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Analysis of the Soil Health of Pastures Over-Seeded with Winter Annuals

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ANALYSIS OF THE SOIL HEALTH OF PASTURES OVER-SEEDED WITH WINTER ANNUALS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The School of Plant, Environmental, & Soil Science

by
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Abstract

Utilizing winter annuals in livestock pastures reduces feed costs of hay, and provides additional vegetative cover that is beneficial to the soil. However, how this practice affects the soil microbial communities has not been well studied. Two sites were included in this study. Both were commercial cattle grazing operations that over-seeded a mixture of grasses, legumes, and brassicas into pastures dominated by bahiagrass (*Paspalum notatum*). Soil health was assessed using a suite of physical, chemical, and biological properties. Soil physical assessments included bulk density and aggregate stability. Soil chemical assessments included macronutrients, pH, soil organic matter (SOM), total carbon (TC), and total nitrogen (TN). Fatty acid methyl ester analysis and enzyme assays were used to determine soil microbial community structure and activity. The objective for site one was to determine how soil health changed with time along a topographic sequence. The objective at site two was to compare the soil health of pastures using varying years of winter annuals and grazing. At both sites, TC and TN did not change with date of sampling although SOM did increase by 6% in site one. Nitrate concentrations decreased by 82% within two years at site one and remained consistent at 2.15 mg kg\(^{-1}\) at site two. Potential soil microbial enzyme activity increased with increasing years of management (grazing and winter annuals) by 27% and over time by 32% at site one and an average of 70% at site two. Total microbial abundance did not change over time at either site. Soil microbial community structure responded to abiotic conditions (e.g. season and topography) but not to years of management. This pasture management technique of over-seeding a diverse mix of winter annuals may, with time, allow for a steady state of C and N while SOM increases and inorganic nitrogen decreases, and enhance the total soil microbial community and their potential enzyme activity.
Chapter 1. Introduction

1.1. Soil Health

The Natural Resource Conservation Service within the USDA uses the term “soil health” interchangeably with “soil quality.” However, not all studies agree with this view. According to Laishram et al. (2012) soil quality is used in describing the functionality of a given soil while soil health is used when the soil is viewed as a living, non-renewable resource. In most research, studying either soil health or quality, there are common indicators that are used even though determination of a healthy soil is still being debated. Although no universal rubric has been accepted, most studies of soil health include chemical and physical soil properties and more are beginning to include soil biological properties as well (Laishram et al., 2012).

Physical indicators of soil health include particle size analysis (PSA) which determines the soil texture or proportion of sand, silt, and clay. Soil texture has been an influential factor in research of soil health indicators (Damsma et al., 2015) and plays a major role in determining soil organic matter (SOM) content (Six et al., 2002). Bulk density is also often used to determine soil health (Logsdon and Karlen, 2004; Damsma et al., 2015; de Paul Obade and Lal, 2016). Bulk density evaluates soil compaction which affects water infiltration rates, pore size, and aeration. Aggregate stability or wet aggregate stability (Idowu et al., 2008) provides information about the soil’s ability to resist erosion. Also, soil aggregates are often sources of populations of living organisms such as bacteria, fungi and other microbial species and are suggested to protect SOM from decomposition (Six et al., 2002).

Chemical indicators of soil health include soil pH, total carbon (TC) and nitrogen (TN), plant essential nutrients such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), and sulfur (S). Analysis of forms of inorganic soil nitrogen (N), nitrate (NO₃⁻) and
ammonium (NH$_4^+$), has also been used as an indicator of soil health (Kang et al., 2005) since these are the plant available forms of N. One soil health indicator that is most often used is SOM (Idowu et al. 2008) or soil organic carbon (SOC) (de Paul Obade and Lal, 2016; Kang et al., 2005). Soil organic matter is of most concern to agricultural producers even more so than soil fertility or pH (Gruver et al., 2006). This is not surprising as SOM greatly influences soil fertility, improves soil structure and aggregate stability, increases water holding capacity and decreases bulk density, provides substrate for microbial growth and activity (Wagner and Wolf, 2005) and is directly related to increased crop production. Recently, this soil characteristic has become of even greater concern due to its importance in carbon (C) sequestration.

Soil biology primarily refers to the soil microbial community and the activity of soil microbes. Unlike soil physical and soil chemical characteristics, soil biological properties fluctuate and change quickly and often. Changing seasons and weather affect soil biology while soil chemical and physical properties are much slower to respond to environmental or management changes (Cardoso et al., 2013). Soil biology is also very site specific (Damsma et al., 2015). Depending on the vegetation, the soil type, and land use history among other things, the soil biology can vary from one field to another. Soil biological indicators of soil health include microbial biomass C and N (Sangha et al., 2005), soil respiration, enzyme activity, fatty acid methyl ester (FAME) analysis, and nucleic acid analysis among others (Cardoso et al., 2013). Enzyme activity is often used due to its reflection of microbial functioning in the soil; however, it is the potential enzyme activity that is measured not the actual activity, and there are close to 500 enzymes that can be measured. Fatty acid methyl ester (FAME) analysis identifies groups of soil microorganisms, but not all fatty acids have been identified within specific groups, and some may not even be from microorganisms. Soil respiration has become more convenient
to measure in the field with the development of the Solvita test (Haney et al., 2008), but even that can be confounded by plant roots and soil temperature and moisture content. There is a lack of standardization which makes using biological properties as soil health indicators difficult to interpret (Laishram et al., 2012). However, measurements of soil biological factors are important in measuring soil health as the activity of soil microbes converts organic nutrients to plant available inorganic forms and can be used in understanding and observing the C cycle. Identifying soil microorganisms can provide information regarding group or even species functionality in the soil environment such as arbuscular mycorrhizal fungi, for example.

Therefore, as this study focuses on soil health, several measurements have been utilized to gather information regarding the physical, chemical and biological factors of the soils under perennial pastures over-seeded with winter annuals. The physical measurements include PSA, bulk density, and aggregate stability. The chemical measurements include TC, TN, SOM, inorganic nitrogen, and other plant essential nutrients. The biological measurements include FAME analysis and enzyme activity of β-glucosidase and N-acetyl-β-glucosaminidase.

1.2. Winter Annuals and Cover Crops

According to Ball, Hoveland, and Lacefield (2015) there is approximately 24 million hectares of perennial pasture land in the South and 8 million hectares of annual pastures. Livestock producers in the southeastern United States have used cool-season annuals as winter forage to extend their grazing season for decades due to the mild winters in the region. Producers who plant cool-season annuals most often use a mixture of a grass usually annual ryegrass (Lolium multiflorum) and a legume usually a clover (Trifolium sp.) over-seeded into a perennial warm-season grass such as bahiagrass (Paspalum notatum) or bermudagrass (Cynodon dactylon). Other cool-season annuals commonly used in the south include grasses, legumes, and
non-leguminous forbs (Ball et al., 2015). While this practice provides an extended grazing season, other benefits include reduced soil erosion and less nutrient loss to runoff or leaching, extended “grazing” for microbial populations, N fixation via legumes which have also been found to improve forage quality (Han et al., 2012; Han et al., 2013; Lithourgidis et al., 2006), and some crucifer species have been found to reduce compaction (Chen and Weil, 2010; Williams and Weil, 2004).

The use of cover crops in row crop agriculture is comparable to the planting of cool-season annual forages, and annual forage species are often used as cover crops. Hubbard et al. (2013) and Wright et al. (2004) found that winter cover crops increased soil C and retained inorganic N compared to fallow. In a study of radish (Raphanus sp.) and cereal rye (Secale cereale) cover crops, Lacey and Armstrong (2015) found that 60-80% of applied fertilizer nitrogen was absorbed by the cover crops. Cover crops have been found to increase inorganic and total P in a maize (Zea mays) cropping system (Dube et al., 2014). Steele et al. (2012) and Hermawan and Bomke (1997) found an increase in aggregate stability and labile C under winter cover crops. Zablotowicz et al. (2010) found that different types of cover crop affected soil microbial community composition. In a review by Brusaard et al. (2007), they discuss the merits of a diverse plant community and indirectly a diverse soil microbial community in resilience and resistance to disturbance and stress.

1.3. Soil Microbiology

Soil microorganisms are an extremely important part of soil health. Bacteria and fungi are the major groups responsible for breaking down organic compounds, mainly plant and animal residues. This results in the cycling of nutrients, the production of carbon dioxide (CO₂) through respiration and a C sink. In 1998, Whitman et al. estimated the total C in prokaryotes in the soil
globally to be 26 Pg which is approximately 4.8% of the estimated total C of prokaryotes. They also estimated that 6.2 Pg of N and .65 Pg of P were stored in soil prokaryotes. A review by Six et al. (2006) determined that bacteria and fungi makeup more than 90% of soil microbial biomass. This study also suggested that soil fungi sequester more C due to the higher C per N requirements and fungi are not as easily degraded as bacteria and are more protected by soil structures.

Soil bacteria are responsible for decomposing SOM. The organic matter that consists of simple compounds, bacteria break down very quickly. What they cannot decompose, recalcitrant compounds, becomes a part of the stable organic soil component called humus. When bacteria decompose organic matter, they produce inorganic nutrients which are available to plants and other soil microbes. This process is called mineralization. However, if the bacteria retain these inorganic nutrients in their own biomass, this is referred to as immobilization. These processes of mineralization and immobilization are key in determining soil nutrient availability and C cycling.

It is through soil microbial action that most organic N is converted to a plant available form, usually NO$_3^-$ or NH$_4^+$. Nitrogen after water is the most limiting nutrient for land plants. Leguminous plants use a mutualistic relationship with a bacteria which live in nodules on the plant roots to fix atmospheric N. These plants utilize the N that is fixed but upon decomposition that N can be incorporated into the soil system. Ammonium is released through ammonification, a secondary result of heterotrophic actions of soil microbes, but this NH$_4^+$ can be volatilized, fixed on soil particle exchanges sites, or converted to NO$_3^-$. Free-living bacteria, *Nitrosomonas* sp. and *Nitrobacter* sp., carry out the process of nitrification. This enzymatic oxidation converts ammonia and ammonium to nitrate.
Actinomycetes are classified as Gram positive (+) bacteria but produce a mycelium much like fungi. These organisms are slow-growing compared to other bacteria, but many degrade chitin, the main component of fungi, and the more recalcitrant compounds of SOM such as lignin. This group of microorganisms is known to decline as soil pH decreases and few exist below pH 5 but are persistent in soils with low moisture and high temperatures (Alexander, 2005).

Fungi are highly diverse which allows this group of organisms to survive in almost any environment. The majority of fungi in the soil are saprophytic, degraders of living or dead organic matter. Fungal saprophytes can degrade substances with a higher C/N ratio such as cellulose and lignin compared to bacteria and are more abundant than bacteria at a lower soil pH. As well as decomposers of organic matter, the hyphae of fungi have been found to act as binding agents of soil particles which increase aggregate stability.

Arbuscular mycorrhizal fungi (AMF) are symbiotic fungi that dwell within the roots of host plants. These fungi extend hyphae into the soil which increases the root zone and root surface area making it easier to acquire soil nutrients. In exchange, the host plant provides photosynthetic carbohydrates to the fungi. Mycorrhizal fungi have been found to produce a gelatinous substance called glomalin that also contributes to aggregate stability in the soil.

1.3.1. Fatty Acid Methyl Ester

In 1995, Cavigelli et al. used Fatty Acid Methyl Esters (FAME) to identify soil microbial communities that were not easily cultured. This technique revealed microbial groups that were previously hidden and their patterns of distribution in the soil. Fatty acids are components of lipids in living organisms and are converted to fatty acid methyl esters during the methylation
process. Classification of fatty acid groups is determined by the number of C atoms from the carboxyl group to the closest double bond and single fatty acids are identified by the number of C atoms from the terminal methyl group (Zilverberg et al., 2015). However, there is some controversy as to whether the fatty acids used for identifying certain groups of microbes may not be produced by other organisms or even more than one group of microbes (Zilverberg et al., 2015).

Another method often used to identify microbial community structure is phospholipid fatty acid (PLFA) analysis. This technique measures the abundance of phospholipids in the soil. On comparison of the two methods (FAME and PLFA), Drenovsky et al. (2004) found that a smaller soil sample could be used for FAME and a greater abundance of fungi register in the FAME method while actinomycetes and bacteria register higher in PLFA. They also found that fatty acids can be found in storage structures in the soil after cell death, but phospholipids do not last long after cell death. The FAME method was also determined to be faster than PLFA (Zilverberg et al., 2015).

1.3.2. Soil Microbial Enzyme Activity

Identifying soil microbial groups may be considered secondary to understanding their function in the soil (Chaparro et al., 2012). Enzyme activity provides information about the nutrient cycling function of soil microorganisms. Soil microbes use enzymes to break down carbon compounds such as plant residue which in turn converts organic nutrients to inorganic forms that are plant available. Measuring these enzymes in the soil provides insight into the potential capacity of the microbial community to cycle nutrients. According to Laishram et al. (2012), there are more than 500 enzymes from which to choose. However, those of greatest
significance are the enzymes used in C, N, and P cycling. β-glucosidase is commonly used as an indicator for soil C cycling, and N-acetyl-β-glucosaminidase (NAGase) has been used as an indicator of C and N cycling.

β-glucosidase is an enzyme which hydrolyzes cellobiose and releases the simple sugar, glucose (Deng and Popova, 2011). Bandick and Dick (1999) found that glucosidase activity in the soil was strongly influenced by soil management. This enzyme assay has been used for many years to understand the C cycling activity of soil microorganisms, and how it is affected by land use and management.

The cell walls of most fungi are made of chitin which contains N-acetylglucosamine (Morton, 2005). This molecule is also found in the cell walls of Gram + bacteria (Alexander, 2005) which is degraded by the enzyme NAGase. NAGase is a chitinase that is used to break down chitin (Deng and Popova, 2011), a complex compound comprising the majority of fungal biomass. This enzyme is also considered important to the nitrogen cycle because of its ability to release amino sugars, a mineralizable form of nitrogen, from the breakdown of chitin (Ekenler and Tabatabai, 2002).

Both glucosidase and NAGase have been found to increase with increasing soil organic carbon (Acost-Martinez et al., 2008; Wallenius et al., 2011; Tischer et al., 2015; Štursová and Baldrian, 2011). As C is the substrate for these enzymes, it follows that there would be a positive relationship. Pasturelands or any agroecosystem that has a constant vegetative cover provide a steady source of C substrate in the form of plant roots and decomposing plant tissue. According to Loranger-Merciris et al., (2006), as plant root biomass and productivity (Broughton and Gross, 2000) increase so too does microbial activity.
1.4. Effects of Winter Annuals/Cover Crops on Soil Microbial Communities

Many studies and reviews have found that using soil biological characteristics as soil health indicators is very site and land use specific (Damsma et al., 2015) and are strongly influenced by land management practices. Land management practices in pasture systems include livestock stocking rate, grazing method, fertilizer application, liming program, tilling, irrigation, and forage species.

1.4.1. Grazing

In a grazing management study, Oates et al. (2012) found that AMF decreased in grazed systems, but that Gram (+) bacteria and actinomycetes were found in greater abundance under grazed pasture compared to an ungrazed harvested hay field. Teague et al. (2011) found that different stocking densities affected the fungi:bacteria ratio. They also found that rotational grazing versus continuous increased the fungi:bacteria ratio. In an integrated crop livestock agroecosystem, Davinic et al. (2013) found that abundance of saprophytic fungi increased under grazed pasture regardless of vegetative species and that AMF were affected by a crop species by grazing interaction. However, Acosta-Martinez et al. (2010) found the greatest abundance of fungi under non-grazed perennial pasture compared to a crop-livestock rotation, but there was no differentiation between AMF and saprophytic fungi. Under high intensity grazing, Hiltbrunner et al. (2012), found a reduction of fungal abundance. AMF root colonization was found to be greater in a crop-pasture rotation and perennial pasture than in a continuous wheat or a crop rotation system (Murphy et al., 2011).

