Abstract
Effects of Nutrient Availability and Disturbance on the Composition and Diversity of Soil Microorganisms
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ABSTRACT

Determining how factors such as disturbance and nutrient availability affect species diversity in a community has been a major goal of community ecology. The purpose of this study was to look at how species diversity and composition of soil bacterial communities are affected by nutrient addition and disturbance. I characterized soil microbial communities at the long-term ecological research site at the West Research Campus (WRC) located in Pitt County, NC.

Briefly, DNA extracted from soils was analyzed using amplicon sequencing of the 16S ribosomal RNA gene. The Illumina Platform was used to sequence the bacterial DNA from each sample, and the Mothur Pipeline was used to analyze the DNA sequences. I hypothesized that changes in nutrient availability and disturbance would impact soil microbial community composition and diversity through direct and indirect effects mediated by plant-soil interactions.

My research complemented previous work carried out in the WRC determining the effects of nutrient addition and disturbance on plant communities. Analysis of 2013 plant data showed that mowing increased plant species richness, and fertilization decreased plant species richness significantly. The experimental treatments as well as the proximity of the blocks to a drainage ditch all had significant effects on plant community composition. Analysis of the microbial community data showed that both fertilization and mowing significantly increased

mean species richness. Relative abundance microbial community composition varied significantly due to the proximity of the blocks to the ditch. Presence/absence microbial community analyses showed significant effects of the treatments, as well as ditch proximity on microbial composition differences. Also, unknown microbial communities showed significant variation of the communities due to the treatments. The results of the presence/absence analysis and the unknown microbial community analysis show the importance of rare taxa and unknown microbial communities to the differences in composition of our soil microbial communities. Analysis of the soil chemical and physical data showed very little variation due to the treatments.

This study will contribute to our understanding of how both plant and soil bacterial community diversity are affected by anthropogenic nutrient addition and disturbances. Maintaining diversity is important for ecosystem stability and functioning.

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EFFECTS OF NUTRIENT AVAILABILITY AND DISTURBANCE ON THE COMPOSITION AND DIVERSITY OF SOIL MICROBIAL COMMUNITIES

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INTRODUCTION

Factors Affecting Community Diversity

A community is an association of interacting species inhabiting some defined area, such as the plant species in a tropical rainforest or the soil microbes in a wetland habitat. The type, number and distribution of the species in a community make up the community's structure. An important aspect of a community is its biodiversity, which can be affected by both abiotic (for example, temperature, light intensity) and biotic factors (for example, other plant communities, animal or microbial communities). Most measures of species diversity take into account both species richness and species evenness. Species richness is the number of different species present in a community, and species evenness refers to the relative abundance or distribution of these species. Communities that are considered to be high in diversity tend to have a high number of species (richness) and a relatively equal distribution of those species (evenness). A major goal of community ecology is to understand the factors that determine species diversity and how diversity affects community processes.

Both nutrients and disturbance strongly influence biodiversity. Nutrients play a vital role in a community because they are a necessity for growth and survival of all living organisms. Ecologists have been studying the effects of nutrient availability on diversity for decades (Newman 1973; Huston 1980; Goldberg and Miller 1990; Hillebrand 2003; Dickson and Foster 2011). In general, increases in nutrient availability have been found to affect species diversity negatively. For example, in a study of rain forest sites in Costa Rica, high levels of nutrients in the soil were strongly correlated with a decrease in tree species richness (Huston 1980). Similar results were seen in another study involving nutrient addition and grazing treatments; in

periphyton communities of three different aquatic ecosystems, nutrient addition had a slightly positive effect on species richness but a negative effect on evenness (Hillebrand 2003). The additional nutrients provided the periphyton species with the needed resources for growth but also increased the dominance of a few species, decreasing species evenness. Negative impacts of nutrients on species diversity was also demonstrated in a three-year experiment increasing the amount of available nitrogen and phosphorus in mixed vegetation wet heathland habitats including *Erica tetralix* and *Molinia caerulia* (Aerts and Berendse 1988). This study concluded that the increase in the amount of nutrients in the environment resulted in *Molinia* increasing in leaf biomass (cover) and outcompeting *Erica,* causing a decrease in species diversity.

In plant communities, nutrient addition can increase the biomass and canopy of a few species disproportionately. Increasing competition for light occurs when the amount of available nutrients in an environment increases (Newman 1973). When nutrients are no longer a limiting factor, plant biomass increases causing a decline in light availability for smaller plants. This will increase the mortality of smaller plants and ultimately cause a decrease in species diversity (Goldberg and Miller 1990). Studies have also shown that even when light limitation is not decreasing plant diversity, nutrient addition continues to decrease plant diversity. In an experiment involving the manipulation of light availability and nutrient addition, fertilization was seen to decrease diversity even in years when light availability did not decrease diversity (Dickson and Foster 2011).

While much of the evidence for a relationship between nutrient availability and diversity has focused on plant communities, nutrient availability also impacts animal communities. Nutrients have been seen to both decrease and increase biodiversity in animal communities. In an aquatic ecosystem, nutrient availability decreased the diversity of macrophytes, which decreased

the diversity of macroinvertebrates (Declerck et al. 2011). Nutrient availability has also been linked to an increase in species diversity. In an experiment looking at the effect of nutrient addition and depletion on freshwater nematode communities, the communities with a greater nutrient supply supported higher nematode diversity. The increase in nematode diversity was also correlated with an higher diversity of larger omnivorous species resulting in an overall increase in ecosystem diversity (Iris and Traunspurger 2005).

The frequency and intensity of disturbance has also been shown to affect community diversity (Connell 1978; Huston 1979). Disturbance is defined as a potentially damaging force being applied to a community or ecosystem (Lake 2000). In the intermediate disturbance hypothesis, Connell (1978) argued that when disturbances are too intense and frequent, only the species that are capable of colonizing quickly and withstanding harsh conditions flourish, and when disturbances are less intense and infrequent, top competitors will competitively exclude the weaker competitors. Therefore diversity will be highest when disturbances are at an intermediate level of both frequency and intensity. The intermediate disturbance hypothesis has been supported by studies of many systems. In a study of tropical rainforests, intermediate levels of disturbance in the form of tree-fall gaps yielded the highest tree species richness (Molino 2001). In another test of the intermediate disturbance hypothesis, 54 stream sites experiencing different intensities and frequencies of disturbance in the form of bed movement were sampled. Out of all the treatments, disturbance was responsible for the most variation in taxon richness of macroinvertebrates, and disturbance at an intermediate level yielded the highest species richness (Townsend et al. 1997). Disturbance in the form of artificial deepening of the mixed layer was applied to plankton communities in their natural aquatic environment. The depth of the mixed layer depends on the

stability of the water surface. Species diversity was the highest at the intermediate level, supporting the intermediate disturbance hypothesis (Floder and Ulrich 1999).

When other factors, such as plant productivity and nutrient addition, interact with disturbance, the effects on diversity can deviate from predictions of the intermediate disturbance hypothesis. Models that include disturbance and productivity indicated that high productivity yielded the highest species richness and that productivity was highest at the highest levels of disturbance (Kondoh 2001). Disturbance in a plant community can counteract the effects of dominant plant species due to nutrient addition. Nutrient addition increases the canopy size of certain plant species. Herbivores and grazers select for dominant plant species and feast on these larger plants decreasing light limitation or competitive exclusion. Eliminating these factors ultimately increased species diversity (Borer et al. 2014).

Diversity is important in the study of communities because it has been shown to be related to a number of ecosystem properties, such as its stability. Stability is defined as the ability of a community to respond after perturbation and persist overtime (Margalef 1969; Lewenton 1969; May 1973). In 1958, Charles Elton introduced the Diversity-Stability hypothesis, which predicts that less diverse communities will be more sensitive to environmental perturbations, and that populations in these simpler communities will fluctuate more intensely in response to environmental variability (Elton 1958). However, in 1973, Robert May challenged this hypothesis by using linear stability analyses and showed that, as the number of competing species increased, population dynamics were progressively less stable. In a more recent study, Tilman et al. (2006) combined both Elton's and May's findings and concluded that greater diversity does lead to a more stable community, but also leads to lower species stability (the ability of a single species to return to equilibrium following a disturbance). Also, diversity

contributes to community stability, but is not the driving force; therefore, if the community has more species that can impact a community in a positive manner, the community is more stable.

Species diversity has important effects on ecosystem services, which are a set of ecosystem processes that are important to humans. Ecosystem processes include climate regulation, crop pollination, and the enhancement of aesthetics (Kremen 2005). Researchers have proposed different theories as to why ecosystem processes are affected by diversity. The precautionary principle argues that because we cannot determine which species are responsible for each ecosystem service, biodiversity must be maintained to avoid the loss of an important ecosystem function (Myers 1993). Isbell et al. (2011) found support for this principle in an experiment testing the effect of different plant species on nutrient uptake and biomass production. Functional diversity can also be important to maintain ecosystem services. Ecosystem function in a diverse community might be less affected by changes in the environment because there are multiple species that can provide that same service to the ecosystem (functional redundancy). Supporting evidence comes from a meta-analysis performed by Worm et al. (2006). They first examined data from 32 published works to see the effects of marine biodiversity on rates of resource collapse, recovery potential, stability, and water quality. It was determined that the increased diversity of primary producers and consumers positively impacted the ecosystem processes. They also examined experiments that altered both species diversity and genetic diversity and found that both genetic and species diversity increased environmental stability, which also maintains and enhances ecosystem processes. This results in a greater ability for a community to cope with stress brought about by humans as well as stressors such as natural disasters (Luck et al. 2003).

Factors Affecting Soil Microbial Diversity

Microbial communities are important drivers of ecosystem functioning (Bardgett and Van Der Putten 2014). They have high genetic diversity due to the magnitude of their populations and their ability to reproduce rapidly (Whitman et al. 1998). Microbes have been linked to ecosystem processes including carbon (C) and nitrogen (N) cycling, decomposition, and stimulation and reduction of plant diversity through symbiotic relationships with microbial communities (Van der Heijden et al. 2008). Microbes contribute about 50 % of the total C on Earth and contain the largest amounts of N and P of any living organism (Whitman et al. 1998). Since microorganisms are responsible for a range of ecosystem functions, microbial diversity and composition must be maintained.

Abiotic and biotic factors influence belowground microbial communities just as they influence the aboveground plant communities. Abiotic factors such as available nutrients, pH, moisture, and temperature have an influence on the diversity and composition of soil microbial communities (Fierer and Jackson 2006; Zhang et al. 2013; Peralta et al. 2012; Pete-Ridge and Firestone 2005). For example, available nutrients affect microbial community composition and diversity in both positive and negative ways. Nutrients can shape microbial communities by speeding up microbial succession (Knelman 2014). Fertilization causes early successional microbial communities to mirror communities that have undergone years of adaptation, which can change the physical and chemical development of the soil ecosystem (Knelman et al. 2014). Although nutrient addition has been seen to positively impact diversity, most studies have found that nutrients decrease microbial diversity and alter composition (Jackson et. al 2009; Carrero-Colon et. al 2006; Campbell et al. 2010). One proposed mechanism for the decrease of microbial diversity is the regeneration of plant communities (Jackson et. al 2009). In an

experiment evaluating the effects of nutrient addition and salinity on microbial community diversity, it was found that N addition decreased microbial community diversity (Jackson et al. 2009). In addition, the N addition caused plant growth to increase, which disturbed the soil structure and decreased microbial diversity (Jackson et al. 2009). Another mechanism by which nutrients decrease microbial community diversity is by decreasing competitive fitness of microbes (Carrero-Colon et. al. 2006). Bacteria are subjected to changes in nutrient availability in their natural environments. In a previous study, supplementing nutrients in nutrient-poor environments caused competition between microbial communities. The microorganisms that quickly responded to nutrient addition were capable of outcompeting slow nutrient responders and eventually competitively excluded these communities. Nutrients not only decrease diversity, but they also change community composition, which alters plant productivity (Campbell et al. 2010). For example, in moist acidic tundra soils, long-term fertilization not only decreased bacterial diversity, but it also changed bacterial community composition. This shift in composition changed the amount of available nutrients in the soil, altering plant productivity (Campbell et al. 2010).

Not only does nutrient availability impact microbial community composition and function, but soil pH is also an important factor influencing the diversity and composition of microbial communities. Bacterial abundance and diversity have been seen to both increase as pH increased, with the diversity almost doubling as the pH rises (Rousk et al. 2010, Hartman et al. 2008). In a study of 98 soil samples collected from across North and South America, it was seen that soils with the higher pH had a greater microbial diversity and greater richness (Fierer and Jackson 2006). It is hypothesized that the effect of pH on bacterial communities is so strong because bacteria have a narrow pH growth tolerance (Rousk et al. 2010). In addition, soil pH impacts soil redox status, which also influence nutrients availability (Husson 2012, Pett-Ridge and Firestone 2005).

Soil moisture is another abiotic factor influencing nutrient availability and redox status leading to changes in microbial diversity and composition in soils. Soil moisture and microbial abundance are often proportional. For example, in a month long soil moisture experiment, respiration rate and moisture were positively correlated and resulted in reduced microbial population size due to experimental drought conditions (Schimel et al. 1998). Studies have also shown that increases in soil moisture can increase microbial biomass, which was measured by the release of C into the environment (Keith et al. 1987). Fluctuations in soil moisture can alter microbial communities and shift soil redox status, contributing to differences in habitats available for microbial communities (Peralta et al. 2012).

Biotic factors, specifically microbial interactions with plants, strongly influence microbial community composition and diversity. Plant diversity loss has being linked to microbial richness and abundance loss (Strecker et al. 2015). Microbial communities can also significantly impact plant community composition and abundance. Specifically, microbial communities can affect plant productivity, diversity, and plant evolutionary processes (Lau and Lennon 2011). Microbial communities also help plants cope with environmental changes (Lau and Lennon 2012). In a previous study, diverse and rapidly evolving microbial communities were the main driver supporting drought tolerance in plants, not the plant's coping strategies (Lau and Lennon 2012).

Plants and microbes develop symbiotic relationships when environmental pressures are detrimental to their survival. A well-studied beneficial relationship between plants and soil microbes is the mycorrhizal relationship. Mycorrhizal relationships are symbiotic associations between plants and fungi that can be found within the roots of most plant species. This interaction can be either an obligate or facultative interaction (Johnson 1993). The symbiotic relationship between plant roots and mycorrhizal fungi is seen in over 80 % of all terrestrial plant

species (Smith and Read 1997). Mycorrhizal fungi act as extensions of plant root systems and increase nutrient uptake (Van Der Heijdin et al. 1998). The symbiotic relationship between plants and mychorrizal fungi mediate plant diversity, allowing different plant species to coexist (Bever et al. 2010; Bolon 1991; Eckhard et al. 1995). Another symbiotic relationship that is well studied is the relationship between legumes and rhizobia. Plants are incapable of fixing atmospheric nitrogen into an inorganic form that they can use for growth. In legume-dominated ecosystems such as grasslands, rhizobia bacteria have accounted for as much as 20% of all the nitrogen taken in by the vegetation (Cleveland et al. 1999, Van der Heijden et al. 2006).

