# NITROGEN DYNAMICS AND ENZYMATIC ACTIVITES OF SHRUB-MILLET SYSTEMS IN SENEGAL

**THESIS**

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## ABSTRACT

Rapid population growth in sub-Saharan Africa has prompted unsustainable agricultural practices such as over-grazing and intensive cropping. This has led to a drastic decrease in soil quality. Generally the soils in this region have high sand content and poor biomass productivity, making maintenance of soil organic matter difficult. However, the continuous decline of soil organic matter is a major driver of the severe degradation processes observed. Indeed, agricultural methods that maintain a quality soil environment while sustaining crop yields are desperately needed.

The Sahel is commonly managed as a parkland agroforestry system, where trees and shrubs randomly grow and establish in farmers' fields. Research has found that woody shrubs in arid and semi-arid environments can create "resource islands" by accumulating nutrients and organic matter underneath and around the shrub canopy. Specifically, there are two native shrub species, *Guiera senegalensis* and *Piliostigma reticulatum*, found in Senegal and throughout much of the Sahel that are known to increase yields when intercropped with pearl millet (*Pennisetum glaucum*) and peanut (*Arachis hypogaea*). Research has provided evidence of both species performing hydraulic redistribution, the movement of water from tap roots to surface roots that is

caused by a water potential gradient from wet subsoil to the dry soil surface. Rainfall is a major limiting factor for crop growth in the Sahel, so this hydraulic redistribution process can have substantial impacts on crop growth and microbial activity surrounding the shrub in times of drought. An increase in microbial activity has major implications regarding nutrient cycling and decomposition in the soil. Therefore, one of the objectives of this study was to investigate soil enzyme activities in the presence and absence of *G. senegalensis* across a rainfall gradient in farmers' fields of Senegal over the course of two growing seasons.

β-glucosidase, acid phosphatase, and β-glucosaminidase activities were positively affected by the presence of shrubs. This shrub effect was observed even during a year with decreased rainfall, suggesting that hydraulic redistribution was supporting microbial processes when rainfall was scarce. The shrub has significant effects on soil chemical properties, with total C and N significantly greater underneath the shrub canopy than away from the canopy across all sampling locations and years. Certain secondary macro- and micronutrients were also elevated in the presence of *G. senegalensis*. These results suggest that the increased concentrations of nutrients and hydraulic redistribution associated with these shrubs are supporting a larger and more active microbial community than the surrounding xeric soil.

Consequently, the second objective of the study was to determine if the shrub rhizospheres are stimulating N fixation by harboring a larger population of diazotrophs. This experiment took place at two long-term research sites in Senegal. Soil samples were collected from millet plus shrub plots and millet alone plots at various sampling dates and $^{15}N$ incubations were set up to determine $\%^{15}N$ incorporation. Samples collected from the

shrub plots revealed higher amounts of incorporation compared to the no shrub plots. However, only *P. reticulatum* exhibited a statistically significant increase in $^{15}N$ incorporation. Furthermore, samples collected within the influence of both shrub species revealed a slight, but statistically insignificant increase in incorporation during the dry season. These results suggest that hydraulic redistribution is sustaining microbial communities capable of performing N fixation throughout the dry season.

Results from both experiments provide evidence that these shrub systems have significant impacts on microbial activity and biogeochemical processes that can lead to higher agricultural productivity.

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# CHAPTER 1: AGRICULTURE AND SHRUB SYSTEMS IN THE SAHEL: A REVIEW

## ABSTRACT

Increasing populations in sub-Saharan Africa has prompted unsustainable agricultural practices that have led to a drastic decrease in soil quality. Sub-Saharan Africa comprises a quarter of the world's malnourished people so food security is a major concern in this region. Much effort has been put forth in West Africa to promote conservation agriculture in order to remediate degraded landscapes, while maintaining crop yields. Green Revolution technologies have largely not been adopted due to infrastructure constraints of the Sahel. However, in Senegal, there is evidence that local woody shrubs, *Guiera senegalensis* and *Piliostigma reticulatum*, increase yields when intercropped with *Arachis hypogaea* (groundnut) and *Pennisetum glaucum* (pearl millet). Shrubs in arid environments can create spatial heterogeneity of important soil biological, physical, and chemical properties, resulting in 'resource islands'. Additionally, these shrubs are capable of performing hydraulic redistribution, bringing water from the wet subsurface to the dry soil surface. This increase in water and nutrient availability is hypothesized to have a positive effect on the microbial communities. Indeed, studies have shown that microbial communities collected from within the shrub influence area are larger and more diverse. However, most studies investigating shrub effects have been on a small scale. Therefore a central objective of this study was to investigate the regional effects that shrub presence has on enzymatic activity, while also investigating how activities are influenced by a rainfall gradient across Senegal's Peanut Basin. Furthermore, there has been little research done regarding the effect of shrubs on the

microbial mediated N dynamics in these agricultural systems. Consequently, another objective of this research is to investigate the shrubs' ability to stimulate N fixation.

# INTRODUCTION

According to estimates from the Food and Agriculture Organization of the United Nations (FAO), world food production would have to double in order to feed a projected population of 9.2 billion by 2050 (FAO, 2009). In 1996, representatives from 185 countries and the European Community united at the World Food Summit (WFS) to address issues concerning undernourishment and sustainable food security. The WFS set the framework for the elimination of hunger worldwide, with an immediate view of reducing the number of undernourished people to half by the year 2015. While the latest estimates from the FAO indicate that overall global hunger is declining, there was not enough time to reach the target set by the WFS. Progress has been particularly insufficient in sub-Saharan Africa, where one in four people suffer from chronic hunger. This region comprises more than a quarter of the world's malnourished people and has the highest prevalence of hunger than any other region in the world (FAO, 2014). Sub-Saharan Africa, in particular, is heavily affected due to the rapid population growth occurring in this region. The population of sub-Saharan Africa was 936.3 million in 2013, and is expected to reach nearly 1.5 billion by 2050 (FAO, 2009). Indeed, the growth in agricultural production observed in the last decade is far from meeting the demands of the steadily increasing population (FAO, 2011).

## Soil Fertility and Degradation in sub-Saharan Africa

Increasing populations in sub-Saharan Africa has prompted unsustainable agricultural practices such as loss or shortening of fallow periods, sedentary agriculture on smaller tracts of land, and the expansion of agriculture onto marginal lands (Buresh and Tian, 1998). As a result, there has been a drastic decline in soil quality. For instance, in semi-arid West Africa, the total extent of soil degradation due to non-sustainable agricultural systems has been estimated at 1.1 million $km^2$ (Vȧgen et al., 2005). The continuous decline of soil organic matter (SOM) is also a major driver of the severe soil degradation processes observed in the region (Lal, 2008). Low levels of SOM cause a reduction in soil structure, making the soil more susceptible to wind and water erosion (Bationo and Buerkert, 2001; Dossa, 2007). Soils in sub-Saharan West Africa have high sand content and poor biomass productivity, making SOM maintenance a difficult task. Furthermore, the high soil temperature leads to rapid decomposition, further decreasing SOM stocks. Nevertheless, maintenance of SOM is crucial for agricultural systems in semi-arid environments (Lahmar et al., 2011).

*Desertification* Declining SOM stocks lead to soil degradation, which can eventually lead to desertification, a common issue in sub-Saharan West Africa (Sanchez et al., 1997). Desertification can be defined as "the diminution or destruction of the biological potential of the land and can ultimately lead to desert-like conditions" (UNCOD, 1977). The region in Africa most affected by desertification is the Sahel. The word Sahel comes from a local word meaning "edge of the desert" and refers to the semi-arid zone that

stretches across the southern edge of the Sahara desert from the Atlantic coast of Senegal and Mauritania to the Red Sea coast of Sudan (Grove, 1978; Bayala et al., 2011). In general, the Sahel is considered the transition zone between the Sahara desert and the savannas to the south (Sotelo Montes et al., 2014).

The main drivers of desertification are overexploitation and improper land use such as overgrazing, lack of water conservation strategies, and human-initiated bushfires (Lambin et al., 2014). The effects of desertification are exacerbated by the climatic variability observed in the Sahel. For instance, minor shifts in precipitation patterns can lead to sensitive plant species being lost, resulting in a loss of plant biodiversity and vegetative cover (Claussen et al., 2013). This loss of vegetative cover triggers a feedback mechanism that has negative impacts on factors such as rainfall regime, soil moisture, soil salinity, and soil erosion. These processes cause a further decline in plant cover, worsening the effects of desertification (D'Odorico et al., 2012). Therefore maintaining vegetative cover in these degraded systems is crucial in order to minimize the effects of desertification.

One of the current approaches used to confront desertification is the extensive planting of trees and other vegetation, which serves as a natural barrier by breaking desert winds, stabilizing the soil, and increasing soil moisture (O'Connor and Ford, 2014; Vetaas, 1992). This is the central idea behind the "Great Green Wall" (GGW) program that was established in 2002 among 11 Sahelian countries. The current objective of the GGW is to plant a 15-km wide forested band that expands the length of Africa, from Senegal to Djibouti, along the southern border of the Sahara desert. A major concern of the GGW program is its reliance on tree species, and in particular, members of the *Acacia*

family (O'Connor and Ford, 2014). *Acacia* trees grow slowly and are valued as a source of cooking fuel in the Sahel. The high demand for fuel wood could compromise the long-term success of the program due to illegal harvesting (Dang, 1993).

However, a study conducted by O'Conner and Ford (2014) proposed using woody shrub species in addition to trees as the basis of the GGW. Similar to trees, shrubs reduce wind speeds, increase soil humidity, and stabilize soil nutrients, allowing other plant life to flourish in surrounding areas (Gómez-Aparicio et al., 2005). Two of the main advantages that shrubs have over trees are a faster growing rate and the ability to reach maturity within a fraction of the time compared to trees. Furthermore, in areas where plant life has been removed, shrubs are often the primary pioneer species and typically establish years before tree species (Dalling and Hubbell, 2002). The rapid establishment and growth rates observed in many shrub species make them an attractive companion plant type for the basis of the GGW, and their utilization could ensure the long-term success of the program and recovery of agricultural land (O'Conner and Ford, 2014).

**Conservation Agriculture in sub-Saharan Africa**

The Sahel is home to about 50 million people, and about 85% of the population relies on subsistence agriculture (Hiernaux and Turner, 2002). The current population growth rate in the Sahel is 3% per year, while the food production growth rate remains at only 2% per year. In order to achieve the Millennium Development Goals developed by the UN, it is estimated that agricultural production in this region would have to increase

by about 6% per annum (Bayala et al., 2011). What is more, this drastic increase in production needs to be accomplished while conserving the region's natural resources. Although Green Revolution techniques have been shown to increase crop yields in the short-term, these practices can decrease soil quality in the long run (Bohlool et al., 1992). Alternatively, techniques that utilize soil conservation would support a more stable ecological environment, while promoting food security in this region.

Conservation agriculture refers to practices that strive to achieve high and sustainable crop yields, while conserving natural resources. Techniques that utilize soil conservation include reducing soil disturbance, maintaining soil cover, and implementing diversified crop rotations (FAO, 2008). While the overall success of conservation agriculture relies on the simultaneous use of these three practices, maintaining organic soil cover is the most prominent aspect in semiarid regions. Soil cover maintenance promotes SOM build-up and fertility replenishment, and has positive impacts on the soil water balance and biological activity. However, this aspect of conservation agriculture is difficult to implement in systems where crop residues are valued more as livestock fodder than as mulch, especially in the Sahel (Lahmar et al., 2011).

The short term benefits of conservation agriculture are important because they largely determine the attractiveness of the system to the farmers. The adoption of conservation agriculture in sub-Saharan West Africa has been difficult due to the variability in the short term crop responses. This variability often discourages farmers to implement these systems (Giller et al., 2009). In order to ensure the success of conservation agriculture on smallholder farms, approaches that utilize locally available

resources and build upon farmers' indigenous practices are needed (Giller et al., 2009; Lahmar et al., 2011).

## Shrub Systems in Senegal

*Parkland Agroforestry* The Sahel is commonly managed as a parkland system, where non-crop species such as trees and shrubs randomly grow and establish in farmer fields (Garrity, 2004; Diedhiou et al., 2009). These parkland agroforestry systems are the primary providers of food, nutrition, income, and environmental services in the Sahel. However, due to decreases in woody biodiversity and vegetative cover, these systems are rapidly deteriorating. Nevertheless, the restoration and protection of these parkland systems is vital in order to sustain the future welfare of Sahelien residents (Bationo et al., 1998).

Although much of the focus has been on the tree component of these agroforestry systems, studies have found evidence of woody shrubs to be effective in promoting crop productivity (Dossa et al., 2013). In particular there are two shrub species, *Guiera senegalensis* and *Piliostigma reticulatum* that are found in Senegal and throughout much of sub-Saharan Africa. These two shrub species are the dominant form of vegetation and coexist with rain fed crops in the Sahel at low densities. Research has shown they can increase crop yields when intercropped with pearl millet (*Pennisetum glaucum*) or peanut (*Arachis hypogaea*) (Diack et al., 2000; Kizito et al., 2006). A study conducted by Dossa et al. (2013) reported that millet and peanut yields were significantly higher when grown in the presence of *G. senegalensis* over 4 growing seasons in Senegal in plots ranging

from zero to 1.5 times the recommended fertilizer rate. If managed properly, these local woody shrubs could provide a sustainable and practical means to increase crop yields in Senegal and much of sub-Saharan Africa.

*Shrub Decomposition and Nutrient Inputs* Traditionally in Senegal, shrubs are pruned back to the soil surface and the residue is burned in order to clear the fields before planting row crops every spring. Farmers will sometimes completely remove the shrubs during the dry season for fuel, fencing, or to increase agricultural land. However, studies have shown rapid decomposition of shrub residue, thus eliminating the need for coppice-burn management techniques (Diack et al., 2000). Diedhou et al. (2009) claim that the presence of shrubs actually enhances decomposition rates. Yet it can be difficult to motivate farmers to mulch if plant residues can be used as livestock fodder. An advantage of using the shrub residue as mulch in these agricultural systems is that it makes poor livestock fodder, giving the farmers more incentive to leave it on the soil surface as an organic input (Wezel and Bocker, 1999).

Studies have found that these local shrubs have a greater potential to provide organic matter inputs to soil than manure, composts, or woody trees (Lufafa et al., 2008). Furthermore, due to the high prevalence of shrubs throughout sub-Saharan Africa, they have the potential to be a significant source of carbon (Lufafa et al., 2008). For example, a short term study demonstrated that incorporating shrub foliage into the soil increases soil organic carbon (Diack et al., 2000). Additionally, in arid environments, shrubs can create spatial heterogeneity of important soil chemical, physical, and biological properties, resulting in ‘resource islands’ (Garner and Steinberger, 1989; Whitford,

2002). However, in order for the organic matter from the shrub residue to be effective, non-thermal management of the shrub systems needs to be implemented by farmers.

*Soil Water Relations* Senegal's climate is semi-arid where rainfall occurs only a few months out of the year, and is often erratic and poorly distributed (Kizito et al., 2007). Furthermore, most of the agriculture in this region is rain fed and therefore very susceptible to climatic changes (Lahmar et al., 2011). As a result, water availability plays a major role in crop production and is often the limiting resource for crops in semi-arid environments (Gregory, 1989). However, in semi-arid regions, deep tree roots can tap into ground-water reserves, helping to sustain surrounding plant life. This happens because of high soil water potential in the subsurface and low water potential at the soil surface (Horton and Hart, 1998). This is known as hydraulic redistribution, a process where deep tap roots bring water from the wet subsurface to the dry soil surface at night when photosynthesis stops. This helps to sustain transpiration during drought periods, decreasing water stress for crops (Moreira et al., 2003; Dawson, 1996).

There is evidence that *G. senegalensis* and *P. reticulatum* are also capable of performing hydraulic lift. Field studies have found that over 90% of *P. reticulatum* and *G. senegalensis* roots are within the 0.2-0.5 meter range, but studies have found that some shrub roots grow deep into the wet subsoil (Wezel and Bocker, 1999; Kizito et al., 2006). For instance, during a preliminary study conducted in Senegal on shrub root patterns, it was reported that 5% of *P. reticulatum* roots extended beyond 3 meters in depth (Kizito, 2003 *Unpublished results*). In 2007, Kizito et al. also observed slight increases in soil moisture in the 0.2 and 0.4 m depths during the dry season, suggesting the occurrence of

hydraulic lift. This may explain why these perennial shrubs continue to flourish during the dry season when soil water is very scarce (Gaze et al., 1998). In addition, Kizito et al. (2007) reported that in crop-shrub plots, drainage losses were about 25-50% lower and soil water storage was about 20% higher when compared to control plots containing crops alone. It is important to note that even during a very dry year, there was a positive crop response, suggesting that the shrubs do not compete with crops for water. These results indicate that the shrubs could supplement soil moisture demands for crops when rainfall is insufficient (Kizito et al., 2007; Dossa et al., 2013).

*Microbial Communities of Shrub Rhizospheres* The nutrients released from shrub litter, root exudates, and root turnover of *G. senegalensis* and *P. reticulatum* have been related to the stimulation and shift of microbial communities in soils beneath shrub canopies (Diedhiou et al., 2009; Debenport et al., 2015). In addition, the hydraulic redistribution of water associated with shrub roots could also be influencing soil microbial communities. Diedhiou et al. (2009) conducted a soil microcosm study that profiled phospholipid fatty acids (PLFAs) and measured enzyme activity during residue decomposition. They found that soil microbial communities beneath the shrubs are more diverse, less stressed, and distinct from soil outside of shrub influence. In a previous study, it was demonstrated that the microbial diversity of shrub rhizosphere soil was maintained over the dry season based on PLFA analysis (Diedhiou, 2007). These results are important because they suggest that biogeochemical processes can proceed during the dry season of semi-arid regions where the plants are performing hydraulic lift.

These results indicate that hydraulic lift, combined with greater availability of C compounds from litter and root exudates, supports a larger and more diverse community that could be increasing crop yields. Dossa et al. (2013) showed that *G. senegalensis* dramatically increased yields of millet and peanut, in both the presence and absence of fertilizer, which suggests that the response could in part be related to the microbial community. Debenport et al. (2015) revealed that intercropping and shrub residue application at two study sites resulted in increased microbial diversity in both fungal and bacterial communities in the root zone of millet. Previous studies examining the effect of intercropping on microbial community structure have used low resolution profiling techniques such as PLFA (Diedhiou et al., 2009; Lacombe et al., 2009). However, Debenport et al. (2015) used Illumina sequencing for fine scale resolution of microbial community structure and found distinct groups of microorganisms associated with improved plant growth in the plots amended with the shrub treatment. Specifically, these distinct groups were found in the *Chitinophaga*, *Aspergillus*, *Fusarium*, *Penicillium*, and *Phoma* genera. Bacteria that promote plant growth and nutrient assimilation and retention can be of extreme importance in low input agricultural systems. Therefore, examining the potential of native shrubs to harbor beneficial organisms is an important step in understanding beneficial shrub-crop relations.

