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EVALUATING THE IMPACT OF NITROGEN FERTILIZATION TREATMENTS AND IRRIGATION ON SOIL HEALTH INDICATORS IN A LONG-TERM CROP ROTATION RESEARCH PLOT

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Abstract

Many agriculturalists have been focusing on the most efficient farming method that would produce the maximum yield while still sustaining the soil ecosystem. Soil samples were collected from the "Old Rotation" area (Auburn University, Auburn, AL), and were assessed for soil biochemical, chemical and biological characteristics related to soil quality. Treatments of the experimental site were a control with no legumes or N fertilizer; cotton every year with winter legumes; a 3-yr cotton-corn-soybean rotation with wheat and winter legumes; and cotton every year with N fertilizer. Impacts of irrigation were also tested between the sites. Assays were performed measuring phosphomonoesterase and phosphodiesterase activity, soil organic carbon, soil pH, and microbial diversity. The 3-year and winter legume rotations showed significant differences in the structure and membership of microbial communities and differences in biochemical activity. These results further demonstrate the ability of crop rotation to enhance the soil health of agricultural ecosystems.

Keywords: Nitrogen Fertilization, Irrigation, Crop Rotation, Soil Ecology, Enzymatic Activity

Introduction

Irrigation has long been seen as a needed but elusive agricultural practice for small farmers to achieve consistency and quality of production. As a result, of its high start-up costs, and the relative underuse in Alabama, farmers have tended to not utilize the method in best management strategies (Shange et al., 2014). As programs and educational efforts leveraged for local producers, there is a need for more research into the greater agroecological impacts of irrigation combined with other management practices. Cultivation practices and nitrogen fertilization have major impacts on soil biology, especially organic storage and metabolism (Doran and Parkin, 1994). These practices have considerable impact on the nutrient cycling processes in the soil which are mediated by soil microorganisms through an array of enzymes (Bandick and Dick, 1999). There is no standard method of determining the soil's ability to perform these functions and to inform decision makers and practitioners to take meaningful action either in the short- or long-term. Given these constraints, significant effort has been focused in the past decade on developing indices for soil health.

Microbial communities are also an important component when looking at soil health and functioning, since they control the potential for enzyme activities (Kandeler et al., 1996). The nutrient cycle in the soil involves biochemical processes that are mediated by microorganisms, plant root and soil animals (Tabatabai, 1994). These biochemical processes are regulated in large part by extracellular enzyme pools that are created by microbiota (bacteria and fungi). Bacteria

acts as a buffer in the soil ecosystem due to their key role in soil processes including nutrient cycling (Acosta-Martinez, 2008). Past studies have established soil bacterial communities as a potential indicator of soil health because of their short life span and its rapid response to a change in the soil environment (Shange et al., 2012; Acosta-Martinez et al., 2008; Shange et al., 2013).

Current methods for evaluating microbial communities, and advances in soil enzymology has opened opportunities of bridging the gap in the ongoing study of soil health and connecting these measures to carbon sequestration and soil management issues. The recent application of molecular methods of measuring soil microbial populations and diversity in the soil is providing opportunities to relate soil enzyme, organic matter and microbial diversity to soil practices (Shange et al., 2012). Long-term field experiments could provide a valuable opportunity to assess different amendments and their effects on soil quality and biochemical dynamics. Therefore, the objectives of this study are to (1) determine the effect of irrigation on soil carbon, microbial, and biochemical dynamics in long-term plots, (2) determine if type of nitrogen fertilization and rotation in long-term cotton plots affect soil phosphate enzyme activities, and (3) assess changes in microbial community composition with respect to the established treatments.

Literature Review

Soil Health and Quality

As a result of the increases in agronomic practices, soil degradation has become a growing issue worldwide. In qualifying the loss of soil health, many have tried to define what soil health and quality exactly are. However, this has generated great concern because there has not been a clear concept regarding soil health and no reliable way of evaluating it. Soil health has been characterized as ecological balance within the soil ecosystem and has been equivocated with soil quality. Doran and Parkin (1994, p.26) defined soil quality as the ability of the soil to function within an ecosystem's boundaries to maintain biological productivity, retain environmental quality, and support plant and animal health. Some indicators have been proposed to evaluate the processes that take place within the soil. For instance, Doran and Parkin (1996) mentioned physical, chemical, and biological properties. He argued that for physical aspects of the soil, texture, root depth, bulk density, and water retention capacity should be evaluated; for the chemical aspects, pH, total C, and nutrient levels should be evaluated, and for biological aspects, C and N microbial biomass, potentially mineralizable N, and respiration should be assessed.