Plants also rely on soil bacteria and fungi to decompose and mineralize soil organic matter for nutrient acquisition. Plant species create positive feedback patterns of nutrient cycling in natural ecosystems (Ingham et al. 1985; Marschner 2007; Chilimba 2002; Van der Putten et al. 2013). Plants take in nutrients, grow by using the nutrients efficiently and effectively, die and decompose. Other plants then use the nutrients from decomposing litter to grow and continue the nutrient cycle (Hobbie 1992).

Not all interactions between plants and soil microbes are beneficial. Plant pathogens negatively affect the plant's ability to acquire nutrients as well as its ability to grow. For example, soil pathogens such as *Phytophthora* and *Pythium* can vary in the impact on plant communities, ranging from decimation of the plant community to a subtle, unrecognizable impact (Burdon et al. 2006). In addition, microbial communities can negatively affect plant communities by competing with the plants for soil nutrients (Van der Heijden et al. 2008). According to Nordin et al (2004), in nutrient limited areas such as the arctic and the alpine tundra, microbial species have been seen to effectively compete with the plant communities, affecting the plants ability to acquire nutrients and to grow.

Abiotic and biotic forms of disturbance can also affect microbial communities. The frequency and intensity of disturbances influence the effect they have on microbial communities (Berga et al. 2012). Disturbance can have very negative impacts on microbial community diversity. `More disturbed soils exhibit low levels of microbial diversity, and undisturbed soils offer the most favorable conditions for microbial growth (Torsvik et al 1996). One way in which disturbance has been seen to negatively impact microbes is through the removal of a large amounts of the plant biomass. Plants are responsible for supplying microbial communities with carbon. Organic carbon is the main energy source for heterotrophic microbial communities; therefore, reduced carbon availability will support a smaller microbial population and in turn decrease microbial diversity (Kowalchuk et al. 2002; Berg and Smalla 2009). Also, specific microbial taxa are associated with certain plant species. The removal of plant biomass in the form of mowing causes plant specific microbes to be lost, which in turn decreases overall microbial diversity (Berg and Smalla 2009). Disturbances not only negatively affect diversity but they also affect microbial community structure (Westergaard et al. 2001).

The addition of nitrogen and phosphorus in combination with disturbance from mowing has been shown to impact soil bacterial communities. Zhang et al. (2013) performed a five-year experiment testing the effect of nutrient addition and disturbance on grassland communities. The nitrogen addition affected the entire soil bacterial communities by significantly decreasing its abundance. Nitrogen addition also significantly decreased the richness and changed the composition of bacterial taxa (operational taxonomic units = OTUs) in the soil microbial community. Although phosphorus alone did not have a significant effect on the microbial communities, the interaction between addition of phosphorus and nitrogen and mowing yielded a significant effect on microbial richness, evenness, and composition (Zhang et al. 2013).

Tools used to study soil microbial communities have historically limited our knowledge about the diversity of bacterial communities. For example, culturing microbial communities is a technique that has been practiced for years. The advantage of culturing microbes is the ability to see the morphological differences between the microbes and also the ability to examine microbial physiology and biochemistry. The disadvantage of this method is only 1 % of soil bacterial populations can be cultured by standard laboratory practices, and it is unknown if this 1 % is a good representation of bacterial communities as a whole (Torsvik et al. 1998). To successfully answer questions concerning soil bacterial diversity, researchers must overcome the challenges associated with studying them.

Defining microbial species is also a challenge to consider when studying microbial communities. There is no clear definition of a bacterial species. For a long time, bacterial species were defined as having one similar diagnostic phenotypic trait and having 70 % DNA cross hybridization (Wayne et al. 1987). In recent years, bacterial species have been characterized by a certain percentage of sequence similarity, usually being greater than or equal to 97 % (Gevers et al. 2005). Bacterial DNA can be transferred horizontally through plasmids, bacteriophages, and transposons, which further complicates the concept of bacterial species (Kirk et al. 2004).

Challenges associated with characterizing microbial communities are compounded by soil heterogeneity and microbial dormancy. Soil biotic communities are highly diverse and contain billions of bacteria cells consisting of tens of thousands of bacterial taxa, as well as other soil organisms including fungi, nematodes, earthworms, and arthropods (Wagg et al. 2014). In one gram of soil there can be an estimated 10^4 different bacterial species (Fierer and Lennon 2011). Microbes are very sensitive to their environments (Lauber et al. 2008). They are dispersed differently throughout the environments they inhabit, and move around rather quickly throughout

these environments (Kirk et al. 2004). Soil environments are spatially heterogeneous in both their physical characteristics and resources (Reynolds et al. 1997), which allows for the coexistence of diverse microbial communities (Magdanova and Golyasnava 2013). When soil is sampled, spatial heterogeneity needs to be taken into account in order to characterize microbial communities more comprehensively. In addition, microbial seed banks can alter microbial community composition making studying the diversity and composition of microbes challenging. In the midst of environmental uncertainty, a subset of soil microbes may become dormant, contributing to microbial seed banks. Dormancy strategies allow some microbial populations to persist in the environment. Seed banks act as a reservoir of dormant individuals that could be awakened under different environmental conditions (Lennon and Jones 2011). Seed banks allow genotypes and populations to remain in a community, which can skew the results of diversity experiments, as well as experiments that look in to ecosystem functions and processes (Lennon and Jones 2011).

Although there are difficulties associated with studying microbial communities, microbial communities are important drivers of ecosystem functioning and must be explored. In a long-term experiment at the West Research Campus, we have been studying the direct effects of two abiotic factors, nutrient addition and disturbance, on the plant communities. We have yet to study how these factors affect the soil microbial communities. My study seeks to determine the direct effects of nutrient addition and disturbance on microbial community composition and diversity, as well as the indirect effects of these abiotic factors, mediated through the plant communities. I hypothesize that changes in nutrient availability and disturbance will impact soil microbial community composition and diversity through direct and indirect effects mediated by

plant-soil interactions. More specifically, experimental plots with higher soil nutrient availability and low disturbance will support the highest levels of species diversity.

MATERIALS AND METHODS

Experimental Design

The long-term experiment is located within a 235 ha tract of land that lies between the Tar River and the Neuse River basins in the Coastal Plains of North Carolina. The land of the site is relatively flat, which causes poor drainage of excess water, classifying over 60 % of the site as jurisdictional wetlands. East Carolina University has leased the site since 1989, and the site comprises the West Research Campus (WRC). The West Research Campus serves as a resource for ecological education and research. Several hectares of the West Research Campus were designated for a long-term experiment, which was initiated in February of 2002. The long-term experiment serves as the basis for my research.

The long-term project involves four experimental treatments replicated on eight 20 m × 30 m blocks in a randomized block design (Figure 1). The purpose of the long-term study is to determine how nutrient availability and disturbance affect the diversity and composition of the plant community. To test the effects of disturbance, half of each block is mowed each winter, and litter is removed by raking. To test the effects of nutrient availability, fertilizer is applied to half of each block in February, June and October of each year. The fertilizer comprises 10 % nitrogen, 10 % phosphorus, 10 % potassium and minor amounts of calcium, magnesium and sulfur. The fertilizer and mowing treatments were randomly assigned to plots within the blocks. Mowing and fertilization are applied in a two factor full-factorial design to yield four treatments: 1) no mowing, no fertilizer; 2) no mowing, fertilizer; 3) mowing, no fertilizer; and 4) mowing, fertilizer. Vegetation is sampled in each plot at three 1 × 1 m^2 permanently marked quadrats located at randomly generated coordinates. The long-term blocks also experience a moisture

gradient due to a drainage ditch located along the side of the blocks that are closest to the road. This drainage ditch causes spatial heterogeneity to the long-term blocks that are not caused by the experimental treatments. Each August, undergraduate students identify and count all of the plant species located within the permanent sampling quadrats.

Plant Data Collection

In August 2013, undergraduate students sampled all plant species located in the 1 m^2 quadrats. They determined the stem count as well as the percent of the quadrat covered by each species. Statistical analyses were performed to test the effects of the treatments on plant community diversity and composition. In univariate analysis, percent cover data were used to determine the species richness for each quadrat. Simpson's Diversity Index was also used as a measure of diversity. The equation for Simpson's Diversity Index is $D = \frac{\sum n(n-1)}{N(N-1)}$, where n is the total number of individuals of a particular species, and N is the total number of individuals of all species. Simpson's Diversity Index takes into account both species richness and evenness; as richness and evenness increase, the total diversity for each plot increases.

Soil Sample Collection

Six soil samples were collected from each treatment plot within each block and combined to represent a single composite sample. The samples were collected at locations 1 m north and 1 m south of the three 1 × 1 m^2 permanent sampling quadrats in each treatment plot. To have uniformity in the soil collections, soil was sampled using a soil corer (3 cm diameter) to a 10 cm depth. The soil samples were placed immediately in sealed plastic zip-locked bags and then stored at 4 °C until processed in the laboratory. Soil samples were passed through a 4 mm sieve,

homogenized, and the plant matter was removed. The homogenized soils from each plot were subsampled for further testing.

Soil Chemical and Physical Properties

Soil moisture for each sample was determined using a gravimetric method. The field-collected soil was weighed and then dried at 105 °C overnight. Soil moisture was measured as the weight of water divided by the weight of dried soil. Total soil organic C and N were determined by elemental analysis using combustion methods. Soil pH was determined by mixing a 1:1 ratio of soil and deionized water (5 g soil: 5 mL deionized water), vortexing the mix and taking the average of three readings using a pH meter. The available ammonium and nitrate was measured on soil extracts. About 5 g of soil and 50 mL of 2M KCl were combined, shaken for an hour, and the soil extracts were collected and analyzed colorimetrically for ammonium and nitrate.

DNA Extraction

Total genomic DNA from each environmental sample was isolated using the Mo Bio Powerlyzer PowerSoil DNA Isolation kit. This process extracted DNA from all organisms present in the soils. Once the DNA was extracted, the NanoDrop spectrophotometer was used to determine the concentration of extracted DNA. Each DNA sample was diluted to a 20 ng/µl concentration and used for downstream molecular analysis.

Microbial Community Analysis

To determine the bacterial composition of each soil sample, we performed targeted amplicon sequencing of the 16S rRNA gene, which is found in all bacteria and highly conserved. The Illumina Miseq platform was used for amplicon sequencing. Illumina is a platform that sequences through synthesis, and was used to determine the base pairs of DNA amplified from the given soil samples. This sequencing method is based on reversible dye-terminators that enable the identification of single bases as they are introduced into DNA strands. The protocol described in Caporaso et al. (2011) was followed with some modification. The diluted DNA samples were mixed with 18.75 µl of water, 5.0 µl of 10x buffer, 10.0 µl of 5x5p solution, 1.0 µl of dNTPs, the 1.0 µl of forward primer and 1.0 µl of barcoded reverse primer and 0.25 µl of Perfect Taq, creating the reaction mixture (modified earth microbiome project). The V4-V5 region of the 16S ribosomal RNA gene was amplified in the PCR reaction using the 515forward/806reverse primer set (Table 1. The primers contain the Illumina adapters, and the reverse primer contains a 12 base pair error-correcting barcode that was unique to each soil sample. This error-correcting barcode allows the samples to be sequenced at the same time (i.e., multiplexed) and then separated downstream based on the unique barcode for each sample. PCR amplification was conducted in triplicate reactions for each soil DNA sample using an Eppendorf Flexlid Mastercycler. The reason for running the PCR amplification in triplicate is to get a concentrated sample. Initial denaturation was at 94 °C for 3 minutes, followed by 30 cycles of 94 °C for 45 seconds, 50 °C for 30 seconds and 72 °C for 90 seconds. The final extension step was 72 °C for 10 minutes. Gel electrophoresis was performed on each DNA sample to check for successful amplification of the 16S rRNA gene. The PCR products were then purified using the Axygen AxyPrep Magnetic PCR cleanup protocol. Once the PCR products were purified, the

Quant-iT kit was used to determine the new concentration of DNA for each sample. The samples were then combined in equamolar concentrations into one tube, and sequenced using the Illumina Miseq Platform at the Center for Genomics and Bioinformatics at Indiana University.

Microbial Sequence Analysis

Mothur, an open-sourced expandable bioinformatics software package, was used to analyze sequences obtained from the Illumina Miseq platform (Schloss et al. 2009). Illumina produces paired-end reads of sequences measuring about 250 bp in length. The first command run was the make.contigs command. This command combined the paired-end Illumina sequences into contigs, which are overlapping sequence reads. The next command used was screen.seqs, which screened the sequences and removed any sequences that were considered ambiguous and that were longer than 275 base pairs. The command unique.seqs was run to identify identical sequences as duplicates and merge the identical sequences leaving only the unique sequences. The count.seqs command was used to create a table in which the rows were sequence names and the columns were group names. Next the pcr.seqs command was used to align the sequences to a reference database. The pcr.seqs command made a database customized to the region of the DNA used in this study. The align.seqs command was used to align the sequences to the Silva reference database (Quast et al. 2013). Next, the screen.seqs command overlapped sequences at the same position, and we used the command filter.seqs to remove overhangs at the both ends of sequences. The unique.seqs was used again to merge identical sequences that formed after the sequences were trimmed in the previous commands.

Additional steps were used to clean up the sequences. The command precluster.seqs illuminated further noise and allowed the sequences to have up to two differences between them.

Next, the command chimera.uchime was used to remove chimeras from my sequences. Chimeras are false sequences that arise from the merger of two distinct sequences. This results in organisms that do not exist being counted in our sequence data. Following the chimera check stage, the remove.seqs command removed the chimeric sequences from the data. To see if there are any undesirable sequences such as Eukaryotes or Archaea, the command classify.seqs was run, and the remove.lineages command removed those undesirable sequences.

Once we had our final sequence data, we determined the error rate of the sequences using a mock community. You can only assess the error rate if you have sequenced a mock community along with your sample data. The error rate allows you to determine if there are any issues with the sequencing set up. Mock communities are a collection of sequences used specifically to assess error rate. The mock communities were pulled out of the community data using the command get.groups. Next, the seq.error command was used to determine the error rate of our sequences.