As previous studies have indicated, the presence of shrub roots and residues has potential to sustain larger and more diverse microbial communities than the surrounding soil. However, little is known about how these shifts in microbial communities affects biogeochemical processes. A microcosm study found that the enzymes cellulase and β-glucosidase were positively affected by the presence of *G. senegalensis* and *P.*

*reticulatum* residues (Diedhiou et al., 2009). Yet, little research has been done about how the presence of these shrubs affects the production of enzymes in the rooting zones of millet. Diakhate et al. (2016) measured urease, arylsulfatase, and dehydrogenase activities in soil collected from millet root zones in the presence and absence of *P. reticulatum* and found that all enzyme activities were higher in the presence of *P. reticulatum*. However, no research has been done concerning the effects *G. senegalensis* has on enzyme activities in the rooting zone of millet. As a result, one objective of this research was to investigate how the presence of *G. senegalensis* in millet fields affects enzyme activities and nutrient availability across a rainfall regime in Senegal.

Little research has been done regarding the effects the shrub rhizospheres have on the nitrogen dynamics of these shrub-crop systems. External inputs of N are rarely added in these agricultural systems so even modest amounts of biologically fixed N could have significant impacts on crop growth. Therefore, another central objective of this research was to determine the ability of *G. senegalensis* and *P. reticulatum* to stimulate nitrogen fixation in millet fields.

# CONCLUSION

The agricultural soils of the Sahel are of inherently low quality and being rapidly depleted of organic matter and nutrients due to unsustainable agricultural practices (Sanchez et al., 1997; Bationo and Buerkert, 2001). Furthermore, this region is experiencing rapid population growth, where one in four people are suffering from chronic hunger (FAO, 2014). In order to promote food production while conserving the region's resources, conservation agriculture practices need to be implemented by farmers. Studies have shown that woody shrubs in arid and semi-arid environments can stimulate crop growth by creating 'resource islands,' which improve soil physical, chemical, and biological properties, which in turn increase crop productivity (Garner and Steinberger, 1989; Whitford, 2002).

In Senegal and throughout much of the Sahel, local woody shrubs *G. senegalensis and P. reticulatum*, dominate the landscape and are known to increase crop yields when intercropped with millet and peanut (Dossa et al., 2013). Both species increase nutrient availability through root exudates and residue decomposition (Diedhiou et al., 2009). In addition, there is evidence that these shrubs can perform hydraulic redistribution which helps decrease water stress for crops during periods of drought (Kizito et al., 2007; Kizito et al., 2012; Dossa et al., 2013). This increase in water and nutrient availability surrounding the shrub canopies is expected to stimulate microbial communities. Results from previous studies have suggested that the shrub rhizospheres support larger and more diverse microbial populations that do not exist in the surrounding xeric soil (Diedhiou et al., 2009).

However, little is known about the microbial communities associated with the shrub rhizospheres. Of particular interest is whether the shrubs promote free-living $N_2$ fixers and nutrient availability in soil. Microbial communities that stimulate plant growth and nutrient cycling can have a significant impact in low yield agricultural systems. Therefore, investigating how the shrub rhizospheres influence the microbial community and biogeochemical processes is the next step in gaining a better understanding of these shrub-crop systems.

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# CHAPTER 2: SOIL ENZYME ACTIVITIES OF SHRUB-MILLET SYSTEMS IN SENEGAL

## ABSTRACT

Studies have found that woody shrub species in arid and semi-arid environments can have significant impacts on soil nutrients and crop yields in the Sahel. In particular, *G. senegalensis* is commonly found in farmers' fields throughout Senegal. This shrub provides organic matter and can stabilize soil water availability through hydraulic redistribution and is known to increase millet and peanut yields. In addition, previous studies have found that this shrub can increase microbial diversity and activity, which could have substantial impacts on biogeochemical processing in agricultural fields across Senegal. Therefore, this study investigated the effects these shrub systems have on soil enzymatic activity and extractable nutrient levels in the presence and absence of *G. senegalensis* across a landscape gradient in farmers' fields in Senegal. β-glucosidase, acid phosphatase, and β-glucosaminidase activities in soil were significantly increased by the presence of the shrub. This shrub effect was observed even during a year with decreased rainfall, suggesting that hydraulic redistribution was supporting microbial communities during times of drought. Urease activity was the only enzyme that did not show a significant treatment effect. However, the shrub had substantial impacts on soil nutrient levels, with total C and N significantly higher underneath the shrub canopy for all sampling locations. Certain secondary macro and micronutrients were elevated in treatments containing *G. senegalensis* as well. Results from this study indicate that the presence of shrubs in farmers' fields have a significant impact on microbial activity and nutrient cycling in soil.

# INTRODUCTION

The soils of the Sahel are seriously degrading due to increasing rural populations, overgrazing, intensive cropping, and scavenging for firewood (Lambin et al., 2014). This is contributing to low yields and food insecurity (Graaff et al., 2011; Niang and Ruppel, 2014). Soil nutrient management is vital for agroecosystems of the Sahel, where chemical fertilizer use is limited due to lack of resources. Even with fertilizer applications, yields are not optimal or even continue to decrease unless there are regular inputs of organic matter and remediation of soil (Sanchez et al., 1997; Badiane et al., 2000a). Besides providing nutrients, organic inputs improve soil quality through decomposition and associated microbial activity by promoting soil aggregation and protecting soil from wind and water erosion (Sanchez et al., 1997; Badiane et al., 2000b; Merckx et al., 2001; Masse et al., 2004). However, animal manure or household refuse do not provide the quantities needed at landscape levels to significantly improve soil quality (Badiane et al., 2000b).

Agroforestry using trees offers a solution when growing in farmers' fields but trees are slow-growing and may be competitive with crops for light and water. In recent years native shrubs (*Guiera senegalensis*, and *Piliostigma reticulatum),* previously an unrecognized component of cropped fields in the Sahel, have been shown to increase soil quality (Dossa et al., 2010) and crop yields (Dossa et al., 2012, 2013). Additionally, Kizito et al. (2012) found that *G. senegalensis* and *P. reticulatum* perform hydraulic

redistribution; the movement of water through tap roots to surface roots that is released at night to rhizosphere soil because of a water potential gradient from wet subsoil to dry surface soil (Caldwell et al., 1998).

*G. senegalensis* has been shown to be more effective than *P. reticulatum* in affecting soils and crop productivity (Dossa et al., 2012, 2013) and it is the most wide spread and dominate species in the cropping regions of Senegal and elsewhere in the Sahel when annual rainfall is less than 800 mm (Lufafa et al., 2008). However, mechanisms for how *G. senegalensis* affects agroecosystems are not fully understood relative to nutrient dynamics and enzymatic activity that drives release of nutrients. Furthermore, the research reported so far (Diedhiou et al., 2009; Dossa et al., 2010; Diedhiou-Sall et al., 2013) has been on small research plots and not representative of what is happening at landscape levels. Therefore, the objective was to determine the impact of *G. senegalensis* on nutrient levels and activities of enzymes involved in organic matter decomposition and nutrient mineralization across the Peanut Basin (the main dryland cropping region of Senegal) along the north-south precipitation gradient.

Soil enzymes play an important role in biogeochemical processes that drives cycling especially for of C, N, P and S (Dick, 1984). Enzymes catalyze many reactions involved in nutrient mineralization and decomposition of organic residues in soil ecosystems. Most soil enzymes are thought to be derived from the soil microbial community, and positive correlations between microbial biomass and enzyme activities are often found. Therefore, enzyme activities can be a good indicator of relative soil microbial activity (Dick, 1984). Additionally, studies have shown that enzyme activities are often positively correlated with soil organic carbon (Frankenberger and Dick, 1983;

Jordan et al, 1995). Enzyme activities can also be used as early indicators to reflect changes in soil properties brought on by changes in management (Ekenler and Tabatabai, 2003; Bandick and Dick, 1999).

While enzymes perform functions that are vital to the cycling of nutrients, it is important to note that enzyme activity is a measurement of potential, and does not reflect actual field conditions (Schloter et al., 2003). Enzyme assays are performed in a laboratory setting under optimal conditions for each particular enzyme. Therefore, enzyme activities are most effective in comparing the differences in management and monitoring trends (Bandick and Dick, 1999).

A few studies have investigated the effects of shrubs on soil enzyme activities. One of these was a microcosm experiment that studied the effects of shrub residue on microbial composition and activity during decomposition (Diedhiou et al., 2009; Diedhiou-Sall et al., 2013). Another study by Diakhate et al. (2016) investigated the impact of *P. reticulatum* on enzyme activities at a long term research plot in Senegal. Both studies found that the presence of the shrubs positively affected enzyme activities. Little is known on whether extractable nutrients (that would be relatively available for crops) are increased in the presence of shrubs. Furthermore, the studies mentioned above relative to enzyme activities were comparing bulk soil from beneath and outside the influence of shrubs with no consideration of whether this effect is conferred on soil closely associated with actively growing crop roots. Consequently, this study investigated the soil enzymatic activity and extractable nutrient levels directly in rhizosphere and intra-root soil beneath pearl millet (*Pennisetum glaucum* ) when

growing beneath the canopy of *G. senegalensis* or outside the influence of *G. senegalensis* along landscape gradients in farmers' fields.

## MATERIALS AND METHODS

### Site Description

The study was done in the Peanut Basin of Senegal, centered at the coordinates N 14° 41' 60", W 16° 0' 0" in a semi-arid savannah with vegetation consisting primarily of shrub land with scattered trees. The mean annual rainfall in the Peanut Basin is 540 mm, with the majority of the rainfall occurring between August and October (Lufafa, 2008). According to the Soil Survey Staff (2003), soils in the area consist mainly of Psamments or Calcids, with greater than 95% sand content.

### Experimental Design & Soil Sampling

The experimental design was a completely randomized 3 X 3 factorial with two replications. The treatments were three rainfall gradient sites and three sampling treatments. The three rainfall gradient sampling sites were chosen along a north-south rainfall gradient in Senegal. Two spatially separated replication sites were sampled per rainfall regime (southern, central, and northern regions of the Peanut Basin), with the southern region receiving more rainfall than the north. Two sites were near Kaolock (N 14° 10' 48", W 16° 15' 0"), two near Theis (N 14° 46' 48", W 16° 54' 60"), and two near Louga (N 15° 16' 48", W 15° 31' 48"), for a total of six sites. All sites were in farmers' fields and are managed in a peanut (*Arachis hypogea*)–pearl millet (*Pennisetum glaucum*)

rotation, with fields receiving no fertilizer or other amendments. However, for this study, both years of sampling occurred during the millet rotation. Furthermore, only *G. senegalensis* was sampled at these sites. At each of the six sites, six *G. senegalensis* shrubs were randomly chosen. The three soil sampling treatments were: 1) two millet plants within the influence of the shrub (<1 meter from the center of the shrub) M-SH; 2) two millet plants outside shrub influence (>4 meters from the shrub base) (M); and 3) soil beneath the shrub canopy (SH).

The two millet plant treatments were sampled by taking a soil core (0 – 20 cm in depth) through the center of the millet root zone. The shrub location treatment was sampled by taking a soil core (0 – 20 cm in depth) from under the shrub canopy. Sampling occurred in 2012 and 2013 during the growing seasons. Soil samples were placed in bags and brought back to the laboratory and stored at -20º C until analysis. All samples were passed through a 2-mm sieve and moisture content was measured prior to analysis.

**Soil Enzyme Activity Analyses**

***β-glucosidase activity*** was determined according to the methods described by Hayano (1973) and Eivazi and Tabatabai (1988) with the following modifications; 0.1 g of field moist soil was mixed with 400 µL of McIlvain buffer solution at pH 5.8 and 100 µL 0.05 *M p*-nitrophenyl-β-D-glucopyranoside (PNG) solution. The solution was incubated at 37° C for 2 hours. After incubation, the solution was centrifuged for 5 minutes at 10,000

RPM. Approximately 0.1 mL of the supernatant was removed and added to 3 mL of $Na_2CO_3$ to terminate the reaction. Color development was measured at 400 nm on a spectrophotometer (Ultrospec 3000, Pharmacia-Biotech). Total concentrations of β-glucosidase were determined in reference to a p-nitrophenol (PNP) standard curve by taking 0, 5, 10, 15, 20 and, 30 µg of PNP. Furthermore, controls were performed by adding PNG substrate solution after the reaction had been terminated. Final β-glucosidase activity is recorded as µg PNP g dry soil$^{-1}$ h$^{-1}$ after correcting for water weight in the beginning soil sample.

***Acid phosphatase activity*** was determined following the method of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) with the following modifications; 0.1 g of field moist soil was mixed with 400 µL of modified universal buffer (MUB) at pH 6.5 and 100 µL of 0.05 *M* *p*-nitrophenyl phosphate (*p*-NPP). The solution was incubated at 37º C for 1 hour. Following incubation, 100 µL of 0.5 *M* $CaCl_2$ and 400 µL 0.5 *M* NaOH were added to the solution to complex humic substances and to terminate the reaction, respectively. Contents of the tubes were centrifuged for 5 minutes at 10,000 RPM, the supernatant was collected, and color developed in the supernatant was measured at 400 nm on a spectrophotometer (Ultrospec 3000, Pharmacia-Biotech). Final concentrations of acid phosphatase were determined in reference to a PNP standards curve by taking 0, 1, 3, 5, 7, and 9 µg of PNP. Controls were measured with each sample by adding the p-NPP substrate solution after the incubation. Final acid phosphatase activity is recorded as µg PNP g dry soil$^{-1}$ h$^{-1}$ after correcting for water weight in the beginning soil sample.

***β-glucosaminidase activity*** was determined as described by Parham and Deng (2000) with the following modifications; 0.25 g field moist soil was added to 1 mL 0.1 *M* acetate buffer at pH 5.5 and 0.25 mL of 0.001 *M* p-nitrophenyl-N-acetyl-β-D-glucosaminide. The solution was incubated at 37º C for 1 hour. Following incubation, the solution was centrifuged for 5 minutes at 10,000 RPM and the supernatant was collected. 1 mL of 0.5 *M* NaOH and 0.25 mL of 0.5 *M* $CaCl_2$ were added to the supernatant to inhibit the reaction and prevent flocculation, respectively. The color that developed was measured at 400 nm on a spectrophotometer (Ultrospec 3000, Pharmacia-Biotech). Final concentrations of β-glucosaminidase were determined in reference to a PNP standards curve by taking 0, 5, 10, 15, 20, and 30 μg of PNP. A control was prepared for each sample by adding the *p*-NPP substrate solution after the incubation. Final β-glucosaminidase activity is recorded as μg PNP g dry $soil^{-1}$ $h^{-1}$ after correcting for water weight in the beginning soil sample.

***Urease activity*** was determined following the method of Kandeler and Gerber (1988) with the following modifications; 0.5 g of field moist soil was mixed with 700 μL of phosphate buffer at pH 7 and 50 μL of 1.2 *M* urea substrate. The solution was incubated at 37º C for 2 hours. After incubation, 1.5 mL of 2 *M* KCl – 0.01 *M* HCl solution was added to stop the reaction. Samples were placed on a rotary shaker for 30 minutes at room temperature and then centrifuged for 5 minutes at 10,000 RPM. To determine the amount of ammonium released, 1 mL of the supernatant was collected and added to 9 mL

of deionized water, 5 mL of 0.3 *M* sodium salicylate – 0.3 *M* sodium hydroxide solution, and 2 mL of 39.1 *mM* sodium dichloroisocyanurate solution. After 30 minutes, color development was determined at 660 nm on a spectrophotometer (Ultrospec 3000, Pharmacia-Biotech). Urease activity was determined in reference to an ammonium chloride standards curve containing 0, 10, 15, 20, and 25 μg N $mL^{-1}$. To account for color development not produced from the urease enzyme, a control was prepared for each sample by adding the urea substrate solution after incubation. Final urease activity is reported as μg $NH_4^+$-N g dry $soil^{-1}$ $h^{-1}$ after correcting for water weight in the beginning soil sample.

**Soil Chemical Characteristics**

Soil pH was determined using a glass membrane electrode in a 1:2 ratio of soil to water. Total soil C and N were measured using Carlos Erba Elemental Analyzer 1108.

The Mehlich 3 method was used to measure available P and secondary macro and micronutrients. The extraction protocol is as follows; 2 g of air-dried soil was mixed with 20 mL of Mehlich 3 extracting solution. Samples were shaken for 5 minutes and immediately filtered through a 0.45 μm filter. (Mehlich, 1984). Extractable soil P, K, Mg, S, Ca, Cu, Fe, and Zn were determined using an Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES).

Ammonium and nitrate were determined colorimetrically from 1 *M* KCl soil extracts by flow injection analysis. After filtration through a glass fiber filter,

ammonium-N and nitrate-N were determined in the extract by the salicylate-nitroprusside and the hydrazine-sulfaniliamide methods, respectively (Mulvaney, 1996).

### Statistical Analyses

All statistical tests were performed using SAS 9.4 (SAS Institute, Inc., 2013). Differences in treatments were examined using analysis of variance (ANOVA) using a general linear model. Pairwise comparisons were calculated using Tukey's honestly significant difference (HSD) comparisons based on a 95% confidence interval.

## RESULTS

### Soil Chemical Analyses

***Landscape Gradient*** During the 2012 growing season, total C and N were significantly higher in the southern and central regions when compared to the northern region. However, extractable P levels showed the opposite trend during the 2012 growing season, with significantly higher levels in the north. 2013 data revealed that extractable P was significantly higher in the southern and central regions than the northern region. During the 2012 growing season, nitrate levels were significantly higher in the southern region, while ammonium was highest at the central sites. Samples from 2013, however, showed a different trend; nitrate was highest at the central sites, while ammonium was significantly higher in the southern and central regions when compared to the north. Soil pH was less acidic in the north than the two other regions, with the central site having the most alkaline soil (Table 2.1).

Secondary macronutrient and micronutrient data were quite variable between sampling years. An exception to this was zinc (Zn) and potassium (K) where, in both years, extractable Zn and K concentrations were significantly highest in the central region. During the 2012 sampling season, iron (Fe) and magnesium (Mg) levels were highest in the south and lowest in the north. In 2013, however, Fe and Mg levels were highest in the central region. For the 2012 soil samples, calcium (Ca), sulfur (S) and copper (Cu) were different from the other nutrients in that the highest levels were reported in the northern region and the lowest in the southern region. Conversely, in 2013, samples collected in the northern region had the lowest levels of these nutrients (Table 2.2)

***Shrub-Millet Sampling Location*** Total N and C levels were significantly higher for the SH and M-SH treatments over the M treatment across all regions in 2012. In the southern and central regions, extractable P was significantly higher for the SH than the M or M-SH treatments for both sampling years. However, no sampling location effect was observed at the northern sites for extractable P during either sampling year.