In regard to soil microbial activity and SOC, Hiltbrunner et al. (2012) found that in alpine regions cattle trampling led to bare ground and high soil bulk density but that erosion and lack of
vegetation led to loss of SOC. Murphy et al. (2011) found that total C and β-glucosidase activity were greatest in pasture systems compared to continuous crop or crop rotation while NAGase activity was found to be greatest under non-grazed perennial pastures compared to continuous crop or crop rotation treatments (Acosta-Martinez et al., 2010; Hewins et al., 2015). Hewins et al. (2015) found that grazing did not affect β-glucosidase activity, but that SOM, pH and soil moisture (Calderon et al., 2016) had the greatest influence on this soil enzyme. Zhou et al. (2012) also found that microbial metabolic activity was most strongly influenced by soil pH and soil moisture.

From the studies mentioned here, it can be deduced that for soil microbial populations and soil enzyme activity continuous cropping is not as favorable as crop-livestock rotations which are not as favorable as perennial pastures. Furthermore, rotational grazing is much more favorable than continuous grazing and low stocking rates are more favorable than high. However, for enzyme activity, it may be that the absence of grazing is more favorable for NAGase than β-glucosidase and that the factors with the strongest influence on enzyme activity are SOM, pH, and soil moisture.

1.4.2. Fertilizer and Liming

In a study of the effect of long term fertilizer applications, Zheng et al. (2013) compared the application of green manure (wheat straw) to the application of synthetic fertilizers. They found that synthetic fertilizer treatments which did not include P had the lowest microbial abundance of both Gram (+), Gram (-) bacteria, and actinomycetes but that the latter increased with the application of green manure. They also found that fungal abundance increased with a green manure but was unaffected by the different synthetic fertilizer treatments. The abundance of
AMF was reduced by synthetic fertilizer application in a crop rotation and fertilizer treatment study (Bakhshandeh et al., 2017). Synthetic N fertilizers were found to reduce the fungi:bacteria ratio and increase the GMp:GMn ratio (Zhou et al., 2017). Fungal species have been found to be strongly affected by liming (Wakelin et al., 2009) which alters soil pH. Goncalves-Lisboa et al. (2014) found that in a low pH and low fertility soil an integrated crop-livestock-forestry practice resulted in a fungi dominant soil system. From these studies, it may be deduced that liming will raise the soil pH which would affect the fungi:bacteria ratio in favor of bacteria. However, the use of synthetic fertilizers may reduce the presence of AMF.

1.4.3. Plant Species and Irrigation

Many studies have been conducted on the effects that plant species may have on soil microbial communities. The following are some examples. Wang et al. (2010) observed a difference in fungal species composition under a legume crop compared to a cereal. Soil microbial community diversity and respiration were found to be greater under legume species (Zhou et al., 2012), and Zhao et al. (2015) found that under wet conditions microbial biomass was greater in a legume monoculture or grass-legume mixture than under a grass monoculture. A combination of oats (Avena sativa) and rye (Secale cereale) used as cover crops in a corn (Zea mays) production system were found to increase mycorrhizal colonization and P uptake in the following corn crop (Kabir and Koide, 2002). Lehman et al. (2012) also found that forage oats (Avena sativa) or mixes of cover crops were best for increasing mycorrhizal inoculum. Calderón et al. (2016) found that microbial biomass was greater under cover crops than fallow treatments. Stephan et al. (2000) found an increase in soil bacterial abundance and activity with increasing plant diversity and functional groups using BIOLOG. They also determined that Trifolium repens had the greatest effect and that this plant species should be considered a “keystone”
species in plant-microbe interactions. Using terminal restriction fragment length polymorphism (T-RFLP) to determine microbial structure, it was found that fungal communities responded to plant diversity (Sayer et al., 2013).

However, in studying bacterial phyla, Kielak et al. (2008) found that plant species diversity had little to no effect on bacterial community structure. Similarly, Singh et al., (2009) found little effect of plant species on bacterial community structure but a slightly stronger effect on fungal community structure. Several studies have found an increase in bacterial abundance and activity with increasing plant diversity due to increasing plant productivity (Broughton and Gross, 2000; Zak et al., 2003; Loranger-Merciris et al., 2006). In a review by Millard and Singh (2010), it was determined that mycorrhizal community structure are particularly influenced by plant species communities, litter inputs were closely tied to saprophytic fungal community structure, and that soil organic quality and quantity have the greatest influence on bacterial community structure.

1.5. Effects Winter Annuals/Cover Crops on Soil Organic Carbon and Total Nitrogen

Soil organic carbon was found to increase under winter cover crops compared to no cover crop and carbohydrate levels were found to be lower in summer likely due to microbial action (Kuo et al., 1997). Cahill et al. (2009) studied C sequestration under warm season (C4) plants and cool season (C3) plants and found that there was greater root biomass under C4 plants suggesting these might be a carbon sink but there were too many uncertainties to confirm this. In a comparison of cropland, conservation reserve program (CRP) land, and native rangeland in west Texas, neither the cropland nor CRP land contained as much total C and N as the native rangeland even after 15 years (Bronson et al., 2004). Perennial pastures in Australia were found to sequester more C than annual pastures (Sanderman et al., 2013). Hubbard et al. (2013) found that cover crops increased soil C, retained N, increased soil moisture and decreased bulk density
compared to fallow. A 2015 meta-analysis by Poeplau et al. determined that cover crops sequester 0.32±0.08 Mg C ha\(^{-1}\) yr\(^{-1}\) and estimated that 16.7±1.5 Mg ha\(^{-1}\) total C to a soil depth of 22 cm could be accumulated within 155 years.

From these studies, it may be deduced that perennial pastures produce greater belowground biomass which may sequester more C than annual pastures. However, annual pastures/cover crops are more productive than fallow fields which may also improve C sequestration and that returning sites to undisturbed systems such as CRP may not sequester as much carbon compared to the native rangeland of arid systems. Therefore, maintaining a living root in the soil at all possible times may be recommended to improve SOM, SOC, TN and C sequestration.

1.6. Topography

In a crop management study in Missouri, Jung et al. (2008) found that SOC was significantly less in the 7.5-15cm depth at the footslope. They suggest it was due to high C mineralization because of the more humid, wetter conditions. Plant productivity was found to decrease from summit to footslope while plant species diversity did the reverse (Broughton and Gross, 2000). In Michigan, over three growing seasons, Ladoni et al. (2015) studied the effect of cover crops on soil nitrate in three different topographical areas: depression, slope and summit. They found that under red clover (\textit{Trifolium pratense}) NO\(_3^-\) increased 35% in depressions, 20% on slopes and 32% at summit positions whereas rye cover crop decreased NO\(_3^-\) by 15% in depressions and had no effect on the other positions. Red clover and rye cover crops were found to affect soil carbon on slopes and summits more than at depressions where cover crop biomass was greatest (Ladoni et al., 2016) but again this was attributed to faster rate of decomposition at the depression compared to the slop and summit topographies.
Therefore, it may be deduced that cover crops at topographical scale will increase SOC and TN especially in depressions, but that they are less likely to increase SOC at the 7.5-15 cm depth in depressions due to a faster rate of decomposition.

1.7. Rationale for Research

Soil health is a topic that has garnered a great deal of attention in recent years. It is considered a measure of a soil’s productive capabilities in the present and for the future. However, there is debate and on-going research to determine how best to measure soil health, but the majority of researchers include some measurement of the physical, chemical and biological soil characteristics. One of the production practices that has grown in popularity in recent years due to its ability to improve soil health is cover crops. These are utilized in row crop agriculture usually in the colder months after the cash crop has been harvested and before the next planting. In pasture systems, these cover crops are termed winter annuals and are often used as forage to extend the grazing season for livestock after the perennial warm-season forage has gone dormant. A plethora of research has been conducted to determine how cover crops or winter annual forages affect soil health. The impacts of grazing on soil health have also been studied especially in environments where soils are most susceptible to degradation such as arid climates. In the southeastern U.S., the warm, humid climate is conducive to maintaining vegetative cover for an extended amount of time compared to drier or colder climates, and livestock producers in the region use this to their advantage. The goal of this study was to quantitatively measure how these management practices, winter annuals and grazing, affected the soil health of these perennial pasture agroecosystems across a toposquence and chronosequence.
1.8. References


Chapter 2. Soil Health in a Perennial Pasture Over-seeded with a Mix of Winter Annuals for Eight Years

2.1. Introduction

Beef cattle producers in the southern United States have often used a mixture of winter annuals, typically annual ryegrass (*Lolium multiflorum*) and a clover (*Trifolium* sp.), to provide forage for their herds through the winter months (Ball et al., 2015). This is especially practical in southern and Gulf Coast states where winters are relatively mild with winter temperatures reaching average lows of 4.4°C. Utilizing winter covers can reduce the expense of feeding hay or stored feeds. A mixture of winter annuals, though requiring more management than a monoculture, has several benefits such as improving forage nutritive quality through the incorporation of legumes (Han et al., 2012; Han et al., 2013; Lithourgidis et al., 2006) and stand resilience in less than ideal conditions such as drought (Corson et al., 2007; Sanderson et al., 2005).

Cover crops and winter annual forages have been found to impact soil health by increasing soil carbon (C) and nitrogen (N) (Hubbard et al., 2013; Kuo et al., 1997; Wright et al., 2004), which can increase microbial activity (Calderón et al., 2016; Loranger-Merciris et al., 2006; Zak et al., 2003) and increase aggregate stability (Hermawan and Bomke, 1997; Steele et al., 2012) reducing runoff and erosion. Additionally, crucifer species, such as turnips (*Brassica* sp.) and radishes (*Raphanus* sp.), have been found to reduce compaction due to the formation of a large taproot (Chen and Weil, 2010; Williams and Weil, 2004). However, little research has been done to quantify or measure the effects of using a diverse mixture of winter annuals on soil health in the southern United States, and more specifically in pasture systems. Soil health is a comprehensive, long term view of the sustainability of agricultural practices. A healthy soil is imperative for continued agricultural production and environmental protection which is one of
the major dilemmas facing agriculture. To determine soil health, three aspects are generally included in an assessment: chemical, physical, and biological factors. Soil chemical characteristics such as inorganic N (Kang et al., 2005) and soil organic matter (SOM) (Idowu et al., 2008) are included as soil health assessments as well as bulk density, a physical factor (Damsma et al., 2015; de Paul Obade and Lal, 2016; Logsdon and Karlen, 2004). Some of the biological parameters used for soil health include enzyme activity (Bastida et al., 2006; Cardoso et al., 2013) which provides information about the nutrient cycling ability of soil microorganisms and fatty acid methyl ester (FAME) analysis (Cardoso et al., 2013) which describes the soil microbial community structure.

The objective of this study was to determine the effects that over-seeding a diverse mix of winter annuals would have on the soil health of a perennial pasture in the Southeast region of the United States over time. It was hypothesized that a diverse mix of winter forage would provide a diverse substrate resulting in increased soil chemical (organic matter, total C, and total N) and biological (microbial community and activity) properties with time.

2.2. Material and Methods

The site for this project was a privately owned commercial grazing operation located in south central Mississippi, 12 km south of Woodville, MS. The soils are a cultivated Loring silt loam the taxonomy of which is a fine-silty, mixed, active, thermic Oxyaquic Fragiudalfs. The site was a bahiagrass (Paspalum notatum) pasture that had been over-seeded with a varying mix of winter annuals including oats (Avena sativa), triticale (Triticum secale), annual rye grass (Lolium multiflorum), hairy vetch (Vicia villosa), radish (Raphanus sp.), turnips (Brassica sp.), AU Red Ace red clover (Trifolium pretense), Ball clover (Trifolium nigrescens), and Dixie crimson clover (Trifolium incarnatum) for the past nine years. Annuals were typically drill seeded in late September or October each year. The grazing regime was mob grazing of approximately 175
head of cattle on 216 hectares with cattle being rotated through paddocks approximately every 12 to 24 hours. There has been no fertilizer or pesticide application to this site since 2010.

Average annual precipitation for the area from 2015-2017 was 183 cm (Figure 2.1). The highest amount of rainfall, 66 cm, occurred in August 2016, and the least amount of rainfall occurred in October 2016 with 0.15 cm. Weather data was reported by the St. Francisville, LA weather station.

Soil samples were collected in October each year as this was when warm-season perennial pasture grasses were entering dormancy and productivity was declining. The winter annuals had just been over-seeded and had not yet germinated. Soil samples were collected in March as this was the time when winter annuals were growing and increasing in productivity.

Soils were collected from topographically distinct areas including summits (2), backslopes (3), and footslopes (2) across approximately 12.14 hectares (Figure 2.2). The elevation of each location ranged from 79 m at Footslope1 to 88 m at Summit1 while the slope ranged from 1.4 to 18%. Soil cores were 10.16 cm wide by 30 cm deep and separated in 7.5 cm intervals. Pseudo-replication was used to increase degrees of freedom. At each site, nine soil cores were taken from within 1 meter, divided according to depth and composited to provide three pseudo-replicates per location for each depth. Soils to be analyzed for biological properties (0-7.5 and 7.5-15 cms) were stored in ice chests at 4°C for travel and then stored at -20°C until analysis. All soils were sieved at 4.75 mm before analysis. An air-dried subsample of soil was analyzed for enzymes and inorganic N.
Figure 2.1. Monthly precipitation from 2015-2017 recorded at the St. Francisville, LA weather station.

Figure 2.2. Map of sampling locations.
Soil organic matter was determined using the percent Loss-on-Ignition method by Nelson and Sommers (1996). Soil samples were oven dried at 105°C for 16 hours then weighed. Samples were then ashed in a muffle furnace at 400°C for 24 hours and weighed. Percent Loss-On-Ignition was then calculated as

\[
\text{% LOI} = \left(\frac{\text{Weight}_{105} - \text{Weight}_{400}}{\text{Weight}_{105}}\right) \times 100
\]

Total C and TN were determined using the dry combustion method by LECO C/N Analyzer. Soil pH (1:1 in deionized water) and TC and TN were analyzed by the LSU AgCenter Soil Testing and Plant Analysis Laboratory. Soil moisture was determined by weighing 5.0 g of field moist sample then drying it in an oven at 105°C for 24 hours and weighing them again. Percent soil moisture was calculated as

\[
\text{% Moisture} = \left(\frac{\text{Field moist weight} - \text{Dry weight}}{\text{Dry weight}}\right) \times 100
\]

Inorganic N was extracted using 10 ml of 2 M KCl per 1 g soil. Colorimetric analysis was done according to the method described by Hood-Nowotny et al. (2010). For ammonium-N, 96-well microplates were filled with a salicylate solution. Then filtered sample was added before the second reagent, bleach/NaOH, was also added. These plates were then incubated in the dark for 50 minutes at room temperature. For nitrate-N, 96-well microplates were filled with a vanadium chloride solution after which the filtered sample was added. These plates were then incubated in the dark for 60 minutes at 37°C. Absorbance was measured at 660 nm for ammonium-N concentration and 540 nm for nitrate-N concentration.

To determine bulk density, soils were collected using soil core rings of a known volume, 267.15 cm\(^3\). Rings were hammered into the soil surface and removed. A second ring was hammered into the same hole to get the bulk density of the second depth. Soils were then oven-
dried for 16 hours at 105°C and weighed. The weight of the soil was divided by the volume to determine bulk density.

Microbial community structure was determined using ester linked FAME analysis according to Shutter and Dick (2000). Field moist soil samples were methylated by the addition of 0.2 M KOH in methanol and subjected to a 37°C water bath for 60 minutes with regular vortexing. Samples were then neutralized by the addition of 1.0 M acetic acid and vortexed. Samples were finally extracted with the addition of hexane inverted and centrifuged at 2200 rpm for 5 minutes. The organic phase was transferred to test tubes and concentrated using N2 gas at 37°C. Finally, samples were rehydrated using hexane and a 19:0 internal standard. Fatty acids were quantified using an Agilent 7890B gas chromatograph equipped with a fused silica capillary column and flame ionization detector. Samples were analyzed using a temperature profile which ramped from 190 to 250°C per minute followed by a ramp to 300°C to clear the column. Fatty acids were identified using the library provided by MIDI (Microbial ID, Inc.). Biomarkers used for identification of soil microbial groups were 17:0 10-methyl for actinomycetes, 16:1 w5c for AMF, 18:3 w6c, 18:1 w9c for saprophytic fungi, 16:1 w9c, 16:1 w7c, 18:1 w7c for GMn bacteria, and 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:0 iso, 17:1 w9c, 18:0 for GMp bacteria (Frostegård and Bååth, 1996; Kroppenstedt, 1992; Madan et al., 2002; Walling et al., 1996; Zak et al., 1996; Zelles, 1997; Zogg et al., 1997).