The final step involved clustering our sequences into Operational Taxonomic Units (OTUs). The mock community was removed from our data using the command remove.seqs. The cluster.split command divided the sequences into bins and clustered the sequences once they were inside the bins. The bins were defined based on similarity to other sequences, therefore we binned sequences based on 97 % similarity. Once this was complete the command make.shared determined how many sequences were in each OTU from each group and the command classify.otu was used to get consensus taxonomy for each OTU. Consensus taxonomy is a standardized definition of measurement properties of taxonomy (Schloss et al. 2009).

Statistical Analyses

Analysis of variance (ANOVA) was used to determine if there was a statistically significant effect of the treatments on the plant diversity of each plot. The statistical program R was used to run the ANOVA using both the species richness and the Simpson's Diversity D value as dependent variables. The treatments and factors tested in the ANOVA were fertilization, mowing, proximity to the ditch as a random variable, and all possible interactions.

To perform multivariate analysis of the plant data, stem counts were first converted to proportional values to standardize the data across all the plots. The data were then used to create a nonmetric multidimensional scaling (NMDS) ordination plot using Bray-Curtis distances to visualize the differences among the 32 samples. The ggplot2 library in the R statistical package was used to create the NMDS plot (R Development Core Team). The adonis function in the R statistical package was used to carry out Permutational Multivariate Analysis of Variance (PERMANOVA) (R Development Core Team). Adonis was used for the analysis and partitioning of sums of squares using semimetric and metric distance matrices (R Development Core Team). The PERMANOVA tested for significant effects of the treatments on community composition. Adonis ran 999 permutations using Bray-Curtis distances. The treatments and factors tested in the PERMANOVA were fertilization, mowing, proximity to ditch, and all possible interactions. To determine the species that contributed the most to the differences in community composition resulting from the treatments, a Simper analysis was performed in R statistical package (R Development Core Team). Simper analyses perform pairwise comparisons of groups of sampling units and find the average contributions of each species to the average overall Bray-Curtis dissimilarity.

ANOVA was used to determine if there was a statistically significant effect of nutrient and disturbance treatments on the soil chemical and physical properties. The statistical program R was used. The treatments and factors tested in the ANOVA were fertilization and mowing as fixed factors, block as a random factor, and all possible interactions.

Similarly to the plant data, univariate analysis of the microbial data began with determining the richness and diversity of the OTU data. Simpson's Diversity Index was used as a measure of diversity for each plot. ANOVA was used to determine the effects of nutrients and disturbance on the richness and D value of the microbial data. The R statistical package was used to run the ANOVA using species richness and the Simpson's Diversity D value as the arguments (R Development Core Team). The treatments and factors tested in the ANOVA were fertilization and mowing as fixed factors, proximity to the ditch, and all possible interactions. Proximity to the ditch was included as a categorical variable to test for possible effects of drainage on the microbial community. For this analysis, the four blocks near the ditch and four blocks away from the ditch were pooled.

PERMANOVA was used to test the effects of each treatment on bacterial community composition, looking at microbial community composition based on relative abundance, presence/absence, and also unclassified microbial taxa. The adonis function in R statistical package was used for the analysis and partitioning sums of squares using semimetric and metric distance matrices (R Development Core Team). The function ran 999 permutations using the Bray-Curtis distances to determine which treatments and factors had a significant effect on the community composition. The treatments and factors tested in the PERMANOVA were fertilization, mowing, proximity to ditch and all possible interactions. NMDS ordination plots were used to visualize differences in bacterial composition due to the treatments using the library

ggplot2 in the R statistical package (R Development Core Team). Again, a Simper analysis was performed in R statistical package to determine the species that contributed the most to the differences between the factors mowing, fertilization, and the interaction of mowing and fertilization, as well as proximity to the ditch in both the relative abundance and presence absence microbial data. Mantel tests were performed to compare patterns in plant and microbial community composition (relative abundance) and soil factors. We measured the extent to which the distance matrix of the plant community data was correlated to the distance matrix of the microbial community data, and if each distance matrix was correlated to that of the soil physical and chemical data. Distance matrices quantify dissimilarity between sample data for numerical computation. Plant and microbial community matrices were based on Bray-Curtis dissimilarity and the soil physical and chemical data matrix was based on Euclidean distances.

RESULTS

Plant Community Analyses

In the analyses of plant community data from 2013 (Table 2), mean species richness was significantly higher in plots that received the mowing treatment (5.51 ± 1.79 species/m^2) than in plots that were not mowed (4.37 ± 1.06 species/ m^2) (Figure 2). Additionally, the mean Simpson's D value of the plots that received the mowing treatment was significantly higher (14.52 ± 0.86) than that of the plots that were not mowed (8.08 ± 0.36) (Figure 3). Mean species richness was significantly lower in fertilized plots (4.51 ± 4.18 species/m^2) than in unfertilized plots (5.37 ± 4.92 species/m^2) (Figure 4). Mean Simpson's D value of the plots that received fertilization was significantly lower (10.29 ± 0.56) than that of the plots that received no fertilization (12.31 ± 1.06) (Figure 5). Species richness and D value varied among block, while the interaction of mowing and fertilization did not have a significant effect on richness or D value. PERMANOVA analysis revealed that both mowing and fertilization significantly influenced plant community composition within the plots (Table 3; Figure 6). The interaction between mowing and fertilization, as well as the proximity to the ditch both had a significant effect on plant community composition.

An indicator species analysis was used to determine which species contributed the most to the differences found between the treatments. The plant species that contributed the most to the differences between the fertilized and unfertilized plots were *Euthamia caroliniana* (Asteraceae), *Chasmanthium laxum* (Poaceae*)*, and *Arundinaria tecta (*Poaceae*)*. These three species accounted for 33.9 % of the difference found between the two factors. The same three

species accounted for 37.6 % of the difference found between the mowed and unmowed plots, as well as 38.8 % of the difference due to an interaction between the treatments.

Nutrient and Disturbance Impacts on the Soil Environment

Mowing did not have a significant effect on many of the soil properties measured in this experiment (Table 4). Fertilizer significantly affected soil pH, with the fertilized plots having a lower pH (5.41 ± 0.16) than the unfertilized plots (5.55 ± 0.05). Additionally, the nitrate levels varied significantly among blocks. Soil carbon content was higher in plots that received fertilization (2.10 ± 0.02 g C kg^{-1}), than plots that did not (1.78 ± 0.14 g C kg^{-1}) (Table 4). Additionally, the carbon to nitrogen ratio in the soil was significantly higher in the plots that were fertilized (11.5 ± 0.41wt:wt), than the plots that were not fertilized (10.5 ± 0.22 wt:wt). The carbon to nitrogen ratio also varied significantly among blocks. Mowing and fertilization significantly affected temperature of each sample (Table 4). Temperatures were significantly higher in the mowed plots (24.6 ± 1.05 °C) than in the non-mowed plots (22.6 ± 0.18 °C). Temperatures were found to be lower in the fertilized plots (23.2 ± 1.02 °C) than in the unfertilized plots (24.03 ± 1.89 °C).

Microbial Community Analyses

Fertilization significantly affected the mean species richness of the microbial communities (Table 5). The plots that received the mowing treatment had a higher species richness (152.88 ± 5.48 OTUs/plot) than those that did not receive the mowing treatment (146.25 ± 5.30 OTUs/plot) (Figure 7). Additionally, the plots that received the fertilization treatment were significantly higher in richness (153.38 ± 4.77 OTUs/plot) than the plots that were not fertilized (145.75 ± 4.60 OTUs/plot) (Figure 8). The Diversity D value was significantly lower in

the mowed plots (7.46 ± 0.23) than in the unmowed plots (8.14 ± 0.50) (Figure 9), but was not significantly lower in the fertilized plots (7.54 ± 0.35) than in the unfertilized plots (8.06 ± 0.62) (Figure 10). Proximity to ditch had no effect on either measure of microbial diversity. Fertilization and proximity to the ditch significantly affected microbial community composition (Table 6; Figure 11). Both experimental treatments, as well as proximity to the ditch had a significant effect on unclassified microbial composition (Table 7; Figure 12). Mowing, fertilization, and proximity to the ditch had significant effects on microbial community composition using presence/absence data (Table 8; Figure 13).

Microbial Sequence Analyses

There were originally 9,189,129 sequences in the Miseq data. After the first screening step in Mothur, the number of sequences was decreased to 7,964,795. Once the sequences were aligned to the database, there were only 1,704,326 unique sequences. After all chimeras were removed and additional screening was performed, the final number of unique sequences used to make the sample by species matrix was 457,790.

Soil Microbial Community Composition

The ten most abundant microbial phyla per treatment were found to be Proteobacteria, Planctomycetes, Acidobacteria, Chloroflexi, Actinobacteria, Verrucomicrobia, Firmicutes, Chlamydiae, Bacteroidetes and Armatimonadetes (Table 9). Each treatment had the same top ten phyla, but in a different order of abundance. Proteobacteria was the most abundant phylum in each treatment encompassing at least 39 % of the total microbial composition. The total number of microbial OTUs was found to be 384 with a total of 53,341 known bacteria in our community. The top ten microbial OTUs based on abundance were Acidobacteria Gp1, Acidobacteria Gp2,

incertae_sedis (Unknown), Acidobacteria Gp3, *Aquicella*, *Gemmata*, *Legionella*, *Ktedonobacter*, *Spartobacteria* and Acidobacteria Gp6. Acidobacteria Gp1 was the most abundant OTU in each treatment (Table10). Gp1 comprised at least 20 % of the total number of bacteria in each treatment. The most abundant bacterial species are almost the same in each treatment, but differ in abundance.

In PERMANOVA analyses of relative abundance data, the top nine microbial OTUs were among the groups that contributed the most to the differences found between the fertilized plots and the unfertilized plots. The indicator species analysis determined that Acidobacteria Gp1 and Acidobacteria Gp2 each had a 5 % responsibility for the differences between the fertilized and unfertilized treatments. Acidobacteria Gp1 and Gp2, *Aquicella*, Gp3, and *Legionella* were among the groups that together contributed over 50 % to the difference between the mowed and unmowed treatments. The microbial species that contributed to differences between the plots that were nearest to the ditch and further from the ditch were Acidobacteria Gp 1, Gp 2, *Aquicella*, Gp 3 and *Legionella* as well. The top nine microbial OTUs contributed to over 60 % of the differences caused by interaction of the two treatments.

In the indicator species analysis of the presence/absence microbial data, it was determined that *Papillibacter, Caulobacter, Silvimonas, Bosea, and Acidothermus* contributed the most to the difference seen among the fertilized and unfertilized plots and *Zymophilus, Desulfovirga, Holophaga, Magnetospirillum, Desulfovibrio* contributed the most to the differences among the mowed and unmowed plots. In the plots that received both mowing and fertilization, *Niastella, Cellulomonas, Hydrocarboniphaga, Dysgonomonas, and Thermogemmatispora* contributed the most to differences seen among these plots. The microbial species that contributed the most to the

differences seen between the near and far plots in proximity to the drainage ditch were *Desulfovibrio, Aneurinibacillus, Anaerobacter, Desulfovirga, and Holophaga.*

Based on patterns of plant and microbial community composition and soil environmental factors, using matrix comparisons in the form of Mantel test (Table 11), there was a significant correlation between the plant community and soil physical and chemical properties. Although there is a significant correlation, the relationship between the plant community and the soil chemical properties is not strong. There was not a significant correlation between the microbial and plant community composition, or between the microbial community composition and soil physical and chemical properties.

DISCUSSION

Understanding the effects of nutrient addition and disturbance on plant and microbial community diversity and composition is important because these communities are integral parts of ecosystem structure and function. The results of the present study showed that the plant communities and microbial communities responded differently to the experimental fertilization and disturbance treatments. Fertilization caused a significant decrease in plant species richness. Plant species richness was 19.1 % lower in the fertilized plots than in the unfertilized plots. In wetland ecosystems, nutrient addition leads to changes in plant community composition, as well as loss of species diversity (Bedford et al. 1999; Clark and Baldwin 2002; Craft et al. 2007). Decreases in plant species richness in nutrient addition experiments are also common in other ecosystems such as grasslands, rain forests, aquatic ecosystems, and heathlands (Clark and Tilman 2008; Huston 1980; Hillebrand 2003; Aerts and Berendse 1988). Recently, Dickson and Gross (2013) published the results of a long-term experiment looking at the effects of fertilization on grassland plant communities. Similarly to our experiment, after 14 years of fertilization there was a sharp decline in plant species richness in this ecosystem (Dickson and Gross 2013). The mechanism that they suggested to be the cause of this decline, and the mechanism that likely contributes to decreased species richness in our plots is competition. Plants compete for resources such as nutrients, water and sunlight. When nutrients are abundant and readily available for uptake, plants compete mainly for light (Newman 1973; Aerts 1999). Plants that have the ability to grow faster and taller and produce larger leaf canopies will have a competitive advantage over smaller plants (Ford 2014). The smaller plants will eventually be competitively excluded from the environment because the larger plants prevent them from obtaining the light necessary for their survival (Goldberg and Miller 1990). A preliminary study

provides evidence for this effect in the WRC plots. *Solidago rugosa*, a perennial angiosperm found in the family Asteraceae, was found to be lower in abundance in the fertilized plots. In order to determine a possible mechanism behind this pattern, I performed removal experiments in the fertilized and unfertilized plots. The results of the experiment showed that in the removal quadrats, *Solidago rugosa* was taller and contained more biomass than those in the control quadrats in the fertilized plots, but this difference was not seen in the unfertilized plots. This study demonstrated that competition was a possible mechanism behind the decrease in species richness in nutrient addition experiments.

In contrast to the plant communities, microbial species richness was 5.23 % higher in the fertilized plots than in the unfertilized plots. The results differ from those of past studies where microbial communities generally declined in response to nutrient additions (Jackson et. al 2009; Carrero-Colon et. al 2006; Campbell et al. 2010). A major cause of microbial community decline in previous studies was the acidification of soils associated with nutrient addition, more specifically N addition (Fierer and Jackson 2006; Zhang et al. 2013). A possible explanation for the increase in species richness with fertilization in our study is the nutrient limited conditions in the wetland soil. When the fertilization treatment occurred, the plant and microbial communities might have assimilated the newly available nutrients before soil environmental conditions had an opportunity to change. The soil did not become more acidified due to the N addition, which ultimately buffered against pH-induced changes in soil microbial communities (Yakov and Xu 2013). Another possible explanation behind microbial richness increasing in the fertilized plots is microbial dormancy. Microbial communities can go into a dormant state and generate a microbial seed bank that can be revived in when environmental conditions become favorable (Lennon and Jones 2011). Dormancy becomes a key mode of survival in nutrient limited

environments because microbes could potentially starve in these environments (Lennon and Jones 2011). Microorganisms exit the dormant state when nutrients are added to the environment, which would increase species richness (Lennon and Jones 2011). Although there was a significant effect of fertilization on microbial communities, the magnitude was not great. Possibly, the time at which we sampled the soils affected the magnitude of the impact that fertilization had on the microbes. We sampled soils at the end of June, which was at least three months after fertilization had occurred. Since the wetland communities were so nutrient limited, the plant communities may have rapidly taken up the nutrients before they affected the microbes in a major way.