In 2012 and 2013, nitrate levels in the central region were significantly higher under the shrub canopy than the surrounding soil. During the 2013, nitrate levels were positively affected by SH in the southern and northern regions. However, this treatment effect was not found in 2012. The only significant effect of the SH treatment on ammonium levels was in the southern region during 2012. With the exception of the northern region during the 2012 growing season, ammonium levels were always highest for SH across all regions and sampling seasons.

A significant difference was observed in soil pH during 2012 in the southern region. Soil taken from the SH location and M-SH was significantly lower at these sampling locations. A similar effect was seen in the northern site during that year, with soil pH being significantly lower for the SH over the other two location treatments. Although the effect was not significant, soil pH was slightly more acidic with the SH than the other sampling locations in the central region (Tables 2.3 and 2.4).

Secondary and micronutrient data during the 2012 growing season revealed only one significant treatment effect. This was observed in the northern region for S levels; which was significantly higher in soil from the M-SH and M treatments. Furthermore, there was a small increase in S levels over the other treatments in the central region. Magnesium, S and Zn were all slightly higher under the shrub canopy in the southern region. In the other regions, however, Mg showed slightly lower levels for the SH treatments while Zn showed slightly higher levels in the M-SH. Iron showed a small increase with SH at the southern and central sites, and was slightly higher within the M-SH in the northern region. Potassium and Ca levels were greater for M for all regions. In the south and north, Cu was slightly higher for SH, while at the central sites Cu was highest outside of shrub influence (Table 2.5).

The 2013 samples showed more significant treatment effects for the secondary and micronutrient data when compared to the 2012 sampling year. For instance, S levels were significantly higher under the shrub canopy across all sampling regions. Furthermore, Ca and K levels were significantly higher with the SH treatment in the south and the north, and both nutrients were slightly higher with the SH treatment for the central sites. In the southern region, Mg was significantly higher for M-SH, and slightly

higher in the north for SH. In the central region, however, the highest levels of Mg were reported in the samples collected outside the influence in the M treatment. Zinc levels were significantly higher for the SH soil at the northern sites. Zinc showed similar trends in the southern and central regions, with the highest levels reported for SH soil and the lowest found in samples collected for M outside of shrub influence. Although not significant, Fe levels were slightly higher for soil from SH, across all regions. No significant differences were observed for Cu during the 2013 sampling season. However, in the south and the north, there was a slight increase in Cu levels for soil from SH soil. The opposite was true for the central sites, with Cu levels highest for M outside of shrub influence (Table 2.6).

**Soil Enzyme Activities**

***Landscape Gradient*** β-glucosidase activity exhibited the same trend during the 2012 and 2013 sampling seasons. The southern and central regions showed significantly higher β-glucosidase activity when compared to the northern region. However, 2013 showed lower overall activity for all regions (Figure 2.1). The acid phosphatase assay performed on the 2012 samples revealed that the southern region had significantly higher activity than the central and northern regions. In 2013, however, acid phosphatase activity was highest in the central region. Nevertheless, the north displayed significantly lower acid phosphatase activity during both sampling years. Similar to β-glucosidase, acid phosphatase activity was lower during the 2013 growing season (Figure 2.2). β-glucosaminidase activity showed the same trend for both sampling years. The central region was significantly greater than the other two regions, while the north showed significantly lower activity for

both years. Again in general, β-glucosaminidase activity was lower in 2013 than 2012 (Figure 2.3). Urease activity was significantly higher in the central region than the two other regions, for both sampling years. For 2012 and 2013, the southern and northern regions showed approximately the same amount of urease activity (Figure 2.4).

***Shrub-Millet Sampling Location*** With the exception of urease, all enzyme assays revealed the lowest activity in samples collected from M (millet root zone soil) growing outside the influence of *Guiera senegalensis*. β-glucosidase in 2012 revealed the highest activity in samples collected under *G. senegalensis* canopies in the southern and central regions (Figure 2.5). Furthermore, samples taken from outside of shrub influence revealed significantly lower β-glucosidase activity in these regions. Samples collected from the northern region in 2012 showed no significant treatment effects. 2013 β-glucosidase activity showed the same trend in the southern region, with the highest activity found in the SH soil. Meanwhile, samples taken outside of shrub influences M had significantly lower activity. In the central region, the shrub effect was more prominent during the 2013 season, with the shrub samples having significantly higher β-glucosidase activity when compared to the other treatments. Unlike the 2012 samples, the 2013 samples from the northern region revealed a significant treatment effect; samples taken far from the shrub canopy had significantly lower β glucosidase activity (Figure 2.5).

The same trend was observed for acid phosphatase activity during 2012 and 2013 in the southern region. Activity was highest in SH soil, while samples taken from M soil outside of shrub influence were significantly lower. In the central region, for both

sampling years, no significant effect was observed. However, samples taken from millet rhizospheres growing near the shrubs displayed slightly higher acid phosphatase activity for both years. In the northern region during the 2012 sampling season, acid phosphatase activity was significantly higher in soil taken from root zone soil of the M-SH than soil collected from the M treatment. In 2013, however, SH soil had significantly higher activity in the north, when compared to soil samples taken outside of shrub influence (Figure 2.6).

β-glucosaminidase activity showed the same overall trend in the southern and central regions for both sampling years. In 2012 at the southern sites, activity was significantly higher in SH soil and M-SH. During the 2013 sampling season the shrub effect was more prominent in the southern region, with the shrub samples showing significantly higher activity than the other treatments. At the central sites in 2012, the SH and M-SH treatments showed significantly higher activities than M soil. In 2013, β-glucosaminidase activity in the central region showed no significant difference between SH and M-SH treatments. However, soil samples collected from M soil growing outside of the influence of the shrub had significantly lower activity. The 2012 samples collected in the north revealed a significant difference between soil from M-SH and M. In 2013, the shrub had a more positive effect on β-glucosaminidase activity in the north; SH samples displayed significantly higher activity than the other treatments (Figure 2.7).

Urease activity revealed less significant treatment effects compared to the other enzymes. In 2012, no significant differences among treatments were observed in the southern or northern regions. However, in the north, there was a slight increase in activity as the sampling location moved away from the shrub base. At the central sites during the

2012 sampling season, urease activity was significantly higher outside shrub influence in M soil when compared to samples collected from beneath the shrub canopy in the SH or M-SH treatments. In 2013, soil collected from M-SH showed the highest activity across all regions. This effect was significant in the northern region, where urease activity was significantly higher in M-SH soil compared to samples collected far away from the shrub in M soil (Figure 2.8).

## DISCUSSION

### Nutrient Status

Previous research in arid and semi-arid regions have documented that woody species accumulate nutrients and organic matter. This has been referred to as "islands of fertility" or "resource islands". In these environments shrubs create distinct ecosystem units that have soils with higher C and N, and improved microclimate and water availability (Schlesinger et al. 1996; Kieft et al. 1998; Van Miegroet et al. 2000). However, these studies were done in natural ecosystems, not cropped fields as is common for *G. senegalensis*. In cropped fields of the Sahel, animal traction is used for shallow tillage to prepare for soil seeding and during the growing season to remove weeds. This disturbance could have the effect of homogenizing soils and reducing the spatially variability of soil chemical properties found in non-cropped arid or semi-arid regions.

For the macro nutrients such as total N, C, and P this does not seem to be the case because across all regions during the 2012 sampling season these nutrients were significantly higher in soil beneath than outside the shrub. These results are consistent

with Dossa et al. (2010) who found both *G. senegalensis* and *P. reticulatum* had higher levels of total N, P, and C beneath shrub canopies over soil outside the influence of shrubs. However, this was only done at 1 location for each species. Our sampling was done in the main cropping region of Senegal and provides much stronger evidence that *G. senegalensis* is important in forming nutrient resource islands across the landscape, as we had north-south gradients and spatially separated replication.

This phenomenon occurs because shrubs accumulate nutrients by retrieving and concentrating nutrients beneath shrubs. This happens as roots explore soil horizontally and vertically for nutrients and water. Then through litter input, root turnover, and root exudates, nutrients would be transformed and released and stored in soils (Gathumbi et al., 2003). In Kenya, Hartemink et al. (2000) showed that the shrub *Sesbania sesban* can retrieve considerable amounts of subsoil inorganic N (mainly as $NO_3^-$). Additionally, *G. senegalensis* may harbor free living N fixing rhizo-microorganisms that further contribute N beneath shrubs.

The regional effects on the secondary macro and micronutrients showed varying trends for 2012 and 2013. For instance, Ca levels were much higher in 2012 than 2013. Cu, Fe, K, Mg, and S showed varying trends as well. These nutrients generally reported higher levels during the 2012 year for at least one of the sampling regions. This could be due to variations in rainfall observed between the two years. For instance, rainfall data collected from 2 research stations in Senegal showed that 2013 was a drier year, which could have implications on nutrient cycling. Conversely, the secondary macro and micronutrients for 2013 showed many more significant shrub treatment effects than 2012. Specifically, calcium, potassium, magnesium, sulfur, and zinc were all significantly

higher within the influence of the shrub at one or more sampling regions in 2013. The significant increase in these nutrients underneath the shrub canopy could be a further indicator of the shrubs performing hydraulic lift and driving biogeochemical processes during times of drought.

**Enzyme Activities**

Overall, enzymatic activity was lower in the northern sites when compared to the southern and central regions. Northern Senegal receives less rainfall and the soils in this region contain a higher sand content than the other two regions studied. Soils that are predominantly composed of sand are generally characterized by a low soil organic content and a low cation exchange capacity. Furthermore, sandy soils lack structural stability and are at a high risk of leaching nutrients through the soil profile (Pieri,1992; Sanchez and Logan, 1992). Indeed, Table 2.1 shows that the northern site had significantly lower total C and N than the other sites further south, which means these soils have lower organic matter and less favorable habitat for microbial communities. Also, this region has less rainfall further impeding microorganisms.

The northern sites having sandy, low organic matter soils, corresponded to lower enzyme activities. First of all this reflects on the less favorable soil environment of the northern sites for microbial growth and functioning that would result in lower production of microbial enzymes. Secondly, hydrolytic enzymes involved in decomposition of organic matter are known to be stabilized in the soil matrix which are known as abiontic enzymes. Extracellular enzymes are thought to be largely of microbial origin and are

stabilized on soil colloids and can maintain their activity for extended periods of time (Burns, 1982; Nannipieri et al., 1996). ß-glucosidase activity has a significant amount of its total activity in soil associated with the abiontic fraction as shown by Busto and Perez-Mateos (1995) and Knight and Dick (2004), where 50 and as much as 75%, respectively, of the total activity is associated with this stabilized fraction. Others have shown that a variety of enzymes including acid phosphatase (Rao et al., 1996; Nanmipieri et al., 1988; Rao et al., 2000), urease (Gianfreda et al., 1995), and others (Sarkar, 1989; Ruggiero et al., 1989; Grego et al., 1990; Lähdesmäki and Pnspasnen, 1992) can be stabilized on humic and/or clay colloids in the soil and retain much of their activity. A key factor for stabilizing enzymes would be clay and organic matter content and as these decrease there is less ability for extracellular enzymes to be protected in soils. Thus, given the sandy and low organic matter soils of the northern region, it would be expected to have less potential stabilized enzymes and this is reflected in the data.

The regional effect on enzyme activities was most pronounced for β-glucosidase, showing significantly lower activity in the northern region for both sampling years in soils with low organic matter (Table 1). This would be consistent with the observation of Hayano and Tabaki (1985) who showed it to be highly correlated with C content in soil. This likely reflects that this enzyme has a greater percentage of activity associated with the abiontic fraction than the other enzymes studied because it has less potential to be stabilized in these low clay and C content soils.

Results from the acid phosphatase and β-glucosaminidase assays had similar regional effects as β-glucosidase; activities of both enzymes were significantly lower in the northern region. However, β-glucosaminidase activity was significantly higher in the

central region. β-glucosaminidase hydrolyzes N-acetyl-β-D-glucosamine, a component of chitin, and releases inorganic N in soils. Chitin is found in the cell walls of insects and fungi and is highly correlated to soil fungal biomass (Ekenler and Tabatabai, 2002). Therefore, the higher presence of this enzyme in the central region could be an indicator of higher levels of fungal biomass at those sites. Furthermore, results from the soil chemical analysis showed significantly higher levels of ammonium in the central region in 2012, and significantly higher levels of nitrate in the central region in 2013. These increased levels of inorganic N in the central region could be due to the release of inorganic N caused by β-glucosaminidase activity.

Unlike the other enzyme activities measured, urease had similar activities in the southern and northern regions for both sampling years. However, similar to β-glucosaminidase, urease activity was significantly higher in the central region during 2012 and 2013. Urease catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide. Urea is an organic compound with a high N content (Bremner and Mulvaney, 1978). Data from the soil chemical analysis showed that total N was significantly higher in the central region during the 2012 sampling (based on one year's data).

In general, the enzyme activities measured showed lower overall activity during the 2013 sampling season compared to the 2012 sampling season, across all sampling regions. This difference could be due to variations in rainfall between the two years. Rainfall data collected from long term research sites, Keur Matar and Nioro, showed a decrease in rainfall in 2013. Nioro, located in southern Senegal received about 34% less total rainfall during the 2013 growing season. Keur Matar, located near the central sites, received 17% less rainfall in 2013. This decrease in precipitation corresponds to the

decrease in enzymatic activity observed in 2013. For instance, β-glucosidase, acid phosphatase, and β-glucosaminidase activities demonstrated the largest decrease in the southern and central regions. Furthermore, acid phosphatase activity experienced the largest decrease across all sites. Interestingly, amounts of extractable P revealed a large increase for each region during 2013. These results indicate that rainfall may have a larger impact on enzymatic activity than nutrient availability.

In most cases, the presence of *G. senegalensis* in millet fields across Senegal had a positive effect on the enzyme activities. For instance, β-glucosidase, acid phosphatase, and β-glucosaminidase activities were higher in soil samples collected within the influence of the shrub. These enzymes showed the same general trend between both sampling years, with the lowest activity observed in samples collected from millet rhizospheres far from the shrub. This enzyme response corresponded to the higher nutrient levels in soil beneath shrub canopies compared to outside the shrub, as discussed in the previous section. An increase in soil nutrients and C in soil influenced by shrubs provides elevated substrates that stimulate microorganisms to produce hydrolytic enzymes.

β-glucosidase is involved in the last step of cellulose degradation and the subsequent release of glucose molecules, which can be used as an energy source by microorganisms (Esen, 1993). This is likely in response to the litter and root inputs that provides elevated levels of cellulose to soil beneath the shrub. Furthermore, the increase in β-glucosamindase activity near the shrub indicates that the shrub rhizospheres could be harboring a higher fungal population. This is consistent with Diedhiou et al. (2009) who found *G. senegalensis* stimulates fungal biomass more so than bacterial.

Although nutrient availability plays a vital role in soil activity and nutrient cycling, the ability of *G. senegalensis* to perform hydraulic lift is an important property of these shrub systems. This hydraulic redistribution can improve soil water relations in the upper soil profile (Kizito et al., 2006, 2012). Redistribution occurs at night when stomata close, which allows water to move through roots along a water potential gradient, from the wet subsurface to the dry soil surface (Scholz et al., 2002; Kizito et al., 2012). This process helps maintain the microbial community and drives biogeochemical processes year round. Additionally, hydraulic redistribution can be vital during the growing season when periods of drought occur (Diedhiou et al., 2009, 2013). This is another mechanism that contributes greater microbial biomass and in turn greater enzyme activity, by maintaining some level of water in the rhizosphere of *G. senegalensis*, even over the 9 month dry period in Senegal (Diedhiou-Sall et al., 2013). Despite the decrease in rainfall during the 2013 season, samples collected beneath the shrub canopy displayed significantly higher amounts of β-glucosidase, β-glucosaminidase, and acid phosphatase activities than soil collected outside of the shrub. These results provide further evidence that the hydraulic redistribution associated with the shrubs is able to maintain microbial activity when rainfall is scarce.

Urease, however, exhibited a different trend between the 2012 and 2013 sampling seasons. For example, in 2012, urease activity was significantly higher in millet rhizosphere soil collected far away from the shrub canopy in the central region. Soil chemical data for the central region exhibited significantly higher levels of nitrate and slightly higher levels of ammonium under the shrub canopy. This could explain why urease activity was significantly lower underneath the shrub canopy. Urease breaks down

urea to release ammonia, which is quickly converted to ammonium in soil (Bremner and Mulvaney, 1978). If ammonium, the product of urease is present, microorganisms will shut down urease production due to feedback inhibition. It may well be that the elevated ammonium levels found beneath the shrub are suppressing urease activity.

Diakhate et al. (2016) conducted a similar experiment to compare enzyme activities in the presence and absence of shrubs in a millet field in southern Senegal. However, this study was conducted on the shrub, *P. reticulatum*. Both *G. senegalensis* and *P. reticulatum* are known to perform hydraulic lift and to have positive effects on surrounding soil chemical and physical properties (Kizito et al., 2012; Diedhiou et al., 2009). Therefore, it would be assumed that the two shrub species would have similar effects on enzymatic activity and nutrient availability. Although their results for β-glucosidase and acid phosphatase activities displayed higher activities in plots containing both shrub and millet plants, this difference was not significant. Unlike the results found in our study, Diakhate et al. found that urease activity was significantly higher when in the presence of the shrub. Furthermore, their data for total N, C, and P revealed only slightly higher levels in the presence of the shrub. Our results for these nutrients show significantly higher levels in the presence of the shrub, across almost every region. These different findings suggest that *G. senegalensis* could be more effective in stabilizing nutrients in the surrounding soil when compared to *P. reticulatum*. This difference in nutrient allocation between the two shrubs, along with difference in soil type, could explain the differing results for the observed enzyme activities.

The shrub effect was generally more significant in the southern region. For instance, β-glucosidase, acid phosphatase, and β-glucosaminidase activities were

significantly higher underneath the shrub canopy or in samples collected within the millet rooting zone soil influenced by the shrub for both sampling years. The southern region of Senegal receives the most rainfall and the soils found in this region have higher clay and organic matter content. In addition, soil nutrients such as extractable P, Cu, Fe, K, Mg, S, and Zn were higher underneath the shrub in the southern region during 2012 and 2013. These elevated nutrients may have led to an increase in microbial activity, which would explain the more significant shrub effect observed in the southern region.

