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Available Nitrogen and Denitrification in Soil Altered by Ground Cover and Nutrient Source in an Organic Apple Orchard

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Abstract

A shift in public demand towards more organic and locally produced fruit and vegetables has been occurring across the United States in recent years. A common practice in organic fruit production is the application of organic ground covers to supply nutrients while enhancing other soil properties. A need for research exists in the southern region of the U.S. examining the effects of regionally applicable ground cover and nutrient management on nitrogen availability and the microbial community to provide information to organic farmers in the region. Two studies were conducted to determine how 12 treatment combinations of four ground covers (compost, wood chips, paper mulch, and mow-and-blow) and three organic fertilizers (poultry litter, organic commercial fertilizer, and a no-fertilizer control) applied every year in April from 2006 to 2013 affected soil properties. In the first study, soils from March 2007 and 2013 were analyzed to determine the long-term effects of the treatment combinations on soil chemical and biological properties at the 0-10 and 10-30 cm depths. In addition, denaturing gradient gel electrophoresis (DGGE) was performed on soil microbial DNA to determine if treatment additions over time had altered the denitrifying community. In the second study, soil biological and chemical properties were measured at the 0-10 cm soil depth before (March) and after (May) yearly ground cover applications (April) to determine how nutrient contents and microbial populations responded to additions immediately (May) and long-term (March) and if responses were the same each year or changed through life of the orchard. Organic matter increased through time regardless of ground cover treatment, with compost resulting in the greatest increase from 1.84 % in 2007 to 5.29 % in 2013. Soil water content, electrical conductivity, microbial biomass nitrogen (N), ammonium (NH_4^+-N), and nitrate-N were all greater in 2013 than in 2007. Microbial species richness (R) was greatest in 2013 in soil receiving compost and

wood chips compared to the other ground cover treatments and R in those two ground covers also increased significantly from 2007 to 2013. Shannon-Weaver index of diversity in 2013 progressed from greatest to least in the order of compost ≥ wood chips ≥ paper ≥ mow-and-blow control with diversity in wood chips significantly increasing from among the lowest diversity in 2007 to among the highest diversity in 2013. The second study revealed many treatment differences that were not apparent in the first study when comparing only the beginning and end of the study. Soil organic carbon (C) and N, microbial biomass C and N, NH_4^+-N, and enzyme activities increased through time, peaked during 2009-2011, and declined to levels with relatively few differences between 2007 and 2013 values. Denitrifying communities (*nirK)* analyzed by DGGE, were a sensitive indicator of treatment effects responding to ground cover treatments in 2007. The trends through time in dissolved nutrients and microbial biomass suggest that the microbial community was not growing continually over time, but shifting in composition and diversity of *nirK*-containing organisms and possibly other groups facilitating N-cycling.

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1. Introduction

Organic management of crops in the United States has increased rapidly in recent years, with sales increasing 69% from 2008-2014 (NASS, 2015a). Apples were the top-grossing organic fruit crop in 2014, with $250 million in sales. However, organic production and organic sales are not evenly distributed throughout the country. Organic crop sales were greatest in the same regions where the most production took place, concentrated in the northeast, north central and western U.S. (NASS, 2015a). The climate in these regions is favorable for apple production and organic management. Consequently, the high density of apple orchards and research programs in place in the northeast and northwest are the major source of organic management data collected in the U.S. to date. In the southeast, naturally occurring soil organic matter (OM) content is generally low in Ultisols, the predominant soil order of the region (Eswaran et al., 2002). In addition, humid and warm temperatures promote insect, disease, and weed growth (Harvell et al., 2002). Although the number of organic farms in Arkansas is less than 50, over 90% of the crops were sold within 100 miles of the farm (NASS, 2015a). Consumer demand for locally produced food has also been increasing; the National Agricultural Statistics Service (2015b) reported nationwide increases in the number of farmers markets (180%), regional food hubs (288%), and farm-to-table programs at schools (430%) since 2006.

Orchards are perennial systems requiring long-term management strategies to ensure their success. Nutrient supply is necessary in all systems, along with disease and pest protection as well as weed control, especially in humid climates such as is found in the southeastern U.S. Ground covers are frequently applied as a management strategy in organic orchards to meet some of these needs and include a wide variety of living and non-living materials, such as nitrogen fixing legumes, animal manure, compost, and plant or plastic based mulch. Organic

matter supply and decomposition by microorganisms provide nutrients essential to the survival of the apple trees. However, management goals must include limiting N losses from soil. Besides losing N, nitrate leaching and/or incomplete denitrification can contribute to atmospheric and aquatic pollution. If managed properly, organic ground covers can provide both immediate and lasting benefits to soil quality, including increased OM, cation exchange capacity (CEC), soil microbial activity, and reduced nitrate concentrations (Marriot and Wander, 2006; Hansen et al., 2001; Yao et al., 2005).

A range of impacts from application of organic amendments has been reported in various cropping systems worldwide. Goyal et al. (1999) reported increases in soil C and N and microbial biomass in integrated systems utilizing a pearl millet/wheat rotation on a sandy loam soil receiving inorganic fertilizer added with wheat straw, farmyard manure, or legume cover crop compared to synthetic fertilizer only. Compost and poultry litter also increased biological activities in a degraded soil in a semi-arid region of Spain and increased potentially mineralizable C and N pools and basal respiration in a citrus orchard in Italy (Canali et al., 2004). TerAvest et al. (2010) reported highest N accumulation and uptake efficiency with ^{15}N labeled compost applied with wood chip mulch compared to a legume cover crop or tillage in a Washington apple orchard on a sandy loam. Kramer et al. (2006) reported decreased nitrate pollution, increased denitrification potentials, denitrification rates, and denitrification efficiency with increased OM and microbial activity when nutrients were supplied by alfalfa or compost additions compared to soils receiving synthetic fertilizers only in another Washington apple orchard.

In contrast, others reported problems with nitrogen loss, through leaching or denitrification. Pimentel et al. (2005) observed a greater percent of added N lost as nitrate in a

silt loam soil from a legume system than an animal based or conventional management of row crops. The legume system provided excess N in some years and loss was exacerbated by environmental factors (heavy fall and winter rains). However, in an orchard system similar to this study, Hoagland et al. (2008) reported a lack of N supply from wood chips compared to legume based ground covers. Wood chip addition caused short-term N immobilization and retained soil moisture which increased tree growth, but also encouraged N loss through denitrification.

Denitrification is a step-by-step pathway that ultimately reduces NO_3^- to N_2, by nitrate, nitrite, nitric oxide, and nitrous oxide reductases (Zumft, 1997). Denitrification has wide-reaching economic and environmental impacts because nitrous oxide is a greenhouse gas and a potential product of denitrification. Bacterial denitrification proceeds when nitrate, soluble organic carbon, limited oxygen and denitrifying organisms are present. Denitrification has been well studied in systems with both conventional and organic management. However, impacts of organic ground cover and nutrient source amendments on denitrification from perennial systems with low organic matter soils characteristic of the southeastern U.S. are not as well studied, and less is known about the long-term effects of repeated soil amendments on the denitrifier community.

An organic orchard was established in Fayetteville, AR in 2006 to obtain regionally applicable data concerning a wide range of challenges experienced by local farmers. Two studies were conducted to assess the effects of seven years of annual ground cover (compost, shredded paper, wood chips, and mow-and-blow as an informal control) and fertilizer (poultry litter, commercial, no fertilizer control) treatment combinations to an organically managed apple orchard soil. The first study compared treatment effects in 2007 and 2013 at 0-10 cm and 10-30

cm on soil properties necessary for denitrification to occur and the denitrifying community, using denaturing gradient gel electrophoresis (DGGE) to investigate *nirK*-harboring organisms change over time. I hypothesized that treatments where substrate availability (OM, DOC, NO_3^-) and soil conditions (pH, temperature, water content) were most conducive to denitrification, microbial biomass and *nirK*-community richness and diversity would be greatest.

The intent with agricultural management is to create an efficient N cycling community that is larger, diverse and more responsive to additions and can adapt to change as well as simultaneously limit N losses. Therefore, the second study was an assessment of treatment effects on available soil C and N and microbial biomass and activity from 2007-2013 before and after annual treatment applications. It was hypothesized that treatments which add more organic matter will have larger and more active soil communities in response to substrate availability and treatments with low C:N ratios will result in more mineralization and greater amounts of inorganic N. Overall, investigating the effects of ground covers and organic fertilizers in a low organic matter soil under climatic conditions of the humid southeastern U.S. will provide much needed insight into management of perennial horticultural systems.

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2. Literature Review

2.1 Nitrogen Cycle

A biogeochemical cycle consists of pools and fluxes. In conceptualizing biogeochemical cycles, pools are organizational units where various forms of nutrients are grouped. Fluxes are the flows between pools, with many biogeochemical processes driven by microorganisms. Investigating the fluxes between these nutrient pools and the processes driving them is paramount in efficient management of a system. Terrestrial nitrogen (N) pools include organic and inorganic N in the soil, N contained in plant biomass, and gaseous forms of N in the soil atmosphere. Pools vary in size and tend to be inversely proportional to the rate of turnover. For example, dinitrogen is an inert gas that is reduced by prokaryotes during dinitrogen fixation, a process that consumes a large amount of energy. Inorganic N, conversely, is a small pool that is rapidly turned over. Inorganic forms of N, ammonium (NH_4^+) and nitrate (NO_3^-) are most readily taken up by plants, but small organic molecules may also be immobilized. Organic soil N originates from plant and animal residues in forms either available to microorganisms or resistant to breakdown. Conversion of organic N into inorganic N by microorganisms is referred to as mineralization.