The enzymes β-glucosidase and N-acetyl-β-glucosaminidase were measured according to Tabatabai (1994) and Parham and Deng (2000) respectively to determine microbial activity. A modified universal buffer (MUB) with pH 6.0 was added to 0.5 g of air-dried soil sample along with substrate of the enzyme being measured. Samples were then incubated for 60 minutes at 37°C. Following incubation, 0.5 M CaCl2 was added along with 0.1 THAM (tris hydroxymethyl
aminomethane) buffer pH 12. This solution was mixed and filtered through Whatman No. 2 folded filter paper. Samples were then pipetted into 96-well microplates and read by a spectrophotometer at 420 nm. Concentrations were determined according to a standard curve.

Analysis of variance (ANOVA) were determined using R statistical software (R Core Team, 2013). Soil depth, date of collection, and topographic site of collection were considered independent variables. Since depth was always significant, the 0-7.5 and the 7.5-15 cm depths were analyzed separately. Using the vegan package in R (Oksanen et al., 2017), principal component analyses of FAME data was conducted using a correlation matrix through the capscale function using the Bray-Curtis dissimilarity index. Vectors were applied through the envfit function.

2.3. Results

2.3.1. Soil physical properties

At the 0-7.5 cm depth, the bulk density increased from 1.25 g cm$^{-3}$ in fall 2015 to 1.31 g cm$^{-3}$ in spring 2017 (P=0.049). Backslope1, Backslope3 and Summit2 averaged 1.38 g cm$^{-3}$ while Summit1, Backslope2 and Footslope2 averaged 1.25 g cm$^{-3}$ and Footslope1 had the lowest bulk density with 1.13 g cm$^{-3}$ (P=0.003). At the 7.5-15 cm depth, the summit locations had the highest bulk density with an average of 1.56 g cm$^{-3}$ followed by Backslope1 with 1.49 g cm$^{-3}$ and all other locations with an average of 1.42 g cm$^{-3}$ (P≤0.0001). An interaction of date and topography at this depth demonstrated that the bulk density of Backslope3 increased from 1.25 to 1.46 g cm$^{-3}$ from fall 2015 to spring 2017 while Footslope2 decreased from 1.50 to 1.40 g cm$^{-3}$ (P=0.008).

Soil moisture was 17% in fall 2016 while it was an average of 29% at all other sampling times (P≤0.0001). An effect of topography revealed that soil moisture was an average of 31% at the footslope locations followed by Backslope1 with 28% and Summit1, Backslope2, and
Summit2 which averaged 24% and finally Backslope3 with 22% which was not different from Summit2 and Backslope2 (P≤0.0001).

2.3.2 Soil Chemical Properties

Measured to a depth of 15 cm, SOM along with TC (P≤0.0001), TN (P≤0.0001), C:N (P=0.009), NH\textsubscript{4}\textsuperscript{+}-N (P≤0.0001), NO\textsubscript{3}\textsuperscript{-}-N (P≤0.0001) except pH (P=0.1788) was affected by depth. SOM was higher at the top depth. At 0-7.5 cm, SOM was lowest in spring 2016 at 59 g kg\textsuperscript{-1} and highest in fall 2016 at 70 g kg\textsuperscript{-1} but had an overall increase of 6% from 2015 to 2017 though there was no significant difference between the fall 2015 and spring 2017 observations. There was no change over time at the 7.5-15 cm depth which averaged 36 g kg\textsuperscript{-1} (P=0.301). As for topography, a consistent pattern could be seen across dates of collection in that SOM was lowest at the Summit2 and Backslope3 compared to all other locations (Table 2.1).

Table 2.1 Interaction of topography and date for SOM (g kg\textsuperscript{-1}) at 0-15 cm. Standard errors in parentheses.

<table>
<thead>
<tr>
<th>Location</th>
<th>Fall 2015</th>
<th>Spring 2016</th>
<th>Fall 2016</th>
<th>Spring 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summit1</td>
<td>53.0 (6.93) B†</td>
<td>54.6 (7.02) B a</td>
<td>71.7 (8.01) A a</td>
<td>58.1 (6.82) B ab</td>
</tr>
<tr>
<td>Backslope1</td>
<td>48.6 (6.99) B b</td>
<td>54.3 (7.51) A b</td>
<td>55.7 (7.39) B b</td>
<td>58.3 (7.91) A b</td>
</tr>
<tr>
<td>Footslope1</td>
<td>42.5 (7.38) A</td>
<td>48.2 (8.91) A</td>
<td>50.4 (9.89) A</td>
<td>49.7 (10.14) A</td>
</tr>
<tr>
<td>Summit2</td>
<td>45.0 (7.85) A</td>
<td>25.4 (4.15) B b</td>
<td>45.4 (7.48) A c</td>
<td>42.0 (8.55) A</td>
</tr>
<tr>
<td>Backslope2</td>
<td>54.4 (7.08) A b</td>
<td>48.7 (7.39) A b</td>
<td>58.1 (8.32) A a</td>
<td>60.1 (7.32) A a</td>
</tr>
<tr>
<td>Footslope2</td>
<td>59.7 (6.03) A a</td>
<td>52.5 (8.64) A a</td>
<td>53.9 (8.05) A</td>
<td>51.8 (6.84) A a</td>
</tr>
<tr>
<td>Backslope3</td>
<td>38.7 (4.17) A e</td>
<td>45.5 (4.23) A a</td>
<td>40.9 (5.05) A d</td>
<td>42.4 (3.91) A</td>
</tr>
</tbody>
</table>

†Uppercase letters denote difference between dates within topography.
‡Lowercase letters denote difference between topography within date.

Both TC and TN were higher at the 0-7.5 cm depth than the 7.5-15 cm depth (Appendix A); however, lower concentrations in TN resulted in a wider C:N ratio at the 7.5-15 cm depth (Table 2.2). An interaction of date of collection and topography at the 0-7.5 cm depth resulted in some locations having higher C:N than others in certain years (P=0.002). These higher observations were due to low TN rather than higher TC (Table 2.2; Appendix A). Contrary to the previous
observations, in spring 2016 at 7.5-15 cm, higher concentrations of TN, 1.8 g kg$^{-1}$, resulted in the lowest recorded C:N at Summit1 (Table 2.2).

Table 2.2 Three way interaction of C:N. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th>Topography</th>
<th>Fall 2015</th>
<th>Spring 2016</th>
<th>Fall 2016</th>
<th>Spring 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summit1</td>
<td>8.5 (0.18) A† b‡</td>
<td>5.4 (0.09) A d</td>
<td>8.0 (0.17) A a</td>
<td>8.6 (0.26) A a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>8.2 (0.18) AB b b</td>
<td>6.1 (0.69) B cd</td>
<td>10.0 (0.95) A a</td>
<td>8.0 (0.04) AB a</td>
</tr>
<tr>
<td>Foootslope1</td>
<td>9.2 (0.68) A ab</td>
<td>6.0 (0.36) A cd</td>
<td>8.2 (0.04) A a</td>
<td>8.1 (0.37) A a</td>
</tr>
<tr>
<td>Summit2</td>
<td>12.2 (1.02) A a</td>
<td>9.5 (0.56) A b</td>
<td>10.7 (0.17) A a</td>
<td>8.8 (0.21) A a</td>
</tr>
<tr>
<td>Backslope2</td>
<td>9.4 (0.34) A ab</td>
<td>8.6 (0.11) A bcd</td>
<td>9.2 (0.30) A a</td>
<td>8.7 (0.10) A a</td>
</tr>
<tr>
<td>Foootslope2</td>
<td>9.1 (1.28) A ab</td>
<td>9.1 (0.30) A bc</td>
<td>8.8 (0.39) A a</td>
<td>8.4 (0.56) A a</td>
</tr>
<tr>
<td>Backslope3</td>
<td>9.1 (0.31) B ab</td>
<td>18.6 (8.04) A a</td>
<td>9.5 (0.17) B a</td>
<td>9.4 (0.18) B a</td>
</tr>
</tbody>
</table>

†Uppercase letters denote difference between dates within topography.
‡Lowercase letters denote difference between topography within date.

Date of collection significantly impacted ammonium-N ($P \leq 0.0001$). Ammonium-N decreased from fall 2015 to spring 2016 by 40%, before increasing in spring 2017 by 39% at the 0-7.5 cm depth (Fig. 2.3a). The pattern was the same in the 7.5-15 cm depth with an overall increase of 28% from 2015 to 2017 (Fig. 2.3b). Nitrate-N, on the other hand, decreased by 58% from spring 2016 to fall 2016 with an overall decrease of 78% from 2015 to 2017 at the 0-7.5 cm depth (Fig. 2.3a, $P \leq 0.001$). At the 7.5-15 cm depth, nitrate-N steadily decreased from fall 2015 to spring 2017 with an overall decrease of 89% (Fig. 2.3b). From 2015 to 2017, total inorganic N measurements, the sum of ammonium and nitrate concentrations, decreased 34% and 41% at the
0-7.5 and 7.5-15 cm depths, respectively (Fig. 2.3). However, at 0-7.5 cm, the decrease in total inorganic N began in fall 2016 whereas, at the 7.5-15 cm depth, the decline began in spring 2016 (Fig. 2.3).

Figure 2.3. Inorganic nitrogen measurements at a) 0-7.5 cm and b) 7.5-15 cm depths across dates of collection. Total inorganic nitrogen (TIN) is the sum of ammonium and nitrate measurements.
An interaction of date of collection and topography was significantly different for both observations of inorganic N. For ammonium-N, the secondary topographic sequence (Summit2, Backslope2, and Footslope2) had the greatest decrease from fall 2015 to spring 2016 (Table 2.3). However, four of the seven locations increased in ammonium concentrations in later samplings with the highest concentration at Footslope1 in 2017 (Table 2.3). There was a significant increase of nitrate-N concentrations at the Footslope1 location from fall 2015 to spring 2016 while concentrations at all other locations decreased or remained unchanged (Table 2.3). Nitrate-N then decreased at all locations in later samplings with Summit2 and Backslope3 having lower concentrations than the other locations (Table 2.3).

Table 2.3. Inorganic nitrogen interaction of date and topography (mg kg\(^{-1}\)) at 0-15 cm. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th>location</th>
<th>NH(_4)-N</th>
<th>NO(_3)-N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall 2015</td>
<td>Spring 2016</td>
</tr>
<tr>
<td>Summit1</td>
<td>6.3 (0.80)</td>
<td>5.6 (0.46)</td>
</tr>
<tr>
<td>Backslope1</td>
<td>5.6 (0.66)</td>
<td>5.7 (0.55)</td>
</tr>
<tr>
<td>Footslope1</td>
<td>8.2 (2.26)</td>
<td>5.8 (0.78)</td>
</tr>
<tr>
<td>Summit2</td>
<td>10.4 (4.25)</td>
<td>4.2 (0.78)</td>
</tr>
<tr>
<td>Backslope2</td>
<td>10.0 (0.94)</td>
<td>5.2 (0.80)</td>
</tr>
<tr>
<td>Footslope2</td>
<td>10.1 (1.33)</td>
<td>5.5 (1.06)</td>
</tr>
<tr>
<td>Backslope3</td>
<td>5.3 (0.56)</td>
<td>5.1 (0.76)</td>
</tr>
</tbody>
</table>

†Uppercase letters denote difference between dates within topography.
‡Lowercase letters denote difference between topography within date.
ND = Not Detectable

The soil pH increased with time from 5.09 in 2015 to an average of 5.43 for the three later samplings, an increase of 6.7%. There was an interaction of depth and topography which
revealed that Footslope1 had the lowest pH at both depths with an average of 4.93, and that the Footslope2 location had a higher pH at the second depth than the top depth, 5.61 and 5.31, respectively. Finally, Backslope3 had a significant difference of pH between the top depth and the second depth, 5.98 and 5.48 respectively.

2.3.3. Microbial Community Response

Potential N-acetyl-β-glucosaminidase (NAGase) activity increased from fall 2015 at 47.12 to an average of 58.88 mg p-nitrophenol kg⁻¹ soil h⁻¹ 2016-2017. While NAGase increased at all locations over time, Footslope1 location was consistently lowest in enzyme activity compared to the other locations (Table 2.4). Potential β-glucosidase activity averaged 72.50 mg p-nitrophenol kg⁻¹ soil h⁻¹ across dates of collection. However, enzyme activity varied significantly by location. At both depths, Summit1 was found to have the highest potential enzyme activity while Footslope1 was lowest at almost all sampling dates except for fall 2016 (Table 2.5). At the 7.5-15 cm depth, β-glucosidase activity varied by year at the Summit1 location while it remained unchanged at all other locations though there were differences among locations (Table 2.5).

Table 2.4. N-acetyl-β-glucosaminidase (NAGase) interaction of date and topography (mg p-nitrophenol kg⁻¹ soil h⁻¹). Standard errors are in parentheses.

<table>
<thead>
<tr>
<th>Location</th>
<th>Fall 2015</th>
<th>Spring 2016</th>
<th>Fall 2016</th>
<th>Spring 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summit1</td>
<td>66.1 (8.85) C† a‡</td>
<td>101.6 (21.89) A a</td>
<td>85.4 (9.55) AB a</td>
<td>72.9 (10.15) BC a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>52.4 (9.88) A ab</td>
<td>49.6 (10.19) A bc</td>
<td>55.7 (15.15) A b</td>
<td>60.0 (13.46) A ab</td>
</tr>
<tr>
<td>Footslope1</td>
<td>27.3 (6.57) A c</td>
<td>30.5 (10.61) A d</td>
<td>37.9 (11.32) A c</td>
<td>41.1 (10.35) A c</td>
</tr>
<tr>
<td>Summit2</td>
<td>48.4 (11.91) B b</td>
<td>59.9 (17.87) AB b</td>
<td>74.0 (18.60) A a</td>
<td>75.0 (19.94) A a</td>
</tr>
<tr>
<td>Backslope2</td>
<td>53.8 (13.19) BC ab</td>
<td>61.7 (13.57) AB b</td>
<td>43.3 (10.32) C bc</td>
<td>73.8 (15.22) A a</td>
</tr>
<tr>
<td>Footslope2</td>
<td>42.1 (8.00) A bc</td>
<td>39.9 (9.74) A cd</td>
<td>50.1 (13.04) A bc</td>
<td>48.0 (11.94) A bc</td>
</tr>
<tr>
<td>Backslope3</td>
<td>39.8 (7.80) B bc</td>
<td>56.6 (10.84) A b</td>
<td>56.1 (12.92) AB b</td>
<td>63.7 (12.72) A ab</td>
</tr>
</tbody>
</table>

†Uppercase letters denote difference between dates within topography.
‡Lowercase letters denote difference between topography within date.
All FAME measurements (absolute and relative abundance) were higher at the 0-7.5 cm depth except for the relative abundance of saprophytic fungi (Fig. 2.4). Total FAME measurements did not change with time at either depth (Table 2.6). At the 0-7.5 cm depth, absolute abundance of GMp and actinomycetes were higher in the fall samplings than the spring samplings by an average of 42.5 and 40%, respectively, while in the 7.5-15 cm depth by an average of 59 and 46.5%, respectively (Table 2.6). The absolute abundance of AMF were higher in the spring by 51.1% on average at the 0-7.5 cm depth and 29.0% higher on average at the 7.5-15 cm depth (Table 2.6).

At the 0-7.5 cm depth in fall 2015, total FAMEs was unchanged across locations and averaged 366.53 nmol g\(^{-1}\). The soil microbial groups were also unchanged except for actinomycetes which were significantly lower at the Backslope3 location with 15.90 nmol g\(^{-1}\) compared to the Footslope2 location with 36.88 nmol g\(^{-1}\). Then in spring 2016, total FAMEs was

### Table 2.5. Three way interaction of β-glucosidase (mg p-nitrophenol kg\(^{-1}\) soil h\(^{-1}\)). Standard errors are in parentheses.