## PERSPECTIVES

The results from this experiment show that the presence of *G. senegalensis* in farmers' fields has positive impacts on soil chemical properties and enzymatic activities. Enzymes play an important role in biogeochemical processing and nutrient availability in soil. Nutrient management is particularly important in arid environments where soil fertility is threatened by issues such as erosion and nutrient leaching (Pieri, 1992; Sanchez and Logan, 1992). Therefore, managing important soil nutrients such as C, N, and P is vital in order for agriculture to be sustained in arid environments such as the Sahel (Manlay et al., 2002). The ability of *G. senegalensis* to stabilize important soil nutrients, and presumably harbor larger and more active microbial communities, is crucial for nutrient availability. The ability to sustain a more active microbial community

leads to the production of more enzymes that are able to breakdown and mineralize compounds that would otherwise be unavailable for plant uptake.

As previous studies have shown, these shrub systems are capable of stabilizing crop yields, while maintaining the soil environment (Dossa 2012; 2013). Our results have shown that *G. senegalensis* is effective in promoting the cycling of N, C, and P in the soil environment. This could be one of the contributing factors to the increased crop yields that are often observed in shrub intercropping systems. The shrubs are locally available and therefore offer a practical means to increase crop yields and SOM in the arid environment of Senegal. However, in order for these shrub systems to be fully effective, coppice and burn techniques need to be discouraged.

## TABLES

Table 2.1 Soil chemical properties by region in 2012 and 2013.

| Region | pH | Total N | Total C | Mehlich Extractable P | $NO_3^-$ | $NH_4^+$ |
|---|---|---|---|---|---|---|
| | | ---------- % ---------- | | ---------- ---mg kg $^{-1}$ ---------- | | |
| | | | 2012 | | | |
| South | 5.65 ab | 0.022 b | 0.291 a | 11.1 b | 0.606 a | 7.47 b |
| Central | 5.57 b | 0.026 a | 0.238 b | 11.4 ab | 0.318 b | 9.51 a |
| North | 5.68 a | 0.011 c | 0.137 c | 13.3 a | 0.355 b | 7.73 b |
| | | | 2013 | | | |
| South | ND | ND | ND | 72.7 a | 2.12 b | 5.18 a |
| Central | ND | ND | ND | 82.4 a | 3.80 a | 4.43 b |
| North | ND | ND | ND | 59.1 b | 2.37 b | 2.91 c |

Means followed by the same letter are not significant using a general linear model univariate analysis of variance (alpha = 0.05)

Table 2. 2 Mehlich extractable nutrients by region in 2012 and 2013.

| Region | K | Mg | Ca | S | Cu | Fe | Zn |
|---|---|---|---|---|---|---|---|
| | ---------- | ---------- | ---------- | mg kg $^{-1}$ | ---------- | ---------- | ---------- |
| | | | | 2012 | | | |
| South | 24.2 b | 49.1 a | 417 c | 2.66 c | 0.226 b | 33.9 a | 0.513 b |
| Central | 42.6 a | 47.1 a | 790 b | 3.45 b | 0.381 ab | 30.4 ab | 1.53 a |
| North | 22.6 b | 27.7 b | 1170 a | 4.94 a | 0.704 a | 27.3 b | 0.763 b |
| | | | | 2013 | | | |
| South | 26.2 b | 39.6 b | 185 b | 3.08 a | 0.236 a | 29.6 b | 0.381 b |
| Central | 37.7 a | 60.6 a | 268 a | 2.49 b | 0.183 b | 33.7 a | 0.546 a |
| North | 26.8 b | 32.4 b | 121 c | 1.66 c | 0.153 c | 21.3 c | 0.295 b |

Means followed by the same letter are not significant using a general linear model univariate analysis of variance (alpha = 0.05).

Table 2.3 Soil chemical properties by treatment in 2012.

| Region | Treatment | pH | Total N | Total C | Mehlich Extractable P | $NO_3^-$ | $NH_4^+$ |
|---|---|---|---|---|---|---|---|
| | | | % | | | mg $kg^{-1}$ | |
| South | SH | 5.58 b | 0.024 a | 0.322 a | 13.7 a | 0.661 a | 8.93 a |
| | M-SH | 5.63 b | 0.023 a | 0.301 a | 11.0 b | 0.538 a | 7.26 b |
| | M | 5.73 a | 0.018 b | 0.251 b | 9.37 b | 0.619 a | 6.25 b |
| Central | SH | 5.47 a | 0.027 a | 0.259 a | 13.3 a | 0.423 a | 9.72 a |
| | M-SH | 5.58 a | 0.027 a | 0.241 a | 10.2 b | 0.282 ab | 9.72 a |
| | M | 5.67 a | 0.024 b | 0.215 b | 9.78 b | 0.248 b | 9.09 a |
| North | SH | 5.56 b | 0.015 a | 0.176 a | 13.1 a | 0.220 a | 7.21 a |
| | M-SH | 5.73 a | 0.011 b | 0.131 b | 12.9 a | 0.482 a | 8.00 a |
| | M | 5.75 a | 0.008 c | 0.104 c | 13.7 a | 0.361 a | 7.76 a |

Means followed by the same letter are not significant using a general linear model univariate analysis of variance (alpha = 0.05).

Table 2. 4 Soil chemical properties by treatment in 2013.

| Region | Treatment | Mehlich Extractable P | $NO_3^-$ | $NH_4^+$ |
|---|---|---|---|---|
| | | ----------mg $kg^{-1}$---------- | | |
| South | SH | 10.7 a | 2.37 ab | 5.41 a |
| | M-SH | 8.61 b | 2.50 a | 5.08 a |
| | M | 7.47 c | 1.50 b | 5.05 a |
| Central | SH | 11.6 a | 5.44 a | 4.41 a |
| | M-SH | 9.45 ab | 3.54 b | 4.69 a |
| | M | 7.37 b | 2.41 b | 4.19 a |
| North | SH | 6.87 a | 3.13 a | 3.08 a |
| | M-SH | 5.85 a | 2.27 b | 2.83 a |
| | M | 5.33 a | 1.71 b | 2.83 a |

Means followed by the same letter are not significant using a general linear model univariate analysis of variance (alpha = 0.05).

Table 2. 5 Mehlich extractable nutrients by treatment in 2012.

| Region | Treatment | K | Mg | Ca | S | Cu | Fe | Zn |
|---|---|---|---|---|---|---|---|---|
| | | ---------- mg kg $^{-1}$ ---------- | | | | | | |
| South | SH | 27.0 a | 55.8 a | 427 a | 5.45 a | 0.240 a | 35.8 a | 0.68 a |
| | M-SH | 24.0 a | 47.7 a | 388 a | 4.66 a | 0.217 a | 33.4 a | 0.46 a |
| | M | 21.5 a | 43.8 a | 437 a | 4.70 a | 0.222 a | 32.6 a | 0.41 a |
| Central | SH | 44.1 a | 41.3 a | 780 a | 3.49 a | 0.299 a | 34.1 a | 1.55 a |
| | M-SH | 41.1 a | 48.8 a | 790 a | 3.38 a | 0.412 a | 28.6 a | 1.87 a |
| | M | 42.7 a | 47.7 a | 802 a | 3.47 a | 0.432 a | 28.7 a | 1.17 a |
| North | SH | 22.9 a | 25.8 a | 1160 a | 2.65 ab | 1.47 a | 27.5 a | 0.701 a |
| | M-SH | 22.6 a | 28.7 a | 1170 a | 2.88 a | 0.33 a | 27.7 a | 0.884 a |
| | M | 22.7 a | 28.6 a | 1180 a | 2.47 b | 0.31 a | 26.7 a | 0.704 a |

Means followed by the same letter are not significant using a general linear model univariate analysis of variance (alpha = 0.05).

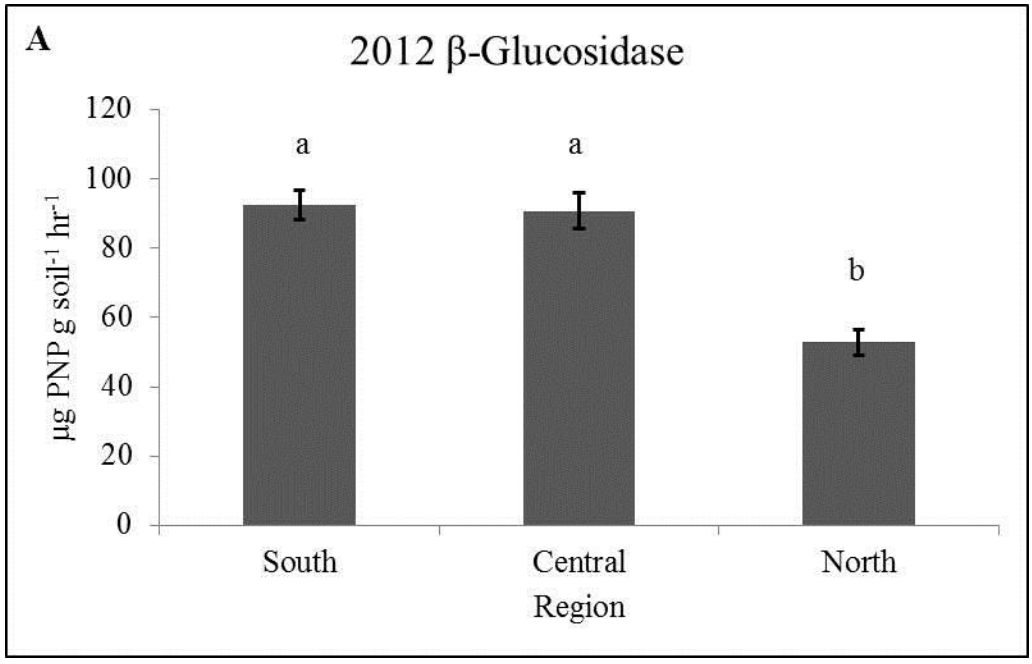
Table 2. 6 Mehlich extractable nutrients by treatment in 2013.

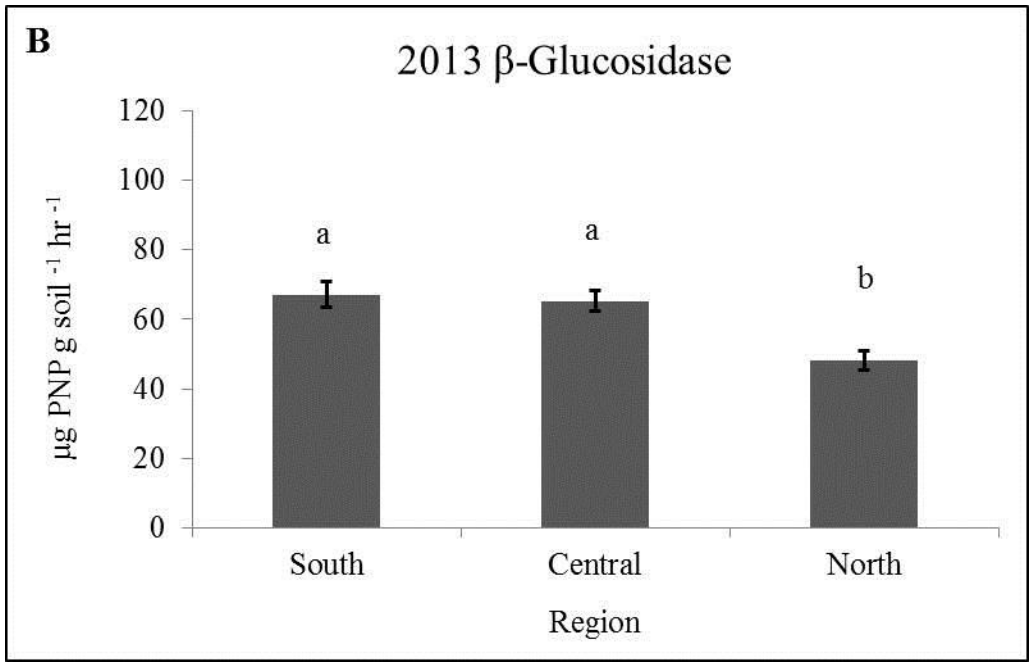
| Region | Treatment | K | Mg | Ca | S | Cu | Fe | Zn |
|---|---|---|---|---|---|---|---|---|
| | | ---------- mg $kg^{-1}$ ---------- | | | | | | |
| South | SH | 33.8 a | 39.9 ab | 192 ab | 3.60 a | 0.241 a | 32.1 a | 0.441 a |
| | M-SH | 27.8 a | 45.3 a | 205 a | 2.83 b | 0.237 a | 28.2 a | 0.385 a |
| | M | 16.8 b | 33.7 b | 160 b | 2.82 b | 0.229 a | 28.6 a | 0.315 a |
| Central | SH | 41.8 a | 56.0 a | 249 a | 2.98 a | 0.171 a | 34.8 a | 0.641 a |
| | M-SH | 36.2 a | 57.8 a | 244 a | 2.32 b | 0.181 a | 33.9 a | 0.582 a |
| | M | 35.1 a | 68.1 a | 301 a | 2.18 b | 0.196 a | 32.4 a | 0.416 a |
| North | SH | 27.6 a | 34.9 a | 136 a | 2.03 a | 0.158 a | 23.2 a | 0.337 a |
| | M-SH | 32.0 a | 33.6 a | 118 ab | 1.60 a | 0.153 a | 20.2 a | 0.304 a |
| | M | 20.9 b | 28.4 a | 108 b | 1.33 c | 0.147 a | 20.4 a | 0.243 b |

Means followed by the same letter are not significant using a general linear model univariate analysis of variance (alpha = 0.05).

## FIGURES

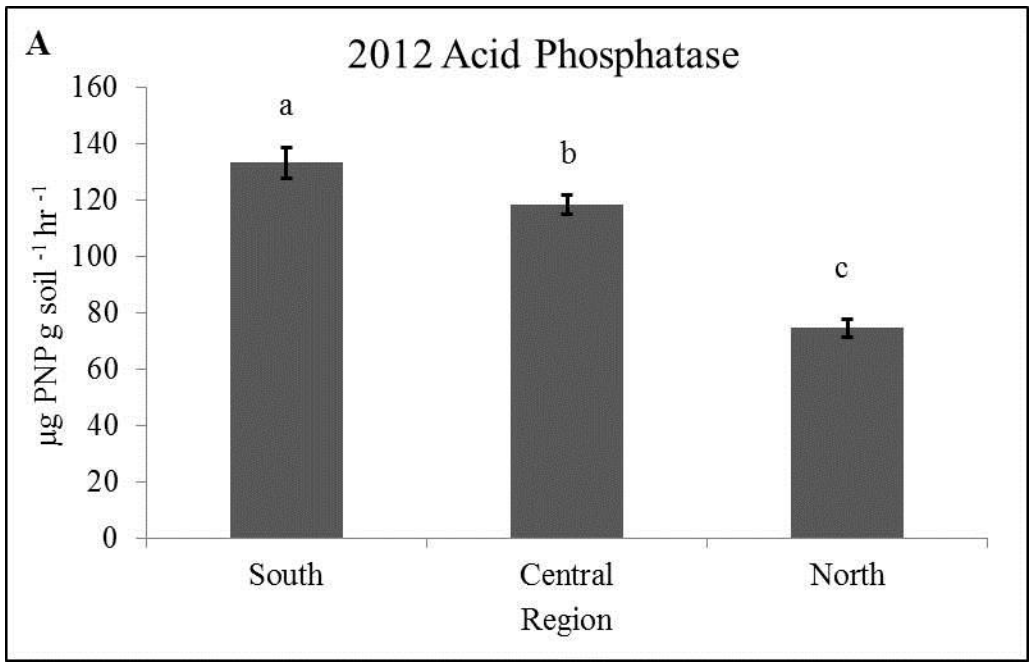
Figure 2.1 β-glucosidase activity by in 2012 and 2013.

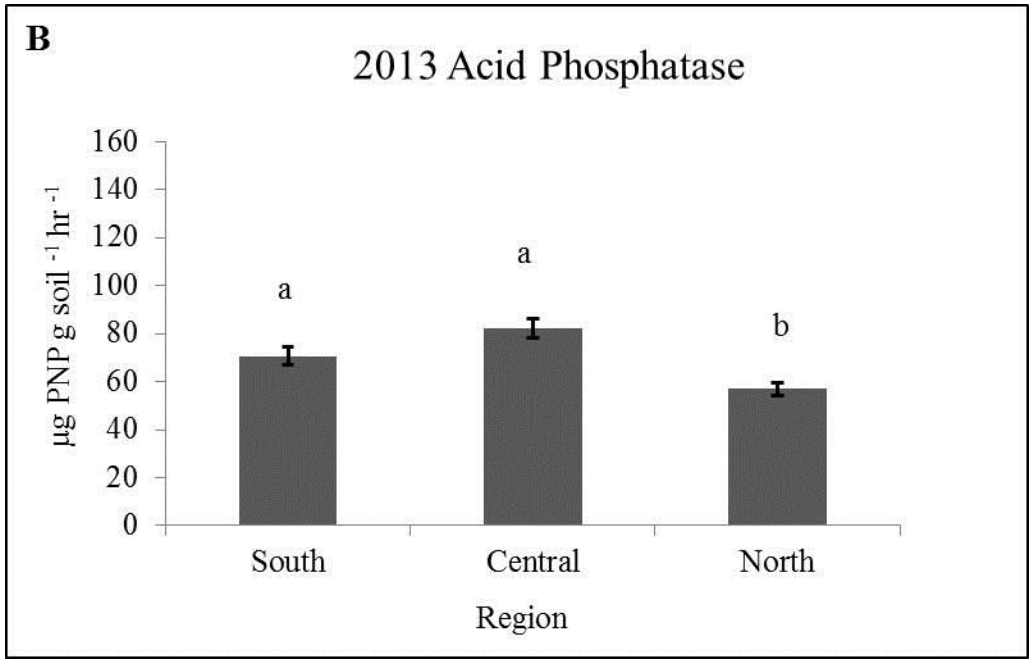



Different letters indicate significant differences between each region using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean (n = 60).