Pascual et al. (1999; 2000) found chemical and physical properties to be slow in response to a change in the soil environment, and require a significant amount of time to assess. Contrary to this, Bandick and Dick (1999) found that biological and biochemical properties provide an accurate and rapid response to a change in soil quality. Soil enzymes are direct mediators for biological catabolism of soil organic and mineral components (Kumar, 2011). Soil enzymes have been known to be a good marker of biological soil fertility since they are involved in microbial cycling of nitrogen, carbon, phosphorus, and sulfur (Pascual et al., 1999). These soil enzymes include dehydrogenase, glucosidases, urease, amidases, phosphatases, arylsulphatase, cellulase, and phenol oxidases.

Abdalla and Langer (2007) conducted a study on soil enzyme activities in irrigated and rain-fed vertisols of the semi-arid tropics of Sudan. In this study, the researchers assessed soil enzyme activity and the affect that short-, medium-, and long-term cultivation has had on the Sudanese

soils. Three enzyme assays were conducted; alkaline phosphatase, protease, and β-glucosidase. In evaluating the three selected enzymes, they were able to observe the influence that the duration of cultivation, crop sequence, water regimes, and soil-climatic conditions had on the carbon (C), nitrogen (N) and phosphorus (P) cycles. Among the three selected enzymes alkaline phosphatase and protease proved to be very sensitive to soil management, whereas β-glucosidase revealed no clear response.

Bending et al. (2004) performed a study on microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. The microbial community metabolism and metabolic diversity were measured by examining the activity of eight key soil enzymes that are involved in the C, N, P, and sulfur (S) cycles. The study showed that the enzymes assayed varied in their sensitivity to management practices, demonstrating that microbial parameters could be more effective in evaluating different soil management practices and the effect that they have on soil health compared to biochemical factors.

A recent study by Acosta-Martinez et al. (2014) examined the impact of the 2011 drought in the southeastern US on the soil health of cotton monocropping and rotational systems. The investigators used eight soil enzyme activities (acid phosphatase, alkaline phosphatase, phosphodiesterase, arylsulfatase, aspartase, urease, L-asparaginase, α-galactosidase, ß-glucosidase and ß–glucosaminidase), and soil organic matter (SOM) as their primary means of measuring soil health. As these enzyme activities were measured across two years (2012 being less extreme drought conditions), all the enzymes except for arylsulfatase and asparaginase showed increased activity in 2011 as opposed to 2012. All enzymes showed increased activity under rotational management as opposed to the monoculture.

In contrast, there have also been studies that have seen both no response and a negative response to enzyme activity in soils to water stress/drought conditions. In 2005, Sardans and Peñuelas published a study that controlled an evergreen oak mountain stand site for runoff and precipitation to simulate drought conditions. In assessing the impact on five soil enzymes (urease, protease, acid phosphatase, alkaline phosphatase, and ß-glucosidase), the investigators found all but one (alkaline phosphatase) were decreased under drought conditions. In another study conducted by Sardans et al. (2006), investigators were interested in the combined impact of warming and drought on soil properties in a Mediterranean shrubland. In an experiment set up in 1999 to simulate night warming, and daily drought, the investigators monitored and analyzed data collected regarding phosphatases (acid and alkaline) and soil chemical characteristics related to phosphorus. Warming treatments had seasonal effects on both acid and alkaline phosphatases (additionally available phosphorus), though drought treatments showed no significance.

Microbial Communities

A variety of different microbial classification methods have been used in past decades, but none of which have the ability to resolve phylogenetic differences in community composition (Dowd et al., 2008a; Dowd et al., 2008b. A more recent method has been derived that grants the opportunity to examine bacteria on various taxonomic specific levels using the 16S rRNA, a technique of next generation sequencing. Next Generation Sequencing is a collection of more recent techniques in sequencing technology that improves the volume, veracity, and velocity of

output. These methods are based on the conserved and hypervariable regions found in the 16S rRNA gene that is ubiquitous to all bacteria. Also, this gene is large enough for informatics and computational purposes (Patel, 2001).

Soil microbial communities are very complex and have an immense impact on soil functioning. To understand them is taking an important step in maintaining sustainable agroecosystems. Many studies have been conducted to evaluate the potential effect that various agricultural practices have on microbial communities. In a study conducted by Shange et al. (2012), microbial communities were assessed from soil under grazed pasture, pine plantation, and cultivated soils under one soil type. When the communities were assessed for structure and membership, each of the systems showed unique characteristics in alpha and beta diversity indicators as well as the major taxonomic groups identified.

Similarly, Jangid et al. (2008) studied microbial communities in three different management types with greater than ten years of practice (conventionally tilled, hay pasture, grazed pasture) under two different fertilizer regimes (inorganic, and poultry litter). Investigators used sequencing and library construction of 16S rRNA genes to compare soil communities under the management practices, which demonstrated that bacterial diversity was always higher in poultry litter amended soils than in inorganic fertilizer amended soils. These changes were seen without respect to season or land management. Also, changes in bacterial membership were seen to be a result of fertilizer amendment rather than a change in land management or season.