2.1.1. Mineralization/Immobilization

During mineralization, heterotrophic microorganisms including bacteria, fungi and actinomycetes release enzymes that aid in the breakdown of large organic molecules. Specifically, proteins and humic compounds are broken down into simpler amino acids, amides, and amines, the amino groups are hydrolyzed, and NH_4^+ is released into the soil solution. Immobilization is the opposite of mineralization; inorganic forms of N are converted into organic

forms of N. The amount of nitrogen mineralized or immobilized may be affected by the C:N ratios of the substrate and microorganisms (Frankenberger and Abdelmagid, 1985), as well as, the microbial respiration rate and ATP content, an indicator of microbial biomass and microbial activity (Bengtsson et al., 2003). Immobilization of soil N by microbes occurs when there is a deficit of N (high C:N ratio) in the substrate being decomposed and thus inorganic N becomes incorporated into biomass of the microorganisms and is not available for plant uptake. Cookson and Murphy (2004) reported that removing the dissolved organic matter pool, a source of N and C, resulted in a decrease in potentially mineralizable N and a decrease in gross mineralization. Barrett and Burke (2000) conducted a study on grassland soils from Texas to Montana and observed that the highest rates of immobilization occurred in soils containing with higher C:N ratios. Microorganisms use inorganic N in the soil solution to synthesize proteins and other N containing compounds essential to their life.

When organic amendments and fertilizers are being added to stimulate plant growth, the transformation of N from organic to plant available inorganic forms can be examined to gain a better understanding of the specific needs of the system. The ability and efficiency of the microbial community to decompose the substrate and transform the organic N into inorganic forms has implications on the amount of fertilization that needs to occur. Measuring organic C and N, potentially mineralizable nitrogen, microbial biomass, and inorganic N will develop a picture of how these processes are unfolding.

2.1.2. Nitrification

Nitrate is the form of inorganic N that is commonly present under oxidized conditions. Nitrification involves two transformations of N, the enzymatic oxidation of NH_4^+ to NO_2^-, and

NO_2^- to NO_3^- occurring quickly thereafter. Rapid nitrite oxidization to nitrate is imperative because even low levels of nitrite can be toxic to plants. Two groups of aerobic bacteria are involved in nitrification. *Nitrosomonas* and *Nitrosopira* are the primary two genera of ammonia oxidizing bacteria (AOB) (Head et al., 1993), while *Nitrobacter* is the most commonly isolated genera of nitrite oxidizing bacteria; all are in the family Nitrobacteraceae (Grigorova and Norris, 1990).

Bacteria are not alone among nitrifying organisms. Heterotrophic nitrification was observed under cultured conditions by Foct and Verstraete (1977) and has since been recognized as important in forest soils (Schimel et al., 1984; Brierley, 2001). Leininger et al. (2006) looked at 12 soils of varying textures, geographic locations, and management and found in all soils sampled that the *amo*A gene copies of the ammonia oxidizing archaea (AOA) were more abundant than the ammonia oxidizing bacteria (AOB) *amo*A gene copies, with the greatest difference in the ratio of AOA:AOB being $> 1000:1$ occurring in the deepest sampled depth (30-40 cm). However, the authors suggest that abundance of AOA's may or may not have a direct relationship to ammonia oxidization. Offre et al. (2009) and Taylor et al. (2012) lend evidence to support that AOA do contribute to ammonia oxidation in soils. Current research will continue to shed light on the importance of AOA in soils. Another group of organisms that can perform ammonia oxidation are in the order Planctomycetes and are capable of anaerobic oxidation—referred to as anammox (anaerobic ammonium oxidation) (Strous et al., 1999). Hu et al. (2011) reported the natural environments that anammox have been identified and include freshwater, terrestrial, extreme environments and the largest contribution in marine ecosystems. The recent developments in ammonia oxidizer diversity add to the excitement and importance of studying soil microbial ecology in order to better manage cropping systems.

Nitrate is of significant importance because nitrate is plant available but is mobile and can be leached from soils creating an environmental hazard. Therefore, understanding the factors that affect these processes such as temperature, moisture and pH is essential. Aerobic processes such as mineralization and nitrification proceed at their maximum rates near 60% water – filled pore space (Linn and Doran, 1984a). Similarly, Mahli and McGill (1982) reported nitrification occurs more quickly in soils at or near field capacity than at either lower or higher moisture contents.

Temperature is another factor that affects nitrification rates as illustrated by a study conducted on three soils in Alberta, Canada. Nitrification rates increased from 4 to 20 °C, with greatest rates of nitrification between 10 and 20 °C. The variation in these findings is evidence that the selection for nitrifiers with either high or low temperature optimums varies with climate (Mahli and McGill, 1982). The high rates observed at these temperatures do not coincide with other studies in warmer climates. For example, Myers (1975) reported the optimum nitrification temperature of approximately 35 $^{\circ}$C in a clay-loam tropical soil.

In addition to temperature and moisture, soil pH affects nitrification. Morrill and Dawson (1967) examined 116 soils over a range of pH values; these were split into four groups with averages of 7.85, 6.38, 5.39, and 5.12. The group with the average pH of 6.38 exhibited the fastest oxidation of ammonium to nitrite with the least nitrite accumulation. These findings have been reproduced many times throughout the years, with culturable nitrifers having an optimal pH of 7.5-8.0 (Prosser, 1989), but without culturing all nitrifiers this may not be an accurate representation of the whole population. Acid tolerant nitrifiers do exist and have been observed in a variety of environments. Pennington and Ellis (1993) observed acidic nitrification in forest and grassland soils and attributed this to autotrophic oxidation as opposed to heterotrophic

oxidation. Nitrification is an acidifying process, and the microbial community existing in different soils under different environmental conditions will respond uniquely. There is a need to analyze soil microbial communities of interest with regards to nitrification and the influence of organic amendments and fertilizers on silt loam soils to gain further understanding of how these processes will proceed in perennial systems given the change in conditions with different ground cover and nutrient source management.

Nitrate can be lost from soil in surface runoff, leached to groundwater, volatized, or denitrified if conditions become anaerobic. When dealing with organic systems, losses of greatest importance are leaching and denitrification. Nitrates leached from the soil can contaminate aquatic systems. Surface runoff is also a major problem in fertilized agricultural fields receiving soluble inorganic N fertilizers. Soluble inorganic fertilizers are easily transported in the soil solution and runoff. It is widely accepted that excess N in surface waters and groundwater cause eutrophication. Eutrophication can lead to algal blooms resulting in decreased oxygen concentrations in the water column followed by potential harm or death of aquatic organisms. In order to avoid costly N losses and environmental hazards, a balanced and efficient N cycling community must be strived after.

2.1.3. Denitrification

Heterotrophic bacterial denitrification is the transformation of nitrate to gaseous forms of N (NO, N_2O, N_2) catalyzed by nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos). Each reduction in the denitrification pathway can be accomplished by a variety of organisms carrying the genes that code for the enzymes responsible for reductions. While heterotrophic bacterial denitrification is generally the most

predominant and has been the most widely studied of the processes in surface soil, there are other denitrifying organisms. Autotrophic bacteria that denitrify use inorganic sources as the electron donor (Straub, 1996). Fungi perform denitrification and have been found to contain Nor (Uchimura et al., 2001) and Nir (Kobayashi et al., 1995). Some have reported that denitrification by fungi dominates denitrification by bacteria in soils (McClain and Martens, 2006; Laughlin and Stevens, 2002). Denitrification by archaea occurs by both assimilatory and dissimilatory pathways (Martinez-Espinosa et al., 2001); however, much less is known about archaeal denitrification comparatively. Nitrifiers have been found to produce N_2O as a by-product of AOB (Hooper and Terry, 1979) as well as a step in the production of N_2 from NO_2^- (Wrage et al., 2001). Aerobic denitrification has also been discovered in the last decade (Su et al., 2004; Okada et al., 2005). This new knowledge will surely change understanding of the nitrogen cycle.

Heterotrophic bacterial denitrifiers use dissolved organic carbon as a carbon source and nitrate as the terminal electron acceptor in anaerobic respiration. Aerobic respiration is at a maximum at 60% water filled pore space and begins to decline above this point (Linn and Doran, 1984a; Linn and Doran, 1984b). Denitrification occurs when the soil water content is great enough to result in sufficient lack of oxygen limiting aerobic respiration, at approximately 80% water filled pore space (Linn and Doran, 1984a; Linn and Doran, 1984b). Soil texture affects denitrification rates and products. Maag and Vinther (1960) exposed soils with six different soil textures to varying water contents and temperatures. They found increasing temperature and soil moisture increased denitrification rates in sandy loam soil, while only temperature increased rates in coarse sandy loam and the ratio of N_2 to N_2O was lower in coarse sandy loam than sandy loam soil. Soil pH also affects denitrification. In a review of 50 years of pH and denitrification dynamics, Simlek and Cooper (2002) stated that acidic soils do not

produce as much N gas as neutral or slightly alkaline soils and suggested that this may be related to acidic effects on nutrient availability or adaptations by the organisms. Acidic soils often had a greater N_2O:N_2 ratio (Simlek and Cooper, 2002).

Denitrification is temporally and spatially variable because of dependence on oxygen level and substrate availability in soils, and can be concentrated in hotspots. These microsites of high rates of denitrification were examined by Parkin (1987) by taking soil samples from a Beltville silt loam. Parkin (1987) optimized all factors that control denitrification in all samples and observed variability that can be attributed to the dispersion of the enzymes controlling denitrification. In the samples where hotspots occurred, 25-85% of the denitrification activities were associated with particulate organic matter content of the sample.

2.2. Challenges in Microbiology

The study of microbial ecology is challenging. The greatest causes for this challenge are that microorganisms cannot be seen with the unaided eye and that they are dynamic in response to changes in the environment. There are a number of methods that can be used to identify, count, classify and analyze microbial actions and interactions. The methods used to study microbial ecology have expanded in the recent past. However, despite advancement with new technologies, ultimately, microbial ecology is a methods-limited discipline.