<table>
<thead>
<tr>
<th>Topography</th>
<th>0-7.5 cm</th>
<th>7.5-15 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall 2015</td>
<td>Spring 2016</td>
</tr>
<tr>
<td>Summit1</td>
<td>144.2 (2.80) B† a‡</td>
<td>178.9 (21.06) AB a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>132.4 (14.95) A ab</td>
<td>84.3 (3.42) B d</td>
</tr>
<tr>
<td>Footslope1</td>
<td>85.6 (5.72) A c</td>
<td>79.9 (6.53) A d</td>
</tr>
<tr>
<td>Summit2</td>
<td>115.9 (16.60) AB abc</td>
<td>150.6 (11.52) A ab</td>
</tr>
<tr>
<td>Backslope2</td>
<td>121.2 (22.00) AB abc</td>
<td>134.4 (16.48) A bc</td>
</tr>
<tr>
<td>Footslope2</td>
<td>107.2 (14.28) A abc</td>
<td>102.3 (3.01) A cd</td>
</tr>
<tr>
<td>Backslope3</td>
<td>92.9 (7.72) AB bc</td>
<td>84.6 (10.90) AB d</td>
</tr>
</tbody>
</table>

†Uppercase letters denote difference between dates within topography.
‡Lowercase letters denote difference between topography within date.
higher at the Summit1 location with 390.25 nmol g\(^{-1}\) compared to the Footslope2 location with 67.70 nmol g\(^{-1}\). The soil microbial groups reflected this pattern. In fall 2016, greater variation revealed total FAMEs was highest at the Summit1 location with 545.49 nmol g\(^{-1}\) while the lowest recorded total FAMEs was the Backslope3 location with 206.10 nmol g\(^{-1}\) compared to the other locations which averaged 325.46 nmol g\(^{-1}\). The soil microbial groups again reflected this pattern. Finally, in spring 2017, the highest total FAMEs were recorded at the Footslope1 location with 357.24 nmol g\(^{-1}\), and the lowest was at the Backslope3 location with 138.12 nmol g\(^{-1}\).

At the 7.5-15 cm depth, the Summit1 location was higher in total FAMEs than all the other locations in fall 2015 and 2016 while in the spring samplings it was not different from the other
Table 2.6. Absolute abundance of fatty acid methyl ester data according to date of collection (nmol g\(^{-1}\)). Standard errors in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Total FAMEs</th>
<th>GMp</th>
<th>GMn</th>
<th>Actinomycetes</th>
<th>AMF</th>
<th>Fungi</th>
<th>F:B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>0-7.5 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall 2015</td>
<td>367 (21.74) †a</td>
<td>87.38 (6.13) a</td>
<td>37.17 (2.30) a</td>
<td>28.32 (2.12) a</td>
<td>16.16 (1.42) c</td>
<td>49.62 (2.84) a</td>
<td>0.34 (0.02) a</td>
</tr>
<tr>
<td>Spring 2016</td>
<td>276 (25.67) a</td>
<td>64.64 (6.96) b</td>
<td>37.65 (3.92) a</td>
<td>20.19 (2.19) b</td>
<td>27.01 (3.58) a</td>
<td>44.00 (4.34) a</td>
<td>0.37 (0.02) a</td>
</tr>
<tr>
<td>Fall 2016</td>
<td>340 (25.62) a</td>
<td>94.96 (6.49) a</td>
<td>38.69 (2.66) a</td>
<td>29.09 (2.08) a</td>
<td>16.74 (1.79) bc</td>
<td>40.00 (3.92) a</td>
<td>0.26 (0.02) a</td>
</tr>
<tr>
<td>Spring 2017</td>
<td>256 (23.43) a</td>
<td>63.14 (5.49) b</td>
<td>34.97 (2.88) a</td>
<td>20.74 (1.72) b</td>
<td>22.71 (2.18) ab</td>
<td>38.91 (2.94) a</td>
<td>0.35 (0.02) a</td>
</tr>
<tr>
<td><strong>7.5-15 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall 2015</td>
<td>157 (19.26) a</td>
<td>34.47 (3.82) a</td>
<td>13.15 (1.62) a</td>
<td>11.56 (1.40) a</td>
<td>7.04 (1.01) bc</td>
<td>28.61 (4.58) a</td>
<td>0.41 (0.05) a</td>
</tr>
<tr>
<td>Spring 2016</td>
<td>110 (11.91) a</td>
<td>21.24 (2.53) b</td>
<td>9.66 (1.29) a</td>
<td>7.19 (0.85) b</td>
<td>9.21 (1.23) ab</td>
<td>17.58 (1.52) a</td>
<td>0.52 (0.06) a</td>
</tr>
<tr>
<td>Fall 2016</td>
<td>134 (13.52) a</td>
<td>31.50 (3.47) a</td>
<td>11.12 (1.37) a</td>
<td>9.57 (1.11) ab</td>
<td>6.24 (0.77) c</td>
<td>20.01 (2.46) a</td>
<td>0.41 (0.05) a</td>
</tr>
<tr>
<td>Spring 2017</td>
<td>101 (8.20) a</td>
<td>20.17 (2.33) b</td>
<td>9.51 (1.06) a</td>
<td>7.20 (0.85) b</td>
<td>9.51 (0.96) a</td>
<td>15.81 (1.35) a</td>
<td>0.51 (0.07) a</td>
</tr>
</tbody>
</table>

†Lowercase letters denote difference between dates within soil microbial groups.

GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; F:B=fungi to bacteria ratio
locations. The soil microbial groups followed the same pattern as the total FAMEs except for AMF which were consistently low at the Footslope1 location averaging 3.98 nmol g\(^{-1}\) compared to the Summit1 location which averaged 14.10 nmol g\(^{-1}\). The bacterial groups were low at the Summit2 location.

The F:B was higher at the 7.5-15 cm depth at 0.50 compared to the 0-7.5 cm depth at 0.33. At the 0-7.5 cm depth, this ratio was increased at the Summit2 location (.74 on average) due to the low bacterial populations except for fall 2016 (.16) when the saprophytic fungi were at the lowest recorded measurement. The same pattern could be seen at the 7.5-15 cm depth.

At the 0-7.5 cm depth, there was a seasonal difference for the relative abundance of GMn bacteria which were higher in the spring samplings with an average of 13.56 mol\%\, than the fall samplings, 10.60 mol\%. However, GMp bacteria and actinomycetes were highest in the fall 2016 sampling at the 0-7.5 cm depth, 26.84 and 8.18 mol\%, respectively, and at the 7.5-15 cm depth, highest in fall 2015 with 22.06 and 7.23 mol\%, respectively.

At the 0-7.5 cm depth, relative abundance of AMF (Fig. 2.5a) and saprophytic fungi were higher at the Backslope3 and Summit2 locations (Fig. 2.5). At the 7.5-15 cm depth, the pattern from the top depth continued for the AMF and saprophytic fungi (Fig. 2.6). The relative abundance of the GMp bacteria and actinomycetes were higher at the Footslope1 location (Fig. 2.5) while at the second depth the bacterial groups were higher at the Backslope1 location as well as Footslope1 (Fig. 2.6). (Missing data resulted in the lack of an ellipses for Fig. 2.5d, Fig. 2.6b, and Fig. 2.6d.)
Figure 2.5. Principle Component Analyses of relative abundance of fatty acid methyl ester data at 0-7.5 cm. a) October 2015 b) March 2016 c) October 2016 and d) March 2017 GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; Bac=total bacteria
Figure 2.6. Principle Component Analyses of relative abundance of fatty acid methyl ester data at 7.5-15 cm. a) October 2015 b) March 2016 c) October 2016 and d) March 2017 GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; Bac=total bacteria
2.4. Discussion

In this project, the effects that the over-seeded winter annuals may have had on this system could not be differentiated from the impacts of other factors such as soil depth, topography, and date of collection. However, use of winter annuals in this agroecosystem has been occurring for the past nine years, and as forage and livestock production have been maintained without fertilizer inputs for the past seven years, it is probable that this management technique is contributing to the sustainability of this system.

Depth affected every measured soil property except pH. Typically, all soil properties affected by depth decreased in samples collected from 7.5-15 cm compared to the 0-7.5 cm except relative abundance of saprophytic fungi. As SOM usually decreases with soil depth, it is not uncommon for soil properties to decrease with depth also especially the soil microbial populations that utilized SOM as a carbon source. Though they did not differentiate AMF and saprophytic fungi, Acosta-Martinez et al., (2010) also found greater relative abundance of fungi at the 5-15 cm compared to 0-5 cm depths in an integrated livestock-cotton system. In this project, the increase of the relative abundance of saprophytic fungi may be due to decomposing root biomass of the bahiagrass which is a deep-rooted perennial (Jesus et al., 2016). It may be that these deeper roots especially bahiagrass are more lignified and therefore more easily decomposed by saprophytic fungi. There may also be less competition for organic substrates at this deeper depth where there is typically a decrease in bacterial abundance.

The extreme difference between the two summit locations may be due to any number of variables such as amount or type of vegetation growing at these locations or preference of cattle grazing. That the two summit locations would be so different from each other and that a backslope would be more similar to a summit location emphasizes the need to consider topography when conducting soil measurements and making farm management decisions.
Topography strongly influenced all of the soil health properties measured in this project, in particular Backslope3, Summit2 and Footslope1. The Backslope3 location had an 18% slope compared to the other locations which ranged from a 1.4 to 7.9% slope. This steeper slope likely subjected this location to greater potential for erosion and therefore the loss of SOM compared to the other locations. This loss of SOM would also explain the low concentrations of TC and TN and subsequently why there was a low abundance of soil microorganisms and contributed to the high bulk density and low nitrate-N. These low measurements were also observed at Summit2 which also had low SOM; however, the slope at Summit2 was approximately 2%, and thus not subject to the same potential erosion expected at Backslope3. Another explanation that may apply to both locations is that the grazing cattle congregated at these particular locations increasing compaction of the soil and subsequently the bulk density and removing vegetative biomass which would decrease the SOM. This could also be the case at Backslope3; however, the slope of that location may discourage cattle from congregating there.

Another location strongly influenced by topography was Footslope1. Contrary to Backslope3 and Summit2, the highest concentration of nitrate-N was observed at Footslope1 in spring 2016 and the same for ammonium-N in spring 2017. It is highly probable that this site, which is located at the base of two hills, essentially a small valley, receives the runoff from the surrounding locations. This is supported by Ladoni et al. (2015) who found increased N concentrations at footslopes compared to depressions and summits under cover crops. Footslope1 is also located downslope from a cattle handling facility located on the west side of the pasture (Fig. 2.2). Footslope1 was also lowest in soil pH which may have been a result of the nitrification of ammonium, possibly from animal excreta, to nitrate. This in turn would explain why there was low NAGase activity at this location. The soil microorganisms may be reducing
production of NAGase enzyme due the concentration of nitrate, or the low pH may be inhibiting the enzyme activity (Hewins et al., 2015). At this same Footslope1 location, the relative abundance of AMF was at its lowest and had consistently high levels of inorganic nitrogen and phosphorus (Chapter 3). The reduction of AMF in response to high concentrations of soil nutrients has been well documented (Smith and Read, 2008) as high P concentrations have been found to reduce the production of root exudates that promote fungal colonization.

The soil bacterial groups followed the pattern of SOM in that they were high where SOM was high and low where SOM was low. Bacteria are typically found in abundance where there is an available carbon source. Saprophytic fungi were less affected by topography though the F:B ratio was widest at the Summit2 location. This was due to low bacterial abundance probably as a result of low SOM rather than a change in the abundance of saprophytic fungi which may be utilizing a more recalcitrant carbon source at the lower depth. While fungi may be less influenced by the amount and type of SOM, they can be strongly influenced by soil moisture. The narrow F:B ratio in fall 2016 at the Summit2 location was likely related to the low soil moisture at that sampling date.

Over time, nitrate-N declined while NAGase and SOM increased. The loss of nitrate is not uncommon in an unfertilized pasture system. It is easily leached through the soil and subject to denitrification and taken up by plants. Though the majority of inorganic N that is taken up by plants is expected to be returned in animal excreta, some of it is incorporated into the animal biomass. Also, as the grazing method is mob grazing, this keeps the vegetation from being grazed too closely and provides time for regrowth and may also encourage more root biomass. Conant et al. (2003) found that management intensive grazing such as mob grazing can increase soil organic C compared to extensive management and haying. This may be why SOM is
increasing. It is possible that the soil microorganisms, in response to the decreasing nitrate-N concentration, may be increasing N cycling enzyme activity in the presence of readily available carbon, to compensate (Bandick and Dick, 1999; Bowles et al., 2014). Also, TC and TN remained unchanged over time. This suggests that this perennial pasture system with incorporated winter annuals is maintaining fertility through microbial action. Possibly, the organic inputs are equaling the inorganic outputs and microbial action is compensating for the loss of inorganic-N, and an equilibrium has been reached.

Also, even though total FAMEs remained unchanged with time, the groups of soil microorganisms were influenced by season. Senesced plant material may have supported larger populations of GMp and actinomycetes in the fall while AMF increased in the spring when living roots would have increased in abundance (Habekost et al., 2008). At fall samplings, winter annual plants had just been over-seeded and had not had time to germinate, but warm season perennials were just going dormant and their aboveground biomass was beginning to degrade. This likely supported the GMp bacterial populations in the fall. However, in spring, day-length and temperature increase, and winter annuals would have been actively photosynthesizing and increasing root biomass. This increase in root biomass and photosynthetic carbon likely increased the AMF abundance and possibly colonization of plant roots. Therefore, though the total soil microbial community is not changing over time, the community composition is responding to changes of season.

2.5. Conclusions

It is common for most soil characteristics to decrease with depth; therefore, it was surprising to find that the relative abundance of saprophytic fungi increased with depth. More investigation is needed to determine what C source these fungi are utilizing and what implications this has for pasture or grassland management and possibly C sequestration. Also to gain a better perspective
on C in this system, it is recommended that future research include measurements of soil respiration to gain perspective on soil microbial outputs. Topography plays a very significant role in all aspects of soil health and must always be considered when determining the best agricultural production techniques. Also, though locations may be similar in topographic location (e.g. summits, footslopes, or backslopes), it does not necessarily indicate that they have similar soil characteristics. As nitrate-N decreased, SOM and NAGase activity increased, and TC and TN remained unchanged over time, it may be that this perennial warm-season pasture over-seeded with winter annuals has reached an equilibrium.
2.6. References


Hubbard, R.K., Strickland, T.C., Phatak, S., 2013. Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. Soil & Tillage Research 126, 276-283.


Chapter 3. Analysis of Changes in Soil Fertility after Eight Years of Over-seeding Winter Annuals into a Perennial Bahiagrass Pasture

3.1. Introduction

There are approximately 24 million hectares of perennial pasture land in the southern United States and an estimated additional 8 million hectares of annual pastures (Ball et al., 2015). Livestock producers in the southeastern United States have used cool-season annuals as winter forage to extend their grazing season for decades due to the mild winters in the region. Producers who plant cool-season annuals most often use a mixture of a grass, usually annual ryegrass (*Lolium multiflorum*), and a legume, usually a clover (*Trifolium* sp.), over-seeded into a perennial warm-season grass such as bahiagrass (*Paspalum notatum*) or bermudagrass (*Cynodon dactylon*). With proper grazing management, utilizing winter forages can be one half to one third the cost per day of feeding hay (Ball et al., 2015).

While winter forage can ameliorate feed costs, other benefits include reduced soil erosion and nutrient loss to runoff or leaching (Dabney, 2001; Dube et al., 2014; Endale et al., 2014), nitrogen (N) fixation via legumes which have also been found to improve forage quality (Han et al., 2013; Lithourgidis et al., 2006; Pirhofer-Walzl et al., 2012), and some brassica species have been found to reduce compaction (Chen and Weil, 2010; Williams and Weil, 2004). In intensely managed systems, like row crop production, winter annuals, or cover crops, have been found to increase soil carbon (C) and increase retention of inorganic N when compared to fallow management (Hubbard et al., 2013; Wright et al., 2004). Kabir and Koide (2002) found that use of rye (*Secale cereale*) and oats (*Avena sativa*) (annual grasses) as cover crops before a corn crop increased mycorrhizal colonization and P uptake.

Perennial grasses typically have a root system consisting of rhizomes, stolons, or tubers which allow the plant to regrow after being dormant during certain times of the year. Annual
grasses on the other hand have a shallower rooting depth and no specialized root structures to allow them to survive dormancy and must therefore be reseeded each season. While there are many benefits of winter annuals, perennial grasses have been found to have a greater impact on soil C and N storage (Mapfumo et al., 2002; Shi et al., 2013). In comparing the perennial grasses (smooth bromegrass and meadow bromegrass) to the annual grass (triticale) Mapfumo et al. (2002) found that perennial grasses had greater root biomass and C and N pools throughout the 0-60 cm soil profile. Shi et al. (2013) compared the decomposition rates of perennial grasses to annuals and found that decomposition of perennial grasses is slower than annuals which may reduce the rate of respiration and allow for carbon sequestration.