Materials and Methods

Study Site

The study site was located in Auburn, AL, called "Old Rotation." The Old Rotation was originally started by Professor J.K. Duggar in 1896 to demonstrate the long-term effects of fertilization and the lack of specific nutrients on non-irrigated crop yield over a100-year period. The Old Rotation is one of the few sites where controlled nutrient deficiencies can be observed on five different crops (cotton, crimson clover, corn, wheat, and soybean) during the year. This experiment preserves a site for monitoring nutrients acclamation and loss and soil quality change, and their effects on the long-term sustainability of an intensive crop rotation system. The site consisted of 13 plots on a one-acre land; the eastern half of the plots were irrigated separately using a system of eight, 8-foot risers in each plot. The timing and the rate of irrigation were based on the weather, crop, and its growth stage. In addition, plots sampled for the study were (control [CON], cotton every year with no soil amendments; winter legume [WL], cotton every year with winter legumes; 3-Year rotation [3YR], 3-year cotton-corn-soybean rotation with winter wheat and winter legumes; and cotton every year with 120 lb. of N-fertilizer per acre per year [Nfert]).

Soil Sampling and Preparation

Samples were collected in October 2012 (after harvest and removal of most biomass) from a depth of 0 to 5cm using a soil auger. Samples collected were put on ice and transported to the Environmental Quality Laboratory at Tuskegee University and stored at 4° C before analysis.

Soil pH and Organic Carbon Determination

Soil pH was determined by the method elucidated by McLean (1982), with a 1:2 soil to water ratio. Soil organic carbon was determined using oxidation method (Walkley and Black, 1934; Walkley, 1947). The results were calculated according to the formula below, using a factor of f = 1.30:

$$\text{Organic C\%} = (\text{meq } K_2Cr_2O_7 \text{ meq } FeSO_4)(0.003)(100) \times f/\text{g water free soil} \quad (1)$$

Enzyme Activity

The method for phosphomonoesterase activity (Acid and Alkaline) was utilized in accordance with the assays elucidated by Tabatabai (1994). To account for non-enzymatic hydrolysis, values for controls were subtracted from sample readings. Toluene was not used as it has been shown that with incubation periods fewer than two hours, the absence of toluene was inconsequential to measured enzyme activity. All enzyme activities reported are expressed on a moisture-free basis.

Bacterial Community Analysis

DNA extraction process was performed on each sample. Two (2) µL of the DNA was quantified using the Nanodrop ND-2000c spectrophotometer. The DNA samples were then sent to a Research and Testing Laboratory (Shallowater, TX) for PCR optimization and tag-encoded FLX amplicon sequencing. The 16S rRNA gene V4 variable region PCR primers 515/806 (Caporaso et al., 2011) were used in a single-step 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on an Ion Torrent PGM following the manufacturer's guidelines. Sequence data were processed using a proprietary analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were depleted of barcodes and primers, then sequences <150bp removed; sequences with ambiguous base calls and with homopolymer runs exceeding 6bp were also removed. Sequences were denoised, Operational taxonomic units (OTUs) were generated and chimeras removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis et al., 2006).

Bioinformatic and Statistical Analysis

The Q25 sequence data derived from the sequencing process was processed using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX) (Dowd et al., 2008a; Dowd et al., 2008b; Edgar 2010; Capone et al., 2011; Dowd et al., 2011; Eren et al., 2011; Swanson et al., 2011; DeSantis et al., 2006) In addition to relative abundance files, an OTU file was provided as well with all the listed OTUs observed. This file was formatted and imported into Estimates software package (Colwell, 2013) to calculate alpha diversity estimates. The generalized linear model (GLM) was used to assess the means of soil physical, chemical, and microbial properties among the systems followed by a Tukey's HSD test for pairwise comparisons. Relative abundance data is presented as percentages/proportions, but prior to subjection to GLM, they were transformed using the arcsine function for normal distribution.

Results and Discussion

Enzyme Activity

Figure 1 shows the influence of irrigation and rotational management on Acid Phosphatase (ACD), Alkaline Phosphatase (ALK), and Phosphodiesterase (PHD). With respect to ACD, a significant interaction was detected ($p = 0.039$) along with treatment differences being detected in the irrigated and non-irrigated plots. The interaction was the result of CON responding negatively to the absence of irrigation, while ACD activity in all of the other plots tended to increase. Though there was the appearance of higher activity in the no irrigation (NIRR) plots, overall there was no significance detected. Within the irrigation (IRR) plots, CON soils distinguished themselves as the ones with the lowest observable ACD activity, while in the NIRR plots CON again had the lowest ($p < 0.01$) ACD activity. WL plots had higher values than the CON in the NIRR plots ($p < 0.05$). The results indicated that the CON plots consistently had smaller values than the other plots and no other differences were observed.

With respect to ALK, a significant interaction was detected ($p = 0.02$) along with treatment differences being detected in the IRR and NIRR plots. The interaction was the result of CON and WL plots performing similarly in the IRR plots, but the WL plots had higher values than the control in the NIRR plots. The results indicated that the CON plots consistently had smaller values than the other plots and no other differences were observed.