Serial dilutions and culturing on selective media is the traditional method of studying soil microorganisms. This method alone cannot be used to grasp the scale of microbial activity in the soil. There are species that exist in the soil that cannot survive under cultivated conditions. Therefore, there are species that have never been cultured because appropriate methods for culturing these organisms have not yet been optimized or discovered (Amann et al., 1995). Soil

is a habitat for a wide range of microorganisms, each unique in many ways. As a result, it is impossible to develop one method to isolate all soil microorganisms (Bakken, 1997). Cultivation conditions do not mimic those of the organism's natural habitat and frequently oversimplify ecological interactions, making it difficult to extrapolate relevance of results to the soil environment. Thus, microbial ecologists often take a multi-pronged approach to studying microbial communities in soil. Methods may focus on microbial abundance and biomass, activity and function, and community structure and diversity.

2.3. Microbial Biomass

In order to understand the N cycle, microbial ecologists are interested in the size of the microorganism pool. Decomposition is largely completed by microorganisms; therefore, it is important to know the amount of microorganisms present. Options to quantify this pool include cell counts or measuring microbial biomass. Counting cells is challenging because it is difficult to differentiate among cells and/or between cells and soil particles. It is possible that an ecologist would just be concerned with a specific group of organisms; however, measuring microbial biomass is a viable option when concerned with the entire pool. Microbial biomass helps to address the functional redundancy of a system. Functional redundancy refers to the ability of many groups of organisms to perform the same ecological function. For example, many bacteria and fungi are heterotrophic decomposers of organic matter performing the same ecological function. It is important to measure microbial biomass when studying soil ecology because microbial biomass is the center for biological activity in soils, an indicator of soil fertility (Beck et al., 1997). Microbial biomass has an approximate turnover time of one to two months (Davidson et al., 1992), and because of this microbial biomass is a sensitive indicator of

fluxes in the system that may not be immediately observable in the passive or stable organic matter pools. Measuring this pool before or and after fertilization or some other environmental change is one way to determine how responsive the community is to the change. Microbial biomass C and N can both be measured as a function of the soil microbial biomass.

Microbial biomass can be measured in a number of ways, but there are three common methods: chloroform fumigation-incubation, chloroform fumigation-extraction method, and substrate-induced respiration method (SIR). Beck et al. (1997) examined ten different variations of three common methods, using 20 different soils, to see how closely the results for microbial biomass C were related. All variations ranked the soils in virtually the same order according to amount of microbial biomass C in the soil suggesting that the results of the three methods are similar enough for comparison. There are advantages and disadvantages to these methods that are worth noting. Relevant to this study, a disadvantage to the chloroform-fumigation extraction technique is the possibility of causing harm to the cells and that would lead to an under-estimation of microbial biomass. However, the chloroform-fumigation extraction method is advantageous when compared to the chloroform-fumigation incubation method because the extraction method takes significantly less time and does not require a carefully controlled incubation and as a result there is no need for correction of loss of N through denitrification (Brookes et al., 1985). Duxbury and Nkambule (1994) stated that the largest disadvantage with the fumigation methods is that results are affected if the C:N ratio is too wide, which is generally not known before the procedure is completed. A general disadvantage of using microbial biomass to indicate soil quality is that the size of the pool is affected by the amount and quality of substrate in the soil and can be temporarily elevated as a response to additions (Duxbury and

Nkambule, 1994). A limitation of the microbial biomass approach is that all microorganisms are treated equally, and thus, there is no differentiation of organisms in the biomass.

2.4. Enzymes

It is not only important to know how much biomass or how many organisms are present but to also know what those organisms are doing. The function of the organisms and the capacity of the system are valuable in understanding nutrient cycles. One way to assess the function of soil microorganisms is to measure the enzymatic activity. Enzymes are proteins that catalyze chemical reactions and each is unique to the type of reaction they are catalyzing. Enzymes catalyze several reactions necessary to microbial life including decomposition of organic materials, formation of organic matter, and nutrient cycling (Dick, 1994). Soil contains free enzymes, immobilized enzymes, and enzymes in microbial cells (Tabatabai and Dick, 2002). Biochemical reactions depend on the enzymes in soil and the available energy sources found in the substrate (Kiss et al., 1978).

Enzyme activities are particularly useful in determining soil health because they change sooner than other measurable variables, can be analyzed using simple procedures, and are closely related to microbial activity (Das and Varma, 2011; Dick et al., 1996). Depending on the process of interest and the enzymes involved in the process, different methods can be used. A particular method of interest in determining the presence and amount of enzyme activities is the colorimetric method. Substrate is added to a solution containing a sample of microorganisms and subjected to non-limiting conditions. When the enzyme present in the sample cleaves the substrate specific to the enzyme of interest, a colored product is formed. The color of the product depends on the substrate that is added to the solution. Once the reaction is complete, the colored solution can be analyzed using a spectrophotometer and compared to standards in order to assess

the amount of enzymatic activity in that sample. In the case of glucosaminidase, a key enzyme involved in the breakdown of large N-containing molecules into labile amino sugars, a *p*-nitrophenyl compound is the substrate added to the soil and the yellow colored product is *p*-nitrophenol (Eivazi and Tabatabai, 1990).

2.5. Denaturing gradient gel electrophoresis

Many molecular based techniques to investigate the microbial community in soil rely on polymerase chain reaction (PCR) to amplify specific DNA fragments, or sequences, within a given genome of interest, including denaturing gradient gel electrophoresis (DGGE). DGGE separates DNA fragments of the same length according to their denaturing points allowing for discrepancies in sequences to be visualized (Fischer and Lerman, 1983). This technique is implemented in order to get a "picture" of the diversity of a given microbial community.

Extraction of DNA is required before PCR or DGGE can be performed. In soils, a bead-beating procedure is commonly performed for DNA extraction. Beads are placed with a sample that is shaken vigorously in lysing buffer; cells are physically lysed and DNA is released into the solution. After the DNA in solution is separated from other compounds, the three-step PCR amplification process takes place. First, samples are heated to denature DNA, temperatures are then lowered to allow for annealing of primers, and the primers will be extended and new DNA strands will be synthesized through the activity of a heat-stable DNA polymerase such as *Taq* polymerase derived from the thermophilic organism *Thermus aquaticus* and free nucleotides. A primer is a sequence of DNA that is designed to bind to fragments of DNA that are unique to the gene that is being amplified. Primers can be designed to target a taxonomically diverse to narrow group of organisms.

Relevant to this study, primers have been designed previously to successfully target genes associated with the processes involved in denitrification. For example, the *nosZ* gene of denitrification is targeted because it encodes the catalytic subunit of nitrous oxide reductase. In a study completed by Henry et al. (2006), a comparison of the *nirK*, *nirS*, and *nosZ* genes was conducted. By targeting the *nosZ* gene it was determined that only 5% of the bacterial community studied contained the nitrous oxide reductase gene.

Denaturing gradient gel electrophoresis consists of a polyacrylamide gel that has a gradient of substances that denature or separate the strands of DNA; commonly this is a mixture of urea and formamide. Denaturing gradient gel electrophoresis should not be confused with agarose gel electrophoresis that is used to visualize PCR products separated by fragment size. In contrast, DGGE separates by sequences of DNA because of the denaturants in the gel. The DNA will reach a point in the gradient where the area in the DNA with the lowest melting point denatures; here, because the DNA gene fragment contains a guanine-cytosine (GC) clamp (i.e. a sequence of about 40 G and C base pairs) the molecule will stop and form a band in the gel (Muyzer and Smalla, 1997). Adding a GC-clamp to the 5' end of the PCR primer will enhance the detection of sequence variants from 50% to nearly 100% (Myers et al., 1985; Sheffield et al. 1989). In order to achieve the most vivid separation of DNA fragments, the optimal gradient and duration must be experimentally determined (Muyzer and Smalla, 1997).

Denaturing gradient gel electrophoresis is often used to study community complexity. DGGE has been used to profile microbial mat and biofilm communities, as well as, communities around hydrothermal vents (Muyzer et al., 1993; Muyzer et al., 1995). DGGE can also be used to detect changes in a microbial community over time, which is helpful when studying how the community is affected after changes occur in the environment. One of the major limitations of

the method is the small size of fragments that can be separated, up to 500 base pairs (Myers et al. 1985). Also, only the dominant species in the sample will be detected. It has been shown that populations that make up 1% or more of the community will be detected with DGGE (Muyzer et al., 1993; Murray et al., 1996).

2.6. Organic Amendments

Orchards are perennial systems requiring long-term management strategies to ensure their success. Many factors affect orchard management decisions, initial characteristics of site location are crucial in making these decisions. Soil physical, chemical and biological characteristics are among factors that need to be addressed. Challenges arise when an orchard is managed organically. In this case, all synthetics including inorganic fertilizers are replaced with organic fertilizers and/or organic amendments. The most apparent benefit of organic soil amendments is the addition of organic matter. Soil organic matter consists of the decomposing plant and animal residues along with the microbial biomass performing decomposition and the chemical products and byproducts of biochemical processes (Lal, 2007).

Research investigating differences between organic, integrated, and conventional systems provide positive feedback for organic management, citing a range of benefits to soil health. Increased OM, and soil microbial activity as well as reduced nitrate leaching are among the benefits observed. Yao et al. (2005) compared pre and post-emergence residual herbicide, mowed-sod, and hardwood bark mulch ground covers in an apple orchard on a silty clam loam. Higher OM (80%), cation exchange capacity (CEC), calcium (Ca) and phosphorus (P) availability, pH and respiration were observed in the mulch treatment. The higher respiration rate was most likely a direct effect of the microbial decomposition of the mulch. Organic farming

added OM resulting in a 14% increase in the soil organic carbon fraction compared to conventional management system and was beneficial in increasing N availability (Marriot and Wander, 2006).