Disturbance through mowing increased plant diversity. Plant species richness in the mowed plots was 26.1% higher than in the unmowed plots. As with fertilization, the effects of mowing in this experiment are likely mediated by plant competition. When disturbance is at an intermediate level, it decreases the chances of competitive exclusion causing diversity to be higher (Connell 1978; Townsend et al. 1997; Molino 2001). Mowing decreases canopy size of taller plants with the larger canopies. This allows smaller plants to get the necessary sunlight for growth, which increases species richness. Mowing can also cause the community composition of plants to shift. Shifting the plant community from annual plants to perennial plants can cause species richness to be higher in mowed plots (Ashok et al. 2006; Frerker et al. 2014; Zhou et al. 2006). For example, in a five-year experiment (Maron and Jeffries 2001), higher species richness was observed in mowed plots, and the plant community composition shifted from annual grasses to perennial forbs. The most abundant plant species in the mowed plots of the WRC are perennials. With the fertilized plots dominated by perennials, there is a good chance that aboveground biomass is similar among the species, and also contributes to increased N

retention is in these plots (Maron and Jeffries 2001). Less competition for nutrients and light availability could be the cause of the higher species richness found in the mowed plots. In the unmowed plots, the dominant species are woody species such as trees. These tree species are strong light competitors. In grasslands we tend to see a shift from annuals to perennials, but in the unmowed we saw a shift to woody tree species. The tree species tower over the rest of the plant species taking in most if the available light. This could be the cause of species richness decline in the unmowed plots.

Mowing had only a minor effect on microbial community composition. Mowing significantly increased the species richness in the microbial communities with the richness being almost 5 % higher in the mowed plots than the unmowed plots (Mowed = 152.88 ± 5.48 OTUs/plot; Unmowed = 146.25 ± 5.30 OTUs/plot). One possible explanation for the minimal effect is the time at which the mowing occurred. Plants provide microbial communities with energy in the form of organic C. Since the mowing occurred at the end of the plant growing season, there is likely less change in C inputs occurring during plant senescence. Microbial communities may have acquired the majority of labile C via plant exudates before the mowing occurred. Similar results were seen in an experiment performed by Zhang et al. (2013). The researchers performed an experiment looking at the effects of mowing and nutrient addition on soil microbial communities. In their experiment, mowing occurred at the end of the plant growing season, resulting in the mowing treatment having little effect on soil microbial communities. Since mowing occurred so late in this experiment, the researchers concluded that if mowing had been applied earlier in the growing season, plant communities would not have provided the microbial communities with the necessary C, potentially causing a decrease in microbial diversity (Zhang et al. 2013). In addition, Antonssen and Olssen (2005) performed a

field experiment in a boreal former hayfield where they were looking at the effect of mowing, among other treatments, on plant and microbial communities. They mowed the experimental site twice a year in mid-June and late August or early September. The late mowing of their plots is similar to the late mowing of our plots, and they saw an increase in soil respiration. They attributed their results to an increase in root C exudation caused by mowing. The decreased foliation in our mowed plots could have increased the C exudation by the plant roots, which could be the reason there was increased microbial diversity in the mowed plots (Antonssen and Olssen 2005).

In terms of overall composition, plant communities were also more affected by experimental treatments than the microbial communities. Plant community composition was significantly affected by fertilization, mowing, block and the interaction between mowing and fertilization. *Euthamia caroliniana, Chasmanthium laxum, Arundinaria tecta, Clethra alnifolia,* and *Smilax glauca* (Smilaceae) were among the most abundant across all treatments. In the comparison of the fertilized and unfertilized plots, and the mowed and unmowed plots, the most abundant species were also the most significant plant species responders (i.e., indicator plant species). *Euthamia caroliniana* was 85 % higher in the fertilized plots, and *Chasmanthium laxum* was 79 % higher in the fertilized plots. This indicates that these species are becoming more dominant in the fertilized plots, which is more than likely what is decreasing diversity in these plots. *Euthamia caroliniana* was almost 870 % in mowed than in unmowed plots. *Arundinaria tecta* was not present in the unmowed plots, but was the second most abundant plant species in the mowed plots. Huberty et al. (1998) looked at the effects of nitrogen addition on plant succession of an old field. They observed that plant communities shifted from annuals to perennials during the nutrient addition experiment. In an experiment testing the effects of

nutrient addition on two different types of plant, a monocarp and a perennial, perennial plants experienced greater plant biomass, seeds per fruit and seeds per plant in the fertilized treatments (Burkle and Irwin 2008). Perennials have also developed adaptations to nutrient limited environments such as long-term investment in the habitat and the ability to explore soils in search of nutrients (Rennenburg and Schmidt 2010). These adaptations could be the driving force behind these plants flourishing in the nutrient depleted wetlands of the long-term blocks. The indicator species of plants were all perennials. Perennials seem to flourish, grow and survive in the conditions of long-term experiment environments (Maron and Jeffries 2001; Burkle and Irwin 2008; Huberty et al. 1998), which may partially explain why these plants dominate all of the treatment plots.

Unlike the plant community composition, microbial community composition was not significantly affected by the fertilization and disturbance treatments but it vary significantly when looking at proximity to the ditch (Table 6). The soil moisture gradients present in the long-term blocks of the WRC could account for the influence that proximity to the ditch had on microbial community composition. Moisture increases as the blocks move further away from the drainage ditch. In the even blocks (wet), the mean soil moisture is 12.78 % higher (28.55 % ± 4.79) than the blocks nearest to the roadside ditch (25.35 % ± 4.16). Soil moisture has a strong influence on microbial community composition (Truu et al. 2009). When unclassified OTUs were analyzed alone, microbial composition was significantly affected by all of the treatments. This demonstrated that the unclassified communities are more affected by the treatments and are could potentially be driving differences in community composition. Also the results of analyses using presence and absence data provides more insight into the composition of the microbial community. The PERMANOVA using relative abundance data was strongly affected by the

most common species. In contrast, presence/absence data gives equal weight to common and rare species. The finding of significant effects of all treatments using these data suggests that rare taxa differ more between treatments than do common taxa. In the simper analysis of the presence/absence microbial data, we get a closer look at the effect of rare species on the differences found in the microbial communities. These species are driving the differences in microbial communities of the samples but their effect gets washed away because they are small in abundance.

Wetland communities are often lacking in oxygen supplies, and therefore alternative electron acceptors must be used. The use of alternative electron acceptors can affect microbial community composition and population size (McClatchey and Reddy 1998). Fluctuations in soil moisture change soil redox status, altering oxygen supplies and ultimately affecting microbial community composition (Peralta et al. 2012).

The microbial phyla with the most sequences in the WRC community were Proteobacteria, Plantomycetes, Acidobacteria, Chloroflexi, Actinobacteria, Verrucomicrobia, Firmicutes, Chlamydiae, Bacteriodetes, and Armatimonadetes (Table 9). These phyla are generally among the most abundant microbial phyla found in soils (Fierer et al. 2007). In a survey of 21 soil libraries with 2920 clones, it was seen that Proteobacteria, Plantomycetes, Acidobacteria, Chloroflexi, Actinobacteria, Verrucomicrobia, Firmicutes and Bacteriodetes are eight of the nine phyla that make up 92 % of these soil libraries (Janssen 2006). In my study, the microbial phyla with the most sequences were similar for all treatments; however, the order in which they were proportionally represented in each treatment differed. This similarity among microbial community composition is possibly due to the fact that pH measures across the treatments are similar. Microbial community composition and diversity are strongly affected by

pH (Fierer and Jackson 2006). Since all the pH levels were similar in our study, the composition of microbial communities remained similar across plots as well. Looking at the other soil chemical and physical properties measured such as total carbon (C) and (N), ammonium and nitrate, and carbon to nitrogen ratio, there seems to be little variation among the treatments. The lack of variation among soil chemical and physical properties could account for the lack of variation among the microbial communities because microbial community composition is related to soil properties (Steenwerth et al. 2002; Dong et al. 2014).

Similarity in soil microbial community across the site is congruent with the finding of similar decomposition function. In a previous study at this site, a litterbag experiment aimed to determine the effect of fertilization treatments in the long-term experimental plots on leaf decomposition rate (H. Vance-Chalcraft, personal communication). This year-long experiment, in which leaf-filled mesh bags were placed in the fertilized and unfertilized plots, resulted in similar decomposition rates in the two treatments. Since the microbial community composition at these plots was found to be similar, it is not surprising that microbial function of decomposition was also similar across the plots. These results are supported by previous studies. Microbial community composition has been linked to its enzyme function and litter decomposition (Waldrop et al. 2000; You et al. 2014; Strickland et al. 2009). Specifically, Strickland et al. (2009) compared litter decomposition rates of distinct microbial communities in different microbial inoculums. They found that decomposition rate, in the form of carbon dioxide production, was strongly dependent on the microbial community. Similarly, You et al. (2014) observed that enzyme functions associated with decomposition and turnover are strongly correlated with bacterial community structure and abundance (You et al. 2014). Since structure and function of bacterial communities are so closely related, the fact that the composition of

microbes in the treatments was not significantly different explains in part why decomposition rate was not significantly different among these same treatments.

Microbial community composition was not only similar at the phylum level but also at the OTU level. The OTU or "species" level represents sequences that were 97% similar. I defined the OTU parameters and the sequences were compared to sequences in the Silva database. The treatments were dominated by different groups of the phylum Acidobacteria such as Gp1, Gp2, Gp3, Gp6, and Gp13. Acidobacteria are highly diverse and make up 20% of all bacteria found in the soil (Naether 2012). In the fertilized and unfertilized plots, Acidobacteria Gp1, Gp2, and Gp3 were indicator species and among the major microbial OTUs that accounted for the differences in the two treatments. The same OTUs were indicator species that accounted for differences in the mowed and unmowed plots. This was expected because these OTUs were dominant in the microbial communities. The dominance of Acidobacteria in our environment can be explained by its physiological make-up. Acidobacteria have many traits that give them the ability to persist in nutrient poor environments. Acidobacteria can use simple sugars as well as complex substrates such as cellulose and lignin as a carbon source (Ward et al. 2009). Also, Acidobacteria are well suited for low nutrient environments because their cells are long lived, and they exhibit low metabolic rates under low nutrient conditions and can handle constant changes in soil moisture (Ward et. al 2009).

The drainage ditch alongside the odd numbered blocks of the long-term experiment at the WRC (Figure 1), contributed to soil heterogeneity that is not the result of our experimental treatments. When looking at the physical appearance of the blocks, it is clear that the blocks closest to the ditch are much dryer than the blocks further away from the ditch. It is evident that the ditch is causing a hydrologic gradient among the blocks. The NMDS plot of the plant

community composition (Figure 6) shows a separation between the plant communities that are near the drainage ditch (odd) and the plant communities further away from the ditch (even). The purpose of this experiment was not to test the effect of the proximity to the ditch, but the ditch seemed to be a major source of the heterogeneity of the soil. The PERMANOVA analyses confirmed that proximity to the ditch was a significant cause of differences seen among plant and microbial community composition. Surprisingly, we did not see a strong effect of the ditch on the moisture content in our sampled soils. In the soil data, proximity to the ditch did not have a significant effect on soil moisture. The time at which we sampled the soils had a big effect on this result. We sampled at an extremely dry time of the summer so the moisture gradient was not present. In order to test truly test the effect of the moisture gradient we would have to collect multiple sample at different times of the year.

The experimental design yielded promising results but slight modification could yield more definitive results. Limitations associated with treatment application may have weakened the effects of the experimental conditions. The use of a 10-10-10 fertilizer made it difficult to identify the effect of one particular nutrient because it contains nitrogen, phosphorus and potassium in equal amounts. If the experiment was designed to test for individual nutrient additions, more variation in plant and microbial community response may have been observed. The mix of nutrients could have also been responsible for the lack of variation seen among the treatments. Some plots could have been lacking in certain nutrients and abundant in others. Different nutrients affect communities in different ways. If we modified individual nutrients, we could assess the effects of specific nutrients and see how these nutrients add variation across the treatments. More dramatic results of nutrient addition are seen when single nutrients are added to the environment. Ramirez et al. (2010) sampled soils from Cedar Creek LTER and the Kellogg

Biological station to determine the effects of nitrogen fertilization on soil microbial communities. The researchers saw that nitrogen fertilization dramatically altered bacterial composition and bacterial community structure was highly responsive to nitrogen fertilization (Ramirez et al. 2010). Similarly in phosphorus addition experiment testing the hypothesis that the addition of phosphorus affected microbial composition, it was observed that phosphorus addition in the form of fertilization shifted microbial communities (Beauregard et al. 2009). When nutrients are added singularly, the effects of the nutrients are more pronounced and easier to interpret.

Humans both intentionally and unintentionally impact natural environments. Anthropogenic nutrient addition such as the fertilization application is common, just as anthropogenic disturbance through mowing, habitat alteration, and climate change are common. My results indicate that plant and microbial community diversity are influenced by nutrient availability and disturbance; however only plant community composition is influenced by these factors. The magnitude of the treatment effects was greater in the plant community than the microbial community. These results are a culmination of 11 years of fertilization and mowing. Although 11 years is a long time, it may not have been long enough for the microbial communities to respond and change due to the treatments.

Maintaining community composition and diversity is important for ecosystem functioning. Ecosystem functions are important for humans because humans garner goods and services from natural ecosystems. The loss of plant and microbial diversity can lead to loss of function in an ecosystem (Philippot et al. 2013; Tilman et al. 1997). Species diversity contributes to resilience and resistance to the anthropogenic changes, and also increases the chance of having a functionally redundant group of species that will ensure continued function of the ecosystem (Allison and Martiny 2008). This experiment can contribute to the established literature on the

effects of nutrient addition and disturbance on communities. In the future I hope to continue looking at the relationship between plants-soil-and microbial communities and how abiotic and biotic factors affect these relationships. I also hope to determine the directionality of plant-microbe species interactions.