When considering PHD activity, both factors and their interactions were significant. Treatment differences were observed in the NIRR plots, but no differences were detected in the IRR plots resulting in the significant interaction. Higher values were observed in the NIRR plots ($p < 0.05$) which also showed more variation in ACD activity. No significant differences were detected among the IRR plots, while within the NIRR plots, distinguishable values included the lowest activity detected in CON soils and the highest in WL. When irrigation regime is not considered, a similar trend was observed, though CON, WL, and Nfert were significantly different from one another ($p < 0.05$).

The soils under study showed comparable activity to earlier studies in acid-neutral soils. With the soils being slightly acidic, ACD were more active than ALK as Eivazi and Tabatabai (1977) reported the optimum pH for soils studied was 6.5 for soils with a pH of 6.4 or less and 11.0 for soils with a pH of 7.4 and above. With respect to the irrigation treatment, PHD was the only enzyme to significantly respond to water supply, while noticeable traces of response could be detected in the ACD activities. Though there have been mixed results in which agricultural soils show decreased (Sardans and Peñuelas, 2005; Sardans et al., 2008), no change (Roldán et al., 2005; Sardans et al. 2006), and increased (Huang et al., 2011; Acosta-Martinez et al., 2014) activity in water stressed soils. A study by Martinez et al. (2013) strongly supports the results here. In their one year study, they measured the activity of ACD, ALK, and PHD (among other enzymes) over the course of drought conditions in Texas in monocropped and rotational systems. The response was increased activity for PHD, ACD, and ALK in the study.

