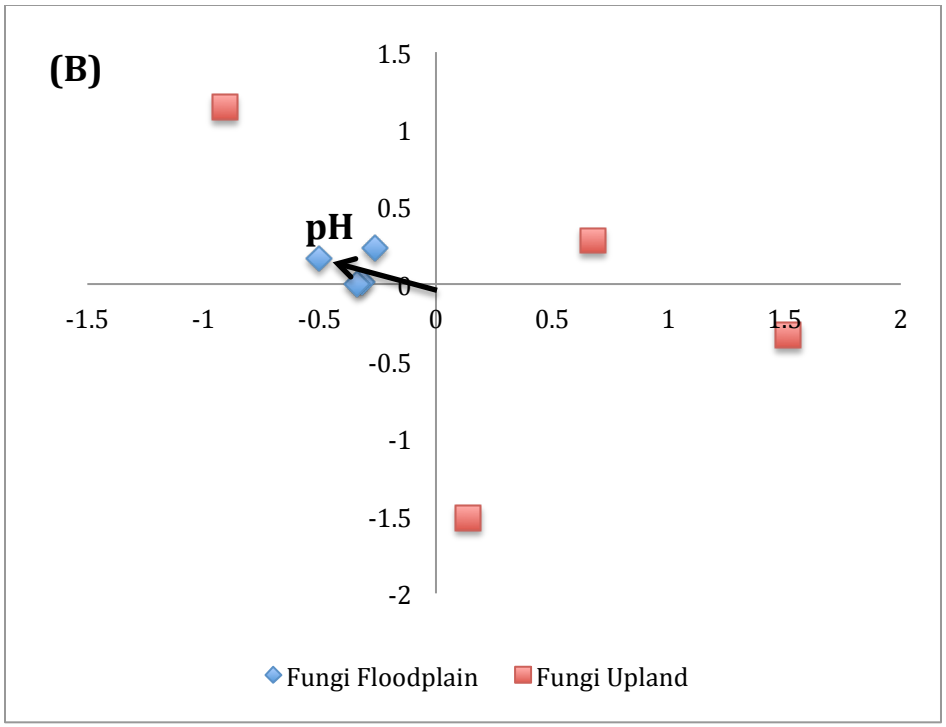
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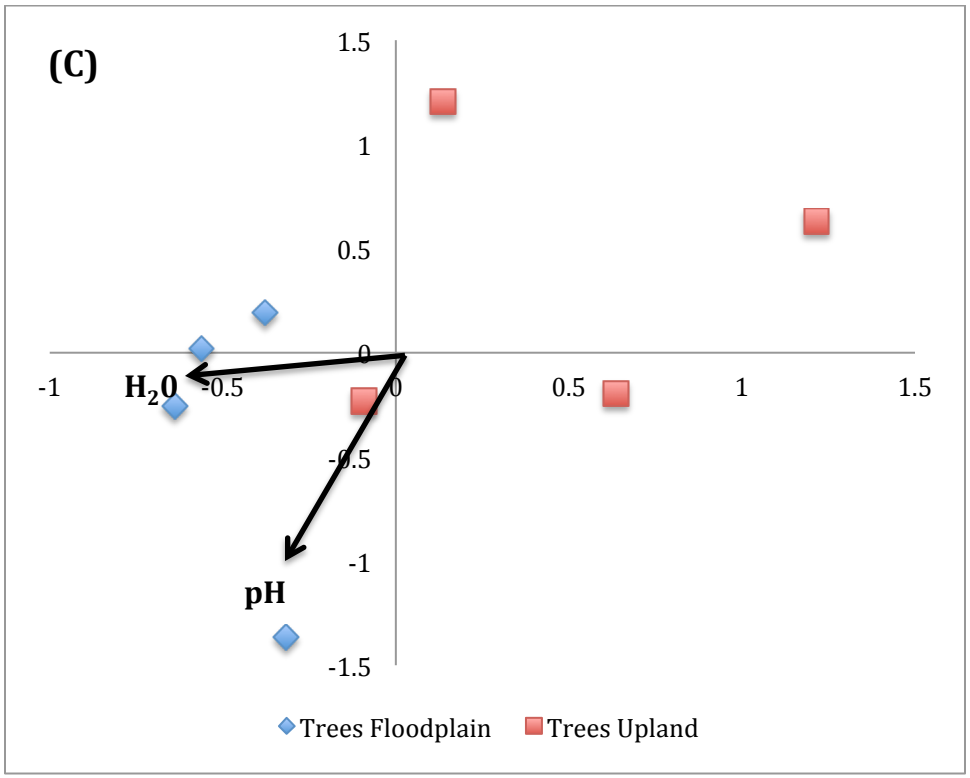
Fungi Upland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