REFERENCES

Aerts, R and F Berendse. "The Effect of Increased Nutrient Availability on Vegetation Dynamics in Wet Heathlands." *Vegetatio* 76.1-2 (1988): 63-69.

Allison, SD and JBH Martiny. "Resistance, Resilience, and Redundancy in Microbial Communities." *Proceedings of the National Academy of Sciences* (2008): 11512-1519.

Antonsen, H and PA Olsson. "Relative Importance of Burning, Mowing and Species Translocation in the Restoration of a Former Boreal Hayfield: Responses of Plant Diversity and the Microbial Community." *Journal of Applied Ecology* (2005): 337-347.

Bardgett, RD and WH Van Der Putten. "Belowground Biodiversity and Ecosystem Functioning." *Nature* (2014): 505-511.

Beauregard, MS C Hamel and M St-Arnaud. "Long-Term Phosphorus Fertilization Impacts Soil Fungal and Bacterial Diversity but Not AM Fungal Community in Alfalfa." *Microbial Ecology* (2009): 379-389.

Bedford, BL, MR Walbridge, and A Aldous. "Patterns in Nutrient Availability and Plant Diversity of Temperate North American Wetlands." *Ecology* (1998): 2151-2169.

Berg, G and K Smalla. "Plant Species and Soil Type Cooperatively Shape the Structure and Function of Microbial Communities in the Rhizosphere" (2009) *FEMS Microbiology Ecology*: 1-13.

Berga, M, AJ Székely, S Langenheder, and CP Slomp. "Effects of Disturbance Intensity and Frequency on Bacterial Community Composition and Function." *PLoS ONE* (2012): E36959.

Bever, JD, IA Dickie, E Facelli, JM Klironomos, J Moora, M. Rillig, MC Stock, WD Tibbett, and M Zobel. "Rooting Theories of Plant Community Ecology in Microbial Interactions." *Trends in Ecology & Evolution* (2010): 468-478.

Bolan, NS "A Critical Review on the Role of Mycorrhizal Fungi in the Uptake of Phosphorus by Plants." *Plant Soil Plant and Soil* (1991): 189-207.

Borer, ET, EW Seabloom DS Gruner et al. "Herbivores and Nutrients Control Grassland Plant Diversity via Light Limitation." *Nature* (2014): 508, 517-520.

Burdon, JJ, PH Thrall, and L Ericson. "The current and future dynamics of disease in plant communities". *Annual Review of Phytopathology* (2006): 19–39.

Burkle, LA and RE Irwin. "The Effects of Nutrient Addition on Floral Characters and Pollination in Two Subalpine Plants, *Ipomopsis Aggregata* and *Linum Lewisii*." *Plant Ecology* (2008): 83-98.

Campbell, BJ, SW Polson, TE Hanson et al. "The Effect of Nutrient Deposition on Bacterial Communities in Arctic Tundra Soil." *Environmental Microbiology* (2010): 1842-1854.

Caporaso, JG, CL. Lauber, WA. Walters D Berg-Lyons et al. "Colloquium Paper: Global Patterns of 16S RNA Diversity at a Depth of Millions of Sequences per Sample." *Proceedings of the National Academy of Sciences* (2011): 4516-4522.

Carrero-Colon, M, CH Nakatsu, and A Konopka. "Effect of Nutrient Periodicity on Microbial Community Dynamics." *Applied and Environmental Microbiology* (2006): 3175-3183.

Chilimba, ADC. "Beneficial Effects of Microbes in Nutrient Recycling in Cropping Systems of Malawi." *Journal of Tropical Microbiology and* Biotechnology (2002): 47-53.

Clark, CM and D Tilman. "Loss of Plant Species after Chronic Low-level Nitrogen Deposition to Prairie Grasslands." *Nature* (2008): 712-715.

Clarke, E and AH Baldwin. "Responses of Wetland Plants to Ammonia and Water Level." *Ecological Engineering* (2002): 257-264.

Cleveland, CC, AR Townsend, DS Schimel et al. "Global Patterns of Terrestrial Biological Nitrogen (N 2) Fixation in Natural Ecosystems." *Global Biogeochemical Cycles* (1999): 623-645.

Connell, J "Diversity in Tropical Rain Forest and Coral Reefs: High Diversity of Trees and Corals Is Only Maintained in a Nonequilibrium State." *Science* (1978): 1302-1310

Craft, C, K Krull and S Graham. "Ecological Indicators of Nutrient Enrichment, Freshwater Wetlands, Midwestern United States (U.S.)." *Ecological Indicators* (2007): 733-750.

Declerck, S, ES Bakker, BV Lith et al. "Effects of Nutrient Additions and Macrophyte Composition on Invertebrate Community Assembly and Diversity in Experimental Ponds." *Basic and Applied Ecology* (2011): 466-475.

Dickson, TL and BL Foster. "Fertilization Decreases Plant Biodiversity Even When Light Is Not Limiting." *Ecology Letters* (2011): 380-388.

Dickson, TL and KLK. Gross. "Plant Community Responses to Long-term Fertilization: Changes in Functional Group Abundance Drive Changes in Species Richness." *Oecologia* (2013): 1513-1520.

Dong, WY, XY Zhang, XQ Dai et al. "Changes in Soil Microbial Community Composition in Response to Fertilization of Paddy Soils in Subtropical China." *Applied Soil Ecology* (2014): 140-147.

Eckhard, G H Marschner and I Jakobsen. "Role of Arbuscular Mycorrhizal Fungi in Uptake of Phosphorus and Nitrogen From Soil." *Critical Reviews in Biotechnology* (1995): 257-270.

Elton, CS. The ecology of invasions by animals and plants. *Methuen* (1958): London.

Fichtner, AG, V Oheimb, W Härdtle et al. "Effects of Anthropogenic Disturbances on Soil Microbial Communities in Oak Forests Persist for More than 100 Years." *Soil Biology and Biochemistry* (2014): 79-87.

Fierer, N and RB Jackson. "The Diversity and Biogeography of Soil Bacterial Communities." *Proceedings of the National Academy of Sciences* (2006): 626-631.

Fierer, N and JT Lennon. "The Generation and Maintenance of Diversity in Microbial Communities." *American Journal of Botany* (2011): 439-448.

Fierer, N MA Bradford and RB Jackson. "Toward An Ecological Classification Of Soil Bacteria." *Ecology* (2007): 1354-1364.

Flöder, S and U Sommer. "Diversity in Planktonic Communities: An Experimental Test of the Intermediate Disturbance Hypothesis." *Limnology and Oceanography* (1999): 1114-1119.

Ford, DE "The Dynamic Relationship between Plant Architecture and Competition." *Frontiers in Plant Science* (2014): 1-13.

Frerker, K, A Sabo and D Waller. "Long-Term Regional Shifts in Plant Community Composition Are Largely Explained by Local Deer Impact Experiments." *PLoS ONE* (2014).

Gevers, D, FM Cohan, JG Lawrence et al."Opinion: Re-evaluating Prokaryotic Species." *Nature Reviews Microbiology* (2005): 733-739.

Goldberg, DE and TE Miller "Effects of Different Resource Additions of Species Diversity in an Annual Plant Community." *Ecology* (1990): 213-225.

Goodwillie C and W Franch. "An Experimental Study of the Effects of Nutrient Addition and Mowing on a Ditch Wetland Plant Community: Results of the First Year." *Journal of the North Carolina Academy of Sciences* 122.3 (2006): 106–117.

Hartman, WH, CJ Richardson, R Vilgalys et al. "Environmental and Anthropogenic Controls over Bacterial Communities in Wetland Soils." *Proceedings of the National Academy of Sciences* (2008): 17842-17847.

Hillebrand, H. "Opposing Effects of Grazing and Nutrients on Diversity." *Oikos* (2003): 592-600.

Hobbie, S "Effects of Plant Species on Nutrient Cycling." *Trends in Ecology and Evolution*. (1992): 7-10

Huberty, LE, KL Gross and CJ Miller. "Effects of Nitrogen Addition on Successional Dynamics and Species Diversity in Michigan Old-fields." *Journal of Ecology* (1998): 794-803.

Husson, O "Redox Potential (Eh) and PH as Drivers of Soil/plant/microorganism Systems: A Transdisciplinary Overview Pointing to Integrative Opportunities for Agronomy." *Plant and Soil* (2012): 389-417.

Huston, M "A General Hypothesis Of Species Diversity." *The American Naturalist*: (1979): 81-101.

Huston, M "Soil Nutrients and Tree Species Richness in Costa Rican Forests. "*Journal of Biogeography* (1980): 147-157.

Ingham, RE, JA Trofymow, ER Ingham et al. "Interactions of Bacteria, Fungi, and Their Nematode Grazers: Effects on Nutrient Cycling and Plant Growth." *Ecological Monographs* (1985): 119-140.

Isbell, F, V Calcagno, A Hector et al. "High Plant Diversity Is Needed To Maintain Ecosystem Services. (2011) *Nature*: 199-202.

Lennon, JT and SE Jones "Dormancy Contributes to the Maintenance of Microbial Diversity." *Proceedings of the National Academy of Sciences* (2011): 5881-5886.

Jackson, CR, and SC Vallaire. "Effects of Salinity and Nutrients on Microbial Assemblages in Louisiana Wetland Sediments." *Wetlands* (2009): 277-287.

Janssen, PH "Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S RRNA and 16S RRNA Genes." *Applied and Environmental Microbiology* (2006): 1719-1728.

Johnson, NC. "Can Fertilization Select for Less Mutualistic Mycorrhizae?." *Ecological Applications*. 3.4 (1993): 749-757.

Jones, SE and JT Lennon "Dormancy Contributes to the Maintenance of Microbial Diversity." *Proceedings of the National Academy of Sciences* (2010): 5881-5886.

Keith, TL, E Soroker and Mk Firestone. "Microbial Biomass Response to a Rapid Increase in Water Potential When Dry Soil Is Wetted." *Soil Biology and Biochemistry* (1987): 119-126.

Kirk, JL, LA Beaudette, M Hart, et al. "Methods of Studying Soil Microbial Diversity." *Journal of Microbiological Methods* (2004): 169-188.

Knelman, JE, SK Schmidt, RC. Lynch et al. "Nutrient Addition Dramatically Accelerates Microbial Community Succession*." PLoS ONE* (2014).

Kondoh, M. "Unifying the Relationships of Species Richness to Productivity and Disturbance." *Proceedings of the Royal Society B: Biological Sciences* (2001): 269-271.

Kowalchuk, G, D Buma, WDB Peter et al. "Effects of Above-ground Plant Species Composition and Diversity on the Diversity of Soil-borne Microorganisms." *Kluwer Academic Publishers* (2002): 509-520.

Lake, PS "Disturbance, Patchiness, and Diversity in Streams." *Journal of the North American Benthological Society* (2000): 573-592.

Kremen, C "Managing Ecosystem Services: What Do We Need to Know about Their Ecology?" *Ecology Letters (2005)*: 468-479.

Lake, PS "Resistance, Resilience and Restoration." *Ecological Management & Restoration* (2013): 20-24.

Lau, JA, and JT Lennon "Evolutionary Ecology of Plant-microbe Interactions: Soil Microbial Structure Alters Selection on Plant Traits." *New Phytologist* (2011): 215-224.

Lau, JA, and JT Lennon. "Rapid Responses of Soil Microorganisms Improve Plant Fitness in Novel Environments." *Proceedings of the National Academy of Sciences* (2012): 14058-14062.

Lauber CL, MS Strickland, MA Bradford et al. "The influence of soil properties on the structure of bacterial and fungal communities across land-use types." *Soil Biology Biochemistry* (2008): 2407–2415

Lennon, JT and SE Jones "Microbial Seed Banks: The Ecological and Evolutionary Implications of Dormancy." *Nature Reviews Microbiology* (2011): 119-130.

Lewontin, RC The meaning of stability. Pages 13–24 in *Diversity and stability in ecological systems* (1969): Brookhaven Symposium in Biology 22. Brookhaven National Laboratory, Upton, N.Y

Luck, GW, GC Daily and PR Ehrlich "Population Diversity and Ecosystem Services."(2003) *Trends in Ecology & Evolution*: 331-336.

Magdanova, LA and NV Golyasnaya "Heterogeneity as an Adaptive Trait of Microbial Populations." *Microbiology* (2013): 1-10.

Margalef, R Diversity and stability: a practical proposal and a model of interdependence. Pages 25–37 in *Diversity and stability in ecological systems* (1969): Brookhaven Symposium in Biology 22. Brookhaven National Laboratory, Upton, N.Y

Maron, JL and RL Jefferies "Restoring Enriched Grasslands: Effects Of Mowing On Species Richness, Productivity, And Nitrogen Retention." *Ecological Applications* (2001): 1088-1100.

Marschner, P "Plant-Microbe Interactions in the Rhizosphere and Nutrient Cycling." *Nutrient Cycling in Terrestrial Ecosystems.* Soil Biology (2007): 159-182.

May, RM Stability and complexity in model ecosystems. *Princeton University Press* (1972*)*: Princeton, N.J

Mclatchey, GP and KR Reddy "Regulation of Organic Matter Decomposition and Nutrient Release in a Wetland Soil." *Journal of Environment Quality* (1998): 1268-1274.

Michiels, IC and W Traunspurger "Impact of Resource Availability on Species Composition and Diversity in Freshwater Nematodes." *Oecologia* (2004): 98-103.

Molino, JF "Tree Diversity in Tropical Rain Forests: A Validation of the Intermediate Disturbance Hypothesis." *Science* (2001): 1702-1704.

Myers, N "Biodiversity and the Precautionary Principle." *Ambio* (1993): 74-79.

Naether, AB, U Foesel, V Naegele et al. "Environmental Factors Affect Acidobacterial Communities below the Subgroup Level in Grassland and Forest Soils." *Applied and Environmental Microbiology* (2012): 7398-7406.

Newman, EI "Competition and Diversity in Herbaceous Vegetation." *Nature* (1973): 310-311.

Nordin, A, IK Schmidt and GR Shaver “Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply.” *Ecology* (2004): 955–962.

Patra, AK, L Abbadie, AC Josserand et al. "Effects of Management Regime and Plant Species on the Enzyme Activity and Genetic Structure of N-fixing, Denitrifying and Nitrifying Bacterial Communities in Grassland Soils." *Environmental Microbiology* (2006): 1005-1016.

Peralta, AL, JW Matthews, DN Flanagan et al. "Environmental Factors at Dissimilar Spatial Scales Influence Plant and Microbial Communities in Restored Wetlands." *Wetlands* (2012): 1125-1134.