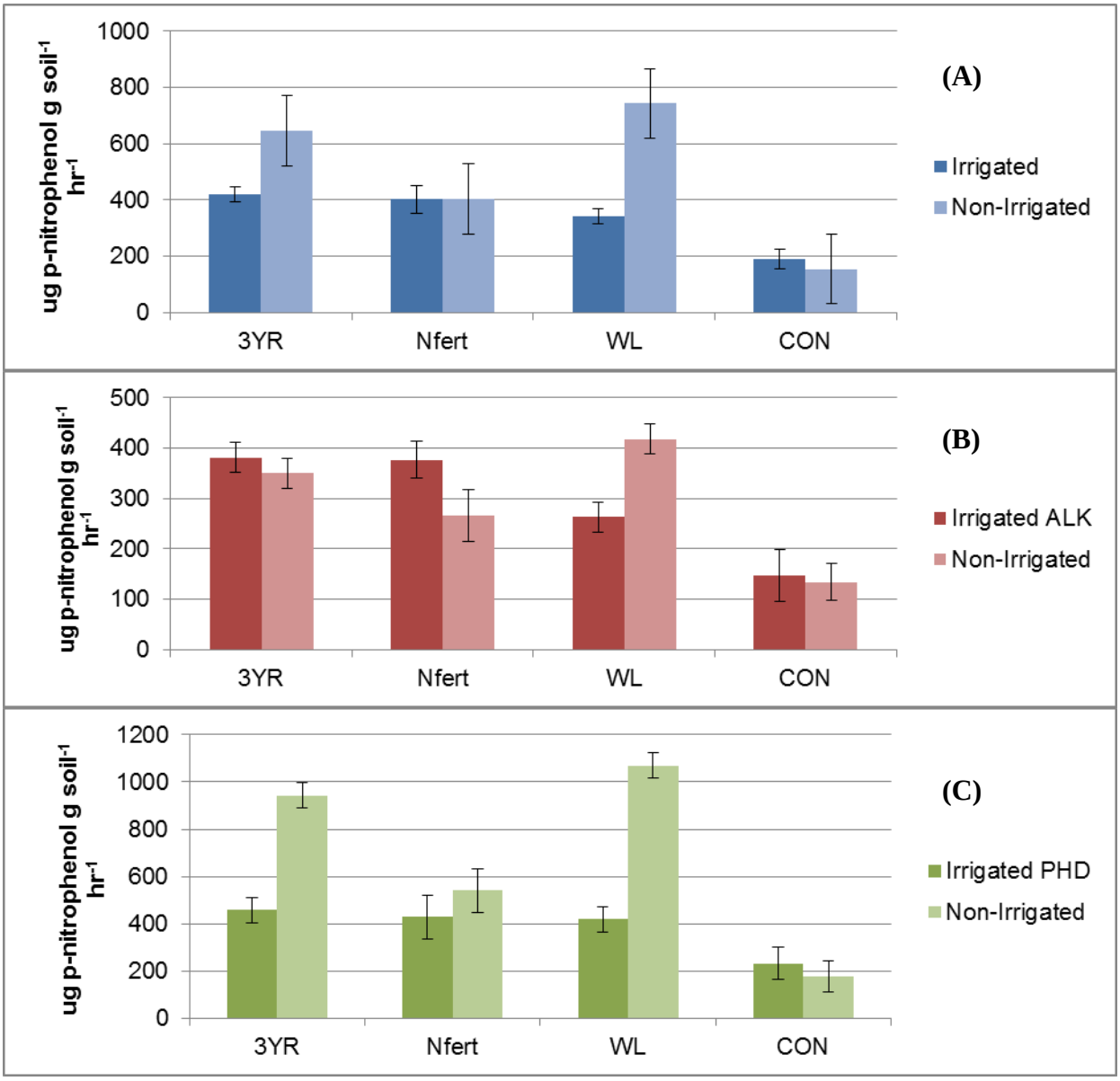

Figure 1. Graphs demonstrating the influence of irrigation and rotational management on ACD (a), ALK (b), and PHD (c) activity

This general trend of higher activity in the presence of no irrigation may be attributed to osmotic stress. Not only were the NI plots not irrigated during the study, but the year of 2012 was one of very limited precipitation, as the study site was contained within an Extreme Drought area for the entire growing season (NOAA, 2016). Osmotic stress in the soil environment has been shown to be responsible for multiple phenomena that could increase extracellular enzyme activity. There could be a release of extracellular enzymes from former Soil Organic Matter (SOM) and clay complexes that were broken up due to excessive drying (Acosta-Martinez et al., 2014). Another reason may be the proliferation of microbes that produce the enzymes (Burns, 1982). These organisms could also be responding to osmotic stress by producing and releasing more of

the enzyme in dry conditions as a mitigation mechanism (Harder and Dijkhuizen, 1983), as other biota have shown comparable strategies (Barrett-Laennard et al., 1982; Ehsanpour and Amini, 2003).

Management and rotational strategies have previously been shown to impact the enzymatic activities in soil communities (Acosta-Martínez et al., 2011; Shange et al., 2012). As stated above in the Introduction, this study's focus was to assess whether the impacts of management history would have an influence on both enzymatic activities and bacterial communities. The results demonstrated differences between cropping regimes with consistent observations of the CON treatment having the lowest enzymatic activity of all the enzymes observed. ACD and PHD demonstrated an increased activity for WL and 3YR plots when compared CON and Nfert. It is important to reiterate that both the WL and 3YR plots were under rotational management, which has been proven to protect soil health (Haynes, 1980), and have specifically been shown to increase enzymatic activity (Dick, 1984; Angers et al., 1992) and more specifically phosphatase activity (Ramos et al., 2010; Acosta-Martinez et al., 2014), regardless of soil type.

Although these soils only have about 1% organic matter content, the increased enzyme activities under WL and 3YR rotation supports the assertion that that an increase in C content under varied rotational management ($p < 0.001$, Table 1, page 12) could help protect the targeted enzymes from denaturation during drought (Acosta-Martinez et al., 2014). Normally, phosphatases more readily respond to changes in organic matter, pH, and disturbance; however, the results did not necessarily follow these trends. It is still possible to see the response of PHD and ACD to rotational strategies as more sensitive indicators of soil quality are ascertained.

Bacterial Communities

Major microbial phyla were observed for their response to the IRR and rotational treatments as well. The phyla *Actinobacteria* and *Proteobacteria* accounted for relative abundance values ranging from 66.7% to 78.8% of all the sequences present and are depicted in Figure 2. In both phyla, a significant difference was detected ($p < 0.05$) between the IRR and NI plots as *Actinobacteria* RA increased with NIRR plots while *Proteobacteria* increased in IRR plots. When all factors are considered, the CON and WL treatments differ with regards to both phyla as well. The dominant phyla were *Proteobacteria* and *Actinobacteria* while the phyla *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia* and *Gemmatimonadetes* showed much less prominence in these soils. All of these phyla have been shown to respond to agricultural management of soils (Jangid et al., 2009, Acosta-Martinez et al., 2008), though the latter showed no significance in the current study. In previous studies by the current authors, the phyla *Proteobacteria* and *Actinobacteria* showed significance in cultivated sites, because of their responsiveness to both disturbance and carbon, with a preference for copiotrophic environments (Shange et al., 2012; Shange et al., 2013).