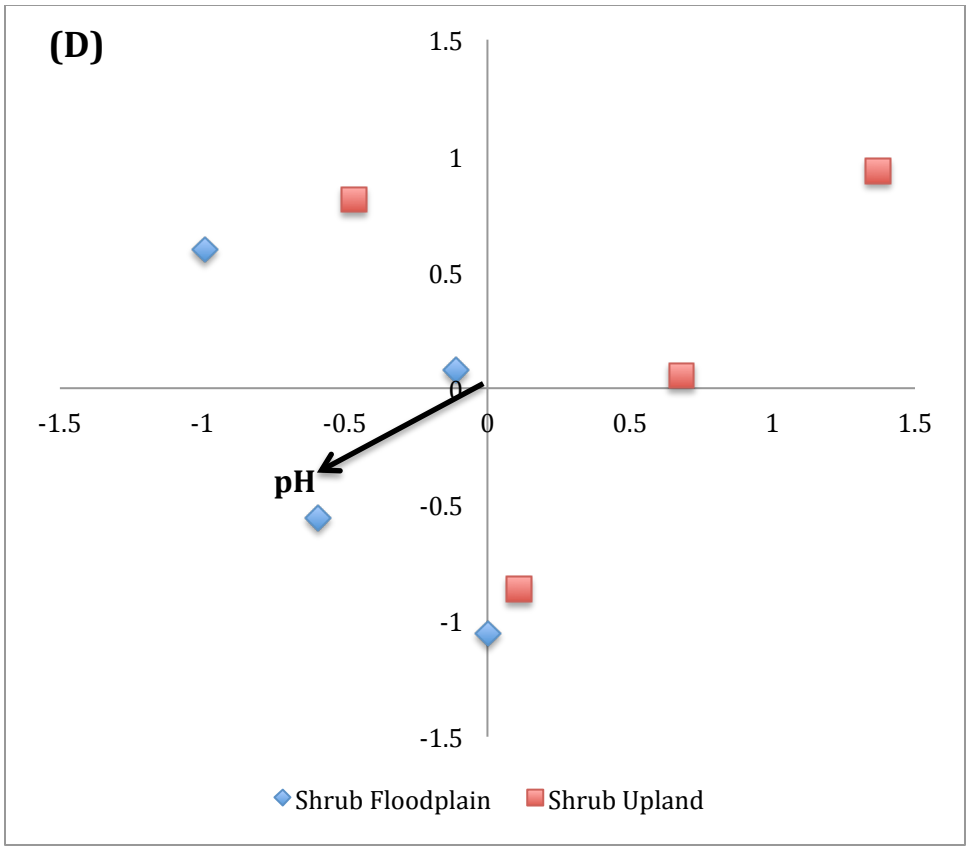
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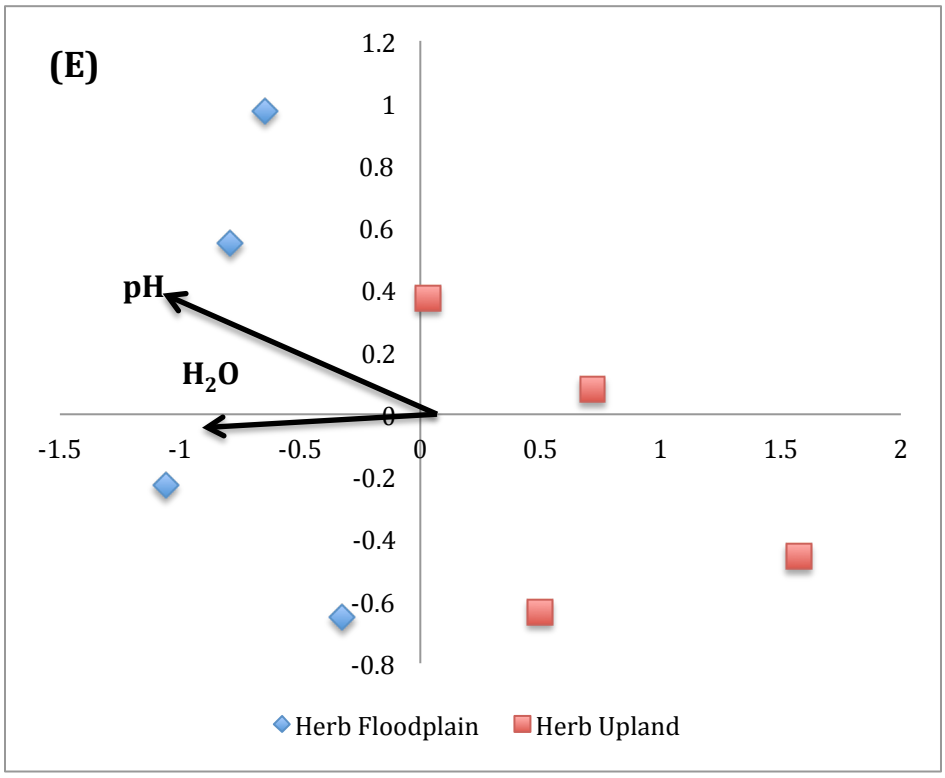
Shrub Upland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Herb Upland