Pett-Ridge, J and MK Firestone. "Redox Fluctuation Structures Microbial Communities in a Wet Tropical Soil." *Applied and Environmental Microbiology* (2005): 6998-7007.

Philippot, L, A Spor, C Hénault et al."Loss in Microbial Diversity Affects Nitrogen Cycling in Soil." *The ISME Journal* (2013): 1609-1619.

Putten, VD, RD Bardgett, JD Bever "Plant-soil Feedbacks: The Past, the Present and Future Challenges." *Journal of Ecology* (2013): 265-276.

Quast C, E Pruesse, P Yilmaz et al. “The SILVA ribosomal RNA gene database project: improved data processing and web-based tools.” 2013

Ramirez, KS, CL Lauber, R Knight et al. "Consistent Effects of Nitrogen Fertilization on Soil Bacterial Communities in Contrasting Systems." *Ecology* (2010): 3463-3470.

Rennenberg, H and S Schmidt. "Perennial Lifestyle--an Adaptation to Nutrient Limitation?" *Tree Physiology* (2010): 1047-1049.

Reynolds, HL, BA Hungate, FS Chapin et al."Soil Heterogeneity and Plant Competition in an Annual Grassland." *Ecology* (1997): 2076-2090.

Rousk, J, E Baath, PC Brookes et al. "Soil Bacterial and Fungal Communities across a PH Gradient in an Arable Soil." *The ISME Journal* (2010): 1340-1351.

Schimel, JP, JM Gulledge, JS Clein-Curley et al. "Moisture Effects on Microbial Activity and Community Structure in Decomposing Birch Litter in the Alaskan Taiga." *Soil Biology and Biochemistry* (1998): 831-838.

Schloss, PD et al. "Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*" (2009): 7537-7541.

Smith, SE and DJ Read. *Mycorrhizal Symbiosis* (1997). 2nd ed. San Diego, Calif. Academic

Steenwerth, KL, LE Jackson, FJ Calderón et al. "Soil Microbial Community Composition and Land Use History in Cultivated and Grassland Ecosystems of Coastal California." *Soil Biology and Biochemistry* (2002): 1599-1611.

Strecker, T, RL Barnard, PA Niklaus et al. "Effects of Plant Diversity, Functional Group Composition, and Fertilization on Soil Microbial Properties in Experimental Grassland." *PLoS ONE* (2015).

Strickland, MS, C Lauber, N Fierer et al. "Testing the Functional Significance of Microbial Community Composition." *Ecology* (2009): 441-451.

Tilman, D, CL Lehman and KT Thomson "Plant Diversity and Ecosystem Productivity: Theoretical Considerations." Proceedings of the *National Academy of Sciences* (1997): 1857-1861.

Tilman, D, PB Reich and JMH Knops. "Biodiversity And Ecosystem Stability In A Decade-long Grassland Experiment." *Nature* (2006): 629-632.

Torsvik, V, FL Daae, RA Sandaa et al. "Review article: novel techniques for analyzing microbial diversity in natural and perturbed environments." *Journal of Biotechnolog*y (1998): 53-62.

Torsvik, V, R Sorheim, J Goksoyr "Total bacterial diversity in soil and sediment communities—a review." *Journal of Industrial. Microbiology* (1996): 170-178.

Townsend, CR, MR Scarsbrook and S Dolèdec. "The Intermediate Disturbance Hypothesis, Refugia, and Biodiversity in Streams." *Limnology and Oceanography* (1997): 938-949.

Truu, M, J Juhanson and J Truu. "Microbial Biomass, Activity and Community Composition in Constructed Wetlands." *Science of the Total Environment* (2009): 3958-3971.

Van der Heijden, MGA, R Bakker, J Verwaal et al. "Symbiotic bacteria as a determinant of plant community structure and plant produc- tivity in dune grassland." *FEMS Microbial Ecology* (2006): 178–187.

Van der Heijden, MGA, JN Klironomos, M Ursic et al. "Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity." *Nature* (1998): 69-72.

Van Der Heijden, MGA, RD Bardgett and NM Van Straalen. "The Unseen Majority: Soil Microbes as Drivers of Plant Diversity and Productivity in Terrestrial Ecosystems." *Ecology Letters Ecology Letters* (2008): 296-310.

Wagg, C, SF Bender, F Widmer et al. "Soil Biodiversity and Soil Community Composition Determine Ecosystem Multifunctionality." *Proceedings of the National Academy of Sciences* (2014): 5266-5270.

Waldrop, MP, TC Balser and MK Firestone. "Linking Microbial Community Composition to Function in a Tropical Soil." *Soil Biology and Biochemistry* (2000): 1837-1846.

Ward, NL, JF Challacombe and PH Janssen "Three Genomes from the Phylum Acidobacteria Provide Insight into the Lifestyles of These Microorganisms in Soils." *Applied and Environmental Microbiology* (2009): 2046-2056.

Wayne LG et al. "Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics." *International Journal of Systematic Bacteriology* (1987): 463–464.

Westergaard, K, AK Müller, S Christensen et al. "Effects of Tylosin as a Disturbance on the Soil Microbial Community." *Soil Biology and Biochemistry* (2001): 2061-2071.

Whitman, WB, DC Coleman and WJ Wiebe "Prokaryotes: The Unseen Majority." *Proceedings of the National Academy of Sciences* (1998): 6578-6583.

Worm, B, EB Barbier, N Beaumont et al. "Impacts Of Biodiversity Loss On Ocean Ecosystem Services. (2006)" *Science*: 787-790.

You, Y, J Wang, X Huang et al. "Relating Microbial Community Structure to Functioning in Forest Soil Organic Carbon Transformation and Turnover."*Ecology and Evolution* (2014): 633-647.

Zhang, X, Q Chen, X Han et al. "Soil Bacterial Communities Respond to Mowing and Nutrient Addition in a Steppe Ecosystem." (2013) *PLoS ONE*: E84210.

Zhou, Z, OJ Sun, J Huang et al. "Land Use Affects the Relationship between Species Diversity and Productivity at the Local Scale in a Semi-arid Steppe Ecosystem." *Functional Ecology* (2006): 753-762.

TABLES

Table 1. Illumina PCR primers. The (x) represents the barcoded portion of the primer that is unique to each sample.

Forward 515f	AATGATACGGCGACCACCGAGATCTACAC TATGGTAATT GT GTGCCAGCMGCCGCGGTAA
Reverse 806r	CAAGCAGAAGACGGCATACGAGAT XXXXXXXXXXXX AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT

Table 2. Summary of ANOVA results showing the contribution of mowing, fertilization, proximity to ditch, and the interaction between mowing and fertilization on plant species richness and Simpson's Diversity D value. Effects were considered significant if $P < 0.05$.

Response Variable	Source	DF	MS	*F*	*P*
Species Richness					
	M	1	994.6	119.242	<0.001
	F	1	98.0	11.750	<0.001
	M×F	1	6.5	0.781	0.3793
	Proximity	1	102.1	12.240	<0.001
	Error	91	759.0	8.3	
Simpson's D Value					
	M	1	31.23	11.778	<0.001
	F	1	17.94	6.766	0.011
	M×F	1	2.99	1.129	0.291
	Proximity	1	19.95	7.524	0.007
	Error	91	241.29	2.651	

Table 3. Summary of Permutational MANOVA (PERMANOVA) results showing the effects of fertilization, mowing, proximity to ditch and the interaction of fertilization and mowing on plant community composition. Effects were considered significant if $P < 0.05$.

Source	DF	SS	MS	*F* Model	R^2	*P* (>*F*)
Fertilizer	1	1.3232	1.3232	7.552	0.05093	<0.001
Mowing	1	6.2997	6.2997	35.955	0.24246	<0.001
Proximity	1	1.8607	1.8607	10.620	0.07162	<0.001
F × M	1	0.5543	0.5543	3.164	0.02133	0.006
Residuals	91	15.9444	0.1752		0.61366	
Total	95	25.9823			1.00000	

Table 4. Mean soil properties (± standard deviation) per treatment and *P* values from ANOVA. Effects were considered to be significant if $P < 0.05$. Mowing and fertilization were treated as fixed factors and block as a random factor. M = mowed, UM = unmowed, F= fertilized, UF=unfertilized

Property	M	UM	F	UF	*P* Mowing	*P* Fertilizer	*P* M×F	*P* Block
pH	5.52 ± 0.02	5.44 ± 0.21	5.41 ± 0.16	5.55 ± 0.05	0.28	0.04	0.56	0.04
Ammonium (μg N kg^{-1})	0.98 ± 0.04	0.94 ± 0.11	0.98 ± 0.04	0.94 ± 0.11	0.75	0.76	0.49	0.32
Nitrate (μg N kg^{-1})	0.26 ± 0.01	0.23 ± 0.03	0.23 ± 0.03	0.26 ± 0.005	0.28	0.21	0.43	0.05
Carbon (g C kg^{-1})	1.99 ± 0.16	1.88 ± 0.29	2.10 ± 0.02	1.78 ± 0.14	0.37	0.03	0.49	0.78
Nitrogen (g N kg^{-1})	0.180 ± 0.003	0.17 ± 0.02	0.180 ± 0.004	0.17 ± 0.01	0.69	0.21	0.34	0.79
Carbon/Nitrogen (wt:wt)	11.2 ± 0.74	10.77 ± 0.56	11.5 ± 0.41	10.5 ± 0.22	0.27	0.04	0.74	0.03
Moisture (%)	27.5 ± 1.58	25.57 ± 0.70	27.3 ± 1.81	25.73 ± 0.93	0.31	0.39	0.73	0.11
Temperature (°C)	24.6 ± 1.05	22.57 ± 0.18	23.2 ± 1.02	24.03 ± 1.89	<0.001	<0.001	0.475	0.35

Table 5. Summary of ANOVA results showing the contribution of mowing, fertilization, proximity to ditch, and the interaction between mowing and fertilization on microbial species richness and Simpson's Diversity D value. Effects were considered significant if $P < 0.05$.

Response Variable	Source	DF	MS	*F*	*P*
Species Richness					
	M	1	552.8	1.594	0.2176
	F	1	1937.5	5.585	0.0256
	M×F	1	148.8	0.429	0.5181
	Proximity	1	30.0	0.087	0.7708
	Error	27	9366	346.9	
Simpson's D Value					
	M	1	3.766	5.775	0.0234
	F	1	2.170	3.329	0.0792
	M×F	1	0.292	0.448	0.5090
	Proximity	1	0.055	0.084	0.7744
	Error	27	17.606	0.652	

Table 6. Summary of Permutational MANOVA (PERMANOVA) results showing the effects of mowing, fertilization, proximity to the ditch, and the combination of mowing and fertilization on relative abundance microbial community composition. Effects were considered significant if $P < 0.05$.

Source	DF	SS	MS	*F* Model	R^2	*P* (>*F*)
Fertilizer	1	0.0522	0.0522	2.5771	0.07432	0.045
Mowing	1	0.0301	0.0301	1.4832	0.04283	0.199
Proximity	1	0.0569	0.0569	2.8084	0.08099	0.039
F × M	1	0.0163	0.0163	0.8045	0.02320	0.505
Residuals	27	0.5476	0.0203		0.77865	
Total	31	0.70321			1.00000	

Table 7. Summary of Permutational MANOVA (PERMANOVA) results showing the effects of fertilization, mowing, proximity to ditch and the interaction of fertilization and mowing on unclassified microbial community composition. Effects were considered significant if $P < 0.05$.

Source	DF	SS	MS	*F* Model	R^2	*P* (>*F*)
Fertilizer	1	0.4122	0.4122	160.10	0.3234	<0.001
Mowing	1	0.3987	0.3987	154.87	0.3128	<0.001
Proximity	1	0.0137	0.0137	5.323	0.0108	0.0279
F × M	1	0.3805	0.3805	147.80	0.2985	<0.001
Residuals	27	0.0695	0.0025		0.055	
Total	31	1.2749			1.00000	

Table 8. Summary of Permutational MANOVA (PERMANOVA) results showing the effects of mowing, fertilization, proximity to ditch, and the combination of mowing and fertilization on microbial community composition using only presence and absence data. Effects were considered significant if $P < 0.05$.

Source	DF	SS	MS	*F* Model	R^2	*P* (>*F*)
Fertilizer	1	0.0432	0.0432	2.0917	0.06027	0.004
Mowing	1	0.040	0.040	1.9528	0.05627	0.016
Proximity	1	0.0483	0.0483	2.3363	0.06732	0.003
F × M	1	0.0273	0.273	1.3227	0.03811	0.126
Residuals	27	0.5581	0.0207		0.77802	
Total	31	0.7173			1.00000	

Table 9. The ten most abundant bacteria phyla in each experimental treatment. Each treatment contains the same phyla but in a different order of abundance.

Control	# of Sequences	Fertilized	# of Sequences	Mowed	# of Sequences	Mowed× Fertilized	# of Sequences
Proteobacteria	15926	Proteobacteria	14036	Proteobacteria	15400	Proteobacteria	14418
Planctomycetes	5244	Planctomycetes	6646	Planctomycetes	5920	Planctomycetes	6083
Acidobacteria	4992	Acidobacteria	5360	Acidobacteria	4333	Acidobacteria	3915
Chloroflexi	2322	Actinobacteria	2095	Chloroflexi	2132	Chloroflexi	2091
Actinobacteria	2246	Chloroflexi	2022	Actinobacteria	2070	Actinobacteria	1987
Verrucomicrobia	2082	Verrucomicrobia	1731	Firmicutes	2015	Verrucomicrobia	1826
Firmicutes	2017	Chlamydia	1690	Verrucomicrobia	1871	Chlamydia	1818
Chlamydia	1856	Firmicutes	1607	Chlamydia	1846	Firmicutes	1737
Bacteroidetes	1567	Bacteroidetes	895	Bacteroidetes	1473	Bacteroidetes	1206
Armatimonadetes	490	Armatimonadetes	523	Armatimonadetes	595	Armatimonadetes	586

Table 10. The nine most abundant microbial OTUs per treatment. Gp1, Gp2, Gp3, Gp6 andGp13 refer to major Actinobacteria groups. The nine most abundant were chosen because they were the most abundant across the treatments, but they were different in their order of abundance for each treatment

Control	# of Sequences	Fertilized	# of Sequences	Mowed	# of Sequences	Mowed + Fertilized	# of Sequences
Gp1	1729	Gp1	1575	Gp1	1280	Gp1	1411
Gp2	1354	Gp2	1185	Gp2	993	*Incertae_sedis*	1071
Gp3	970	*Incertae_sedis*	1098	*Incertae_sedis*	789	*Aquicella*	994
Incertae_sedis	824	*Aquicella*	957	*Aquicella*	788	Gp2	847
Gemmata	708	Gp3	898	Gp3	737	Gp3	826
Aquicella	620	*Legionella*	692	*Legionella*	590	*Legionella*	600
Ktedonobacter	523	*Gemmata*	549	*Gemmata*	568	*Gemmata*	596
Spartobacteria	504	*Ktedonobacter*	505	*Ktedonobacter*	508	*Ktedonobacter*	515
Legionella	419	Spartobacteria	428	Spartobacteria	408	Spartobacteria	456
Gp 6	293	Gp 13	301	*Chthonomonas* Armatimonadete	285	*Chthonomonas* Armatimonadete	270

Table 11. Summary of results from Mantel test used to correlate dissimilarity matrices. Correlations between microbe and plant communities, microbe communities and soil characteristics, and plant communities and soil characteristics were based on Pearson's product-moment correlations using the Mantel test. Community matrices were based on Bray-Curtis dissimilarity and soil characteristics matrix was based on Euclidean distances.