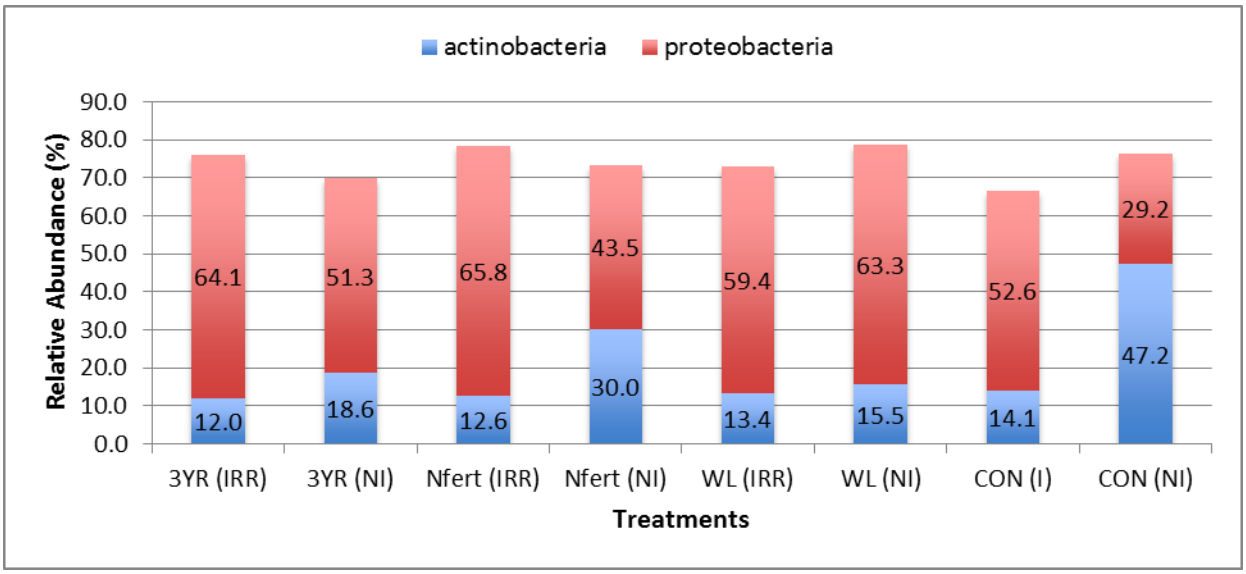

Figure 2. Stacked bar graphs depicting the relative abundance of the two most dominant phyla (*Actinobacteria* and *Proteobacteria*) in response to irrigation and rotational management

Microbial classes of the dominant phyla identified were also observed to respond to the treatment factors of IRR and rotational treatment. *Actinobacteria* (class) were significantly increased in both the CON and Nfert treatments with no irrigation (Figure 2). Consistently, the most dominant class was *gammaproteobacteria* which significantly decreased without IRR in CON and Nfert treatments (Figure 3). Overall, the treatments that showed significant difference were WL and CON. Prior to the use of non-cultural methods of bacterial identification, *alphaproteobacteria* were assumed to be associated with soil and root systems of nitrogen-fixing bacteria. This has begun to change with the association of taxa from *Betaproteobacteria* (Chen et al., 2001; Moulin et al., 2001; Valverde et al., 2003; Chen et al., 2005), and now *gammaproteobacteria*. These *gammaproteobacteria* have been described not as true symbionts, but as opportunistic bacteria that could be residents of the bulk soil as well (Ibanez et al., 2009). The rotation with legumes in the current soil systems under study could be the reason for the predominance of the *gammaproteobacteria* class, as it is common to see this class in soils, but has not shown to be predominant when considering the other classes of Proteobacteria.

As a result of the assumed importance of *gammaproteobacteria* to the identified ecosystem, the investigators took a closer look at the descending levels of taxonomy to identify important genera and species that may have functional ties to the soil ecosystems under study. At the genera level, significant changes were detected in *pseudomonas, nitrosomonas, and nitrosococcus* taxa. For *nitrosomonas*, highest values were observed under the 3YR (1.16%) and the lowest under the CON (0.91%). *Pseudomonas* and *Nitrosococcus* showed significant increases in relative abundance ($p = 0.05$) with the presence of irrigation. Species identified that belong to these genera are *Nitrosomonas aestuarii*, *Nitrosomonas communis*, *Pseudomonas putida*, *Pseudomonas putida tp2*, *Pseudomonas umsongens*, *Pseudomonas taiwanensis*, and *Pseudomonas spp, and Nitrosococcus spp*.