The objective of this study was to determine how the use of winter annuals over-seeded in a perennial warm season pasture in southern Mississippi affected the soil fertility of the system at both 0-15 cm and 0-30 cm. It was hypothesized that soil organic matter (SOM), total carbon (TC), and total nitrogen (TN) would increase with time due to the belowground accumulation of root biomass while soil macronutrients would remain stable due to the deposition of cattle excreta and mineralization activity of soil microorganisms.

3.2. Materials and Methods

The site for this project was a commercial cattle grazing operation located 12 km south of Woodville, MS (31.012794, -91.317935). The soils were a cultivated Loring silt loam. The soil taxonomy is fine-silty, mixed, active, thermic Oxyaquic Fragiudalfs. The perennial warm-season grass was bahiagrass and the over-seeded winter annuals included oats, triticale (Triticum secale), annual ryegrass (Lolium multiflorum), hairy vetch (Vicia villosa), radish (Raphanus sp.), turnips (Brassica sp.), AU Red Ace red clover (Trifolium pretense), Ball clover (Trifolium nigrescens), and Dixie crimson clover (Trifolium incarnatum) which had been incorporated into the management system for the past nine years. Annuals were drill seeded in the fall of each
year. Fields were grazed using mob grazing in which approximately 175 head of cattle moved across an estimated 217 hectares with herds being rotated to the next paddock every 12 to 24 hours. No fertilizer or pesticides had been applied since 2010. The average annual precipitation from 2015-2017 was 183 cm. Over the course of the project, the greatest precipitation event, 66 cm, occurred in August 2016, and the lowest recorded rainfall occurred in October 2016 with 0.83 cm of rain (Figure 2.1).

The area of the site sampled included topographically distinct locations including summits (2), backslopes (3), and footslopes (2) from which soil samples were collected (Figure 2.2). Summit1, Backslope1, Footslope1 and Backslope3 comprised the primary topographic sequence while Summit2, Backslope2, and Footslope2 were considered the secondary sequence. Soil samples were collected using soil cores that were 10.16 cm wide by 30 cm deep and divided into 7.5 cm intervals. To reduce the degrees of freedom, pseudo-replication was implemented. At each of the seven locations, nine soil cores were taken from within 1 meter of each other, separated according to depth, and composited to provide three pseudo-replicates per location for each depth. To remove plant matter and other large debris, all samples were sieved to 4.75 mm before analysis and air-dried for 36 hours.

The only sites in which both forage and soil samples were collected were the Summit1, Backslope1 and Footslope1 locations. Forage samples were collected from February 2015 to August 2016. For determining proportions of vegetative diversity, “date” was used as replication as forage samples were not collected from traditionally replicated plots. Vegetative diversity was divided into three groups: grasses, legumes, and weeds. Grasses were a sum of all grasses whether cool season or warm season or annual or perennial depending on when the sample was collected. Legume included basic classifications of clover and vetch. Plants which did not fit into
either category were designated weeds, for example bur clover (*Medicago polymorpha*) and little barley (*Hordeum pusillum*). Season was used as a variable and consisted of spring and summer 2015 and 2016.

Soil organic matter was measured using the method described by Nelson and Sommers (1996). For 16 hours, soil samples were oven dried at 105°C and weighed. For the next 24 hours, soil samples were then ashed in a muffle furnace at 400°C and weighed. To calculate percent Loss-On-Ignition the following formula was used:

\[
\% \text{ LOI} = \left( \frac{\text{Weight}_{105} - \text{Weight}_{400}}{\text{Weight}_{105}} \right) \times 100
\]

To determine soil moisture, 5.0 g of field moist sample were oven-dried at 105°C for 24 hours and weighed. Percent soil moisture was calculated as

\[
\% \text{ Moisture} = \left( \frac{\text{Field moist weight} - \text{Dry weight}}{\text{Dry weight}} \right) \times 100
\]

To determine inorganic N, first, soil samples were extracted by shaking 1 g of sample in 10 ml of 2M KCl for 1 hour on the low setting. Second, the first reagent for NH\textsubscript{4}\textsuperscript{+}-N, salicylate solution was pipetted into a 96-well plate before the extracted sample was added followed by the second reagent, bleach/NaOH solution. Samples were incubated for 50 minutes in the dark at room temperature. For NO\textsubscript{3}\textsuperscript{-}-N, a vanadium solution was pipetted into the 96-well microplates followed by the extracted sample which were incubated for 60 minutes in the dark at 37°C. Following incubation, the 96-well microplates were analyzed on an EON spectrophotometer by BioTek according to the method from Hood-Nowotny et al. (2010).

Using a Mehlich-3 extraction solution and inductively coupled plasma, the macronutrients (P, K, S, Ca, Mg, Na, Cu, and Zn) were analyzed by the LSU AgCenter Soil Testing and Plant Analysis Laboratory (STPAL). Soil pH was measured in 1:1 in deionized water:soil solution. Also processed by STPAL, TC and TN were determined using the dry combustion method by
LECO C/N Analyzer (St. Joseph, MI). Total C and TN were converted to soil C content and soil N content using bulk density at 0-15 cm and reported in Mg ha⁻¹.

Water aggregate stability was determined using an Eijkelkamp Wet Sieving Apparatus. Four grams of air dried soil per sample was pre-moistened to prevent slaking during the sieving process. Soils were subjected to repeated immersion in deionized water for 3 minutes in a 0.25 µg sieve. The sieved sample was collected and oven-dried at 110°C for 16 hours and weighed (W1). To correct for sand content, the remaining soil was immersed in a NaOH dispersing solution until remaining aggregates were disrupted and passed through the sieve. The dispersed soil was collected, oven-dried at 110°C for 16 hours, and weighed (W2). The aggregate stability was determined using the following formula

\[
\text{% Aggregate Stability} = \frac{W2}{W2 + W1}
\]

Analysis of variance (ANOVA) and Principal Component Analysis were determined using R statistical software. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Soil depth, date of collection, and topographic site of collection were considered independent variables. ANOVAs of each response variable (each soil nutrient and forage yield) was determined using the lm function in R. For forage diversity, data were analyzed using the Proc Glimmix procedure of SAS 9.4 after exploring this along with Proc Mixed using various covariance structures for repeated measures then choosing the approach with the best fit (smallest BIC). Significance was always p-value <0.05. Using the vegan package in R, principal component analysis was used to determine the relationships of each response variable to the independent variables using a correlation matrix through the capscale function using the Bray-Curtis dissimilarity index. There were 1000 permutations.
3.3. Results

3.3.1. Soil chemical and physical analysis at 0-15 cm

Soil moisture was consistent with precipitation (Figure 2.1) with the highest soil moisture in fall 2015 and spring 2016 averaging 62% and the lowest in fall 2016 at 33%. Soil moisture was highest at the footslopes with an average of 62.5% and lowest at the Backslope3 and Summit2 locations with an average of 44%. Aggregate stability (P=0.001) averaged 90% at the Backslope2, Backslope3, and Summit1 locations while all other locations averaged 85%. Pearson’s correlation revealed a positive relationship between SOM and aggregate stability to be 0.44.

Total N content (P≤0.0001) was lowest at the Summit2 and Backslope3 locations (Table 3.1). Soil P (P≤0.0001) was highest in the first fall and spring samplings and decreased by 44% in the fall 2016 but increased by 33% in spring 2017 (Table 3.2). Soil P (P≤0.0001) concentration was highest at Footslope1 (Table 3.1). Soil K (P=0.001) declined from fall 2015 to fall 2016 then began to recover in spring 2017 (Table 3.2). Soil K (P≤0.0001) concentrations were highest at the Summit1 location with 435.97 mg kg⁻¹ compared to an average of 255.67 mg kg⁻¹ at all other locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>TN (Mg ha⁻¹)</th>
<th>NO₃-N (mg kg⁻¹)</th>
<th>P (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summit1</td>
<td>1.05 (0.02) a</td>
<td>23.6 (2.24) a</td>
<td>46.9 (2.94) b</td>
</tr>
<tr>
<td>Backslope1</td>
<td>1.09 (0.02) a</td>
<td>12.8 (1.09) bc</td>
<td>25.8 (1.12) c</td>
</tr>
<tr>
<td>Footslope1</td>
<td>0.90 (0.03) ab</td>
<td>27.5 (3.20) a</td>
<td>62.0 (2.69) a</td>
</tr>
<tr>
<td>Summit2</td>
<td>0.69 (0.02) c</td>
<td>5.9 (1.42) c</td>
<td>25.4 (2.24) c</td>
</tr>
<tr>
<td>Backslope2</td>
<td>1.03 (0.01) a</td>
<td>19.7 (2.13) ab</td>
<td>33.1 (1.75) c</td>
</tr>
<tr>
<td>Footslope2</td>
<td>1.05 (0.02) a</td>
<td>24.2 (4.07) a</td>
<td>34.7 (3.12) bc</td>
</tr>
<tr>
<td>Backslope3</td>
<td>0.79 (0.02) bc</td>
<td>7.1 (1.08) c</td>
<td>28.7 (2.41) c</td>
</tr>
</tbody>
</table>

†Different lowercase letters denote difference between dates across locations.
Table 3.2. Soil nutrients according to date at 0-15 cm. Standard errors in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>NO$_3$-N (mg kg$^{-1}$)</th>
<th>P (mg kg$^{-1}$)</th>
<th>K (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall 2015</td>
<td>25.2 (2.23) a†</td>
<td>45.2 (2.45) a</td>
<td>325.5 (13.08) a</td>
</tr>
<tr>
<td>Spring 2016</td>
<td>27.0 (2.22) a</td>
<td>44.1 (1.92) a</td>
<td>268.5 (10.05) bc</td>
</tr>
<tr>
<td>Fall 2016</td>
<td>12.2 (1.30) b</td>
<td>24.6 (1.83) b</td>
<td>232.8 (10.13) c</td>
</tr>
<tr>
<td>Spring 2017</td>
<td>4.6 (0.35) b</td>
<td>32.7 (1.95) b</td>
<td>299.5 (7.85) ab</td>
</tr>
</tbody>
</table>

†Lowercase letters denote difference between dates.

There was an interaction between date of collection and topography for SOM (P=0.002), TC content (P=0.004), NH$_4^+$-N (P=0.019), and S (P=0.019). Summit2 and Backslope3 were lowest in SOM throughout the duration of the project while the highest measurement of SOM was in fall 2016 at the Summit1 location (Table 3.3). Soil C content decreased from fall 2015 to spring 2017 at Summit2 and Backslope3 only (Table 3.3). Ammonium-N was low in spring 2016 across locations but lowest at the Summit2 location. Ammonium-N concentrations were highest in spring 2017 at Footslope1 (Table 3.3). Sulfur (S) concentrations was high at Footslope1 regardless of date but was highest in fall 2015. The lowest S concentration was measured at Summit2 in spring 2017 (Table 3.3).

Table 3.3. Interaction of date and topography for soil nutrients at 0-15 cm. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>SOM (g kg$^{-1}$)</th>
<th>C (Mg ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall 2015</td>
<td>Spring 2016</td>
</tr>
<tr>
<td>Summit1</td>
<td>106.0 (3.59) B† abc‡</td>
<td>109.2 (1.85) B a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>97.1 (4.74) B bcd</td>
<td>108.6 (2.00) AB a</td>
</tr>
<tr>
<td>Footslope1</td>
<td>85.1 (3.85) B de</td>
<td>96.4 (0.88) AB ab</td>
</tr>
<tr>
<td>Summit2</td>
<td>89.9 (5.26) A cde</td>
<td>50.8 (1.23) B c</td>
</tr>
<tr>
<td>Backslope2</td>
<td>108.8 (1.41) AB ab</td>
<td>97.3 (4.79) B ab</td>
</tr>
<tr>
<td>Footslope2</td>
<td>119.5 (3.80) A a</td>
<td>105.0 (3.55) A ab</td>
</tr>
<tr>
<td>Backslope3</td>
<td>77.5 (2.57) A e</td>
<td>86.2 (3.97) A b</td>
</tr>
</tbody>
</table>

‡Lowercase letters denote difference between dates.
Backslope3 | 9.9 (0.35) A a | NA | NA | 7.5 (0.24) B bc
---|---|---|---|---
| NH$_4^+$-N (mg kg$^{-1}$) | Fall 2015 | Spring 2016 | Fall 2016 | Spring 2017
Summit1 | 12.6 (0.71) B ab | 11.1 (0.35) B a | 25.9 (0.71) A a | 24.8 (1.08) A ab
Backslope1 | 11.3 (0.15) B b | 11.3 (0.26) B a | 19.6 (0.28) A abc | 24.6 (1.15) A ab
Footslope1 | 16.4 (1.65) BC ab | 11.5 (0.36) C a | 19.8 (3.02) AB abc | 26.4 (2.59) A a
Summit2 | 20.8 (4.68) A a | 8.4 (1.06) B a | 12.9 (0.44) AB cd | 18.1 (1.45) A bc
Backslope2 | 20.0 (0.56) A a | 10.4 (1.08) B a | 16.8 (0.52) AB bcd | 23.1 (1.11) A ab
Footslope2 | 20.2 (0.31) AB a | 11.0 (0.56) C a | 22.9 (0.73) A ab | 14.1 (1.97) BC c
Backslope3 | 10.7 (0.60) B b | 10.3 (0.97) B a | 10.8 (0.40) B d | 20.2 (1.33) A abc

| S (mg kg$^{-1}$) | Fall 2015 | Spring 2016 | Fall 2016 | Spring 2017
Summit1 | 32.2 (2.69) B c | 41.3 (1.55) A b | 28.0 (0.77) B bc | 6.3 (1.87) C bc
Backslope1 | 30.0 (0.25) A c | 36.1 (0.95) A bcd | 31.8 (0.58) A b | 8.8 (0.65) B bc
Footslope1 | 64.6 (1.71) A a | 60.3 (0.85) A a | 44.5 (0.97) B a | 40.2 (1.96) B a
Summit2 | 20.0 (1.60) A c | 30.5 (0.80) A d | 19.5 (0.84) B d | 2.8 (0.43) C c
Backslope2 | 42.6 (2.59) A b | 39.2 (1.01) A bc | 28.9 (0.47) B bc | 12.0 (0.68) C b
Footslope2 | 28.3 (1.25) A c | 32.4 (2.13) A cd | 27.5 (1.62) A bc | 4.1 (0.65) B c
Backslope3 | 35.9 (1.86) A bc | 30.6 (1.09) A d | 22.0 (0.71) B cd | 8.8 (1.46) C bc

†Uppercase letters denote difference among dates within topography
‡Lowercase letters denote difference among topography within dates
NA is not available as bulk density was not measured for these dates

3.3.2. Soil chemical analysis at 0-30 cm

Total C (P≤0.0001) was lowest at Summit2 compared to the other locations (Table 3.4) and tended to be higher in the fall samplings with an average of 61.45 mg kg$^{-1}$ compared to spring samplings which averaged 55.45 mg kg$^{-1}$. Nitrate-N (P≤0.0001) decreased from fall 2015 to fall 2016 by 52% and continued to decrease 67% in spring 2017 (Table 3.4). The highest NO$_3^-$-N (P≤0.0001) concentrations were at the Footslope locations and lowest at the Summit2 and Backslope3 locations (Table 3.4). Soil P (P≤0.0001) concentration followed the same pattern as the 0-15 cm measurements according to date (Table 3.4). Phosphorus (P≤0.0001) concentration was highest at Footslope1 and Summit1 with an average of 68.50 mg kg$^{-1}$ compared to an average of 39.42 mg kg$^{-1}$ at all other locations (Table 3.4). Similar to the measurements at 0-15 cm, soil K (P≤0.0001) was 22% higher in fall 2015 and spring 2017 compared to spring and fall...
2016 (Table 3.4). The Summit1 location had the highest measured K ($P \leq 0.0001$) while the lowest measurements were at the Footslope locations (Table 3.4).

Table 3.4. Soil nutrients according to topography 0-30 cm. Standard errors in parentheses.