Comparison	Pearson's correlation	
	r statistic	*P*
Microbe - Plant	0.06301	0.18
Microbe - Soil	0.05121	0.273
Plant - Soil	0.1108	0.046

FIGURES

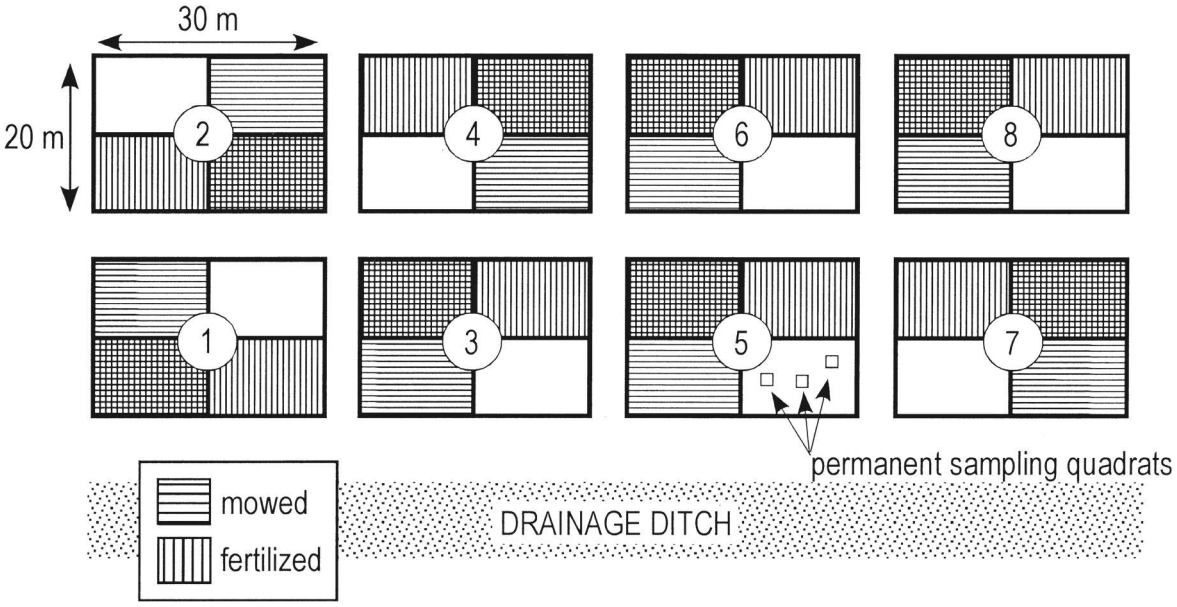

Figure 1: Experimental Design of the Long-Term Experiment. Experimental treatments are replicated on eight blocks measuring 20m × 30m. The blocks include a 2 × 2 factorial design with mowing and fertilization as the two factors. Figure adapted from Goodwillie and Franch, 2006.

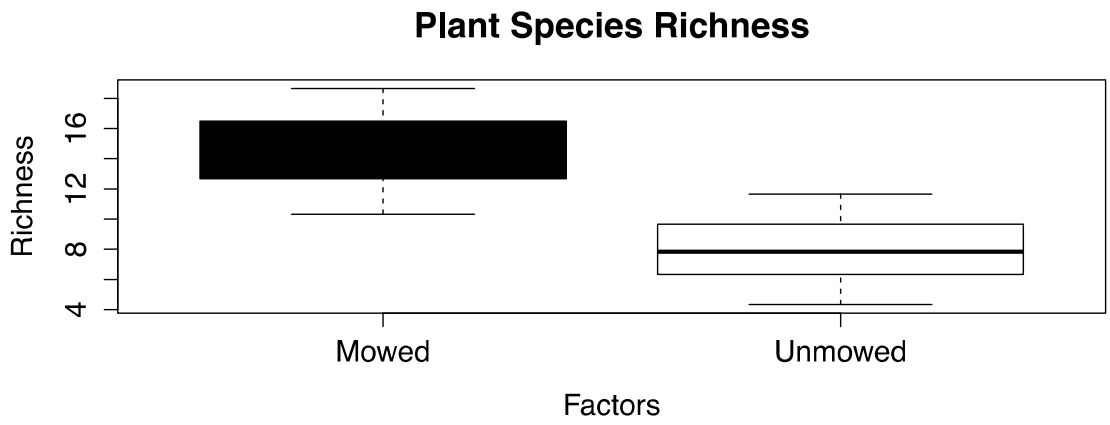

Figure 2. Box and whisker plot of the effects of mowing on plant species richness. The box and whisker plot divides the data into quarters with the two lines representing the two extremes of the data. The filled bar indicates that the mowing treatment was applied to these sequences. The open box did not experience the fertilization treatment. The dark line in the middle represents the mean value. Plant species richness was significant effected by mowing $P < 0.05$.

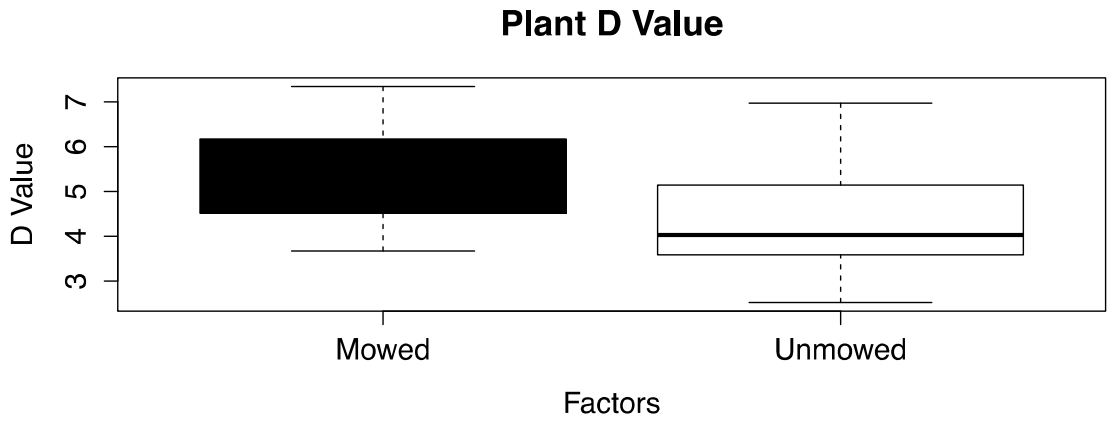

Figure 3. Box and whisker plot of the effects of mowing on plant D value. The box and whisker plot divides the data into quarters with the two lines representing the two extremes of the data. The filled bar indicates that the mowing treatment was applied to these sequences. The open box did not experience the fertilization treatment. The dark line indicates the mean value. Mowing had a significant effect on plant D value $P < 0.05$.

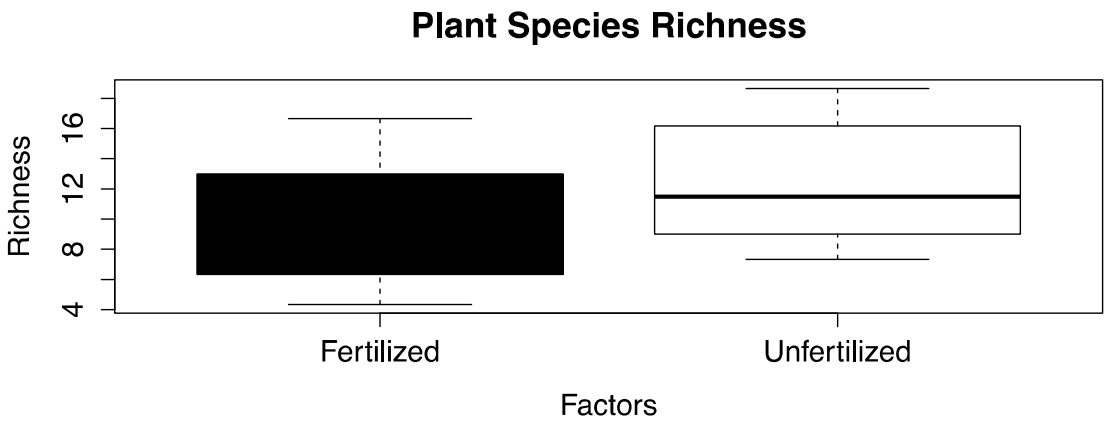

Figure 4. Box and whisker plot of the effects of fertilization on microbial species richness. The box and whisker plot divides the data into quarters with the two lines representing the two extremes of the data. The filled bar indicates that the fertilization treatment was applied to these sequences. The open box did not experience the fertilization treatment. The dark line indicates the mean value. Fertilization had a significant effect on plant species richness $P < 0.05$.

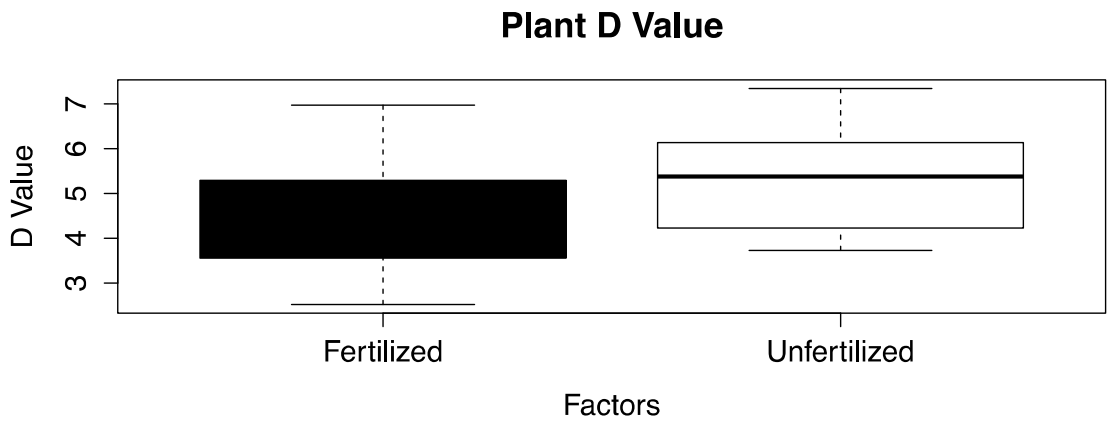

Figure 5. Box and whisker plot of the effects of fertilization on plant D value. The box and whisker plot divides the data into quarters with the two lines representing the two extremes of the data. The filled bar indicates that the fertilization treatment was applied to these sequences. The open box did not experience the fertilization treatment. The dark line indicates the mean value. Fertilization had a significant effect on plant D value $P < 0.05$.

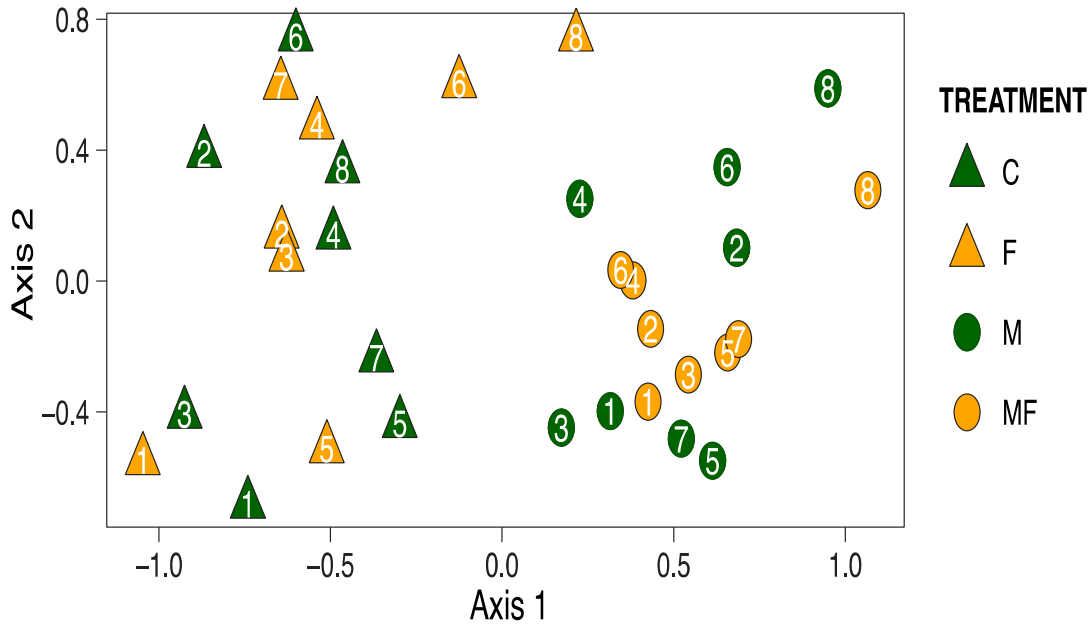

Figure 6. Nonmetric Multidimensional Scaling plot of plant communities showing the community composition of different treatment plots. A circle indicates mowed and triangle indicates unmowed treatments. Green indicates fertilized and yellow indicates unfertilized treatments.