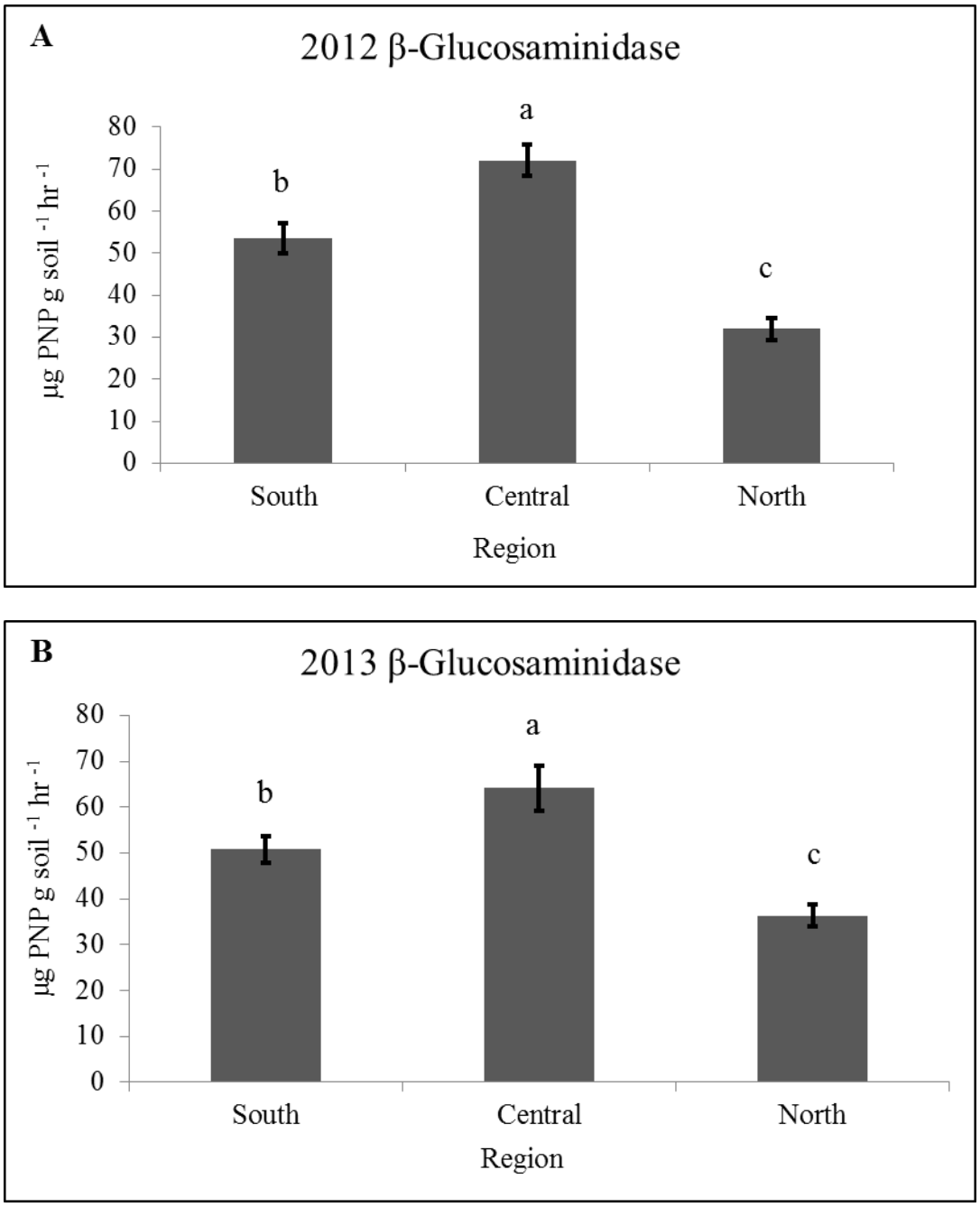
Figure 2.2 Acid phosphatase activity by region in 2012 and 2013.



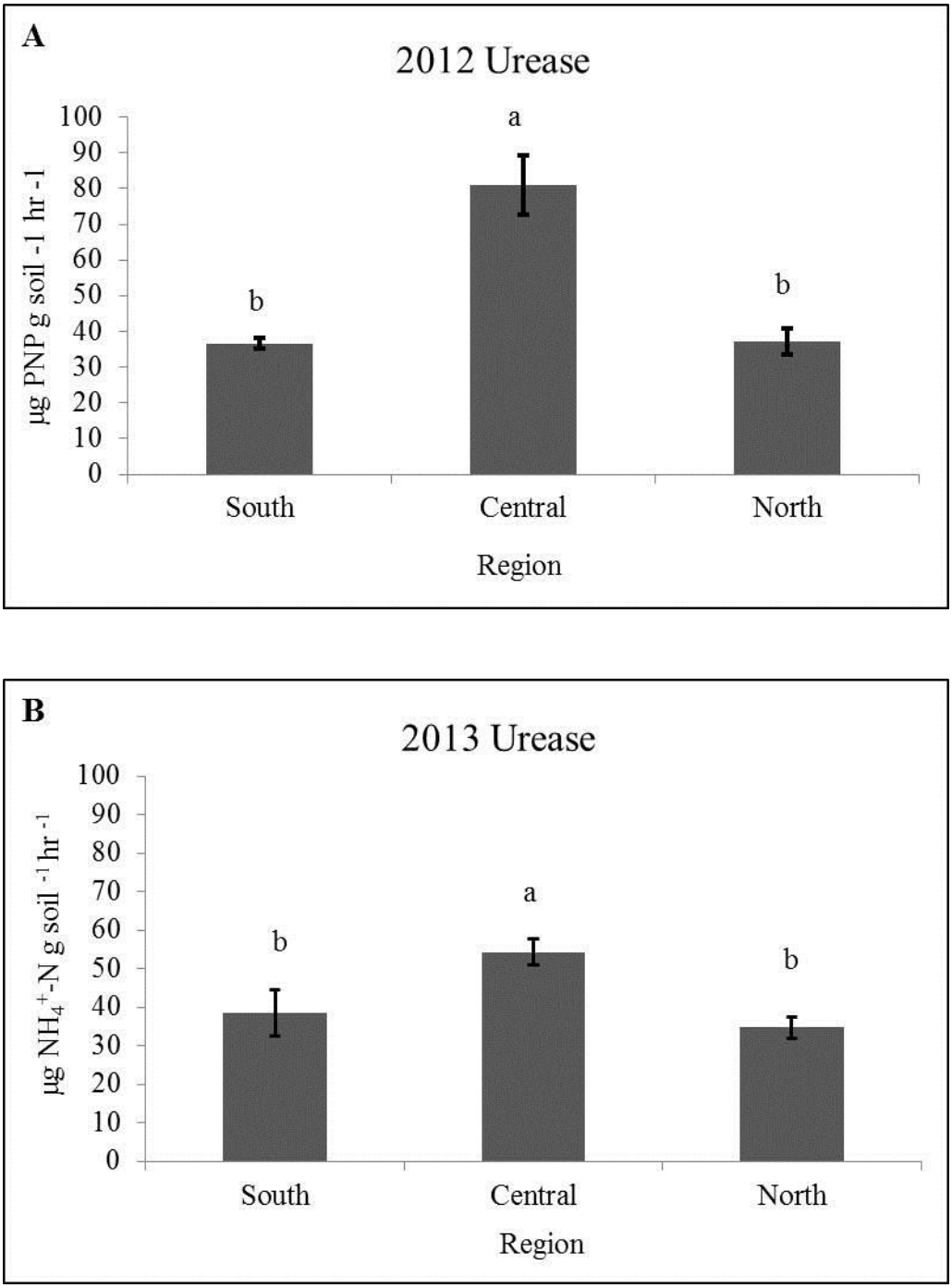

Different letters indicate significant differences between each region using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean (n = 60).

Figure 2. 3 β-glucosaminidase activity by region in 2012 and 2013.



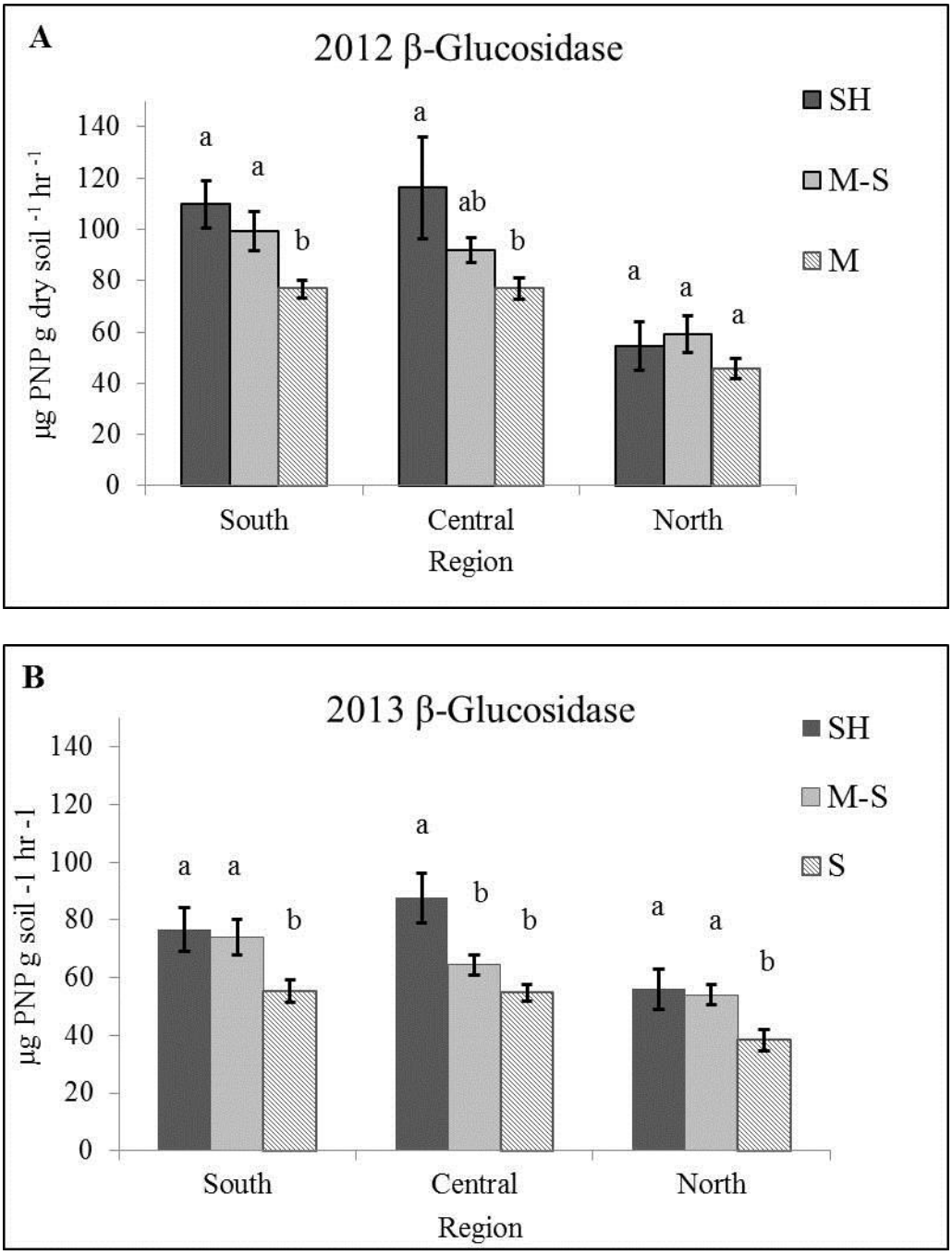

Different letters indicate significant differences between each region using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean (n = 60).

Figure 2.4 Urease activity by region in 2012 and 2013.



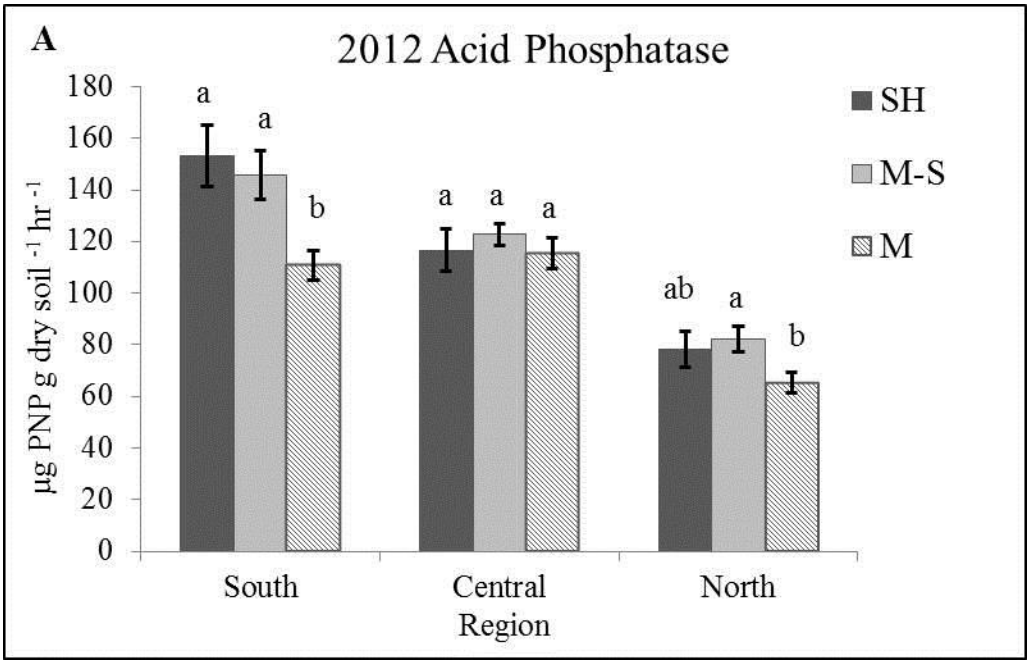

Different letters indicate significant differences between each region using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean (n = 60).

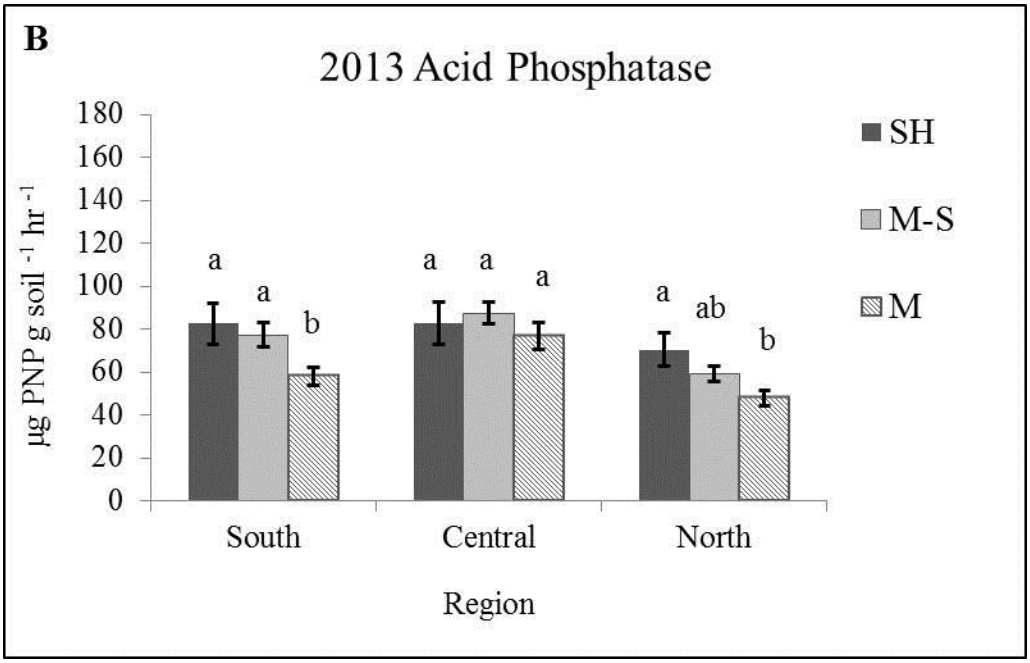
Figure 2.5 Treatment response of β-glucosidase activity in 2012 and 2013.




Different letters indicate significant differences between sampling locations relative to the shrub using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean. For each region, replication is as follows: shrub treatment (SH): n = 12, near shrub treatment (M-S): n = 24, far from shrub treatment (S): n = 24.

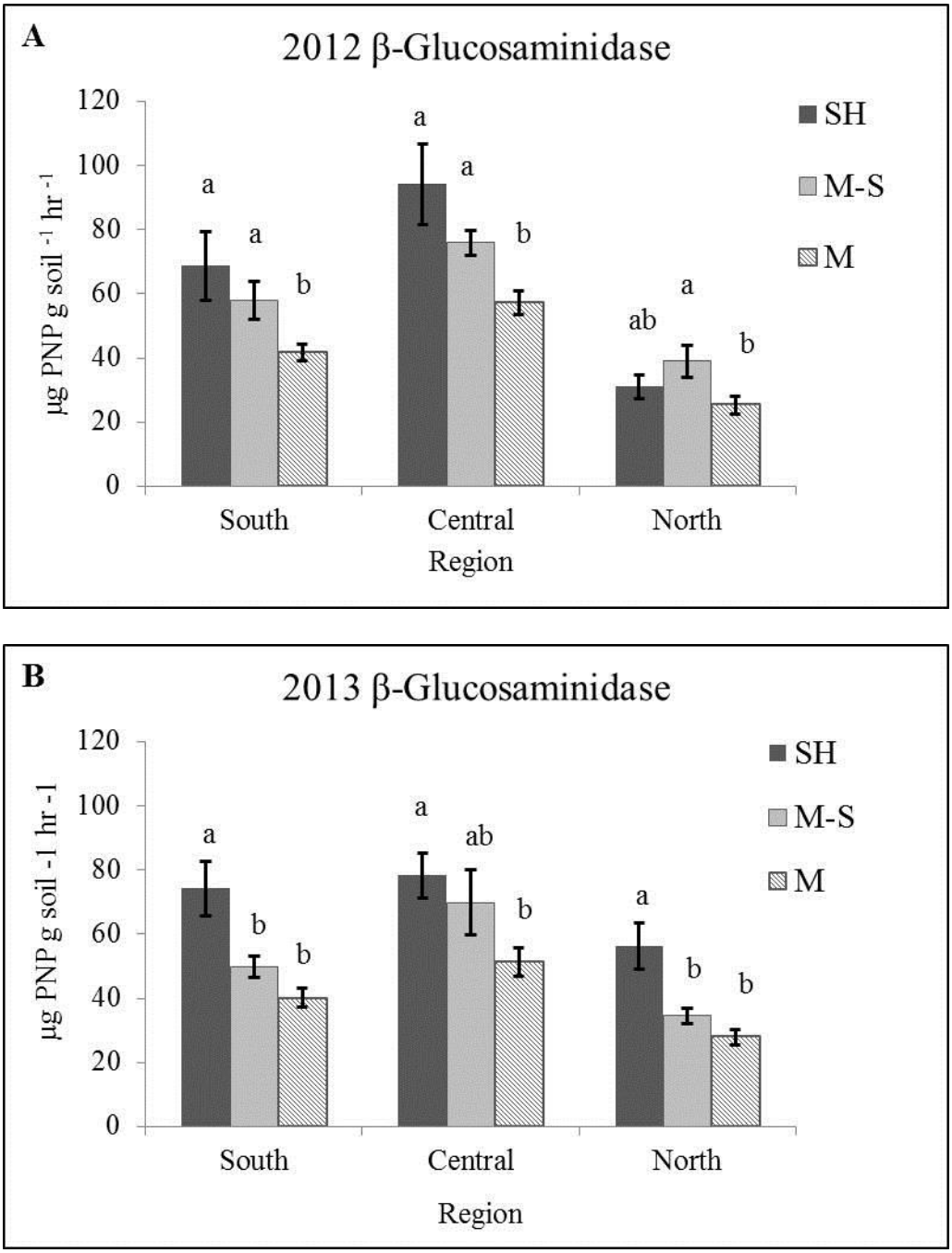
Figure 2.6 Treatment response of acid phosphatase activity in 2012 and 2013.