<table>
<thead>
<tr>
<th>Location</th>
<th>TC (g kg$^{-1}$)</th>
<th>NO$_3$-N (mg kg$^{-1}$)</th>
<th>P (mg kg$^{-1}$)</th>
<th>K (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summit1</td>
<td>68.0 (1.75) a†</td>
<td>32.9 (3.32) ab</td>
<td>66.0 (3.83) a</td>
<td>780.0 (30.04) a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>59.8 (1.03) bc</td>
<td>20.1 (1.37) bc</td>
<td>35.6 (1.46) b</td>
<td>479.6 (15.70) bc</td>
</tr>
<tr>
<td>Footslope1</td>
<td>56.0 (1.00) cd</td>
<td>38.1 (3.31) a</td>
<td>71.0 (2.89) a</td>
<td>406.2 (10.06) cd</td>
</tr>
<tr>
<td>Summit2</td>
<td>42.5 (1.36) e</td>
<td>6.4 (1.49) d</td>
<td>43.2 (2.88) b</td>
<td>467.6 (14.67) bc</td>
</tr>
<tr>
<td>Backslope2</td>
<td>65.5 (1.03) ab</td>
<td>25.3 (2.27) abc</td>
<td>39.6 (2.07) b</td>
<td>508.9 (17.93) b</td>
</tr>
<tr>
<td>Footslope2</td>
<td>66.5 (1.39) a</td>
<td>36.9 (5.10) a</td>
<td>41.4 (3.75) b</td>
<td>354.8 (13.45) d</td>
</tr>
<tr>
<td>Backslope3</td>
<td>51.0 (1.18) d</td>
<td>11.9 (2.01) cd</td>
<td>37.4 (2.99) b</td>
<td>466.4 (18.69) bc</td>
</tr>
</tbody>
</table>

Date of collection

<table>
<thead>
<tr>
<th>Date</th>
<th>TC (g kg$^{-1}$)</th>
<th>NO$_3$-N (mg kg$^{-1}$)</th>
<th>P (mg kg$^{-1}$)</th>
<th>K (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall 2015</td>
<td>62.5 (1.24) a</td>
<td>36.8 (3.12) a</td>
<td>58.8 (3.12) a</td>
<td>546.4 (20.63) a</td>
</tr>
<tr>
<td>Spring 2016</td>
<td>54.7 (1.28) c</td>
<td>31.4 (2.36) ab</td>
<td>58.1 (1.99) a</td>
<td>459.9 (17.97) b</td>
</tr>
<tr>
<td>Fall 2016</td>
<td>60.4 (1.57) ab</td>
<td>22.5 (1.99) b</td>
<td>32.3 (1.86) b</td>
<td>407.6 (18.24) b</td>
</tr>
<tr>
<td>Spring 2017</td>
<td>56.3 (1.09) bc</td>
<td>7.4 (0.62) c</td>
<td>41.6 (2.13) b</td>
<td>565.3 (15.25) a</td>
</tr>
</tbody>
</table>

†Lowercase letters denote difference between topography or dates
Table 3.5. Interaction of date and topography for soil nutrients at 0-30 cm. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>SOM (g kg$^{-1}$)</th>
<th>TN (g kg$^{-1}$)</th>
<th>pH</th>
<th>NH$_4^+$-N (mg kg$^{-1}$)</th>
<th>S (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall 2015</td>
<td>Spring 2016</td>
<td>Fall 2016</td>
<td>Spring 2017</td>
<td>Fall 2015</td>
</tr>
<tr>
<td>Summit1</td>
<td>164.3 (3.51) B† a‡</td>
<td>168.8 (1.56) B a</td>
<td>218.7 (4.00) A a</td>
<td>187.0 (4.72) B a</td>
<td>7.1 (0.32) B ab</td>
</tr>
<tr>
<td>Backslope1</td>
<td>148.7 (6.80) A ab</td>
<td>166.9 (1.88) A a</td>
<td>162.6 (4.51) A bc</td>
<td>173.3 (2.10) A a</td>
<td>6.8 (0.15) AB ab</td>
</tr>
<tr>
<td>Footslope1</td>
<td>130.4 (3.67) AB b</td>
<td>139.4 (1.06) A b</td>
<td>146.1 (0.65) A cd</td>
<td>112.1 (11.45) B c</td>
<td>5.7 (0.36) B b</td>
</tr>
<tr>
<td>Summit2</td>
<td>127.5 (3.70) A b</td>
<td>77.3 (1.37) B c</td>
<td>132.4 (3.95) A d</td>
<td>116.4 (3.14) A bc</td>
<td>3.8 (0.32) A c</td>
</tr>
<tr>
<td>Backslope2</td>
<td>174.9 (1.44) AB a</td>
<td>153.5 (4.70) B ab</td>
<td>181.7 (1.15) A b</td>
<td>185.2 (4.24) A a</td>
<td>6.8 (0.22) AB a</td>
</tr>
<tr>
<td>Footslope2</td>
<td>173.5 (5.36) A a</td>
<td>148.4 (4.05) AB ab</td>
<td>163.3 (4.81) A bc</td>
<td>130.8 (9.76) B bc</td>
<td>7.3 (0.10) A ab</td>
</tr>
<tr>
<td>Backslope3</td>
<td>131.2 (1.65) A b</td>
<td>131.0 (5.85) A b</td>
<td>124.7 (6.86) A d</td>
<td>140.5 (2.93) A b</td>
<td>6.3 (0.10) A ab</td>
</tr>
</tbody>
</table>

Notes: †, ‡, and § indicate significance levels for comparisons.
Soil organic matter (P=0.001) was highest in fall 2016 at Summit1 location and lowest at the Summit2 location in spring 2016 (Table 3.5). Total N (P=0.040) was similar to the pattern of SOM; however, TN in the Summit2 location was consistently one of the lowest across all sampling dates (Table 3.5). Soil pH (P=0.002) was lowest in fall 2015 at all locations and Footslope1 had the lowest soil pH across dates (Table 3.5). The highest recorded observation for $\text{NH}_4^+$-N was at the Summit1 location in fall 2016, and, while all other locations varied with time, Backslope3 remained unchanged across dates (Table 3.5). The Footslope1 location was consistently higher in S than any other location except Summit1 which decreased between spring and fall 2016 (Table 3.5). Sulfur concentrations were low in the spring of 2017 across locations with the lowest concentration measured at Summit2 (Table 3.5).

3.3.3. Forages

Average forage yield across topography was higher in spring 2015 than in spring 2016, 1732 and 797 kg ha$^{-1}$, respectively, and, although not statistically significant, tended to be higher at Footslope1 (Table 3.6). An interaction of topography by season was significant for grasses (P=0.002) and weeds (P=0.029) but not for legumes (P=0.87). Legume production tended to be higher in spring compared to summer, but was not statistically significant (Table 3.7). In spring 2015, the proportion of grasses was 22% higher at the Footslope1 location than the Summit1; however, in spring 2016 there was a 44% greater proportion of grasses at the Summit1 location compared to Footslope1 (Table 3.7). In spring 2016, the proportion of weeds at Footslope1 was 65% higher than at Summit1 (Table 3.7).
Table 3.6 Forage yield along primary topographic sequence in kg ha\(^{-1}\). Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Summit1</td>
<td>1752 (262.94) a†</td>
<td>712 (176.06) a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>1640 (105.34) a</td>
<td>645 (136.14) a</td>
</tr>
<tr>
<td>Footslope1</td>
<td>1804 (255.26) a</td>
<td>1034 (345.11) a</td>
</tr>
</tbody>
</table>

† Lowercase letters denote difference among topography within dates.

Table 3.7 Proportion of plant functional groups according to a date by topography interaction reported as % dry weight of forage collected.

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Spring 2015</th>
<th>Summer 2015</th>
<th>Spring 2016</th>
<th>Summer 2016</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass Composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Footslope1</td>
<td>71 a†</td>
<td>44 b</td>
<td>40 b</td>
<td>56 ab</td>
<td>10.96</td>
</tr>
<tr>
<td>*Lower</td>
<td>63 ab</td>
<td>56 a</td>
<td>56 a</td>
<td>62 ab</td>
<td>10.96</td>
</tr>
<tr>
<td>*Upper</td>
<td>47 c</td>
<td>61 a</td>
<td>57 a</td>
<td>49 b</td>
<td>10.96</td>
</tr>
<tr>
<td>Summit1</td>
<td>56 bc</td>
<td>55 a</td>
<td>58 a</td>
<td>63 a</td>
<td>10.96</td>
</tr>
<tr>
<td>SEM</td>
<td>7.75</td>
<td>6.33</td>
<td>5.48</td>
<td>7.75</td>
<td></td>
</tr>
</tbody>
</table>

| Legume Composition |             |             |             |             |     |
| Footslope1        | 18          | 0.6         | 17          | 3.3         | 3.80|
| Lower             | 19          | 1.4         | 13          | 5.9         | 3.80|
| Upper             | 23          | 1.5         | 14          | 6.0         | 3.80|
| Summit1           | 23          | 2.2         | 16          | 5.0         | 3.80|
| SEM               | 8.55        | 6.98        | 6.04        | 8.55        |     |

| Weed Composition  |             |             |             |             |     |
| Footslope1        | 6 b         | 44 a        | 38 a        | 33 ab       | 10.74|
| Lower             | 15 ab       | 34 ab       | 27 b        | 24 b        | 11.32|
| Upper             | 25 a        | 29 b        | 24 b        | 40 a        | 12.40|
| Summit1           | 18 ab       | 32 b        | 23 b        | 23 b        | 11.32|
| SEM               | 8.77        | 7.16        | 6.204       | 8.773       |     |

† Within columns, least square means followed by similar letter are not different (\(P > 0.05\)).

*Upper and Lower were forage observations from the Backslope1.

3.4. Discussion

Though it is likely that winter annuals had an impact in this system, it could not be differentiated from the strong influences of abiotic factors such as topography and climate. Also, any impacts of these shallow rooted plants were likely in the top 15 cm of the soil while the perennial plants likely impacted the top 30 cm of soil.
At the 0-15 cm depth, SOM increased over time especially at the primary topographic sequence while soil C content did not change overall. One possibility is that soil microaggregates are physically protecting the SOM from decomposition as suggested by Lal (2007). Soil aggregates form when soil particles cohere to each other and increase in size as more and more particles or other aggregates cohere or are bound by root exudates or soil microbial biofilm, glomalin, or fungal hyphae. Soil aggregates can contain microsites of SOM that are protected from soil microbial contact and enzyme activity. In a perennial grassland system such as this one, water aggregate stability would be high due to the almost constant vegetative cover and abundant root biomass which would promote a diverse and abundant soil microbial population (Chapter 1). The positive relationship between SOM and aggregate stability supports this possibility. Another explanation would be spatial heterogeneity. In a meta-analysis by Poeplau and Don (2015), they found that cover crops increase SOC sequestration but found 13 studies in which SOC decreased with added C inputs. Their explanation was priming when low C:N plant material is suddenly added to the system causing a microbial response that degrades both the new and old C in the system which does not fit the perennial pasture system of this project. They also suggested spatial heterogeneity in which SOC stocks of a site are so varied that any slight effects of cover crops is difficult to detect. Measuring certain fractions of SOM such as the light fraction or particulate organic matter might provide a clearer perspective of why SOM is increasing at 0-15 cm depths, but TC is not. However, there were only two measurements for TC at the 0-15 cm depth at two different seasons which may be hiding a closer relationship with SOM.

While TC did not decrease overall, it did decrease at two particular locations, Backslope3 and Summit2. However, SOM though usually low at these locations, did not decrease with time.
As mentioned in Chapter 1 of this dissertation, Backslope3 has an 18% slope which makes it very vulnerable to erosion. This is likely why TC is decreasing here though it does not explain why SOM does not decrease at this location. It may be that degradation of SOM is slower at this location, compared to the others, due to the lower soil moisture which would result in lower soil microbial abundance. Summit2 also had lower soil moisture than the other locations, but it has a slope of approximately 2% (Chapter 1). The similarity of the Summit2 to Backslope3 could possibly be due to vegetative production or species which were not measured or an unmeasured soil characteristic.

Unlike at the 0-15 cm soil depth, TC and SOM at 0-30 cm had higher measurements in fall than spring but did not increase with time overall. Seasonal fluctuation may be due to perennial plant roots which grow deeper into the soil profile than annual plant roots. In the fall, the bahiagrass of this pasture system is beginning to enter into dormancy for the winter and may therefore be devoting more carbon to root systems while at the time of spring sampling these perennial plants are dormant. Mapfumo et al. (2002) found that perennial grasses impacted soil C and N pools at 0-60 cm. However, the lack of change with time suggests a steady state of SOM and TC at deeper depths. Six et al. (2002) determined that soil physiochemical characteristics such as being chemically bound to silt and clay minerals inhibit SOM degradation resulting in a lack of increasing SOC in spite of increasing SOM inputs which suggests that soils can reach a C saturation point. It may be the case in this system as the soil is a silt loam with average clay content of 20% and silt of 63%.

At 0-15 and 0-30 cm, TN varied by location over time but remained unchanged over time. However, inorganic N, NO₃⁻-N in particular, decreased in the two years of this project. This suggests that while inorganic N is being lost, organic N is increasing. Sources of organic N
would be plant biomass, soil microbial or faunal biomass, and livestock manure. Leaching was most likely responsible for the loss of NO$_3^-$-N as well as denitrification due to the low soil pH and high rainfall of the area. Loss of soil fertility is not uncommon in pastures not receiving fertilizer applications (Haynes and Williams, 1993) due plant uptake and removal by grazing though it would seem that in this agroecosystem the removal of N from the system by livestock is not greater than their contribution through manure inputs.

At both 0-15 and 0-30 cm, S and P decreased with time though P showed signs of recovery in spring 2017. Sulfur responds very similarly to NO$_3^-$-N in the soil and is likely being lost in the same way through plant uptake and leaching. As for P and K which both decreased in fall 2016, this may have been due to a plant uptake or, in the case of P, a changing pH. Potassium, a base cation, is almost entirely inorganic and typically made more available in a more neutral pH. It is also readily taken up by plants from the soil or can be lost to runoff or erosion, and in this study, the lowest measured K was in fall 2016 following the severe rain event.

While the forage yield decreased from 2015 to 2016 this may not be an accurate representation of the actual forage yield in those years. The timing of forage collection was not always consistent with how long before or after the cattle had grazed the area. However, the trend of higher yield at the footslope location is noticeable in both years. This is probably due to the accumulation of nutrients, NO$_3^-$-N, S, and P, at this location which is the lowest point at the site. In spring 2015, the proportion of grasses was high whereas the proportion of weeds was low, which is opposite of the trend observed in spring 2016, when the proportions were almost equal to each other. It is possible that loss of NO$_3^-$-N, S, and P is contributing to loss of yield (if yield numbers are considered to be accurate) and promoting weed populations which can out-compete grasses in low nutrient soils.
3.5. Conclusions

Due to the influences of abiotic factors such as topography and climate, any effects that winter annuals may have had in this agroecosystem were not detected. Although, continued use of winter annuals in a perennial pasture and rotational grazing may continue to increase SOM at the 0-15 cm depth. At the 0-30 cm depth, it is possible that perennial grass root biomass is affecting SOM, C, and N pools. Without fertilizer applications, \( \text{NO}_3^- \cdot \text{N} \) and S concentrations will likely continue to decline, and, based on the data, forage yield may also decline. However, the producer has been utilizing these management techniques for the past eight years, and livestock production has not declined. If at some point the forage production decreases below the requirements of the livestock grazing these pasture, liming and fertilizer application may be necessary to increase forage biomass and quality. An even distribution of soil nutrients and subsequent forage production may not be achievable due to the topography of this site.
3.6. References


Hubbard, R.K., Strickland, T.C., Phatak, S., 2013. Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. Soil & Tillage Research 126, 276-283.


Chapter 4. Analysis of the Soil Health of Pastures under Differing Years of Winter Annual Covers and Grazing

4.1. Introduction

In the southeastern United States, there are approximately 24 million hectares of perennial pasture and an additional 8 million hectares of annual pasture (Ball et al., 2015). Livestock producers especially in the southeastern United States have often used winter annuals as forage for their livestock in cooler months in order to extend the grazing season and reduce the amount of hay or concentrate feeds that would need to be purchased (Utley and McCormick, 1978). Grazing livestock instead of feeding hay can lower costs by one third to one half per day (Ball et al., 2015).