Soil ammonification and nitrification in conventional and organic tomato cropping systems in California were compared. Burger and Jackson (2003) reported that the greater OM supply increased microbial activity and led to a greater N supply that lasted into the growing season. Kramer et al. (2006) reported organically managed soil decreased nitrate pollution, increased denitrification potentials, denitrification rates, and denitrification efficiency while increasing the OM and microbial activity of the soil. Nitrate leaching was greater in the conventionally managed soil, and the highest N_2 emissions were observed in the organically managed soils while the N_2O emissions were not significantly different. In regards to N cycling, the ability of a microbial community to fully carry out denitrification, with N_2 as the end product, has many environmental implications. Hansen et al. (2001) also reported nitrate leaching was greater in conventional than in organic systems. In contrast, Pimentel et al. (2005) observed similar and more nitrate leaching when comparing two organic plots with conventional management. An organically managed legume supplied twice as much N as needed and heavy winter rains along with summer drought stunted corn and led to more N loss because of reduced N uptake by the corn (Pimentel et al., 2005).

Despite these research data, the evidence that organically grown fruit production produces more or higher fruit quality is not as strong or clear as the evidence for increased soil health. Peck et al. (2006) compared productivity and fruit quality in organic, integrated, and conventionally managed apple orchards. All productivity and fruit quality parameters were variable among the treatments and over time, possibly as a result of a biennial bearing pattern in

the organic system. The only parameters that were significantly better in organically managed fruit were higher anti-oxidant levels and better and longer storage capacity. Roussos and Gasparatos (2009) also found variable results in their comparison and concluded that the conventional and organic systems exhibited similar quality characteristics and lower yield. However, greater market value for organic apples could possibly make up the difference in the lack of yield. Reganold et al. (2001) compared organic, integrated, and conventional apple orchards in Washington State from 1994 to 1999. Along with increased soil quality and less negative environmental impact, sweeter apples and higher profitability were reported. There was no significant difference in the yield of apples among the three systems.

A variety of organic amendments exist that may contain any combination of C, N, potassium (K), P, micronutrients or other molecules, which may be helpful for plant growth when released during decomposition. If managed properly, organic amendments such as ground covers can provide a slow release of essential nutrients, increase infiltration, reduce erosion, provide weed control, and improve soil structure and tilth. Not every amendment will provide the needs of a particular system in the same way, some may actually provide excess beyond requirements and losses will occur. The goal of organic N management is to facilitate efficient internal terrestrial N cycling without promotion of N losses, especially as those can result in atmospheric and aquatic pollution. Ground covers are applied to the surface of the soil and have both immediate and lasting effects. Ground covers protect against wind, buffer temperature fluctuations by protecting the soil surface from drying out (Snyder and Connell, 1993). Ground covers block seeds from entering the soil and germinating, and thus provide some weed management. Ground covers have also been reported to have effects in reaching below the

topsoil. Larney et al. (2004) reported increased nitrate from compost, manure, alfalfa, and straw amendments at the 15-30 cm depth one year after application.

Ground cover and organic fertilizer treatments are being tested around the world to determine which locally available materials work well in a particular system and produce desired results. Tejada (2006) added two organic amendments, a crushed cotton gin compost and poultry manure, to a degraded soil in a semi-arid region of Spain. Both of the treatments increased biological activities of the soil, but the poultry manure exhibited a greater increase in microbial biomass and enzymatic activities. Canali et al. (2004) showed limited effects of compost and poultry litter applications in a citrus orchard in Italy, but did observe greater amounts of potentially mineralizable C and N pools and a greater basal respiration rate. Baldi et al. (2010) compared compost applied at two rates (10 and 5 t/ha/yr), cattle manure (10 t/ha/yr) and a mineral fertilizer in a peach orchard and concluded that the higher rate of compost applied (10 t/ha/yr) restored fertility and could be used in the management of the orchard. Excess nitrate in is a concern when a large amount of compost is applied, but nitrate concentrations were not significantly different among the treatments for most of the sampling dates, even though SOM was different (Baldi et al., 2010). Mineralization and N release of SOM may not only be dependent on the concentration of SOM, which would explain the similar levels of nitrate across treatments.

Granatstein and Mullinix (2008) compared a variety of inert and living mulches with the goals of weed control, increasing water retention and providing N to organic fruit trees in the Pacific Northwest. Wood chips, shredded paper, and clover provided the best weed control and mulched plots retained 15-20% more moisture than unmulched plots. Alfalfa hay, wood chips, and paper contributed to the highest infiltration rates. Alfalfa provided the most N of all of the

treatments with the clovers having similar results; the high N treatments resulted in negative effects on fruit quality and the alfalfa induced alternate bearing patterns. The moisture benefits of wood chips are also described in Hoagland et al. (2008), but wood chips did not provide enough N to the apple trees as determined by foliar samples and did not stimulate microbial activity. However, the results are over a two-year period in a newly established orchard and more time may be needed to see the full benefits of the mulch. Wood chips provided better conditions for apple trees than legume cover crops, which outcompeted the new trees for nutrients (TerAvest et al., 2010). From the literature, there is a consensus for the need for more research on ground covers and organic systems in general, to fully understand these systems and processes and aim to increase production and soil health without accumulating excess nutrient concentrations and contributing to non-point source pollution.

Ground covers and organic fertilizers have significant impacts on the microbial system to which they are applied. Microorganisms mediate N cycling and the processes affected by of the integration of environmental, physical, chemical, and biological factors. Organic matter increases overall soil health and is the backbone of organic systems with the potential to create an environment that can sustain plant life without the addition of inorganic fertilizers. However, excess N can pollute water and the atmosphere. Soil and molecular analyses can diagnose the demand for N revealing inefficiencies within the system and how the treatments are affecting microbial communities at various depths in the profile. The intent is to create an efficient N cycling community that is diverse and responsive to additions and can adapt to change and simultaneously limit N losses. Investigating the effects of the ground covers and organic fertilizers in a specific soil under conditions unique to the southern U.S. will give much needed insight into management of these perennial orchard systems.

2.7. References

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3. Denitrifier Community Response to Seven Years of Ground Cover and Nutrient Management in an Organic Apple Orchard Soil

Keywords: denitrification, microbial community, nitrogen, organic, ground cover, DGGE

3.1. Abstract

Ground cover addition is common in organic orchard management and can serve multiple purposes such as providing a nutrient source while protecting the soil surface. In the current study, twelve treatment combinations of one of four ground covers (compost, wood chips, paper mulch, and mow-and-blow) combined with one of three organic fertilizers (poultry litter, organic commercial fertilizer, and no-fertilizer control) were applied every year in April from 2006 to 2013. Soil was sampled in March 2007 and 2013 to determine the short and long-term effects of the treatment combinations on soil chemical and biological properties at the 0-10 and 10-30 cm depths. In addition, denaturing gradient gel electrophoresis (DGGE) was performed to determine if treatment additions over time had altered the denitrifying community. Organic matter (OM) increased through time regardless of ground cover treatment, though compost increased OM the most from 1.84 % in 2007 to 5.29 % in 2013. Soil water content, electrical conductivity, microbial biomass N, ammonium-N, and NO_3^--N, which were all greater in 2013 than in 2007. Microbial species richness (R) was greatest in 2013 in soil receiving compost and wood chips compared to the other ground cover treatments, and R in those two ground covers also increased significantly from 2007 to 2013 (P <0.0001). Shannon-Weaver index of diversity (H) in 2013 progressed from greatest to least in the order of compost ≥ wood chips ≥ paper ≥ mow-and-blow control with diversity in wood chips significantly increasing from among the lowest diversity in 2007 to among the highest diversity in 2013. At the 10-30 cm soil depth, there was a main effect of ground cover on organic matter content (P = 0.0445) ranging in order from compost (1.78 %) ≥ wood chips (1.61 %) > paper (1.56 %) ≥ mow-and-blow control (1.46 %). There were no

significant interactions or main effects of ground cover, fertilizer or year on the ecological indices calculated from the DGGE profiles at the 10-30 cm soil depth. In the 0-10 cm soil depth, microbial species richness (R), diversity (H and D), and evenness (J and E) in compost treatments was greater than wood chip or mow-and-blow treatments, which coincided with elevated DOC concentrations in compost treatments in 2007. The *nirK* community responded to wood chip treatments, significantly over time with greater species richness and diversity in 2013.

3.2. Introduction

Orchards are perennial systems requiring long-term management strategies to ensure their success. Nutrient supply is necessary in all systems, along with disease and pest protection and weed control in many systems, especially humid climates such as those found in the southeastern United States. One way to meet some or all of these needs in organic systems is with the application of ground covers, which encompass a wide variety of living and non-living materials, such as nitrogen fixing legumes, animal manure, compost, and plant or plastic based mulch. If managed properly, organic amendments including organic, ground covers such as those used in this study (compost, shredded paper, wood chips, and a mow-and-blow control) can have both immediate and lasting effects, including a slow release of essential nutrients, increased infiltration, wind protection and reduced erosion, weed control, and improved soil structure. The goal of organic nitrogen (N) management is to facilitate efficient internal terrestrial N cycling without promotion of N losses, especially as those can result in atmospheric and aquatic pollution. Microbial decomposition and nutrient cycling are the driving force behind the supply of nutrients to the crop in organically managed agriculture. Through the addition and subsequent decomposition of organic matter, microbial activity is stimulated. Processes involved in the N cycle can be altered, depending on the inputs of organic substrates and the resultant changes in microbial community composition. Microbial activity and the soil nutrient cycling services they provide play a significant role in healthy soil functioning.

Denitrification is a microbial mediated process and a mechanism of soil N loss having wide-reaching economic and environmental impacts. Nitrous oxide is a greenhouse gas and a potential product of denitrification. Denitrification has been well studied in both conventional and organic management systems. However, impacts of organic ground cover and nutrient

source amendments on denitrification from perennial systems with low organic matter soils characteristic of the southeastern U.S. are not as well studied, and even less is known about long-term effects on the denitrifier community.