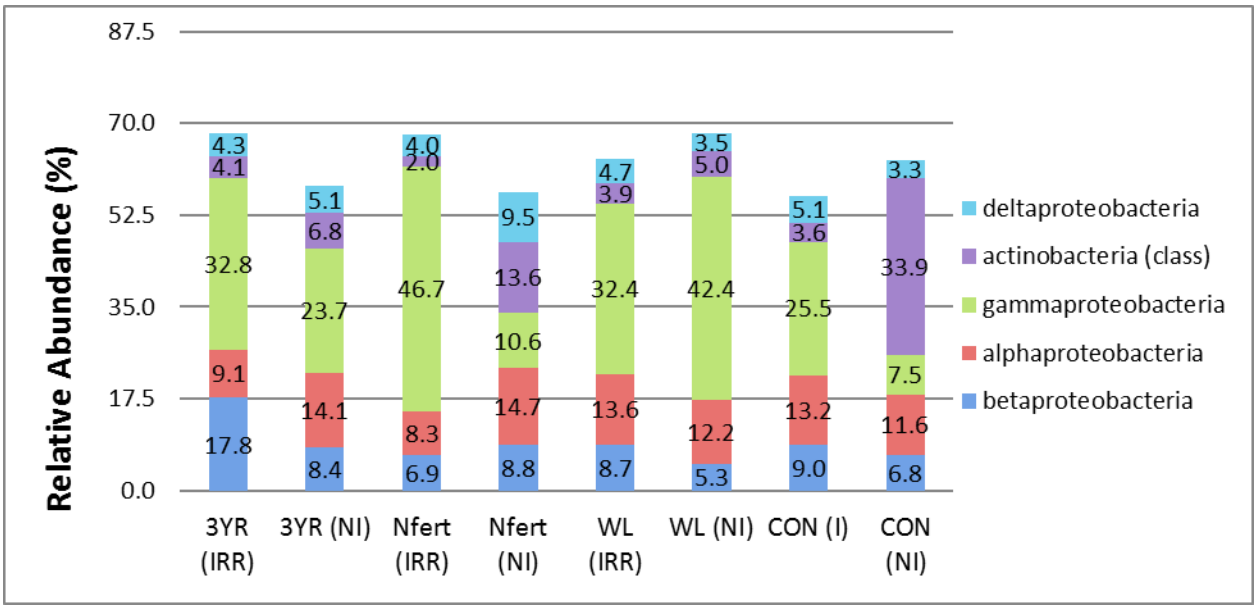

Figure 3. A stacked bar chart depicting the relative abundance of the various classes of Proteobacteria, as well as the most dominant *Actinobacterial* class

With respect to the genera identified, they have been established as key members of the nitrification process and plant growth promotion in the rhizosphere. It is assumed that the function, in addition to the presence of these groups, is enhanced in the presence of water as the genera has been shown to be beneficial to Plant Growth Promoting Rhizobacteria (PGPR) with insecticidal functionality towards agricultural pests (Liu et al., 2010; Chen et al., 2014), indole acetic acid production for enhanced nutrient uptake, siderophore production, ammonia production, phosphorus solubilization, and heavy metal solubilization (Tripathi et al., 2005; Pandey et al., 2006; Rajkumar et al., 2006; Joseph et al., 2007; Rajkumar and Freitas, 2008; Ahemad and Khan, 2011; Ahemad and Khan, 2012a; Ahemad and Khan, 2012b). Both *Nitrosomonas* species are established lithotrophic ammonia oxidizers (Koops et al., 1991), and *Nitrosococcus* have prior been established in soil systems as an autotrophic ammonia-oxidizing bacterium (Sharma, 2005). With the presence of legumes in both the 3YR and WL rotational strategies, it may be that a legacy community of nitrogen-fixing bacteria is left for nitrifiers to actively nitrify.

Alpha diversity (richness) estimates were calculated for each of the treatment factors. Values between IRR and NIRR were not different and varied little, which is why values presented in Figure 4 are not considering irrigation regime. Though no significance was found, IRR plots always identified more individuals and predicted more OTUs than NIRR. For all the indices calculated, the lowest values were observed in the Nfert. The most individuals were found in the CON treatment (17,809 sequences), but the most species predicted seemed to be in the WL (6,872 OTUs). Treatments WL and Nfert were significantly distinguished ($p < 0.05$). Not displayed, but salient to the results of the study are values for Shannon Wiener Diversity Index in which the descending order of diversity found across the plots was: WL (7.28), 3YR (7.25), CON (7.16), and Nfert (6.97); with NIRR being more diverse than IRR.

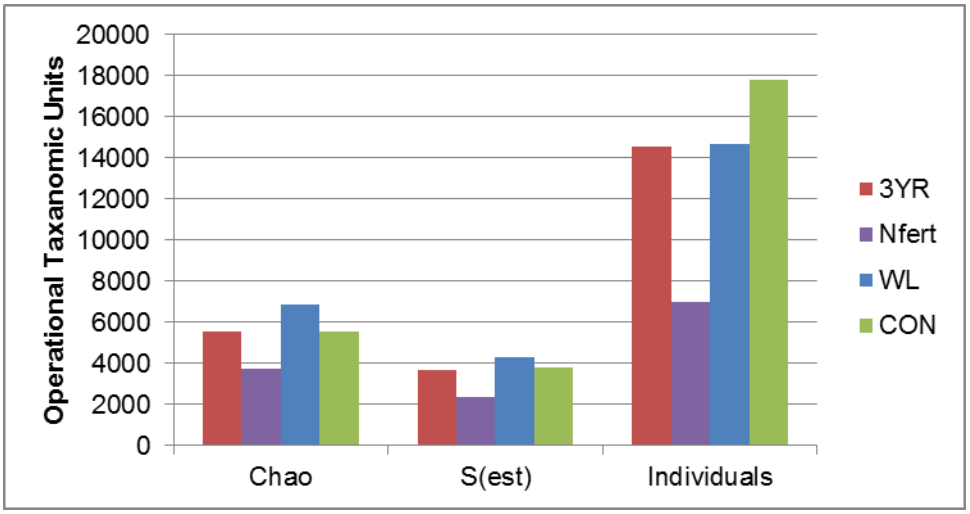

Figure 4. A clustered bar graph for the three measures of bacterial community richness with respect to the rotational management treatments