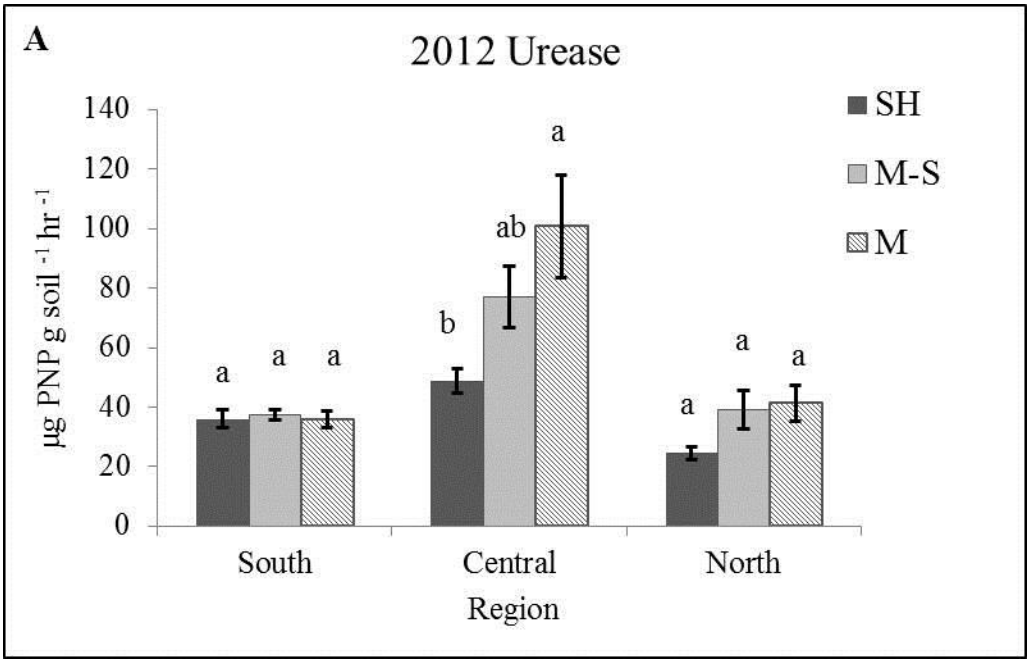

Different letters indicate significant differences between sampling locations relative to the shrub using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean. For each region, replication is as follows: shrub treatment (SH): n = 12, near shrub treatment (M-S): n = 24, far from shrub treatment (S): n = 24.

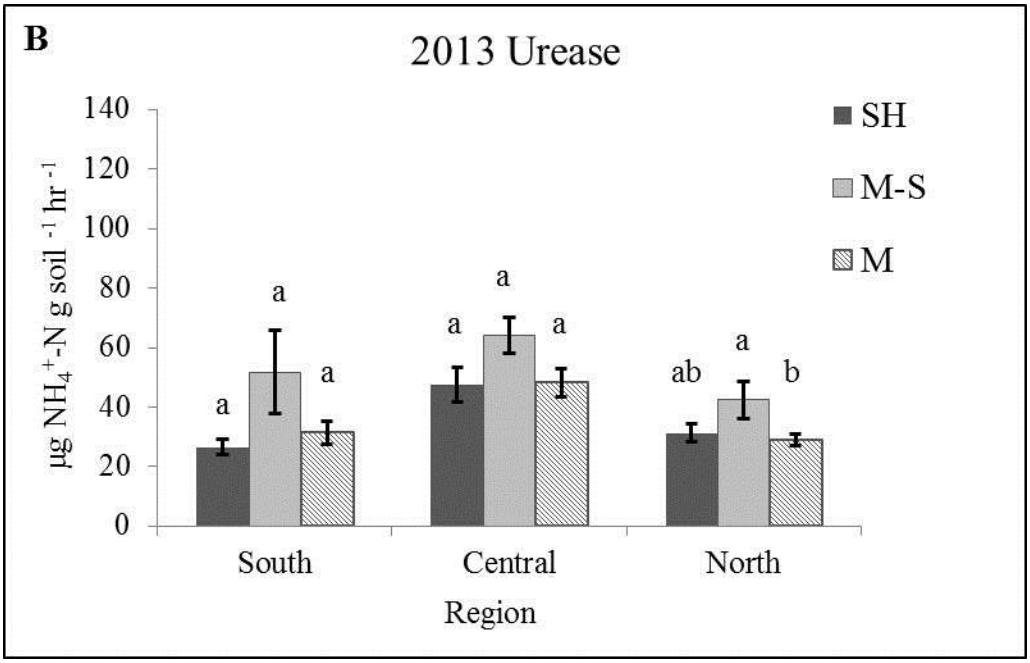
Figure 2.7 Treatment response of β-glucosaminidase activity in 2012 and 2013.




Different letters indicate significant differences between sampling locations relative to the shrub using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean. For each region, replication is as follows: shrub treatment (SH): n = 12, near shrub treatment (M-S): n = 24, far from shrub treatment (S): n = 24.

Figure 2.8 Treatment response of urease activity in 2012 and 2013.




Different letters indicate significant differences between sampling locations relative to the shrub using a general linear model univariate analysis of variance (alpha = 0.05). Vertical bars represent ± standard error mean. For each region, replication is as follows: shrub treatment (SH): n = 12, near shrub treatment (M-S): n = 24, far from shrub treatment (S): n = 24.

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# CHAPTER 3: NITROGEN DYNAMICS OF SHRUB-MILLET SYSTEMS IN SENEGAL

## ABSTRACT

Unsustainable agricultural practices, along with inherently low quality soil, have led to a drastic decrease in soil quality in the Sahel. Of particular concern is the depletion of N from these systems. Studies have reported that local woody shrub species, *Guiera senegalensis* and *Piliostigma reticulatum* significantly increase soil nutrients and are known to perform hydraulic redistribution, helping to provide water to nearby crops in times of drought. In addition, *G. senegalensis* has been shown to drastically increase crop yields and N uptake when intercropped with millet, even in the absence of fertilizer. The increase in N uptake suggests that the shrub may harbor N fixing microorganisms, which could be a contributing factor to the increase in crop yield. Consequently, the objective of this research was to investigate the ability of *G. senegalensis* and *P. reticulatum* to stimulate N fixation when intercropped with millet. The study took place at two long term research sites in Senegal, where soil was collected from millet plus shrub plots and sole millet plots. Sampling occurred three times at each site, and incubations were set up after each sampling to determine $^{15}N$ incorporation. Samples collected from the shrub plots exhibited higher amounts of incorporation over the no shrub plots, suggesting that the shrubs are harboring a more active diazotroph community. However, only samples collected from underneath *P. reticulatum* in October 2013 showed a statistically significant increase in $^{15}N$ incorporation compared to the other treatments. Samples collected from within the influence of both shrub species showed greater amounts of incorporation during the dry season sampling when compared to samples collected from

no shrub plots. These results indicate that hydraulic redistribution is sustaining microbial processes during times of drought.

# INTRODUCTION

Nitrogen (N) is an essential nutrient for life on earth. It accounts for almost 80% of the Earth's atmosphere and is the fourth most abundant element in living biomass. However, most N is in the form of $N_2$ gas, a relatively stable structure. Therefore, the availability of N can present major limitations on plant growth in terrestrial ecosystems (Myrold, 2005). The main sources of plant available N are from the mineralization of organic matter, biological N fixation, and external inputs (Giller et al., 1997). Conventional agriculture relies heavily upon the use of external inputs in order to manage N deficiencies in cropping systems (Oberson et al., 2007). However, due to the high cost of fertilizer and lack of infrastructure required for transportation, average fertilizer rates in sub-Saharan Africa are less than 10 kg $ha^{-1}$ (Sanchez, 2002; Vanlauwe et al., 2011). Fortunately, microbial driven processes can fix $N_2$ gas and convert it to forms more readily available to plants (Moir, 2011).

Biological $N_2$ fixation (BNF) is a microbial mediated process found only in prokaryotes that converts atmospheric dinitrogen gas into a form more easily utilized by plants (Singh et al., 2010; Peoples and Craswell, 1992). Specifically, BNF is the conversion of $N_2$ gas into $NH_3$ by specific enzymes, such as nitrogenase (Singh et al., 2010). Symbiotic N-fixation, such as with legumes and *Rhizobia*, can serve as a major source of N in certain cropping systems. In this relationship, *Rhizobia*, infect root hair of leguminous plants and form nodules. The nodules then serve as areas where *Rhizobia* can proliferate. The *Rhizobia* are able to use plant nutrients for energy and fixing N, while the

bacteria supply the host plant with available N fixed from the atmosphere (Sanchez et al., 2011). Nodulated legumes have been utilized to supplement crop yields for centuries (Sanginga 2003; Bohlool, 1992). However, it is important to note that diazotrophs can develop associations with non-leguminous plants, leading to similar amounts of N being fixed (Singh et al., 2010). For instance, *Azospirillium*, in association with plant roots, can fix atmospheric $N_2$ (Steenhoudt and Vanderleyden, 2000). Sugarcane is one of the few known non-leguminous field crops that have been reported to obtain a substantial amount of total plant N from atmospheric $N_2$ through BNF (Boddey et al., 1995; Reinhold-Hurek and Hurek, 2011). For many years, only a limited number of organisms were thought to fix N. However, research has shown that many different phyla in both bacteria and archaea are capable of BNF (Young, 1992). This process can be very valuable in low input agricultural systems, such as the ones often found in the Sahel.

Nitrogen depletion in Africa is a serious issue due to large amounts of nutrients being removed from the soil without adding sufficient amounts of manure or fertilizer to replenish the soil (Sanchez, 2002). For instance, in African soils, 4.2 million tons of N are lost per year, but only 0.8 million tons are reapplied by fertilization (Hai et al., 2009). However, in the Sahel, agroforestry using woody shrub species has been shown to enhance soil quality by adding organic matter and nutrients through litter input, root exudates, and root turnover (Gathumbi et al., 2003). *Guiera senegalensis* and *Piliostigma reticulatum* are native shrubs commonly found in farmers' fields throughout the Sahel that are known to improve soil properties (Diedhiou et al., 2009). Studies have shown evidence of these shrubs performing hydraulic redistribution. This refers to the movement of water through tap roots to surface roots caused by a water potential gradient (Caldwell

et al., 1998). The increase in water availability and nutrients caused by these shrub systems has been shown to sustain a larger and more diverse microbial community (Diedhiou et al., 2009; Debenport et al., 2015). Microorganisms associated with shrub rhizospheres could be providing beneficial functions, such as N fixation, that could improve crop growth.

Indeed, *G. senegalensis* and *P. reticulatum* have been shown to increase yields when intercropped with millet (*Pennisetum glaucum*) or peanut (*Arachis hypogeaea*) (Diack et al., 2000; Kizito et al., 2006, Dossa et al., 2012). Dossa et al. (2012) demonstrated that millet grown in the presence of *G. senegalensis* significantly increased crop yields over 4 growing seasons when compared to no shrub plots. This yield response was observed in plots containing zero fertilizer as well. Furthermore, nutrient uptake was significantly impacted as well in plots receiving zero fertilizer, with the shrub plots taking up 12.1 kg N $ha^{-1}$ and the no shrub plots only taking up 4.3 kg N $ha^{-1}$. These results suggest that *G. senegalensis* rhizospheres are harboring a larger amount of free living $N_2$ fixers.

The ability of these shrubs to stimulate N fixation could have substantial impacts on the amount of plant available N around the shrub. Most subsistence farmers in Senegal cannot afford fertilizer, so intercropping with shrubs could provide an alternative method to manage N in agricultural fields (Sanchez, 2002). Results from Dossa et al. (2012) strongly indicate that the shrubs are capable of promoting $N_2$ fixation. Increases in microbial diversity associated with shrub rhizospheres further suggest that the shrubs could be hosting a greater amount of diazotrophs. Therefore, a primary objective of this

study was to investigate if the presence of *G. senegalensis* and *P. reticulatum* can stimulate N fixation in millet fields in Senegal

## MATERIALS AND METHODS

### Site Description

The study was conducted at two long term research sites that have been under management since 2003. During the dry season of 2003, all shrubs were removed to establish no-shrub plots. Shrub plots were established by randomly, but evenly planting shrub seedlings. At each site, there were four replicate blocks, measuring 46 m X 11 m each. Within each block there were 8 subplots, each measuring 10 m X 6 m. There was a 3-m gap between blocks, and a 2-m gap between adjacent plots. Millet was planted on all plots in the summer of 2004 and fertilized with 68.5 kg N, 15 kg P, and 15 kg K $ha^{-1}$ to allow the plots to equilibrate for one year before the start of the experiment. All plots were subjected to a peanut-millet crop rotation from 2004 to 2013, representative of the dominant agricultural system used in the region.

The first study site, Keur Matar, is located in the northern region of the Peanut Basin (N 14° 45’ 00”, W 16° 51’ 00”) and has a mean annual precipitation of 450 mm. The mean annual minimum temperature is 20°C and the mean annual maximum temperature is 33°C. The dominant shrub species at Keur Matar is *G. senegalensis*, with a density of 888 to 1555 shrubs $ha^{-1}$ (Kizito et al., 2006). The site lies on a Dior soil (Rubic arenosol with 95% sand) (FAO, 2006).

The second site, Nioro du Rip, is located in the southern region (N 13° 45' 00", W 15° 47' 00" W) of the Peanut Basin and has a mean annual precipitation of 750 mm. The mean annual minimum and maximum temperatures are 20°C and 37.5°C, respectively. The dominant shrub at Nioro du Rip is *P. reticulatum*, with a density of 1500 - 1833 shrubs $ha^{-1}$ (Kizito et al., 2006). The site lies on a Deck-Dior soil (fine sandy, mixed Haplic Ferric Lixisol with 87% sand) (FAO, 2006 and Diack et al., 2000).

**Experimental Design and Soil Sampling**

Sampling at Keur Matar and Nioro followed a randomized complete block design. Only non-fertilized plots containing shrub and no-shrub were sampled across the four blocks. Five soil cores of 2.5 cm diameter (0 – 30 cm depth) were collected from under the shrub canopy (SH) and placed into a single bag to form a composite sample. Similarly, a millet plant growing within the influence of the shrub (<1 meter from shrub base) was sampled by taking soil cores from the millet rhizosphere zone (M-SH) to form a composite sample. The shrub and millet sampling described above was replicated twice per shrub plot. In the no-shrub plots, 2 millet plants were sampled by taking 2.5 cm diameter soil cores from the millet rhizosphere zones (M). Sampling occurred once during the growing season of 2013, once at the end of the 2013 growing season and once in the middle of the dry season of 2014 at both Keur Matar and Nioro. Since the millet plants had already been harvested during the December 2013 sampling for both sites, soil was not sampled from millet rhizospheres. Rather, the soil cores were taken from bulk soil less than a meter from the shrub base for the 'M-SH' samples. The 'M' samples

were soil cores taken from bulk soil in the no shrub plots. The same sampling method was utilized for the March 2014 samplings at each site, which took place in the middle of the dry season. Samples were brought back to the laboratory the day of sampling, and homogenized and passed through a 4-mm sieve and stored at 4º C until analysis.

### $^{15}$N Incubation

Incubations were set up the day after sampling. 20 g of each soil sample was placed in a 60 mL bottle and brought to 2/3 field capacity. Samples were placed under modified ambient atmosphere concentrations of 20% $O_2$ and 80% $N_2$. In order to account for isotopic fractionation of N, each soil sample was split, with half receiving labelled $^{15}N_2$ and the other half receiving unlabeled $N_2$. Samples were incubated horizontally at 30º C in the dark for 30 days. After incubation, samples were air dried and $\delta\ ^{15}N / ^{14}N$ [‰] was measured using the Delta V Advantage Isotopic Ratio Mass Spectrometer.

To determine $\%^{15}N$ incorporation in each sample, the following equations were used based on Moore-Kucera and Dick (2008):

Equation 1: $$R = \left(\left(\delta \frac{^{15}N}{^{14}N} \Big/ 1000\right) + 1\right) \times R_{air}$$

Where,

$R_{air}$ = 0.0036762

Equation 1 is used to convert the delta values to $^{15}N/^{14}N$ (R).

Equation 2: $F = {}^{R}/_{(R+1)}$

Equation 2 is used to calculate the fraction of $^{15}N$ before incubation ($F_{t0}$) and after incubation ($F_{tx}$).

Equation 3: $\%^{15}\text{N} = \left( (F_{tx} - F_{t0}) * \left( {}^{N_i}/_{15\ \text{N}_{added}} \right) \right) \times 100$

Where,

$N_i$ is the initial amount of N in the sample (μg N $kg^{-1}$ soil)

$N_{added}$ is the amount of $N_2$ added to the sample (μg $N_2$ $kg^{-1}$ soil)

Equation 3 is used to calculate the amount of $^{15}N$% incorporated by subtracting the fraction of $^{15}N$ before the incubation (using the control sample receiving unlabeled $^{14}N$) from the fraction of $^{15}N$ after the incubation (using the duplicate sample receiving the labeled $^{15}N$). The difference is then multiplied by the ratio of the initial amount of N in the sample to the amount of $^{15}N$ added.

**Soil Chemical Characteristics**

Soil pH was determined using a glass membrane electrode in a 1:2 ratio of soil to water. Total soil C and N were measured using Carlos Erba Elemental Analyzer 1108. The Mehlich 3 method was used to measure extractable P and secondary macro and micronutrients. The extraction protocol is as follows; 2 g of air-dried soil was mixed with 20 mL of Mehlich 3 extracting solution. Samples were shaken for 5 minutes and immediately filtered through a 0.45 μm filter (Mehlich, 1984). Total soil P, K, Mg, S, Ca,

Cu, Fe, and Zn were determined using an Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES).

Ammonium and nitrate were determined in 1 *M* KCl soil extracts (ADD RATIO). After filtration through a glass fiber filter, ammonium-N and nitrate-N were determined by the salicylate-nitroprusside and the hydrazine-sulfaniliamide methods, respectively (Mulvaney, 1996).