Research investigating differences between organic, integrated, and conventional systems provides positive feedback for organic management, citing a range of benefits to soil health. Increased organic matter (OM), cation exchange capacity (CEC), soil microbial activity, and reduced nitrate leaching are among the benefits observed (Marriot and Wander, 2006; Hansen et al., 2001; Yao et al., 2005). Kramer et al. (2006) reported apple orchard soil in Yakima, Washington supplied with nutrients from alfalfa or compost additions had decreased nitrate pollution and increased denitrification potentials, rates, and efficiency with increased OM and microbial activity when compared to soils receiving synthetic fertilizers only. In contrast, Pimentel et al. (2005) compared two organic management treatments, a legume based row crop rotation and an animal based row crop rotation with a conventionally managed row crop rotation system, and observed more added N lost as nitrate in a silt loam soil from the legume system, which was exacerbated in some years by environmental factors.

Ground covers used in organic systems have included a multitude of materials. There are also a vast amount of organic fertilizers available. Ground cover and organic fertilizer treatments are being studied to determine which combinations of materials work well. Tejada (2006) added crushed cotton gin compost and poultry manure to a degraded soil in a semi-arid region of Spain. While both increased biological activities of the soil, there was a greater increase in microbial biomass and enzymatic activities with poultry manure addition.

Others also reported positive impacts with compost addition, Canali et al. (2004) observed greater potentially mineralizable C and N pools and a greater basal respiration rate in a

citrus orchard in Italy. Baldi et al. (2010) compared compost applied at two rates (10 and 5 t ha $^{-1}$ yr $^{-1}$), cattle manure (10 t ha $^{-1}$ yr $^{-1}$) and a mineral fertilizer to a silty loam soil in an Italian peach orchard and concluded that the higher rate of compost applied (10 t ha $^{-1}$ yr $^{-1}$) restored fertility by increasing soil OM and supporting fruit production. Even though various amounts of OM were added to the soil, nitrate levels were not significantly different among the treatments for most of the sampling dates, indicating mineralization of OM was not solely dependent on amount applied (Baldi et al., 2010).

Granatstein and Mullinix (2008) compared a variety of inert and living mulches with the goal of weed control, increasing water retention and providing N to organic fruit trees in the Pacific Northwest. Alfalfa mulch and white clover mowed and burned treatments contributed to greater percent leaf N in the last two years of the study, while wood chips, shredded paper, and clover provided the best weed control, and mulched plots retained 15-20% more moisture than unmulched plots. TerAvest et al. (2010) applied wood chips mulch, legume cover crop, and tillage fertilized with ^{15}N labeled composted poultry litter in an apple orchard on a sandy loam in central Washington State and observed the greatest benefits in N accumulation and uptake efficiency from wood chip additions. However, in a similar study on organic apple orchard management strategies conducted also conducted in central Washington, Hoagland et al. (2008) reported a lack of N supply from wood chips compared to legume based ground covers. Addition of wood chips caused short-term N immobilization and retained soil moisture which increased tree growth but also encouraged N loss through denitrification. From the literature, there is a consensus for the need for more research on ground covers and organic systems in general in order to fully understand these systems and processes with the overall goal of increasing

production and soil health without accumulating excess nutrient concentrations and contributing to non-point source pollution.

Denitrification is a step-by-step pathway that ultimately reduces NO_3^- to N_2, by nitrate, nitrite, nitric oxide, and nitrous oxide reductases (Zumft, 1997). Each reduction in the denitrification pathway can be accomplished by a variety of organisms carrying the genes that code for the enzymes responsible for reductions. Bacterial denitrification proceeds when nitrate, soluble organic carbon, limited oxygen and denitrifying organisms are present. Denitrifiers use dissolved organic carbon as a carbon source and nitrate as the terminal electron acceptor in anaerobic respiration. Aerobic respiration is at a maximum at 60% water filled pore space and begins to decline above this point; thus, denitrification occurs when the soil water content is great enough to result in sufficient lack of oxygen limiting aerobic respiration, at approximately 80% water filled pore space (Linn and Doran, 1984a; Linn and Doran, 1984b). Soil texture affects denitrification rates and products. Maag and Vinther (1960) exposed soils with six different soil textures to varying water contents and temperatures. They found increasing temperature and soil moisture increased denitrification rates in sandy loam soil, while only temperature increased rates in coarse sandy loam and the ratio of N_2 to N_2O was lower in coarse sandy loam than sandy loam soil.

Soil pH also affects denitrification. In a review of 50 years of pH and denitrification dynamics, Simlek and Cooper (2002) state that acidic soils do not produce as much N gas as neutral or slightly alkaline soils and suggest this may be related to acidic effects on nutrient availability or adaptations by the organisms. Acidic soils often had a greater N_2O:N_2 ratio (Simlek and Cooper, 2002).

To gain insight into the community diversity and the relative contribution of organisms to the functional potential of denitrification, diversity and abundance of denitrification genes can be analyzed. There are two nitrite reductase genes, copper containing (*nirK*) and cytochrome cd1 containing (*nir*S) which are functionally the same and are each found in a large number of diverse organisms in soils (Coyne et al., 1989; Braker et al., 1998; Hallin and Lindgren, 1999; Heylen et al., 2006). In this study, I targeted *nirK,* to better understand the denitrifier community diversity and potential to contribute to terrestrial N loss; *nirK* has been shown to work well in environmental samples and with denaturing gradient gel electrophoresis (DGGE) (Throbäck et al., 2004).

Soil and molecular analyses can diagnose the demand for N and reveal inefficiencies within the system and how the treatments are affecting microbial communities at various depths in the profile. The intent with agricultural management is to create an efficient N cycling community that is diverse and responsive to additions and can adapt to change and simultaneously limit N losses. Investigating the effects of ground covers and organic fertilizers in a low organic matter soil under climatic conditions of the humid southeastern U.S. will provide much needed insight into management of perennial horticultural systems. The objective of this study was to determine if seven years of annual ground cover (compost, shredded paper, wood chips, and mow-and-blow as an informal control) and fertilizer treatment combinations (poultry litter, commercial, no fertilizer) to an organically managed apple orchard soil changed the soil denitrifying community or the potential for denitrification over time. This objective was achieved by using DGGE to investigate *nirK* harboring organisms and to determine how the community structure and diversity have changed over time. Soil biological and chemical properties measurements were analyzed to determine if available nutrients and soil conditions

necessary for denitrification to occur had changed over time. I hypothesized that treatments where substrate availability (OM, DOC, NO_3^-) and soil conditions (pH, temperature, water content) were most conducive to denitrification, microbial biomass and *nirK* community richness and diversity would be greatest.

3.3. Materials and Methods

3.3.1. Experimental Design

The 0.30-ha organic apple orchard is located at the University of Arkansas Main Agriculture Experiment and Extension Center in Fayetteville, Arkansas (36°N, 94°W). Enterprise apple trees (*Malus domestica* Borkh.) with M26 rootstock were planted in 2006 on Captina (Fine-silty, siliceous, active, mesic Typic Fragiudult) and Pickwick (Fine-silty, mixed, semiactive, thermic Typic Hapludult) silt-loam soils (NRCS, 2014). Before planting, the area was tilled and leveled, and lime and manure were added to adjust pH and organic matter. Soil properties at the beginning of the experiment in 2006 are shown in Table 1 and orchard preparation details can be found in Choi et al. (2011a). The orchard was managed following the National Organic Program Standards (AMS, 2012). The ground covers included urban compost (C), white shredded paper (P), wood chips (W), and a mow-and-blow control (M). The compost applied was composed of vegetative waste (i.e., grass clippings, wood pruning, and yard waste), composted for 90 to 120 d, obtained from the City of Fayetteville, AR until 2011, and beginning in 2012 was obtained from PC Turnkey in Springdale, AR, and composted using an active-pile process. Wood chips were also obtained from the City of Fayetteville, AR and consisted of mainly hardwood species. The shredded paper was obtained from the University of Arkansas, Fayetteville, AR. Mow-and-blow treatments consisted of tall fescue (*Festuca arundinacea* Schreb. 'KY 31') planted between rows and other naturally occurring native, herbaceous species.

Each ground cover treatment was applied to the surrounding tree area (2 x 2 m²) and two guard trees every April at a depth of 7.5-12 cm. Each ground cover also received locally available poultry litter or commercial pelletized organic fertilizer, a pelletized poultry manure (Perdue AgriRecycle, Seaford, DE) was used through 2011 until discontinued and was replaced with an alfalfa (*Medicago sativa*) based commercial organic product (Bradfield Organics Feed Solutions, St. Louis, MO) in 2012. Fertilizers were applied at a rate of 50 g of N per tree per year of tree age (450 g N per tree maximum), or no fertilizer (control). Full details of management of ground covers and fertilizers are described in Mays et al. (2014) and Mays et al. (2015). Average carbon (C), nitrogen (N), phosphorus (P), and potassium (K) concentrations for ground covers and fertilizer treatments are shown in Table 2, characterization details are described in Choi et al. (2011b).

3.3.2. Soil Sampling, Storage and Characterization

Samples were collected in March 2007 and 2013 from 0-10 and 10-30 cm depths. Composite samples were obtained by sterilized soil probe by collecting 8 cores randomly at least 15 cm from the trunk and within 60 cm between trees in a row and 45 cm between rows and soils were stored in sterile bags. Soil temperature at 10 cm depth was measured around each tree at the time of sampling. Soils were immediately placed on ice in the field, stored at 4 °C upon return to the laboratory, sieved through a sterilized 2-mm sieve, and stored moist at 4 °C with a subsample frozen at -80 °C until DNA was extracted.

Gravimetric soil water content was determined from soil (10 g) oven dried at 105 °C for at least 24 hr until a constant weight was reached. All soil properties are expressed per gram of oven-dry soil. Electric conductivity (EC) and pH of the soil were measured potentiometrically

using 1:2 soil-to-water ratio. Organic matter content was determined using loss-on-ignition (6 hr at 550 ^{o}C).