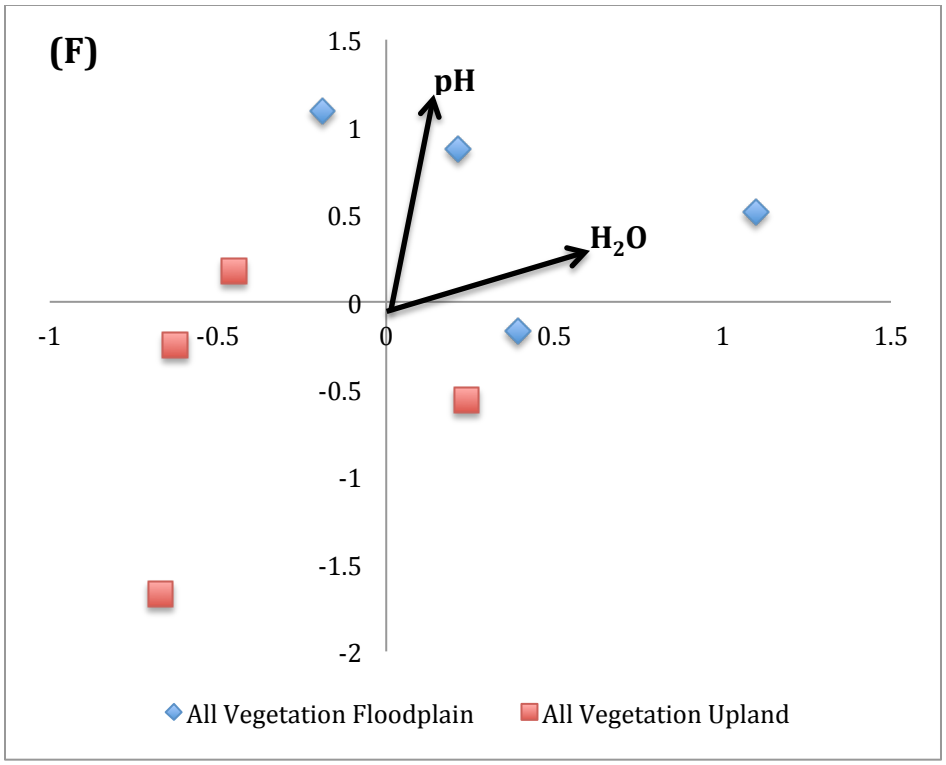

Figure 8: NMDS separation of biotic communities (by strata) with soil chemistry overlaid as a second matrix. Panels represent: A. bacterial communities, B. fungi communities, C. trees, D. shrubs and E. herbaceous plants, F. all vegetation together.

Table 1. Site location and soil chemistry measurements. NTR = Near Toms River, DM = Dove Mill, BBL = Blacks Branch, VH = Van Hiseville.