### Statistical Analysis

All statistical tests were performed using SAS 9.4 (SAS Institute, Inc., 2013). Differences in treatments were examined using analysis of variance using a mixed model. Pairwise comparisons were calculated using Tukey's honestly significant difference (HSD) comparisons based on a 95% confidence interval.

## RESULTS

### Soil chemical analyses

***Nioro*** Total N and C were both significantly higher in the SH treatment during the December 2013 sampling. Extractable P levels were always slightly higher in the SH treatment for all 3 sampling dates. This difference was significant during the March 2014 sampling, with significantly higher extractable P levels for SH when compared to the M-SH treatment. During the October and December 2013 sampling, there was a significant increase in nitrate levels for SH samples. In December 2013, $NO_3^-$ levels were also significantly higher in M-SH when compared to the M treatment. Although not

significant, $NO_3^-$ was higher in the SH and M-SH treatments during the March 2014 sampling. In October 2013, $NH_4^+$ levels were significantly higher in M samples when compared to SH. Conversely, $NH_4^+$ was slightly higher in the SH treatment during the December 2013 and March 2014 sampling. There was no significant treatment effect on soil pH (Table 3.1).

For all three sampling dates, Ca and Cu levels were significantly higher in the SH treatment. Iron, on the other hand was significantly higher in M samples during the October 2013 and March 2014 samplings, and slightly higher in December 2013. Potassium and Mg showed similar trends; both nutrients were significantly higher in SH and M-SH in October 2013 and March 2014. However, during the December 2013, K and Mg were significantly in SH samples. The only significant interaction observed for S levels was during the March 2014 sampling date. Sulfur was significantly higher for SH when compared to M-SH. The other sampling dates revealed no treatment effect on S levels. The December 2013 sampling revealed that Zn was significantly for SH. However, for the other two sampling dates, there was only a slight increase in Zn for SH (Table 3.2).

***Keur Matar*** Total N and C were significantly higher for SH during the October 2013 sampling than the other two treatments. No significant interactions were observed for extractable P levels during any of the sampling dates. Nitrate levels were significantly higher in the SH treatment for all sampling dates. Ammonium levels, however, were significantly higher in M samples in October 2013. On the contrary, $NH_4^+$ was significantly higher in SH during the December 2013 sampling. Although not significant,

$NH_4^+$ was higher in SH and M-SH during the March 2014 sampling. Soil pH was slightly lower in SH and M-SH samples (Table 3.3).

Calcium, Cu, Mg, and Zn levels were all significantly in SH during December 2013. Although not significant, Ca, Mg, and Zn were also in SH during the March 2014 sampling. Furthermore, there was no observed treatment effect on Cu during this sampling date. The only significant treatment effect observed in the nutrient analysis for the March 2014 sampling was for K; levels were significantly higher in SH and M-SH. Potassium was slightly higher in these treatments in the December 2013 samples as well. For either sampling date, there was no significant treatment effect observed for S or Fe. However, there was a small increase in Fe levels in the M treatment (Table 3.4).

## $^{15}$N Incubation

***Nioro*** The October 2013 incubation revealed a significant effect of sampling location on %$^{15}$N incorporation; SH treatment was significantly higher than M. Soil samples collected for the SH treatment showed a larger amount of $^{15}$N incorporation when compared to M. Although not significant, the December 2013 and March 2014 incubation results revealed similar trends with SH showing the highest amount of $^{15}$N incorporation. In contrast to the October 2013 incubation, the lowest amount of $^{15}$N incorporation for the December 2013 incubation was found in M-SH. However, for the Mach 2014 incubation, the lowest amount of $^{15}$N incorporation was shown in the M treatment (Figure 3.1). Table 3.5 shows that *P. reticulatum* is estimated to add more kg N ha-1 through biological N fixation, when compared to *G. senegalensis*.

***Keur Matar*** All the incubations that were set up using soil collected from the Keur Matar site showed lower amounts of $^{15}N$ incorporation compared to the Nioro incubations. Furthermore, no significant interactions were revealed for these incubations. However, the incubations set up during October 2013 and March 2014 showed a similar trend, with the highest incorporation in the SH treatment, and the lowest in the M treatment. Conversely, the December 2013 incubation revealed the highest amount of $^{15}N$ incorporation M-SH, and the lowest for SH (Figure 3.2).

## DISCUSSION

Soil collected from Nioro for the $^{15}N$ incubations exhibited higher overall amounts of $^{15}N$ incorporation for each sampling period when compared to Keur Matar. However, determining why this difference is occurring is confounded by major differences in soil type, climate, and shrub species.

From a climate and soil type perspective, Nioro has more favorable conditions to support microbial communities. The Nioro soil has 87% sand content, while Keur Matar soil is 95% sand (FAO, 2006). Which in turn, Nioro's greater clay content has higher organic matter, as reflected in the higher total C and N (Table 3.1). This provides the Nioro soil with more potential to sequester C and nutrients and better habitat because it has more clay and greater aggregation. Additionally, climate is more favorable at Nioro because it receives about 40% more rainfall per year than Keur Matar (Kizito et al.,

2006). Also, the rainy season starts sooner and ends later than at Keur Matar, which likely has a large impact on the soil microbial communities, a community that likely harbors a larger diazotroph population.

Another biotic factor is the difference in shrub species present at each site; *G. senegalensis* is the shrub located at Keur Matar, while *P. reticulatum* is present at Nioro. One difference is the biomass production of each species, which is important because litter inputs, root turnover, and root architecture will affect the size and composition of microbial communities. In extensive studies of both species in farmers' fields, Lufafa et al. (2008) found that *G. Senegalensis* averaged 33% more above ground biomass than *P. reticulatum*. Conversely, average belowground root biomass was 36% greater for *P reticulatum* over *G. Senegalensis*. Other factors are root exudates that would also affect microbial communities. There is no clear interpretation to separate the shrub effects from climate and soil type.

As expected there was an effect of season on $^{15}N_2$ fixation. The October 2013 sampling occurred during the rainy growing season and soil was collected from millet and shrub rhizospheres. However, during the December and November 2013 samplings, the millet had already been harvested. Therefore, soil was not collected from the millet rhizospheres, but from bulk soil located near and far from the shrub canopy. This was also done for the March 2014 sampling, which occurred in the middle of the dry season. This is important because the millet rhizosphere effect has a large impact on the communities present, which likely impacts the amount of $^{15}N$ incorporated during the incubations. For instance, the amount of $\%^{15}N$ incorporated was highest in samples collected from millet rhizospheres within shrub influence during the Nioro October 2013

sampling. However, for the November 2013 and March 2014 sampling dates, the shrub samples displayed the highest amount of $\%^{15}N$ incorporated. This is probably because the millet rhizospheres growing near the shrub are harboring a larger population of diazotrophs when compared to the bulk soil near the shrub.

The relative amounts of $NO_3^-$ and $NH_4^+$ for each sampling date at Nioro and Keur Matar indicates that nitrification slows down after the October 2013 sampling as the soil becomes drier. For instance, levels of $NH_4^+$ increase as the soil dries, while $NO_3^-$ levels decrease. This suggests that the nitrifying community is slowing down as the soil dries, and the amount of $NH_4^+$ oxidized to $NO_3^-$ is decreased. Amounts of $NO_3^-$ are higher under the shrub canopy for every sampling at both sites. This difference is significant every time except the March 2014 sampling at Nioro. These results suggest that the shrub rhizospheres are harboring a more active nitrifying community than the surrounding bulk soil.

Soil chemical properties are improved by shrub presence as well. For instance, total N and C are significantly higher under the shrub canopy for Keur Matar and Nioro. During the dry season at Nioro, extractable P was positively affected by the presence *of P. reticulatum*. Furthermore, *P. reticulatum* had a significant impact on all of the micronutrients measured for at least one of the sampling dates. *G. senegalensis* had a similar effect on micronutrients as well, except iron was not significantly impacted by shrub presence. While S was significantly higher underneath *P. reticulatum*, S levels were unaffected by the presence of *G. senegalensis*. These results show that the litter and root exudates from both shrub species have a positive impact on nutrient levels in the surrounding soil. This increase in available nutrients allows the shrub rhizospheres to

sustain larger and more diverse microbial communities than the surrounding bulk soil. This phenomenon was demonstrated by a study conducted by Debenport et al. (2015). They found an increase in microbial diversity in plots containing the shrubs at both Keur Matar and Nioro. Similarly, PLFA results from a different study found higher microbial diversity in soil amended with residues from both shrub species as well (Diedhiou et al., 2009). Additionally, data from the $^{15}N$ incubation suggests that the enhanced chemical properties associated with these shrub systems are allowing for a larger and more active community of diazotrophs.

The $^{15}N$ incubation using soil from the Nioro October 2013 sampling exhibited a significant treatment effect. The soil samples collected near the shrub had the most $^{15}N$ incorporation, while the samples collected far from the shrub had a significantly lower amount of incorporation. The results from the incubations using soil from the November 2013 sampling and March 2014 displayed the highest amount of $^{15}N$ incorporation in the shrub samples. However, the difference was not significant, which was due to high variability. This could be due to the heterogeneous nature of the soil during times of drought. Hydraulic redistribution by *P. reticulatum* could be creating spatial heterogeneity, where certain microenvironments contain a more active population capable of fixing N.

The incubations using soil from Keur Matar didn't show any significant treatment effects. However, the lowest amount of $^{15}N$ incorporation was always found in samples collected far from the canopy of *G. senegalensis*. Similar to the Nioro results, soil from the December 2013 and March 2014 sampling showed a large amount of variation in $^{15}N$ incorporation, across all treatment types. Keur Matar's higher sand content is not able to

support as active of a community as the soil at Nioro. Furthermore, Keur Matar receives less rainfall and it is often erratic. Both of these factors are likely contributing to the lower amount of N fixation occurring at Keur Matar.

During the dry season, biogeochemical processes generally slow down. However, in this study dry season incubations had the highest amount of $^{15}N$ incorporation underneath the shrub canopy for both Keur Matar and Nioro. These results indicate that microbial populations capable of performing N fixation are sustained throughout the dry season. This is likely due to hydraulic redistribution which has been confirmed for both *G. senegalensis* and *P. reticulatum* (Kizito et al., 2007; Kizito et al., 2012). In addition, nutrients and organic C are higher in soil beneath the shrub canopy which contributes to supporting the microbial communities. Also, at this time crop is senescing which would be a rich substrate that may also contribute to the elevated N fixation rates.

Results from both Keur Matar and Nioro exhibited the highest rate of incorporation in samples collected within the influence of the shrub, suggesting that both shrub species are harboring a larger population of N fixing bacteria. The $\%^{15}N$ data from the incubation experiments can be extrapolated out to estimate amounts of N fixation brought on by the microbial communities associated with these shrub systems. Specifically, using the results from the October 2013 sampling, the amount of N fixed per hectare can be estimated to predict how much N these shrub systems can add during the growing season. This can be done by taking into account sampling depth, shrub stand density, diameter of the shrub, length of incubation, and $\%^{15}N$ incorporation. Lufafa et al. (2008) did a survey across the major dryland cropping region of Senegal and found that the shrub density for *G. senegalensis* ranged from 228 – 409 shrubs $ha^{-1}$ in farmers' fields

and 134 – 288 shrubs $ha^{-1}$ for *P. reticulatum*. Lufafa et al. (2009) also determined that the average canopy diameter of *G. senegalensis* and *P. reticulatum* was 226.2 and 187.7 cm, respectively. Using these numbers, 9 to 20 kg N $ha^{-1}$ is being fixed in soil beneath *P. reticulatum* over a 3 month growing period. On the other hand, only about 0.05 – 0.09 kg N $ha^{-1}$ would be fixed by the bacterial communities associated with *G. senegalensis*. However, the shrub densities at Nioro and Keur Matar are 1500-1833 and 888-1555 shrubs $ha^{-1}$, respectively. Using these more optimized shrub densities, it is estimated that *P. reticulatum* has the potential to add 108 - 132 kg N $ha^{-1}$ over the course of 3 months during the growing season, while *G. senegalensis* has the potential to add 0.61 – 1.08 kg N $ha^{-1}$.

According to FAO, the total average fertilizer use in Senegal in 2013 was only about 11 kg per hectare of arable land, while the United States' average fertilizer use was about 132 kg per hectare (F.A.O., 2006). Nitrogen is generally the most limiting nutrient for plant growth, and therefore biological nitrogen fixation (BNF) can be a valuable process, particularly in agricultural systems that are low input (Wild, 1988). Subsistence farmers in the Sahel can rarely afford chemical fertilizers to increase crop yields (Sanchez, 2002; Vanlauwe et al., 2011). Therefore, BNF is very valuable in these low input systems.

The amount of plant available N that these shrub systems could add to the soil during the growing season could have substantial impacts on crop growth and yields. Based off the results found in the $^{15}N$ incubation, *P. reticulatum* has greater potential to increase levels of plant available N. *P. reticulatum* is more dominant in southern Senegal, where soils are of better quality and more precipitation. Therefore, the better

quality soil that is generally associated with *P. reticulatum* is expected to host a more active and diverse microbial community.

However, the relatively high rates of N fixation for soil beneath *P. reticulatum* must be interpreted with caution. Despite the high variability within each shrub species the relative consistency in the range of values between soils from each species, lends confidence to the data generated under the conditions of the incubation. Stock et al. (1995) conducted a study investigating nutrient cycling affected by *Acacia* species through a $^{15}N$ field incubation method. Similar to our results, they obtained $\delta^{15}N$ values that showed a lot of variation between and within treatments. However, the $\delta^{15}N$ values from their study were overall lower than the $\delta^{15}N$ shown in our data. Our results likely overestimate N fixation because the incubations were performed under optimal conditions in a lab setting. Therefore, these conditions may not be fully representative of field conditions. Another enigma is that the yield response to shrubs at the long-term sites (the places where soils were obtained for the $^{15}N_2$ fixation incubations) is very significant for the northern Kuer Matar site which has *G. senegalensis* (Dossa et al., 2012) but less so and mixed at the southern Nioro site which has *P. reticulatum* (Dossa et al., 2013). Thus the site where shrubs increase crops the most (even in the absence of any fertilizer) actually had very low rates of N fixation. Of course many factors go into yield response and no doubt *G. senegalensis* helps crops disproportionately at this site because the soil is sandy and the climate is drier. So that organic inputs and hydraulic lift by *G. senegalensis* are major factors in driving the crop response.

Clearly more studies are needed to determine rates of N fixation by these shrub intercrop systems and to parse out the factors that contribute to the crop response

conferred by shrubs. Ideally $^{15}N$ studies should be done in situ, at more sites and that with plants to determine actual uptake of fixed N.

# CONCLUSIONS

Shrubs in farmers' fields throughout the Sahel have been shown to increase crop yields when intercropped with pearl millet (*Pennisetum glaucum*) and groundnut (*Arachis hypogaea*). Specifically, *Guiera senegalensis* and *Piliostigma reticulatum* are woody shrubs found in Senegal and throughout much of the Sahel that can significantly increase millet yields. Both shrubs are capable of performing hydraulic redistribution, the movement of water from tap roots to surface roots that is caused by a water potential gradient from wet subsoil to the dry soil surface. Water availability if a major limiting factor for crop growth in the Sahel so this process can reduce water stress for crops during times of drought. Furthermore, litter inputs and root exudates from the shrubs have positive impacts on organic matter and nutrients in the surrounding soil. The increase in organic matter and nutrients associated with these shrub systems, along with hydraulic redistribution, are thought to harbor larger and more diverse microbial communities than the surrounding soil. Larger and more active microbial communities will drive biogeochemical processes, which in turn can have substantial impacts on crop yields.

Results from this study provide evidence that these shrub systems harbor a more active microbial community that drives biogeochemical processes. For example, β-glucosidase,

acid phosphatase, and β-glucosaminidase activities were significantly higher in the presence of *G. senegalensis* when compared to soil taken outside of shrub influence. The other study conducted in this project provided evidence that these shrub systems are capable of driving biogeochemical processes as well. For instance, rates of biological N fixation were greater in the presence of both *G. senegalensis* and *P. reticulatum*, suggesting that the shrub rhizospheres are harboring a larger population of diazotrophs. However, the increase was only significant in the presence of *P. reticulatum*. Additionally, rates of fixation were maintained throughout the dry season, indicating that hydraulic redistribution could be sustaining biogeochemical processes in times of drought. N is generally the major limiting crop nutrient. Therefore, biological N fixation could be a valuable process in low input agricultural systems.

It is also important to note that total N and total C were significantly higher in the presence of *G. senegalensis* and *P. reticulatum* across all sampling dates and locations. Phosphorus and other nutrients were positively affected by shrub presence as well. These results provide further evidence that these shrub systems could be very valuable in agricultural systems where fertilizers are rarely used. However, in order for these systems to be fully effective, coppice and burn practices need to be stopped and shrub litter needs to be left on the soil surface.

## TABLES

Table 3. 1 Soil chemical properties at Nioro.

| Treatment | pH | Total N | Total C | Mehlich Extractable P | $NO3^-$ | $NH4^+$ |
|---|---|---|---|---|---|---|
| | | ---------- %--------- | | ---------- mg $kg^{-1}$---------- | | |
| | | | October 2013 Sampling | | | |
| SH | 5.34 a | 0.029 a | 0.321 a | 10.8 a | 6.89 a | 0.428 b |
| M-SH | 5.30 a | 0.022 b | 0.278 b | 8.99 a | 4.85 b | 0.578 ab |
| M | 5.33 a | 0.022 b | 0.269 b | 9.91 a | 3.51 b | 0.707 a |
| | | | December 2013 Sampling | | | |
| SH | ND | ND | ND | 12.8 a | 3.58 a | 1.83 a |
| M-SH | ND | ND | ND | 11.2 a | 3.53 a | 1.53 a |
| M | ND | ND | ND | 11.2 a | 1.30 b | 1.60 a |
| | | | March 2014 Sampling | | | |
| SH | ND | ND | ND | 13.6 a | 2.58 a | 2.17 a |
| M-SH | ND | ND | ND | 10.1 b | 2.14 a | 1.51 a |
| M | ND | ND | ND | 11.7 ab | 1.04 a | 0.952 a |

For one given sampling date, means followed by the same letter are not significant using a mixed model univariate analysis of variance (alpha = 0.05).