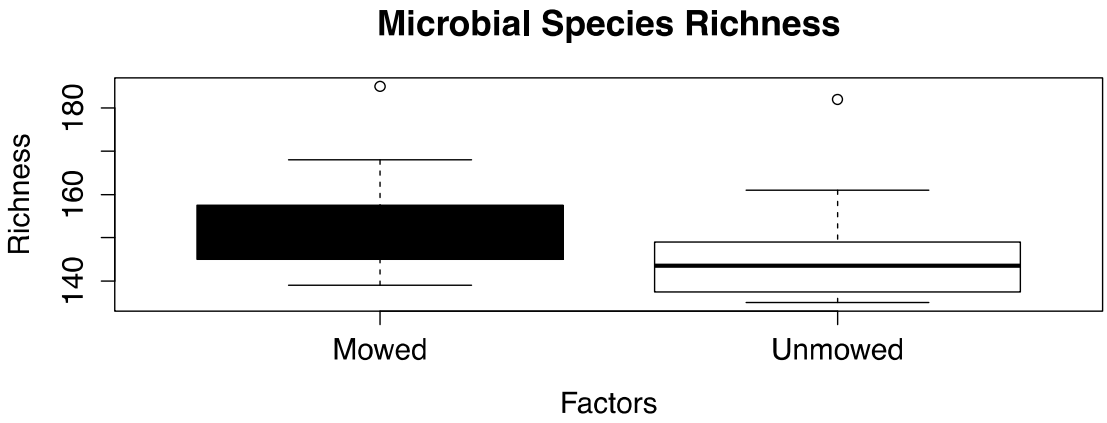

Figure 7. Box and whisker plot of the effects of mowing on microbial species richness. The box and whisker plot divides the data into quarters with the two lines representing the two extremes of the data. The filled bar indicates that the fertilization treatment was applied to these sequences. The open box did not experience the fertilization treatment. The dark line represents the mean value.

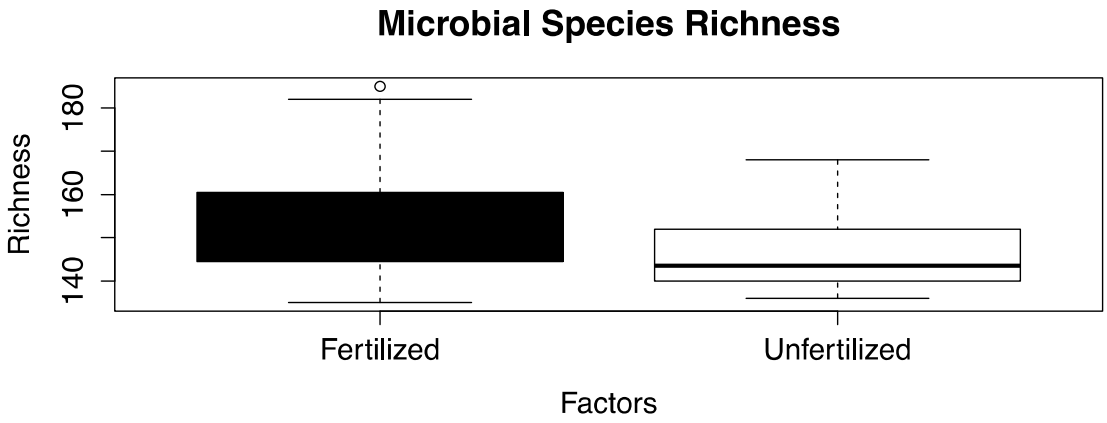

Figure 8. Box and whisker plot of the effects of fertilization on microbial species richness. Box and whisker divide the data into quarters. The filled bar indicates that the fertilization treatment was applied to these sequences. The open box did not experience the fertilization treatment. The dark line indicates the man value Fertilization had a significant effect on microbial species richness $P < 0.05$.

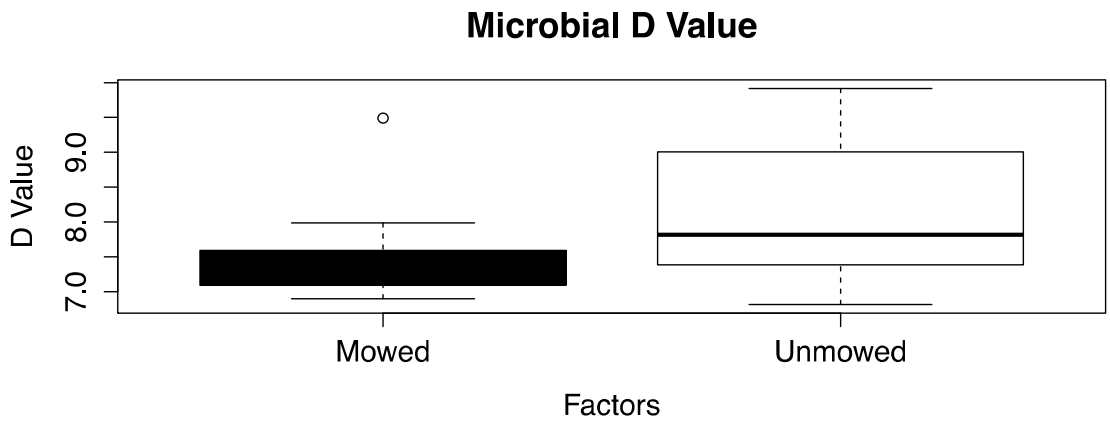

Figure 9. Box and whisker plot of the effects of mowing on microbial D value. The box and whisker plot divides the data into quarters with the two lines representing the two extremes of the data. The filled bar indicates that the mowing treatment was applied to these sequences. The open box did not experience the treatment. The dark line indicates the mean value. Mowing had a significant effect on microbial D value $P < 0.05$.

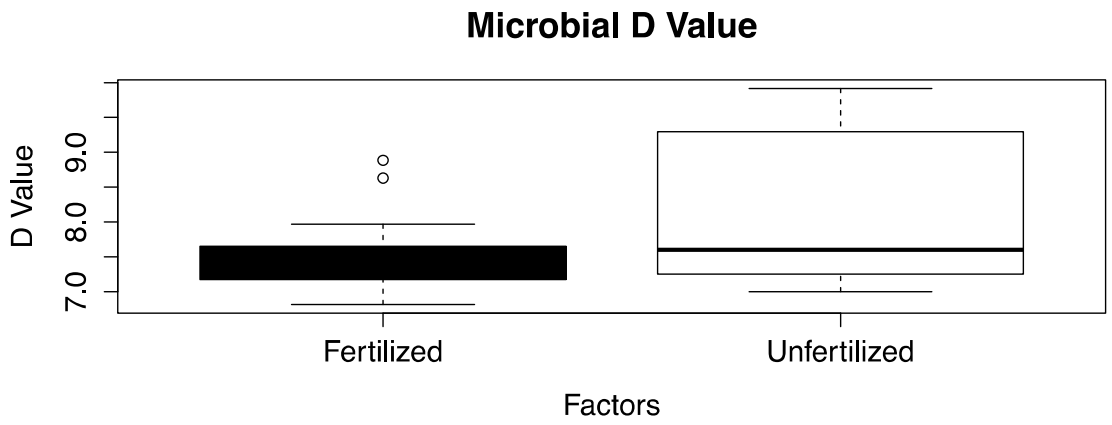

Figure 10. Box and whisker plot of the effects of fertilizer on microbial D value. The box and whisker plot divides the data into quarters with the two lines representing the two extremes of the data. The filled bar indicates that the fertilization treatment was applied to these sequences. The dark line indicates the mean value. The open box did not experience the treatment.

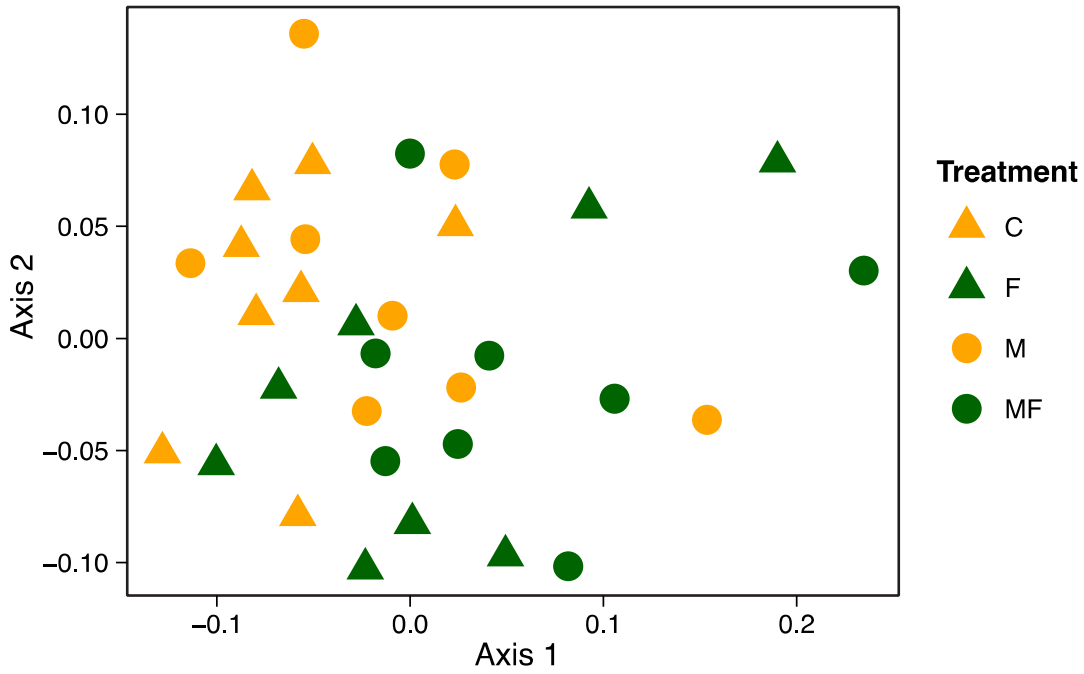

Figure 11. Nonmetric Multidimensional Scaling plot of microbial communities showing the community composition of different treatment plots. A circle indicates mowed and triangle indicates unmowed treatments. Green indicates fertilized and yellow indicates unfertilized treatments.

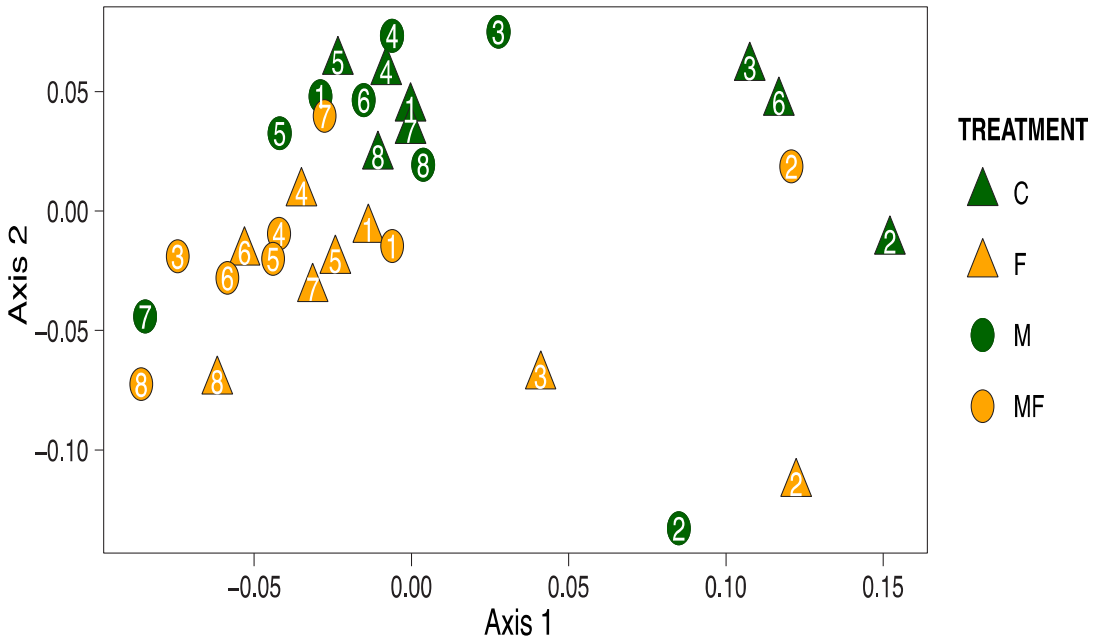

Figure 12. Nonmetric Multidimensional Scaling plot of bacterial community composition (unclassified) showing the community composition of different treatment plots. A circle indicates mowed and triangle indicates unmowed treatments. Green indicates fertilized and yellow indicates unfertilized treatments.

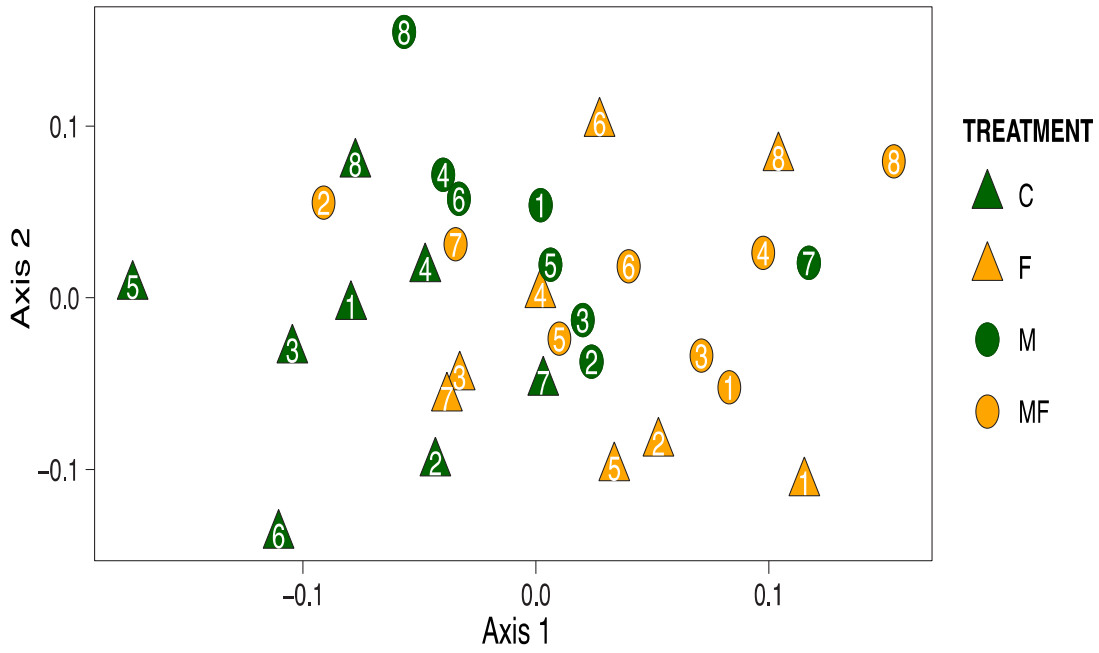

Figure 13. Nonmetric Multidimensional Scaling plot of bacterial community composition (presence/absence) showing the community composition of different treatment plots. A circle indicates mowed and triangle indicates unmowed treatments. Green indicates fertilized and yellow indicates unfertilized treatments.