Site and Zone	Lat and Long	pH	NH4 mg/kg	NO3+NO2 mg/kg	Total %N	Total %C
NTR Floodplain	39° 58’ 12’’ N, 74° 12’ 55’’ W	4.17	38.61	bdl	.48	10.61
NTR Upland	GPS 39° 58’ 54’’ N, 74° 12’ 32’’ W	3.81	10.33	bdl	.15	4.77
DM Floodplain	40° 3’ 39’’ N, 74° 16’ 23’’ W	4.61	36.56	bdl	.31	5.41
DM Upland	40° 3’ 37’’ N, 74° 16’ 24’’ W	3.7	24.12	bdl	.59	13.55
VH Floodplain	40°, 6’, 1”N, 74°, 21’,31”W	4.14	12.25	bdl	.21	3.97
VH Upland	40°, 5’, 52”N, 74°, 22’, 6”W	3.55	9.01	bdl	.17	4.52
BBL Floodplain	40°, 0’, 24”N, 74°,, 19’, 41”W	3.99	107.00	bdl	1.42	39.89
BBL Upland	40°, 0’, 38”N, 74°, 19’, 23”W	3.5	7.65	bdl	.21	7.57

Table 2. Frequency of plant species found in wetland (24 plots total) and upland (12 plots total) in four wetland and associated uplands along the Toms River and its tributaries.

Scientific Name	Wetland	Upland
Acer rubrum	24	7
Aronia arbutifolia	5	1
Betula nigra	2	0
Carex atlantica	4	0
Carex bullata	5	0
Carex collinsii	3	0
Carex formosa	1	0
Carex intumescens	3	0
Carex louisianica	7	0
Carex nigromarginita	0	1
Carex seorsa	2	0
Carex spp.	7	3
Carex stricta	8	0
Carex trisperma	1	0
Chamaecyparis thyoides	9	3
Chimaphila maculata	0	5
Cinna latifolia	4	0

Clethra alnifolia	20	9
Cornus spp.	1	0
Crataegus spp.	1	0
Cyperus spp.	1	0
Dichanthelium clandestinum	1	0
Dioscorea villosa	2	0
Galium asprellum	1	0
Gaultheria procumbens	0	4
Gaylussacia baccata	5	8
Gaylussacia dumosa	3	1
Gaylussacia frondosa	6	9
Ilex glabra	1	0
Ilex opaca	5	2
Iris spp.	1	0
Leersia oryzoides	1	0
Lilium spp.	0	1
Lindera benzoin	2	2
Liquidambar styraciflua	2	0
Lobelia spp.	0	1
Lycopus virginicus	1	0
Lyonia ligustrina	10	2
Lyonia mariana	2	0

Lysimachia nummularia	3	1
Magnolia virginiana	3	3
Maianthemum canadense	1	1
Maianthemum racemosa	1	0
Melampyrum lineare	0	2
Microstegium vimineum	5	0
Mitchella repens	0	1
Moss species	18	4
Myrica pennsylvanica	2	4
Nyssa sylvatica	13	5
Oenothera spp.	1	0
Onoclea sensibilis	4	0
Osmunda cinnamomea	3	4
Osmunda regalis	1	1
Oxalis spp.	1	0
Oxydendrum arboreum	2	1
Panicum spp.	5	0
Parthenocissus quinquefolia	5	2
Peltandra virginica	5	0
Pilea pumilla	1	0
Pinus rigida	10	5
Polygonum spp.	2	0

Potentila spp.	1	0
Prunus avium	2	0
Prunus serotina	0	2
Prunus virginiana	2	0
Pteridium aquilinum	0	2
Quercus alba	1	0
Quercus bicolor	4	1
Quercus ilicifolia	3	3
Quercus marilandica	1	1
Quercus montana	3	1
Quercus pallustris	0	2
Quercus phellos	1	0
Quercus rubra	3	0
Quercus spp.	3	1
Quercus veluntina	2	0
Photinia pyrifolia	1	0
Rhododendron periclymenoides	7	4
Rhododendron viscosm	5	2
Rubus hispidus	4	2
Rubus spp.	1	1
Sassafras albidum	1	3
Smilax glauca	3	3

Smilax laurifolia	1	0
Smilax rotundifolia	18	8
Solidago spp.	8	1
Sphagnum spp.	21	0
Symplocarpus foetidus	1	0
Toxicodendron radicans	7	2
Trientalis borealis	2	1
Ulmus spp.	1	0
Vaccinium atrococcum	2	0
Vaccinium corymbosum	21	9
Vaccinium spp.	5	0
Verbena spp.	3	0
Viburnum dentatum	1	0
Viola lanceolata	2	0
Viola spp.	1	0

*Non-native species to NJ.

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