There are several studies focused on grazing, some of which found increased saprophytic fungal abundance and decreased soil organic matter (SOM) (Davinic et al., 2013), increased mycorrhizal % colonization (Damsma et al., 2015), greater bulk density (Mapfumo et al., 1999), and altered enzyme activity (Hewins et al., 2015). These studies were conducted using various stocking rates with higher stocking rates typically having a negative effect on most soil health parameters and lower stocking rates benefitting soil health. The use of winter annuals may ameliorate some negative effects particularly in areas where hay or concentrate feeds would be supplemented in the winter months as these are areas where cattle may congregate. A high concentration of cattle in one area would increase bulk density by trampling and concentrate manure deposition leading to a decrease of plant growth at that location.

Studies of the benefits of winter annuals to the soil in the southeastern United States have focused on carbon (C) and nitrogen (N) inputs in row crops (Hubbard et al., 2013) or integrated cropping livestock (ICL) systems (Franzluebbers and Stuedemann, 2015). Few studies, if any, have focused on how grazing and winter annual forages affect soil health in pastureland alone.
The objective of this study was to determine how varying durations of grazing and winter annual utilization affected the soil health of a perennial warm season pasture compared to no management at all.

4.2. Materials and Methods

The site for this project was a privately owned commercial grazing operation located in 27 km north of St. Francisville, LA (30.898961, -91.316006). Three pastures (approximately 4 hectares each) with varying years of management were selected. The pastures had been under cattle grazing for different lengths of time: 10 years, 4 years, and 0 years. Additionally, the pastures at 10 years and 4 years had been over-seeded with winter annuals for the past four years while the ungrazed (0 years) pasture had no over-seeding of winter annuals during the course of this study. The soil in all three fields was classified as a cultivated Loring silt loam (fine-silty, mixed, active, thermic Oxyaquic Fragiudalfs). The unmanaged pasture had a vegetative cover of weeds such as buttercup (*Ranunculus* sp.). The grazed sites were bahiagrass (*Paspalum notatum*) pasture that were over-seeded with a varying mix of winter annuals including oats (*Avena sativa*), triticale (*Triticum secale*), annual ryegrass (*Lolium multiflorum*), hairy vetch (*Vicia villosa*), mustard greens (*Brassica juncea*), collard greens (*Brassica oleracea*), AU Red Ace red clover (*Trifolium pretense*), Ball clover (*Trifolium nigrescens*), and Dixie crimson clover (*Trifolium incarnatum*) for the past four years. The grazing regime was rotational grazing with rotations every 24 to 48 hours of approximately 120 head of cattle across 101 hectares. There had been no fertilizer or pesticide application to this site since 2010.

Average annual precipitation for the area from 2016-2018 was 203 cm according to the St. Francisville, LA weather station. The highest amount of rainfall, 66 cm, occurred in August 2016 two months prior to sampling, and the lowest rainfall occurred in October 2016 with 0.84 cm of rain throughout October (Figure 2.2).
Each pasture was divided into three pseudo-replicates from which five soil cores were collected to a depth of 30 cm in increments of 7.5 cm. Within each pseudo-replicate five soil cores were composited to provide a representative sample. Sampling occurred in October 2016 and 2017 and March 2017 and 2018. Pseudo-replication was used to decrease degrees of freedom as true replication of the management practices was not available. Soils to be analyzed for biological properties were stored in ice chests at 4°C for travel and then stored at -20°C until analysis. All soils were sieved at 4.75 mm before analysis. An air-dried subsample of soil was analyzed for enzymes and inorganic N.

To determine bulk density, soils were collected using soil core rings of a known volume, 267.15 cm$^3$. Rings were hammered into the soil surface and removed. A second ring was hammered into the same hole to get the bulk density of the second depth. Soils were then oven-dried for 16 hours at 105°C and weighed. The weight of the soil was divided by the volume to determine bulk density.

To determine soil moisture, 5.0 g of field moist soil sample were weighed out then dried in an oven at 105°C for 24 hours and weighed again. The formula used for calculation of soil moisture was

\[
\text{% Moisture} = \frac{(\text{Field moist weight} - \text{Dry weight})}{\text{Dry weight}} \times 100
\]

To determine SOM, soil samples were oven dried at 105°C for 16 hours then weighed. Samples were then subjected to 400°C in a muffle furnace for 24 hours and weighed. This is referred to as the percent Loss-on-Ignition method developed by Nelson and Sommers (1996). Percent Loss-On-Ignition was calculated as

\[
\text{% LOI} = \frac{(\text{Weight}_{105} - \text{Weight}_{400})}{\text{Weight}_{105}} \times 100
\]
Soil pH (1:1 in deionized water) and TC and TN as well as extractable P, K, and S were analyzed by the LSU AgCenter Soil Testing and Plant Analysis Laboratory. Total C and TN were determined using the dry combustion method in a LECO C/N Analyzer. Soil concentrations of P, K, and S were determined by a Mehlich-3 extraction solution and inductively coupled plasma.

According to the method from Hood-Nowotny et al. (2010), to determine both nitrate (NO$_3^-$) and ammonium (NH$_4^+$), inorganic N was extracted using 10 ml of 2M KCl per 1 g soil. For NH$_4^+$-N, a salicylate solution was the first reagent pipetted into a 96-well microplate. Extracted sample was added before the second reagent, a bleach/NaOH solution. These plates were incubated in the dark at room temperature for 50 minutes. For NO$_3^-$-N, the only reagent, a vanadium solution was pipetted into 96-well microplates after which the extracted sample was added. For 60 minutes, these plates were incubated in the dark at 37°C. After incubation, all microplates were read on an EON microplate spectrophotometer from BioTek.

Microbial community structure was determined using ester linked fatty acid methyl ester analysis according to Shutter and Dick (2000). Field moist soil samples were methylated by the addition of 0.2 M KOH in methanol and subjected to a 37°C water bath for 60 minutes with four intervals of vortexing. Samples were then neutralized by the addition of 1.0 M acetic acid and vortexed. Samples were finally extracted with the addition of hexane, inverted, and centrifuged at 2200 rpm for 5 minutes. The organic phase was transferred to test tubes and concentrated using N$_2$ gas at 37°C. Finally, samples were rehydrated using hexane and an internal standard. Biomarkers used identification of soil microbial groups were 17:0 10-methyl for actinomycetes (Actino), 16:1 w5c for arbuscular mycorrhizal fungi (AMF), 18:3 w6c, 18:1 w9c for saprophytic fungi, 16:1 w9c, 16:1 w7c, 19:0 cyclo w6c, 18:1 w7c for Gram negative (GMn) bacteria, and
14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:0 iso, 17:1 w9c, 18:0 for Gram positive (GMp) bacteria (Frostegård and Bååth, 1996; Kroppenstedt, 1992; Madan et al., 2002; Walling et al., 1996; Zak et al., 1996; Zelles, 1997; Zogg et al., 1997). Identification of fatty acids was done using MIDI software on a gas chromatograph (Agilent Technologies 7890B GC).

The enzymes N-acetyl-β-glucosaminidase (NAGase) and β-glucosidase (glucosidase) were measured according to Parham and Deng (2000) and Tabatabai (1994), respectively, to determine potential microbial activity. Half a gram of air-dried soil sample was mixed with a modified universal buffer (MUB pH 6.0) along with substrate of the enzyme being measured. This soil solution was incubated for 60 minutes at 37°C. Following incubation, 0.1 THAM (tris hydroxymethyl aminomethane) buffer pH 12 and 0.5 M CaCl2 were added to the soil solution and mixed. This solution was filtered through Whatman No. 2 folded filter paper. Samples were then pipetted into 96-well microplates and read by a spectrophotometer at 420 nm. Concentrations were determined according to a standard curve.

Analysis of variance (ANOVA) were determined using R statistical software (R Core Team, 2013). Years of management and date of collection were considered independent variables. Soils were analyzed at 0-15 and 0-30 cm depths. For the 0-15 cm values, the 0-7.5 and 7.5-15 cm were summed for all the soil characteristics except pH, bulk density, and C:N and F:B ratios. The same summation across all depths was used for 0-30 cm values. Using the vegan package in R (Oksanen et al., 2017), principal component analyses of FAME data was conducted using a correlation matrix through the capscale function using the Bray-Curtis dissimilarity index. Vectors were applied through the envfit function. The slice function in SAS 9.4 was utilized to determine Tukey’s LSD for interactions.
4.3. Results

4.3.1. Years of management

At the 0-15 cm depth, P (P=0.032), K (P=0.023), pH (P≤0.0001) and bulk density (P=0.034) varied under years of management. There was 32.49 mg kg$^{-1}$ of P in the 4-year pasture compared to an average 22.04 mg kg$^{-1}$ in the 0- and 10-year pastures. Potassium concentration was 188.91 mg kg$^{-1}$ in the 4-year pasture compared to an average of 155.08 mg kg$^{-1}$ in the 0- and 10-year pastures. Soil pH was highest in the 10-year pasture at 5.95, an increase from pastures grazed for 0 and 4 years (average 5.33). Bulk density was lowest in the 10-year pasture at 1.33 g cm$^{-3}$ compared to the 0- and 4-year pastures which averaged 1.42 g cm$^{-3}$.

At the 0-30 cm depths, K (P=0.021) was higher in the 4-year pasture than the 0- or 10-year pasture. Soil pH was 5.88 in the 10-year pasture compared to an average of 5.47 under the other pastures. An interaction of dates of collection and years of management affected K concentrations (P=0.007). Potassium was highest in the March samplings across all pastures; however, measurements in the 4-year pasture in the October 2016 sampling were not different from the March samplings (Fig 4.1).

Measured to depth of 0-15 cm, soil microbial groups and the total FAMEs (P=0.053) did not change with years of management; however, the glucosidase (P=0.002) and NAGase (P≤0.0001) enzymes did. The potential activity of glucosidase was 25% higher under the 10-years of management compared the 0- and 10-year pastures (Figure 4.2). The NAGase enzyme increased from the 0- to 4-year pasture by 24% and from the 4 to 10 year pasture by 29% (Figure 4.2).
At 0-15 cm, TC (P≤0.0001) decreased by 40% from October 2016 to October 2017 before returning to previous concentrations in March 2018 (Table 4.1). Ammonium-N (P≤0.0001)
concentrations followed the same pattern as TC, decreasing by 65% before increasing to previous concentrations. Total N (P=0.0003) remained unchanged from October 2016 through October 2017 before increasing by 40% from October 2017 to March 2018 (Table 4.1). Potassium (P≤0.0001) concentrations were higher in the March samplings compared to those collected in the October (Table 4.1). Sulfur (P=0.005) concentrations were highest in the March 2018 sampling compared to the previous samplings (Table 4.1). At 0-30 cm, TC (P=0.0003) was highest in October 2016 compared to the following three sampling dates (Table 4.2). Total N at 0-30 cm (P=0.001) was similar to 0-15 cm with the highest measurement in March 2018 though it was not different from October 2017. At 0-30 cm, K (P≤0.0001) concentrations were higher in March samplings than October (Table 4.2).

Measured in 0-15 cm samples only, the absolute abundance of GMp (P=0.0003), GMn (P≤0.0001), saprophytic fungi (P≤0.0001), and AMF (P≤0.0001) decreased from October 2016 to March 2017 and again in October 2017. Absolute abundance of these groups did tend to increase in March 2018 (Table 4.3). Actinomycetes (P=0.0006) decreased from March 2017 to October 2017 (Table 4.3). The fungi to bacteria ratio (F:B) (P≤0.0001) decreased 5-fold from October 2016 to March 2017 (Table 4.3). Both enzymes increased their potential activity from March 2017 to March 2018 (Table 4.3). An interaction of years of management and dates of collection affected total FAMEs only (P=0.043). In October 2016 total FAMEs were highest regardless in March 2018 total FAMEs rebounded by 67% in 4-year pasture and 292% in the 10-year pasture (Table 4.4).
Table 4.1. Soil nutrients according to dates of collection (0-15 cm). Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>SOM (g kg(^{-1}))</th>
<th>TC (g kg(^{-1}))</th>
<th>TN (g kg(^{-1}))</th>
<th>C:N</th>
<th>NH(_4^+)-N (mg kg(^{-1}))</th>
<th>K (mg kg(^{-1}))</th>
<th>S (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2016</td>
<td>72.7 (2.78) a†</td>
<td>35.2 (0.13) a</td>
<td>3.4 (0.01) b</td>
<td>10.6 (0.32) a</td>
<td>31.0 (0.54) a</td>
<td>138.0 (11.33) b</td>
<td>28.4 (0.80) b</td>
</tr>
<tr>
<td>March 2017</td>
<td>70.4 (1.29) a</td>
<td>31.1 (0.06) a</td>
<td>3.4 (0.01) b</td>
<td>9.0 (0.08) b</td>
<td>25.8 (0.64) b</td>
<td>209.5 (2.44) a</td>
<td>24.5 (0.80) b</td>
</tr>
<tr>
<td>October 2017</td>
<td>66.1 (0.84) a</td>
<td>22.0 (0.11) b</td>
<td>3.0 (0.01) b</td>
<td>7.2 (0.15) bc</td>
<td>11.8 (0.22) c</td>
<td>110.0 (6.06) b</td>
<td>27.6 (1.31) b</td>
</tr>
<tr>
<td>March 2018</td>
<td>80.6 (1.68) a</td>
<td>33.7 (0.06) a</td>
<td>4.2 (0.01) a</td>
<td>7.9 (0.09) c</td>
<td>31.2 (1.26) a</td>
<td>207.9 (6.18) a</td>
<td>34.2 (0.49) a</td>
</tr>
</tbody>
</table>

†Lower case letters denote difference between dates of sample collection.
SOM=soil organic matter; TC=total carbon; TN=total nitrogen; C:N=carbon to nitrogen ratio; NH\(_4^+\)-N=ammonium; K=potassium; S=sulfur

Table 4.2. Soil nutrients according to dates of collection (0-30 cm). Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>TC (g kg(^{-1}))</th>
<th>TN (g kg(^{-1}))</th>
<th>C:N</th>
<th>K (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2016</td>
<td>54.9 (1.44) a†</td>
<td>5.3 (0.10) bc</td>
<td>10.4 (0.22) a</td>
<td>283.4 (19.56) c</td>
</tr>
<tr>
<td>March 2017</td>
<td>44.8 (0.72) b</td>
<td>4.8 (0.08) c</td>
<td>9.4 (0.10) b</td>
<td>417.0 (7.50) a</td>
</tr>
<tr>
<td>October 2017</td>
<td>38.4 (1.51) b</td>
<td>5.5 (0.17) ab</td>
<td>7.0 (0.10) c</td>
<td>233.2 (7.63) c</td>
</tr>
<tr>
<td>March 2018</td>
<td>43.1 (0.71) b</td>
<td>5.9 (0.05) a</td>
<td>7.2 (0.08) c</td>
<td>360.7 (8.08) b</td>
</tr>
</tbody>
</table>

†Lower case letters denote difference between dates of sample collection.
TC=total carbon; TN=total nitrogen; C:N=carbon to nitrogen ratio; K=potassium
Table 4.3. Absolute abundance of fatty acid methyl esters and potential enzyme activity. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>GMp</th>
<th>GMn</th>
<th>Actino</th>
<th>Fungi</th>
<th>AMF</th>
<th>F:B</th>
<th>NAGase</th>
<th>Glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2016</td>
<td>72.34 (4.86) a†</td>
<td>40.30 (2.50) a</td>
<td>23.97 (1.59) a</td>
<td>53.49 (3.13) a</td>
<td>34.46 (2.75) a</td>
<td>0.42 (0.02) a</td>
<td>66.0 (3.92) bc</td>
<td>65.8 (4.75) bc</td>
</tr>
<tr>
<td>March 2017</td>
<td>42.85 (3.29) bc</td>
<td>29.42 (1.89) b</td>
<td>23.23 (2.80) a</td>
<td>8.78 (0.63) b</td>
<td>8.22 (0.61) b</td>
<td>0.08 (0.003) b</td>
<td>61.9 (2.75) c</td>
<td>61.9 (1.67) c</td>
</tr>
<tr>
<td>October 2017</td>
<td>23.66 (1.21) c</td>
<td>8.32 (0.26) c</td>
<td>5.56 (0.30) b</td>
<td>3.30 (0.11) b</td>
<td>0.47 (0.02) b</td>
<td>0.08 (0.003) b</td>
<td>76.1 (3.33) ab</td>
<td>78.8 (2.54) b</td>
</tr>
<tr>
<td>March 2018</td>
<td>58.35 (7.60) ab</td>
<td>17.17 (1.77) c</td>
<td>12.52 (1.13) b</td>
<td>6.97 (0.78) b</td>
<td>2.57 (0.31) b</td>
<td>0.08 (0.003) b</td>
<td>84.8 (3.11) a</td>
<td>126.0 (4.63) a</td>
</tr>
</tbody>
</table>

†Lower case letters denote difference between years of management.

GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; F:B=fungi to bacteria ratio; NAGase= N-acetyl-β-glucosaminidase; Glucosidase=β-glucosidase

Table 4.4. Interaction of date of collection and years of management for total fatty acid methyl esters. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>October 2016</th>
<th>March 2017</th>
<th>October 2017</th>
<th>March 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol g⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>350 (42.25) A† a‡</td>
<td>197 (7.25) B a</td>
<td>102 (5.01) B a</td>
<td>112 (3.92) B b</td>
</tr>
<tr>
<td>4</td>
<td>374 (12.05) A a</td>
<td>158 (27.17) B a</td>
<td>104 (3.05) B a</td>
<td>174 (33.85) B b</td>
</tr>
<tr>
<td>10</td>
<td>328 (46.37) A a</td>
<td>260 (18.11) A a</td>
<td>100 (2.18) B a</td>
<td>393 (18.51) A a</td>
</tr>
</tbody>
</table>

†Upper case letters denote difference between dates of collection.
‡Lower case letters denote difference between years of management.
Figure 4.3. Principle component analysis of absolute abundance of fatty acid methyl ester data a.) according to years of management and b.) according to date of collection. Vectors are relative abundance of soil microorganisms. Bac=total bacteria; GMn= Gram negative bacteria; GMp=Gram positive bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi.
Principle component analysis of relative abundance of FAME data demonstrated that years of management (Fig 4.3a) had no influence on relative abundance of soil microbial groups while dates of collection did (Fig 4.3b). Sample sets separated more distinctly when considering dates of collection (Fig 4.3b) demonstrating the increase of GMp bacteria in October 2017 and March 2018 while AMF were opposite with their highest measurements in October 2016 and March 2017. GMn and Actino were highest in March 2017 (Fig 4.3b).

4.4. Discussion

The majority of the measured soil variables in this project were not affected by years of management. This may be due to the fact that although there is some difference in how these pastures are all managed, they are all still grassland pastures and have had minimal disturbance (median stocking rate and rotational grazing) for several years. However, most measured variables were affected by date of collection especially the microbial community. It is likely that climate drove the shifts in the microbial community, but this is indicative of resilience of the system. Since the soil microorganisms are responding to sudden climatic effects and not to long-term disturbance, this suggests that the microbial community will respond to sudden change but then return to average behavior which signifies a resilient and healthy soil community probably due to the constant and abundant vegetative cover. Also, though winter annuals likely contribute to this resilience, the effects of winter annuals in this system could not be differentiated from the effects of other factors such as climate or cattle grazing.

The increasing enzyme activity is likely tied to a higher pH in the 10-year pasture. A review by Sinsabaugh et al. (2008) found that OM followed by soil pH most affected enzyme activity. Since OM was the same across these pastures, it is more than likely the soil pH of the 10-year pasture being closer to neutral that is contributing to increasing enzyme activity. Hewins et al., (2015) found a strong correlation between extracellular enzyme activity and pH, specifically that
glucosidase activity increased with greater pH. As to why the pH is higher in the 10-year pasture, this may be due to the length time these fields have been grazed. While a meta-analysis by Abdalla et al. (2018) found no change in soil pH after two and more years of grazing, Martins et al. (2016) found that crop land that incorporated grazing had a higher pH than non-grazed. They suggest that grazing causing plants to mine base cations from deeper in the soil to replace those lost in grazed plant biomass. Jenkins et al., (2008) found that using manure fertilizers increased soil pH compared to synthetic fertilizers and suggested that this was due to the presence of base cations neutralizing soil acidity. Measurement of the base cations in this project may provide more information as to whether or not 10 years of grazing affected the soil pH.

High P and K levels in the 4-year pasture may be due to runoff from the 10-year pasture and 4 years of manure deposition from grazing cattle. This is supported by the interaction effect that revealed high K concentrations in the October 2016 sampling of the 4-year pasture. Heavy rainfall (70 cm) two months before this sampling may have led to runoff of K and P in manure from the 10-year pasture to this low point.

Typically, grazing had been found to increase soil bulk density; however, Taboada et al., (2011) identified several studies that found grazing impacts can be ameliorated in soils with high OM, profuse plant root biomass and a low stocking rate. As there was no change in SOM or TC among the pastures it is not likely that there was greater plant root biomass in the 10 year pasture than the others. However, the low bulk density in the 10-year pasture may be due to being under perennial grass pasture for a longer duration than the other pastures in that a decade of root growth and decomposition may have left channels and pores in the soil reducing bulk density.

The high TN and S concentrations in March 2018 are likely due to forage biomass production at that time. These nutrients follow the same pattern as SOM which was also highest
in March 2018 though not significantly (P=0.07). The changes in TN and TC across dates of collection affected the C:N which decreased in October 2017 and March 2018 likely influencing the potential activity of both enzymes which increased at those collection dates.

High total FAME and absolute abundance of soil microbial groups in October 2016 was likely due to above average rainfall which occurred two months prior to sampling. As the soils dried out, it is likely the oxygen that entered the soil environment promoted aerobic soil microbial activity and consequent growth. As this system was a perennial pasture, there would likely have been little to no limit of C substrate such as decomposing aboveground and belowground plant biomass resulting in a peak of soil microbial growth in October 2016. Other studies have found this pattern of microbial growth after flooding (Orchard and Cook, 1983; Rinklebe and Langer, 2006; Bossio and Scow, 1997). Another possibility is that the heavy rainfall combined with warmer temperatures caused a flush of vegetative growth, supported by the relatively high TC and NH$_4^+$-N, which contributed to the increase of soil microbial growth. This second possibility is supported by the high relative abundance of AMF which typically increase with increased plant roots. We hypothesize that the samples collected following October 2016 demonstrate more of the typical system conditions with fall normally being lower in most soil parameters compared to spring. In spring, day-length increases, temperature begins to increase and plants are actively photosynthesizing and growing both aboveground and belowground. This also typically results in vegetation with low C:N ratios which are readily consumed by soil microorganisms. The high total FAME measurement in March 2018 in the 10-year pasture may have been influenced by the presence of cattle in the pasture at the time of sampling which would have added fresh inputs of manure, an easily degraded C and N source.
Since years of management had no effect on the relative abundance of the soil microbial community composition, it would seem that these soil microorganisms have reached an equilibrium across the pastures. They were unaffected by vegetative species since there was definitely a difference in vegetation in the 0 year pasture or by presence or duration of grazing. However, the community composition did change with date of collection. This shift in soil microbial community composition is indicative of a resilient soil system in this agroecosystem.

4.5. Conclusions

Due to the long duration (up to 10 yrs) and relatively low disturbance of these management activities, the soil system seems to have reached a steady state of inputs and outputs. The response to the abiotic (rainfall and temperature) changes to the system may be indicative of the resilience of this site. This management technique of maintaining a vegetative cover and utilization of rotational grazing at low stocking rates was not detrimental to soil health and may even enhance the resilience of the system over time.
4.6. References


Hubbard, R.K., Strickland, T.C., Phatak, S., 2013. Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. Soil & Tillage Research 126, 276-283.


Chapter 5. Conclusions

In this study of grazed perennial warm-season pastures over-seeded with winter annuals, the effects of winter annuals could not be differentiated from the effects of other factors such as topography, climate, and cattle grazing; however, it is likely that they are contributing positively to the soil health of these pastures. Also, soil fertility may be decreasing in these systems but organic inputs and soil microbial activity appear to be maintaining productivity suggesting that an equilibrium has been established. Topography has a major effect on soil fertility and soil biology and must be considered when making management decisions. Also, though locations may be topographically similar that does not equate to similar soil characteristics. Long-term rotational grazing with low stocking rates which maintain vegetative cover for the majority of the year promote good soil health and can result in a resilient soil system that though affected in the short term by climate will maintain productivity and return to average levels with time.
### Appendix A: Three-way interaction of TC and TN

#### Table A.1. Three way interaction of TC (g kg\(^{-1}\)). Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Fall 2015</th>
<th>Spring 2016</th>
<th>Fall 2016</th>
<th>Spring 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-7.5 cm</td>
<td>0-7.5 cm</td>
<td>0-7.5 cm</td>
<td>0-7.5 cm</td>
</tr>
<tr>
<td>Summit1</td>
<td>32.29 (3.38) A† a‡</td>
<td>29.00 (2.00) AB ab</td>
<td>36.10 (2.43) A a</td>
<td>21.75 (9.44) B a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>29.22 (2.80) A a</td>
<td>28.44 (2.10) A ab</td>
<td>31.80 (2.68) A ab</td>
<td>28.09 (1.87) A a</td>
</tr>
<tr>
<td>Footslope1</td>
<td>25.48 (3.90) A a</td>
<td>27.59 (1.71) A ab</td>
<td>31.14 (0.28) A abc</td>
<td>31.03 (4.18) A a</td>
</tr>
<tr>
<td>Summit2</td>
<td>26.20 (4.44) A a</td>
<td>20.46 (5.46) A bc</td>
<td>23.81 (4.04) A bc</td>
<td>22.86 (1.83) A a</td>
</tr>
<tr>
<td>Backslope2</td>
<td>31.02 (1.72) A a</td>
<td>34.57 (4.03) A a</td>
<td>29.31 (1.96) A abc</td>
<td>28.44 (2.73) A a</td>
</tr>
<tr>
<td>Footslope2</td>
<td>30.49 (1.58) A a</td>
<td>31.10 (4.25) A a</td>
<td>26.05 (5.31) A bc</td>
<td>27.60 (1.02) A a</td>
</tr>
<tr>
<td>Backslope3</td>
<td>24.27 (1.01) A a</td>
<td>12.51 (2.78) B c</td>
<td>21.72 (3.35) A c</td>
<td>22.37 (2.53) A a</td>
</tr>
<tr>
<td></td>
<td>7.5-15 cm</td>
<td>7.5-15 cm</td>
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<td>7.5-15 cm</td>
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<tr>
<td>Summit1</td>
<td>15.82 (1.24) A b</td>
<td>12.84 (0.48) A bc</td>
<td>19.20 (0.65) A a</td>
<td>19.59 (3.67) A a</td>
</tr>
<tr>
<td>Backslope1</td>
<td>13.92 (1.60) A bc</td>
<td>12.55 (0.43) A bcd</td>
<td>14.21 (0.97) A b</td>
<td>13.96 (0.13) A bc</td>
</tr>
<tr>
<td>Footslope1</td>
<td>10.20 (1.99) A c</td>
<td>10.00 (0.18) A cd</td>
<td>11.85 (0.49) A b</td>
<td>12.72 (1.12) A c</td>
</tr>
<tr>
<td>Summit2</td>
<td>11.73 (1.05) A bc</td>
<td>7.75 (2.58) A d</td>
<td>10.03 (0.42) A b</td>
<td>7.14 (1.24) A d</td>
</tr>
<tr>
<td>Backslope2</td>
<td>16.11 (1.06) A b</td>
<td>13.66 (1.67) A bc</td>
<td>14.03 (0.04) A b</td>
<td>17.66 (1.50) A ab</td>
</tr>
<tr>
<td>Footslope2</td>
<td>21.85 (4.70) A a</td>
<td>15.82 (1.23) AB ab</td>
<td>10.46 (1.98) B b</td>
<td>16.25 (0.75) AB abc</td>
</tr>
<tr>
<td>Backslope3</td>
<td>11.77 (1.00) AB bc</td>
<td>19.23 (2.33) A a</td>
<td>11.28 (2.41) B b</td>
<td>12.05 (1.52) AB c</td>
</tr>
</tbody>
</table>

†Uppercase letters denote difference between dates within topography.  
‡Lowercase letters denote difference between topography within date.

#### Table A.2. Three way interaction of TN (g kg\(^{-1}\)). Standard errors are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Fall 2015</th>
<th>Spring 2016</th>
<th>Fall 2016</th>
<th>Spring 2017</th>
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<tr>
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<td>0-7.5 cm</td>
<td>0-7.5 cm</td>
<td>0-7.5 cm</td>
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<tr>
<td>Summit1</td>
<td>3.81 (0.33) B† a‡</td>
<td>5.32 (0.29) A a</td>
<td>4.55 (0.39) AB a</td>
<td>2.48 (1.07) C b</td>
</tr>
<tr>
<td>Backslope1</td>
<td>3.56 (0.29) B a</td>
<td>4.74 (0.60) A ab</td>
<td>3.21 (0.08) B bc</td>
<td>3.50 (0.25) B ab</td>
</tr>
<tr>
<td>Footslope1</td>
<td>2.86 (0.59) B ab</td>
<td>4.66 (0.53) A ab</td>
<td>3.81 (0.03) AB ab</td>
<td>3.82 (0.41) AB a</td>
</tr>
<tr>
<td>Summit2</td>
<td>2.22 (0.47) A b</td>
<td>2.23 (0.74) A c</td>
<td>2.24 (0.41) A c</td>
<td>2.59 (0.18) A ab</td>
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<tr>
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<td>3.33 (0.30) A ab</td>
<td>4.03 (0.42) A b</td>
<td>3.20 (0.31) A bc</td>
<td>3.29 (0.33) A ab</td>
</tr>
<tr>
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<td>3.64 (0.40) A b</td>
<td>3.01 (0.71) A bc</td>
<td>3.32 (0.14) A ab</td>
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<td>1.07 (0.61) C c</td>
<td>2.29 (0.38) AB c</td>
<td>2.41 (0.32) A b</td>
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<tr>
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<td>7.5-15 cm</td>
<td>7.5-15 cm</td>
<td>7.5-15 cm</td>
</tr>
<tr>
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<td>1.79 (0.09) A ab</td>
<td>2.38 (0.16) A a</td>
<td>2.33 (0.43) A a</td>
</tr>
<tr>
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<td>1.67 (0.12) A ab</td>
<td>1.24 (0.06) A bc</td>
<td>1.70 (0.05) A bc</td>
</tr>
<tr>
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<td>0.81 (0.03) A c</td>
<td>1.22 (0.03) A bc</td>
<td>1.51 (0.16) A bc</td>
</tr>
<tr>
<td>Summit2</td>
<td>0.75 (0.15) A b</td>
<td>0.59 (0.28) A c</td>
<td>0.95 (0.04) A c</td>
<td>0.76 (0.19) A d</td>
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<td>1.43 (0.22) A b</td>
<td>1.50 (0.03) A b</td>
<td>2.02 (0.18) A ab</td>
</tr>
<tr>
<td>Footslope2</td>
<td>1.78 (0.15) A a</td>
<td>1.60 (0.04) A ab</td>
<td>1.24 (0.18) A bc</td>
<td>1.83 (0.09) A abc</td>
</tr>
<tr>
<td>Backslope3</td>
<td>1.43 (0.39) A a</td>
<td>2.08 (0.30) A a</td>
<td>1.16 (0.28) A bc</td>
<td>1.31 (0.18) A c</td>
</tr>
</tbody>
</table>

†Uppercase letters denote difference between dates within topography.  
‡Lowercase letters denote difference between topography within date.
Vita

Kathleen Marie Bridges was raised in Haynesville, LA. She attended Louisiana State University from 2004-2008 for her Bachelor of Science degree in Animal, Dairy, & Poultry Science. For her Master of Science degree in Conservation and Sustainable Development, she attended the University of Georgia from 2008-2011. After completion of her Master’s degree, she began work for the University of Tennessee Agriculture Extension Service in Fayette County as a 4-H extension agent. In 2015, she was accepted into the doctoral program in the School of Plant, Environmental, and Soil Sciences at Louisiana State University under the direction of Dr. Lisa M. Fultz working in soil health of perennial pasture systems. The title of her dissertation is “Analysis of the soil health of pastures over-seeded with winter annuals.”