Table 3. 2 Mehlich extractable nutrients at Nioro.

| Treatment | K | Mg | Ca | S | Cu | Fe | Zn |
|---|---|---|---|---|---|---|---|
| | ---------- mg kg $^{-1}$ ---------- | | | | | | |
| | | | | October 2013 Sampling | | | |
| SH | 43.9 a | 49.0 a | 233 a | 3.85 a | 0.390 a | 19.4 c | 0.729 a |
| M-SH | 33.9 b | 42.5 ab | 194 bc | 3.62 a | 0.331 bc | 21.0 b | 0.570 a |
| M | 19.6 c | 31.4 c | 160 c | 3.77 a | 0.318 c | 22.5 a | 0.557 a |
| | | | | December 2013 Sampling | | | |
| SH | 23.8 b | 34.6 b | 68.2 a | 5.67 a | 0.451 a | 20.1 a | 0.834 a |
| M-SH | 67.3 a | 47.7 a | 67.3 a | 6.29 a | 0.367 b | 20.3 a | 0.670 ab |
| M | 47.8 a | 55.1 a | 28.8 b | 6.04 a | 0.374 b | 22.3 a | 0.462 b |
| | | | | March 2014 Sampling | | | |
| SH | 57.7 a | 53.8 a | 259 a | 4.37 a | 0.468 a | 21.3 b | 0.627 a |
| M-SH | 44.4 a | 58.1 a | 252 a | 3.84 b | 0.409 ab | 22.4 b | 0.622 a |
| M | 19.9 b | 30.8 b | 154 b | 4.26 ab | 0.386 b | 25.3 a | 0.274 a |

Means followed by the same letter are not significant using a mixed model univariate analysis of variance (alpha = 0.05).

Table 3. 3 Soil chemical properties at Keur Matar.

| Treatment | pH | Total N | Total C | Mehlich Extractable P | $NO3^-$ | $NH4^+$ |
|---|---|---|---|---|---|---|
| | | ---------- % ---------- | | ---------- mg $kg^{-1}$ ---------- | | |
| | | | October 2013 Sampling | | | |
| SH | 4.94 a | 0.030 a | 0.370 a | ND | 7.13 a | 3.40 b |
| M-SH | 4.97 a | 0.021 c | 0.233 bc | ND | 6.33 a | 3.19 b |
| M | 5.07 a | 0.021 bc | 0.248 b | ND | 2.63 b | 4.21 a |
| | | | December 2013 Sampling | | | |
| SH | ND | ND | ND | 3.08 a | 4.27 a | 1.70 a |
| M-SH | ND | ND | ND | 3.16 a | 2.76 b | 1.23 b |
| M | ND | ND | ND | 3.08 a | 0.517 c | 1.06 b |
| | | | March 2014 Sampling | | | |
| SH | ND | ND | ND | 26.9 a | 2.90 a | 0.905 a |
| M-SH | ND | ND | ND | 26.2 a | 2.24 ab | 0.355 a |
| M | ND | ND | ND | 26.4 a | 0.561 b | 0.075 a |

Means followed by the same letter are not significant using a mixed model univariate analysis of variance (alpha = 0.05).

Table 3. 4 Extractable nutrients for Keur Matar.

| Treatment | K | Mg | Ca | S | Cu | Fe | Zn |
|---|---|---|---|---|---|---|---|
| | | ---------- mg kg $^{-1}$ ---------- | | | | | |
| | | December 2013 Sampling | | | | | |
| SH | 20.0 a | 40.8 a | 314 a | 5.82 a | 0.33 a | 43.4 a | 1.27 a |
| M-SH | 19.2 a | 27.6 b | 133 b | 4.39 a | 0.33 a | 43.7 a | 0.50 b |
| M | 13.2 a | 14.4 c | 93.2 b | 4.69 a | 0.28 b | 47.9 a | 0.45 b |
| | | March 2014 Sampling | | | | | |
| SH | 26.9 a | 43.75 a | 275 a | 4.83 a | 0.24 a | 38.9 a | 1.03 a |
| M-SH | 27.7 a | 31.03 a | 142 a | 4.35 a | 0.24 a | 38.7 a | 0.60 a |
| M | 16.0 b | 19.28 a | 106 a | 4.49 a | 0.25 a | 39.6 a | 0.30 a |

Means followed by the same letter are not significant using a mixed model univariate analysis of variance (alpha = 0.05).

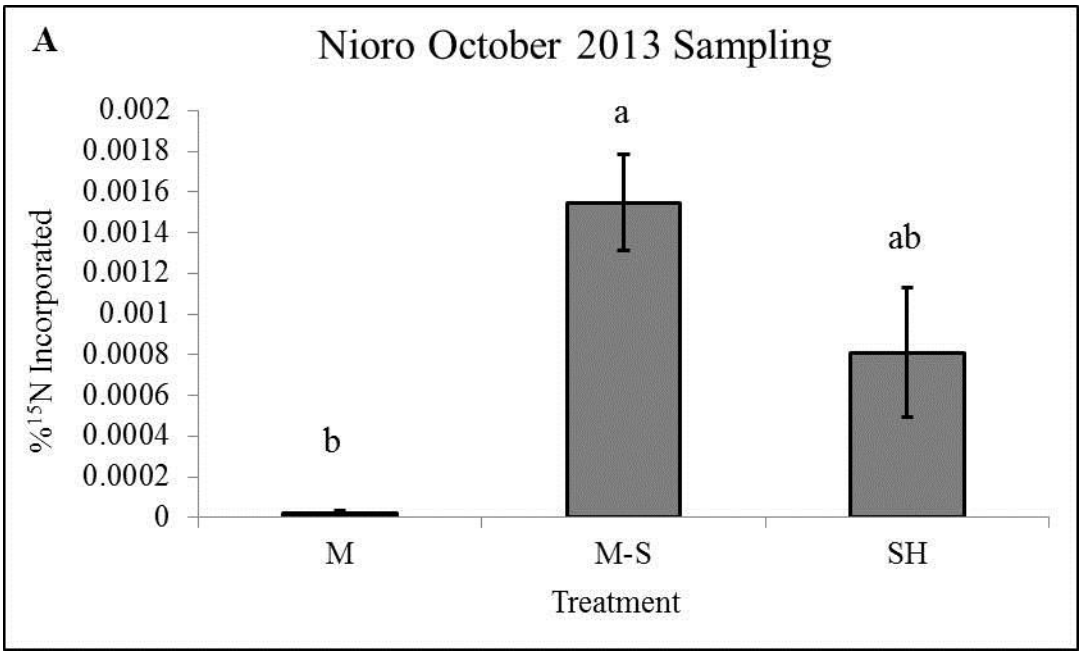


Table 3. 5 Estimated N added by the shrub systems through BNF.

| Shrub | Shrub Density | N added |
|---|---|---|
| | shrubs ha$^{-1}$ | kg N ha$^{-1}$ |
| *G. senegalensis* | 228 – 409 | 0.05 - 0.09 |
| *P. reticulatum* | 134 – 288 | 9 - 20 |

## FIGURES

Figure 3. 1. $^{15}N$ incubation results for Nioro.



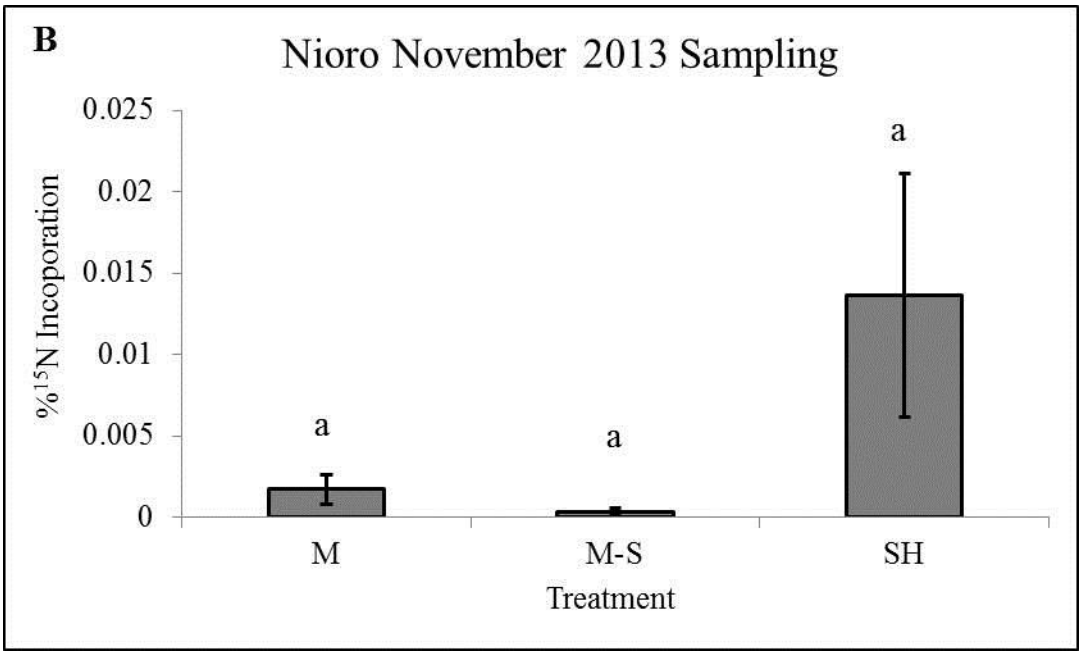



Figure 3. 2. $^{15}N$ incubation results for Nioro.

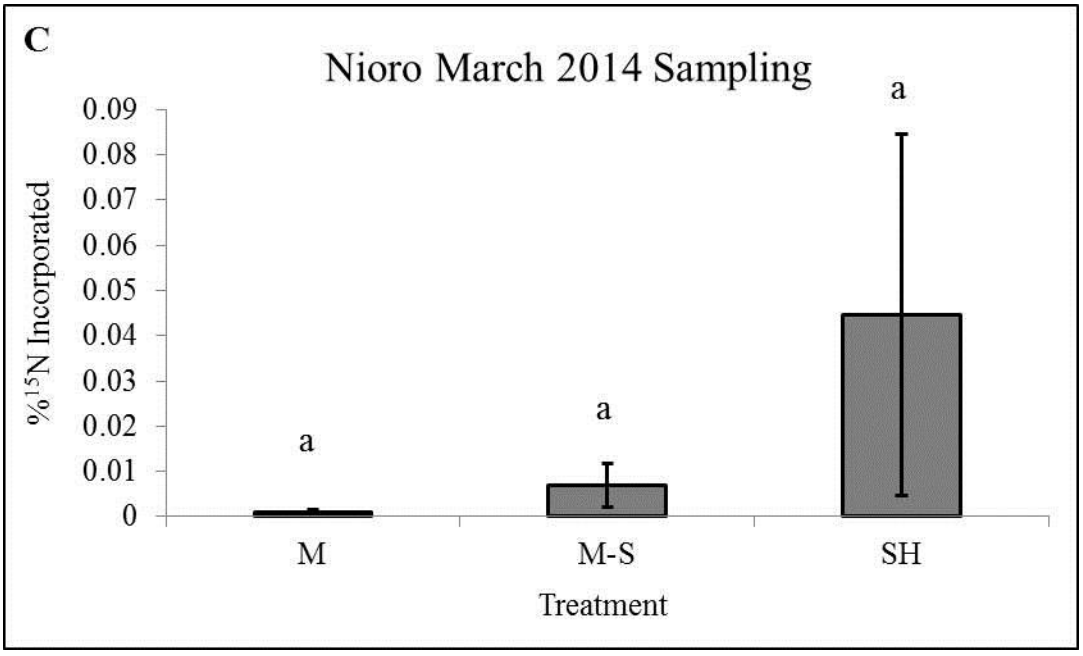
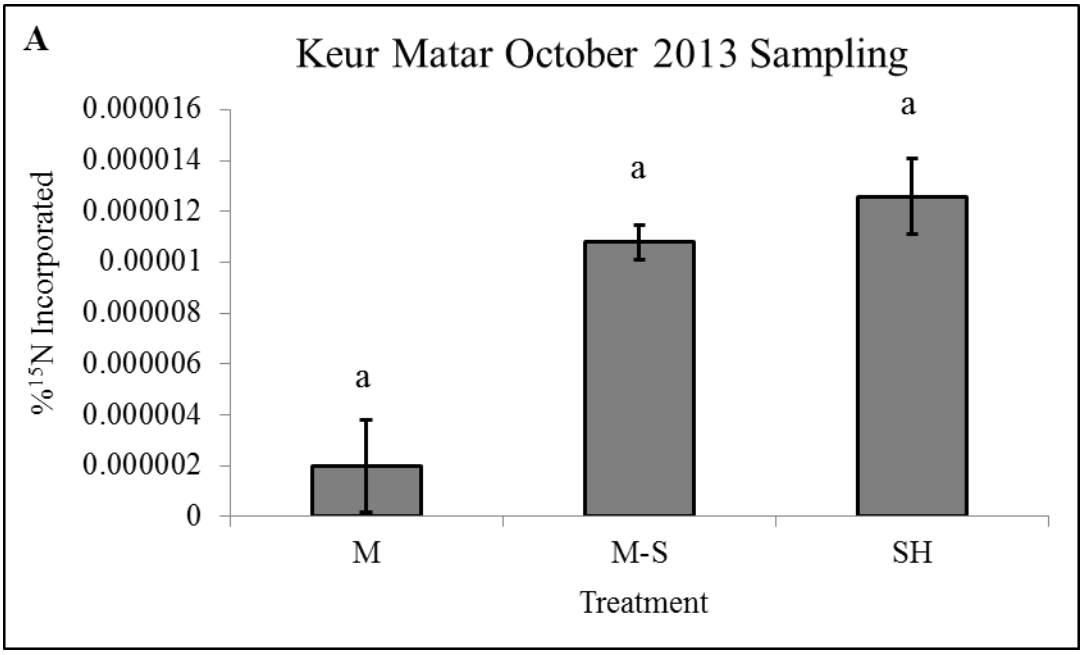



Figure A is during the rainy growing season, Figure B is at the end of the growing season, and Figure C is during the dry season. Columns with different letters indicate a significant difference between treatments (alpha = 0.05). Vertical bars represent ± standard error mean (n = 8).

Figure 3. 3 $^{15}N$ incubation results for Keur Matar.



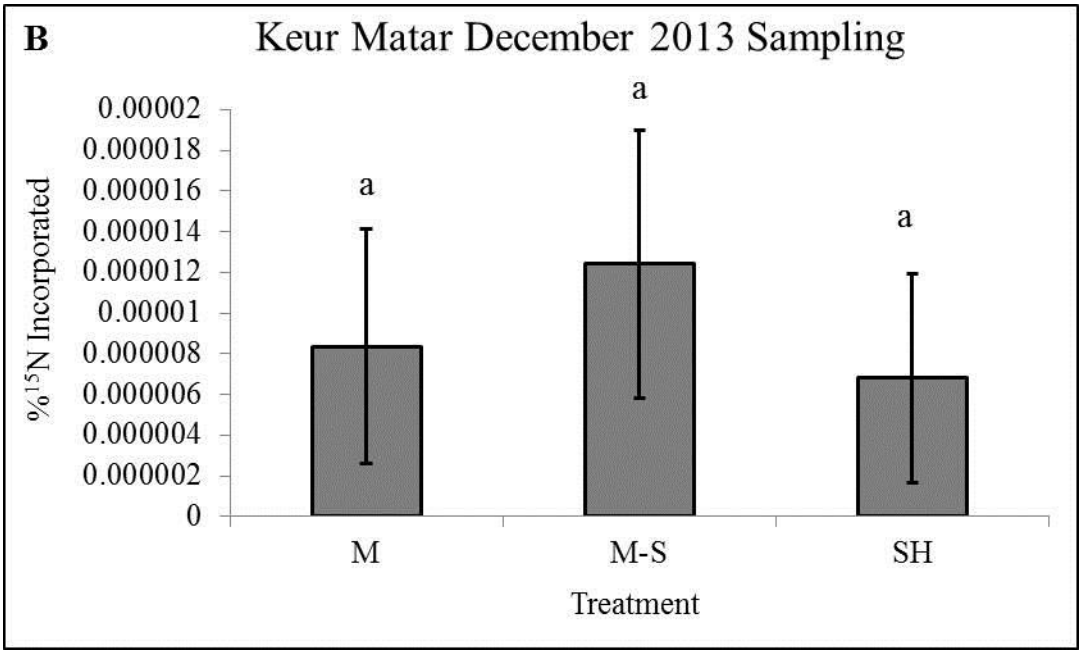



Figure 3. 4 $^{15}$N incubation results for Keur Matar.

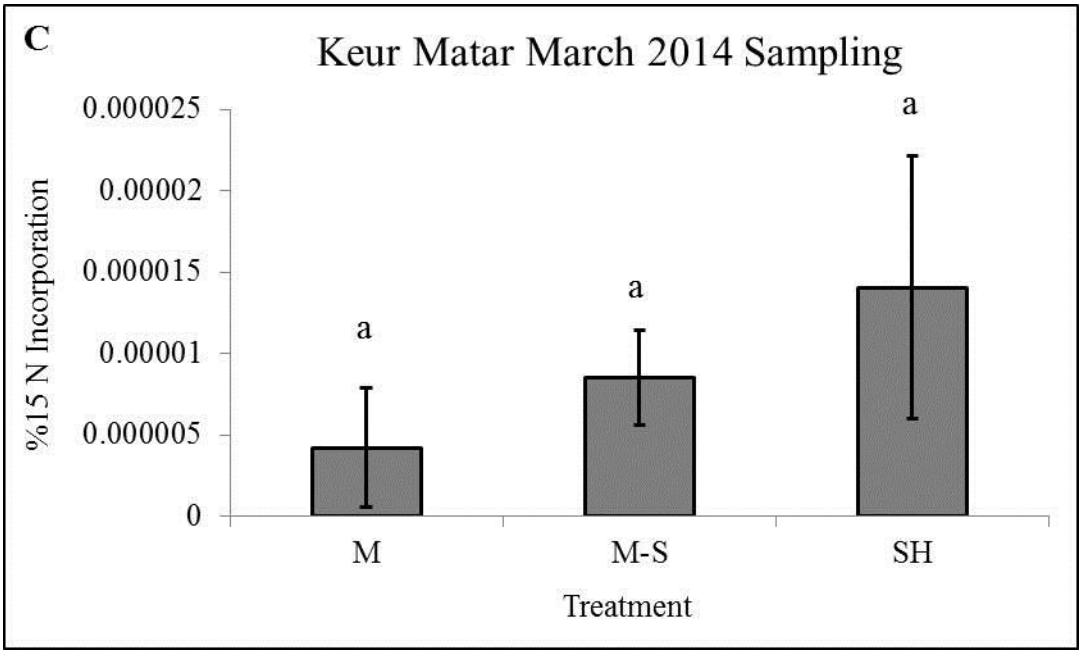



Figure A is during the rainy growing season, figure B is at the end of the growing season, and figure C is during the dry season. Columns with different letters indicate a significant difference between treatments (alpha = 0.05). Vertical bars represent ± standard error mean (n = 8 for graphs A and B, and n = 4 for graph C).

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