3.3.3. Extractable C and N, Microbial Biomass C and N and Total C and N

Microbial biomass C and N were measured using chloroform-fumigation extraction (Vance et al., 1987). Unfumigated and 24-hr chloroform-fumigated soil samples were extracted at 1:5 ratio (wt:vol) in 0.5 *M* K_2SO_4, shaken for 30 min, and filtered through Whatman #42 filters. A Shimadzu TOC-V PC-controlled total organic carbon and attached total N analyzer (Shimadzu, Columbia, MD) was used to determine the dissolved organic carbon (DOC), and dissolved total nitrogen (DTN) solution concentrations and microbial biomass was calculated from the difference in C and N concentrations between fumigated and unfumigated samples.

A single extraction approach (Jones and Willet 2006) was used to calculate DOC, DTN, nitrate-N (NO_3^--N), and ammonium-N (NH_4^+-N) per g dry soil from unfumigated soil samples. Concentrations of NH_4^+-N and NO_3^--N were determined colorimetrically using a Skalar segmented-flow autoanalyzer (Skalar Inc., Norcross, GA). The salicylate hypochlorite procedure was used to measure NH_4^+-N (Mulvaney, 1996). Using a modified Greiss-Illosvay procedure, NO_3^--N was determined by utilizing Cd/Cu reduction of NO_3^- to NO_2^- (Mulvaney, 1996). Nitrate-N and ammonium-N were summed to calculate inorganic N (N_i), and dissolved organic nitrogen (DON) was calculated by subtracting N_i from DTN (Jones and Willet, 2006).

3.3.4. PCR

DNA was extracted from soil (~500 mg) using the NucleoSpin Soil DNA Extraction Kit (Clontech Inc., Mountain View, CA) according to manufacturer's protocol. Extracted DNA was

quantified using spectrophotometry (ND2000; Thermo Fisher Scientific Inc., Waltham, MA), and diluted to the same concentration before use in PCR. The *nirK* gene fragment was amplified with primers F1aCu [ATC ATG GTS CTG CCG CG] and R3Cu [GCC TCG ATC AGR TTG TGG TT] with a 33-bp GC-clamp (5' GGC GGC GCG CCG CCC GCC CCG CCC CCG TCG CCC 3') (Hallin, 1999; Throbäck et al., 2004). Reactions (25 µL) contained a final concentration of 1× PCR buffer (10 m*M* tris- HCl, 50 mM KCl, 0.01% (wt:vol) gelatin) 1.5 m*M* $MgCl_2$, 200 µ*M* each dNTP, 600 ng/µL BSA, 0.5 µM of each primer, 1.25 units of Taq polymerase (GoTaq®; Promega, Madison, WI) and 1 µL of 5 ng μL^{-1} template DNA for 0-10 cm samples, and 2 µL of 5 ng μL^{-1} template for 10-30 cm DNA. A PTC-200 DNA Engine (MJ Research Inc., Waltham, MA) thermal cycler was used to carry out PCR reactions. The following conditions were determined experimentally to optimize target amplification: initial denaturation at 94 °C for 2 min, 30 (0-10 cm) or 35 (10-30 cm) cycles of 94 °C for 30 sec, 59 °C for 45 sec, and 72 °C for 45 sec, and final extension at 72 °C for 7 min. DNA template and cycle number were increased in 10-30 cm samples because of otherwise limited amplification.

Amplification was confirmed by gel electrophoresis in 1.5% agarose gels stained with ethidium bromide and were digitally visualized with a Kodak EDAS 290 system and ID software package (Kodak, New Haven, CT). DNA mass standards (Bio Rad Laboratories, Hercules, CA) were used with each gel to confirm distance of migration and determine DNA concentration.

3.3.5. DGGE

The PCR amplified products (~ 20 µL) were loaded onto polyacrylamide DGGE gels for community profile creation. Protocols for F1aCu:R3Cu from Throbäck et al. (2004) were modified to suit the samples being analyzed. Vertical gels 1.0-mm thick containing 7%

polyacrylamide (acrylamide:bisacrylamide ratio of 37.5:1) were electrophoresed in 1.5X TAE buffer (40 m*M* Tris-acetate and 1 m*M* EDTA, pH 8.0) at 90V and 60 °C for 16 hr in a D-code system (Bio Rad Laboratories). Linear gradients were created using 45% and 75% denaturing solutions (100% equal to 7 *M* urea and 40% deionized formamide). Gels were stained for 20 min with SYBR Green and visualized using a Kodak EDAS 290 system and ID software package (Kodak).

Digital pictures of the gels were imported to Gel Compar II (Applied Maths Inc., Austin, TX) to analyze the presence or absence of bands and migration distances. Band detection and lane width were set to default values, with the exception of disk size to subtract background which was set to 10.0%. Software determination values for migration distances of detected bands were manually converted into a presence-absence table, where 0 designated the absence of a band, and 1 designated the presence of a band. A 3% optimization and 0.5 position tolerance were selected for the band matching settings.

Total band number per lane represents species richness (R), and band intensities were normalized by dividing the individual band intensity by the greatest intensity on the gel to reduce potential differences due to staining or picture quality. Diversity indices including Shannon-Weaver (H), Shannon-Weaver index of equitability (J), Simpson's (D) and Simpson's index of equitability (E) were calculated using the equations described in Wakil et al. (2008). In addition to the traditional diversity indices, a more recent approach to community analysis was also used and is based on calculating range-weighted richness (Rr) and functional organization (Fo) according to Marzorati et al. (2008). Range-weighted richness is a calculation that considers the width of the gradient used in DGGE, and this is considered to be related to the quality of

environment for the microorganisms. The expectation is that a wider range in gradient is needed to capture a larger amount of genetic variability.

The functional organization analysis uses Pareto-Lorenz evenness curves (Lorenz, 1905) to plot DGGE data to determine the distribution of species in a community or the distribution of band intensity within a lane. The cumulative proportion of abundances (intensity) is plotted on the y-axis and the cumulative proportion of species (bands) is plotted on the x-axis. The 20% vertical axis line is used to score each curve. A 25% curve would represent a community with high evenness and low functionality. A 45% curve represents communities that are balanced, because some species are present in high numbers, but the majority of the species are present in decreasing amounts. This structure would be best suited to handle disruptions to the system. An 80% curve represents a community with few dominant species with the remaining species present at low proportion; this community type can be highly functional under current conditions but is susceptible to changes in the environment.

3.3.6. Statistical Analyses

An analysis of variance (ANOVA) using SAS (version 9.4, SAS Institute Inc., Cary, NC) was performed to determine the effects of ground cover and fertilizer treatments and year on measured variables. The 0-10 cm and 10-30 cm depths were analyzed separately. The design was a 4 x 3 randomized complete block (i.e. 12 total treatment combinations of ground cover by fertilizer) with three replications, which was treated as the whole plot portion with a split plot for year. Interactions were only presented when they were significant ($P < 0.05$), otherwise only significant main effects were presented. During the DGGE analysis, some replications in the 0-10 cm depth were excluded because of low quality results, resulting in uneven sample sizes and a

large number of least significant differences (LSD) to compare treatment means. To simplify the explanation of treatment effects, the most conservative LSD for each factor level was chosen to perform mean separations. Unless specifically noted in tables, significant differences at the whole plot level encompass differences at the split plot level. In the 10-30 cm depth, some DNA samples did not amplify well or at all during PCR, resulting in treatment combinations and/or blocks with only one replication for DGGE related measurements. Here an ANOVA was performed to determine the effects of ground cover and year on diversity indices excluding blocks and fertilizer.

3.4. Results

3.4.1. 0-10 cm Soil Depth

There was a main effect of ground cover on pH (P = 0.0007, Table 4), but no interactions were significant. Soil pH was highest in the paper treatment, followed by compost, with lower and similar soil pH under wood chips and in the mow-and-blow treatments (Table 4). Nitrate-N (NO_3^--N) concentrations were also affected by the main effect of ground cover (P = 0.0256, Table 3) with concentrations highest in compost and wood chips (Table 4). Nitrate-N concentration in the mow-and-blow soil was not different from soil receiving wood chips, while soil nitrate-N concentration with paper was significantly lower than with wood chips. A main effect of year was observed for EC, water content, Bio N, NH_4^+-N, and NO_3^--N, which were all greater in 2013 than in 2007 (Tables 3 and 5).

There was a significant ground cover by fertilizer interaction on DOC, Bio C, Bio N and OM, and temperature (Table 4). Compost in combination with any fertilizer resulted in more DOC than any other treatment combination (Table 6). However, in the compost treatment,

poultry litter had a negative effect on microbial biomass C concentrations and soil organic matter, with measurements lower than the no-fertilizer control. Commercial fertilizer significantly increased DOC, Bio C, Bio N, and OM in soil receiving wood chips compared to the no-fertilizer control in that ground cover. Poultry litter fertilizer increased DOC, Bio N, and OM, but not Bio C in soil receiving wood chips compared to the no-fertilizer control in that ground cover. There were no differences observed in mow-and-blow or paper treatments in response to fertilizers with the exception of Bio N in paper treatments increasing compared to the control with the addition of poultry litter and commercial fertilizer. Microbial biomass C and N followed the same numerical trends as each other in response to fertilizers across ground covers although differences were not always significant. There was no significant differences in soil temperature with fertilizer addition within a ground cover, and no consistent trends across ground covers.

The interaction of ground cover and fertilizer had a significant effect on many of the diversity indices (Table 7). Richness was greater in communities where compost was applied in combination with any of the three fertilizer treatments compared to the other three ground covers in the absence of fertilizer (Table 8). Richness increased significantly in soil receiving commercial fertilizer and wood chips or the mow-and-blow compared to the absence of fertilizer, but not paper, and it decreased in the compost treatment compared to the absence of fertilizer. Soil receiving wood chips was the only treatment to increase in both richness and Shannon-Weaver diversity (H) in the presence of poultry litter or commercial fertilizer compared to the soil in that ground cover but the absence of fertilizer. Shannon –Weaver index of equitability (J) did not change much with fertilizer within a ground cover except with the mow-and-blow where, although richness increased, J decreased with commercial fertilizer compared to the absence of

fertilizer. Functional organization did not show many large differences; however, Fo was lower in soil with poultry litter and paper compared to commercial fertilizer and paper.