Established above was the idea that the shift seen in ACD and PHD for greater activity was due to the increased extracellular enzyme pool under NIRR regimes. However, changes in microbial community composition and structure may have also contributed to the higher enzymatic activities compared to the other irrigation and rotational regimes. With respect to irrigation, induced drought has prior established shifts in the microbial community (Berard et al., 2011). The authors also noted these shifts in key taxa within the current study, but the diversity and richness results present another aspect of the community. As measured, there seems to be no impact of irrigation on the richness or diversity of the soil microbial communities. Though not significant, the trend in rotational strategies for richness and diversity seem to mirror that of the values for pH (Table 1). This furthers the notion put forth by Ramirez et al. (2010) that stated pH was more influential on the structure of microbial communities than the membership.

Soil pH and Carbon

Soil pH across the plots ranged between 6.7 and 7.1 (Table 1). The rotational treatments were shown to have a significant impact on the means. Also, a significant effect was seen in the interaction of factors, where treatment differences were detected in IRR and NIRR plots. Overall, the only distinguishable plots were the 3YR and the Nfert ($p < 0.05$) as they were the lowest and highest values, respectively. Soils under the 3YR regime were consistently the lowest measured, while the CON was highest in irrigated soils and Nfert in NIRR soils. Rotational strategies seemed to impact soil pH without respect to irrigation. Previous studies suggest the strong influence of soil pH on both community membership and structure (Hartman et al., 2008; Lauber et al., 2009). A strong influence of community membership means the expectation of the relative abundance of *Acidobacteria* to increase at low pH, and the expectation of the relative abundance of *Actinobacteria* and *Bacteriodetes* to decrease at high pH (Jones et al., 2009; Lauber et al., 2009). However, the results presented do not reveal such predictions, suggesting that soil pH, may not play as prominent a role in community membership across the rotational strategies.

Table 1. Responses of Soil pH and Organic Carbon to Rotational Management within Irrigation Strategies

	Treatment	pH	Std. Error	Carbon*	Std. Error
IRR	3 Yr	5.82b	0.13	0.87a	0.09
	CON	6.66a	0.16	0.68a	0.11
	WL	5.98b	0.13	0.99a	0.09
	N-fert	6.21ab	0.22	0.94a	0.15
NIRR	3 Yr	5.99b	0.13	0.87a	0.08
	CON	5.83b	0.16	0.36b	0.09
	WL	6.21ab	0.13	0.84a	0.08
	N-fert	6.82a	0.22	0.34b	0.13

*Carbon values are expressed as % organic carbon

Soil organic carbon (SOC) was measured in very low concentrations throughout the study area, as the highest measure was in the IRR Nfert site (0.99%) and the lowest in the NIRR WL site (0.34%). Both irrigation and the rotational treatments proved to be significant factors in the presence of SOC in the observed plots. NIRR plots were significantly lower in organic carbon than IRR plots ($p < 0.05$), while overall CON soils were lowest in organic carbon. When separated into IRR and NIRR, no differences were seen amongst the IRR soils, while NIRR soils showed a distinction between WL and 3YR and CON & Nfert ($p<0.05$). Overall, CON soils were lower in organic carbon content ($p < 0.05$). Cotton monocropping (CON) treatments have traditionally shown decreases in soil organic carbon leading to other issues of soil health (Acosta-Martinez et al., 2008). Although these soils have only less than 1% organic matter content, the findings demonstrate that an increase in C content under rotations with legumes compared to cotton monoculture and inorganic fertilization ($p < 0.05$, Table 1) could have helped to protect enzymes from denaturation or protease hydrolysis during the drought conditions.

Conclusion

Dynamic soil properties, such as enzymatic activity and bacterial community structure, are important when assessing impacts to soil health with regards to irrigation practices and rotational management. Irrigation seemed to serve as a stabilizing mechanism, because it did not cause much variation between the dynamic soil properties; however, the lack of irrigation had the opposite effect. As promoted and established in scientific agriculture, rotational strategies that include plants that are engaged in symbiotic relationships with specific groups of nutrient cycling bacteria add to the long-term health of the soil. Also, based on climate change data, scholars and climatologists have predicted for there to be more drought/flood conditions for large portions of the Southeastern US. This trend requires the need for additional research into best management practices with regards to irrigation and other agronomic practices that are based on the science of soil health and conservation. This has implications for the average producer who could use such techniques or methods to improve his or her cultivation practices; hence, a long-term benefit for the environment.

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