There was a significant fertilizer by year interaction on richness (Table 7). Fertilizer affected richness after only one application in April 2006, with commercial fertilizer increasing richness compared to no fertilizer and poultry litter in 2007 (Table 9). By 2013, richness increased such that it was greater than measured in 2007 and was similar across fertilizer treatments.

Ground cover treatments had varying effects on DOC, DON, Bio C, and OM depending on year (Table 3). Dissolved organic carbon showed a short-term response to ground cover in the compost treatment in 2007, measuring about twice the concentration as found in other ground covers (74 μg C g^{-1} compared to 32 - 44 μg C g^{-1}; Table 10). By 2013, ground covers, with the exception of the mow-and-blow, increased DOC from the 2007 values, with largest concentrations continuing to be measured in the compost treatment. In contrast to DOC, DON decreased significantly through time in all ground covers except compost which was greater in DON than other ground covers in both years. Microbial biomass C was lower in 2013 than 2007 in mow-and-blow and paper treatments, decreasing by about half in each treatment. Microbial biomass C did not vary by year with the addition of compost or wood chips. Organic matter increased over time regardless of ground cover treatment. There was an increase of organic matter over time in compost from 1.84 % in 2007 to the greatest overall organic matter of 5.29 %. Conversely, OM in mow-and-blow control increased to a lesser extent and mow-and-blow was significantly lower in OM than in the wood chips and compost treatments in 2013.

In addition to the significant interactions of fertilizer and ground cover and fertilizer and year, all seven ecological indices were also significantly affected by the interaction of ground

cover and year (Table 7). Species richness (R) was greatest in 2013 in soil receiving compost and wood chips compared to the other ground cover treatments in 2013, and R in those two ground covers increased significantly compared to richness in each respective ground cover in 2007 (Table 11). Shannon-Weaver index of diversity (H) in 2013 progressed from greatest to least in the order of compost ≥ wood chips ≥ paper ≥ mow-and-blow control with diversity in wood chips significantly increasing from among the lowest diversity in 2007 to among the highest diversity in 2013. Equitability either did not change through time or, in the wood chips treatment, decreased. Compost and paper were more even (J) than mow-and-blow and wood chips in 2013. The community in the compost treatment was more diverse than in mow-and-blow and wood chips in 2007 and was more diverse than in mow-and-blow and paper treatments in 2013. Simpson index of diversity (D) was affected similarly in 2007; communities where compost was applied had greater Simpson's index value than other ground covers. The community in the wood chip treatment was only similar in Simpson's index value to that in compost in 2013. Simpson's diversity ranged in order from compost = wood chips > paper ≥ mow-and-blow treatments in 2013.

While richness increased markedly in the wood chips treatment, communities in 2007 were more even than 2013, according to both Shannon's index of equitability (J) and Simpson's index of equitability (E) (Table 11). Simpson's index of equitability did not change through time in paper and the mow-and-blow treatments, but it decreased in compost and wood chips. Communities in compost and wood chips were more evenly distributed (E) than in mow-and-blow or paper treatments in 2007. Conversely, communities in paper showed greater evenness (E) than compost or wood chips in 2013. Mow-and-blow and paper treatments did not alter communities from 2007 and 2013 in calculations of the Shannon and Simpson indices. In 2007,

compost communities had lower functional organization than other communities. Functional organization (more species at greater abundances) increased from 2007 to 2013 in soil with compost, mow-and-blow and wood chips, but decreased during that time in soil from the paper treatment.

There was a ground cover by fertilizer by year effect on range-weighted richness (Rr) (P = 0.0069, Table 7). The only difference in Rr in 2007 was soils where compost was combined with poultry litter or no fertilizer were significantly greater than the same fertilizer treatments in the wood chips ground cover treatment (Table 12). The poultry litter with wood chip combination and compost with no-fertilizer control combination were similar in 2013 and had the greatest range-weighted richness measured. Regardless of fertilizer, both compost and wood chip communities had greater range weighted richness in 2013 than in 2007. Range-weighted richness did not vary in mow-and-blow and paper communities within a fertilizer combination between 2007 and 2013.

3.4.2. 10-30 cm Soil Depth

A main effect of year was observed for multiple soil properties (Table 13). Soil OM and ammonium-N (NH_4^+-N) concentrations increased through time (Table 14). Microbial biomass C and N concentrations were significantly lower in 2013, with a large difference (56.6 μg C g^{-1}) observed in Bio C over time (Table 14). Water content was also lower in 2013 than in 2007 (Table 14). There was a main effect of ground cover on OM content (P = 0.0445, Table 13) ranging in order from compost (1.78 %) ≥ wood chips (1.61 %) > paper (1.56 %) ≥ mow-and-blow control (1.46 %).

There was a ground cover by year interaction on electrical conductivity (EC), pH, DOC, DON, and NO_3^--N (Table 13). There were no differences in EC among ground cover treatments

in 2007 (Table 15). Electrical conductivity increased in all ground cover treatments through time such that in 2013 EC was highest in compost followed by wood chips, then paper, and finally the mow-and-blow control. The soil pH was similar in the paper and compost treatments in 2007, which was greater than in soils with mow-and -blow and wood chip additions. Compost, paper and wood chip additions all increased soil pH over time. Soil receiving paper had the highest pH in 2013, followed by compost and wood chips which were similar and greater in pH than the mow-and-blow. The only significant change in DOC over time within a ground cover was an increase with the addition of compost. There was both a short and long-term response in DOC in soils with compost addition, which increased DOC concentrations compared to mow-and-blow and paper additions in 2007 and in 2013. In 2007, there were no differences in DON concentrations. Compost, paper and wood chip additions all increased DON concentrations in soils over time, but DON concentrations were greater with compost applications compared to all other ground covers in 2013. Ground cover additions did not result in significantly different NO_3^--N concentrations in 2007. Compost followed by wood chips increased NO_3^--N through time and those treatments had greater concentrations in 2013 than soils with mow-and-blow and paper treatments.

Fertilizer and ground cover interactions had significant effects on DON and pH (Table 13). Soil DON concentrations were greater without the addition of fertilizer within all four ground cover treatments (Table 16). Wood chip additions in combination with poultry litter produced greater concentrations of DON than wood chips with commercial fertilizer. Although the same pattern of fertilizer effects on DON concentrations was present in other ground cover treatments, differences were not significant. Soil pH was highest with the addition of paper in the absence of fertilizer directly followed by commercial fertilizer. Compost and paper applied with

any fertilizer had greater soil pH than all wood chips and mow-and-blow treatment combinations.

Fertilizer addition had significant effects on soil DON and NO_3^--N concentrations over time (Table 14). There were no differences in DON or NO_3^--N concentrations in 2007 among fertilizer treatments. In 2013, DON concentration was greatest in soils with no fertilizer addition, increasing 23.7 μg N g^{-1} from 2007 (Table 17), while the DON concentrations with commercial fertilizer and poultry litter were about 20 and 40%, respectively, of the value measured in the absence of fertilizer. Nitrate-N was greater in 2013 than 2007 and was greater in the presence, as opposed to the absence, of fertilizer.

There were no significant interactions or main effects of ground cover, fertilizer or year on the ecological indices calculated from the DGGE profiles (Table 18). There was a main effect of ground cover on organic matter content (P = 0.0445, Table 13) ranging in order from compost (1.78 %) ≥ wood chips (1.61 %) > paper (1.56 %) ≥ mow-and-blow control (1.46 %).

Only 25 and 23 measurements were useable in 2007 and 2013, respectively, out of 36, limiting ability to detect significance in treatment effects. Richness, ranged from 24.43 to 35.17 in the 10-30 cm depth; in comparison, richness ranged from 22.2 to 45.0 in the 0-10 cm depth (Tables 9 and 19). On average, lower but not drastically different diversity index values were observed in the 10-30 than in 0-10 cm (Tables 9 and 19). Results may be indicating greater spatial variability in the movement of ground cover and fertilizer treatment effects below the surface 10 cm, making it more difficult at our sample size to detect treatment effects.

3.5. Discussion

Considering the physical density and chemical complexity, it is not surprising that compost was responsible for large increases in OM (Neilsen et al., 2003; Cayuela et al., 2004; Yao et al., 2005). Baldi et al. 2010 also reported large increases in OM of 169% after 9 years with the highest rate of compost applied, 10 t ha $^{-1}$ yr $^{-1}$, in a peach orchard. The building of soil organic matter, the rate of decomposition and nutrient cycling, depends upon the substrate quality, the microbial community and soil conditions (temperature, moisture and pH). A determining characteristic of substrate quality is the composition of the materials being added (Bengtsson et al., 2003; Mungai and Motavalli, 2005); sugars, proteins and starches are easily decomposed, while hemi-cellulose, cellulose and lignin are increasingly difficult to decompose (Tisdall and Oades, 1982). In our study, the most difficult ground cover to breakdown, according to lignin content of similar materials used by others was compost, wood chips, mow-and-blow and paper (~ 39%, 26%, 12%, and 6.5%, respectively) (Francou et al., 2008; Holland et al., 1991; Chen et al. 2002; Komilis and Ham, 2003).

The C:N ratio of the of the ground covers and/or fertilizer added are also important because microorganisms incorporate the carbon, nitrogen and other nutrients to create proteins and other key building blocks of their biomass during the decomposition process, and the C:N ratio of microorganisms is 5:1-15:1 and if N is limiting it will be immobilized (Frankenberger and Abdelmagid, 1985; Nicolardot et al., 2001). Barrett and Burke (2000) conducted a study on grassland soils from Texas to Montana and observed that the highest rates of immobilization occurred in soils containing with greater C:N ratios. Lower C:N residues (< 20:1) are considered greater quality resources. In this study, compost C:N ratio averaged 13.1, wood chips was 39.2, mow-and-blow was 15.9 while the C:N ratio of paper was 205.9 (Table 2). However, there are

exceptions to the rule, benefits have been reported by others with the use of high C:N additions, for example Yao et al. (2005) measured soil microbial community responses to ground covers, including grass and mulch in a New York orchard on a silty clay loam over 12 years. In the mulch treatment with a high C:N of 98:1, OM doubled, less N leached, respiration was greater, and bacterial counts increased (Yao et al., 2005). Huang et al. (2008) compared mulch to no mulch treatments in two hardwood plantations in Australia, and found that mulch had greater immobilization rates than the non-mulch, more SOM and H (calculated form BIOLOG plates) than non-mulch treatments. In contrast in the current study, a low C:N ratio treatment, mow-and-blow had few significant differences overall, except increased in OM and denitrifier species richness over time. Yao et al. (2005), also saw few differences in the grass treatment in their study except that soil N retention was greater than in herbicide treatments.

Without fertilizer, nitrogen could be limiting and decomposition and cycling in these treatments could be slowed. It would be expected that adding paper or wood chips would immobilize N and combining a fertilizer would increase the decomposition by increasing the N available to the microorganisms (Singh and Gupta, 1977). However, results of lower decomposition with nitrogen additions to litter have also been reported (Hobbie, 2008; Berg, 2008; Fang et al., 2007) related to the biochemical interactions of N containing compounds during decomposition. The commercial fertilizer had a greater percentage of nitrogen (4.4%) and a lower C:N ratio (7.8) than the poultry litter (1.9% N, 19.4 C:N) (Table 2). Fertilizers increased OM, DOC, Bio C and N in the wood chip treatments, and surprisingly differences in paper treatments were not significant from the no-fertilizer control except for an increase in Bio C and N. The larger biomass with the addition of fertilizer in paper treatments suggests that the total community may be getting larger but the response is not being measured in the *nirK* gene

targeted. Residue quality may be impacting microbial biomass in this case, as paper has the lowest lignin content of the ground covers. Species richness (R) and diversity (H) of *nirK* containing denitrifiers in the soil receiving wood chips and fertilizers were greater compared to wood chips and no fertilizer. Poultry litter and compost combination had a significant negative effect on OM and biomass C and N compared the no-fertilizer control. The combination of compost and poultry litter applications has not been widely studied, so it is possible there is a synergistic effect occurring with this treatment combination; however, the governing mechanisms are beyond the scope of the current study but pose an interesting research question for future investigation.

Even though compost and wood chip treatments increased OM and DOC over time, and changed the microbial community, the response was different. With the addition of resources available in the soil (OM, DOC, and DON), a large microbial community is expected in 2013 (Kramer et al., 2006; Baldi et al., 2010). However, there was lack of increase in microbial biomass carbon concentrations from 2007 to 2013, which could have been a result of the significantly cooler temperatures at the time of sampling in 2013. This decrease was not measured in wood chips and compost treatments and may have resulted from a buffering effect of those ground covers (Pickering et al., 1998). Soil DNA concentrations increased from 2007 to 2013, and a similar increase in the total community seemed likely throughout the DGGE analysis of the soil denitrifier community but community composition was impacted differently by ground cover and nutrient source treatments.

Denitrification is highly dependent upon DOC, nitrate and pH. Total microbial community richness and diversity has been strongly linked to soil pH (Fierer and Jackson, 2006) and specifically, denitrifiers are positively correlated with soil pH (Hallin et al. 2009). Barta et

al. (2010) reported a strong correlation of quantity of denitrifiers (*nirK* and *nir*S) and dissolved organic carbon and nitrogen concentrations as well as pH in spruce forest soils. Dandie et al. (2011) conducted a study in Canada on soils with either agricultural (maize) or riparian (mixed wood understory) zones and found that *nirK* harboring organisms were most strongly correlated with soil pH and found along with Attard et al. (2011) that *nirK* are more important in agricultural ecosystems compared to their *nir*S counterparts. However, in the current study the soil pH was 7.01 (compost), 6.83 (mow-and-blow), 7.32 (paper), 6.84 (wood chips), and these values were similar to those with highest diversity and abundances reported by others (Fierer and Jackson, 2006; Barta et al., 2010) suggesting that in this study, pH is not limiting the *nirK* community and that other factors, such as DOC or soil water content, are most likely having a greater impact on *nirK* communities.

Dissolved organic carbon and nitrogen are not necessarily labile; however, they are the soluble fraction of organic matter that is physically available for use by microorganisms (Cook and Allan, 1992; Aiken and Costaris, 1995; Chantigny, 2002).The rapid increase in dissolved C and N could have immediately impacted species richness and Shannon and Simpson diversity with the addition of compost in 2007 and may explain the lack of response over seven years, because the response was already being measured. Suzkics et al., (2009), reported changes in *nirK* abundance after only a 4 day incubation of two forest soils under varying soil moisture contents, which supports the findings in the current study of changes within the *nirK* community in 2007 in the compost treatment after only 1 year of treatment applications. Correlations between dissolved organic carbon and *nirK* have been reported by many others (Jin et al., 2014; Barta et al., 2010). Attard et al. (2011) reported that soil organic carbon content explained 76% and 53% of the variability of the annual crop/grassland rotation and the till/no till rotation sites

studied, respsectively; water-filled pore space and nitrate contributed to the variability as well, but the nitrate correlation was weak. Kandeler et al. (2006) examined denitrifier abundance in a glacier foreland and reported that organic carbon was the most important factor contributing to differences in that ecosystem. All treatments in the current study increased in DOC concentrations through time except the mow-and-blow treatments, but the only treatment that did not increase through time in species richness was paper, possibly because this treatment contributed the least amount of nitrate-N of the four ground covers. The consistently high DON with compost addition may be a result of the large amount of organic matter being added in the compost treatment where concentrations remain elevated or it could be an indication that mineralization is not proceeding efficiently. Even though there is smaller biomass in the compost treatments, nitrate concentrations measured in decreasing order of compost ≥ wood chips ≥ mow-and-blow control ≥ paper suggest that in addition to high DON concentrations in both 2007 and 2013, mineralization and nitrification are occurring at rates sufficient to accumulate excessive nitrate concentrations. However, there is not less DNA in the compost treatments, which may indicate that although the community is not growing it is changing to a community that is more specialized in the breakdown of the complex molecules in the compost, or that the microbial biomass is responding quickly to additions and the flux biomass is not being captured. Rousk et al. (2015) reported that changes in, sandy clay loam SOM after the addition of glucose similarly affected the mineralization of varying ages of soil organic matter (2-13 months) in a perennial rye grass/white clover, and increased SOM mineralization could not be explained by microbial growth dynamics.

The response in nitrate follows the same trend as DOC, which are both necessary for denitrification, and these responses were also similar to trends in denitrifier diversity indices,

high in compost and wood chips followed by paper and mow-and-blow. Although nitrate has not been as strongly linked to denitrifiers (Attard et al. 2011) compared to organic carbon or pH, it is central to denitrification. Nitrate-N was greater in 2013 than in 2007 in this study, possibly contributing to the increase in richness observed over time in the compost, mow-and-blow and wood chip *nirK* communities.

The *nirK* community structure has not been studied extensively with DGGE in orchard soils, but some have investigated *nirK* communities with DGGE in other agricultural soils. Wertz et al., (2009), used DGGE to investigate the *nirK* community in a potato-spring wheat rotation over a potato growing season in Canada and found the communities were spatially variable between the soil in the potato hills, the furrow soil, and soil from areas close to the potato plants on the hill and varied over time in the hill soil. The richness of *nirK* communities in the potato field soil (< 20 DGGE bands) was less than the richness of *nirK* in the current study (> 20 DGGE bands), however different primers were used during PCR which could have an effect on species richness (Wertz et al., 2009). Clark et al. (2012) investigated the impacts of N management to activity and diversity in the Broadbalk wheat experiment established 160 years ago. They calculated H from DGGE completed using the same primers used in this study. Their H values ranged from 2.9-3.1 with R range of 18-23 and in comparison our H values ranged from 3.0-3.5 with R ranging from 31-45. They reported a positive relationship between *nirK* and N_2O flux but, overall, physiochemical properties may have more influence on emissions with high OM soil.

3.6 Conclusion

All ground covers increased organic matter over time, and all ground covers except mow-and-blow increased DOC over time but compost added the most OM and DOC. There was more

NO_3^--N, NH_4^+-N, and DON in 2013 than in 2007. I hypothesized that treatments where substrate availability (OM, DOC, NO_3^--N) and soil conditions (pH, temperature, water content) were most conducive to denitrification, microbial biomass and *nirK* community richness and diversity would be greatest. The hypothesis regarding microbial biomass was not supported by the results, mow-and-blow treatments added less OM and DOC, while compost and wood chip treatments added more OM, DOC, and NO_3^- -N but microbial biomass did not differ across ground covers within a year. The treatments with the biggest impact on denitrifying *nirK* organisms were compost and wood chips. The change in the compost communities was apparent in 2007 by greater species richness and diversity compared to the other ground cover treatments. Wood chips had the greatest change through time of the ground cover additions and in 2013 had similar species richness and diversity when applied in combination with either fertilizer even though DOC concentrations were less than in compost treatments.

3.7. References

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3.8. Tables

Table 1. Initial properties of an organically managed apple orchard silt loam soil, Fayetteville, AR, 2006 (n=36).

Year	pH	EC[a] (μmhos cm $^{-1}$)	Bulk Density (g cm$^{3\ -1}$)	OM (%)	N	C	P	K
					----------(mg kg $^{-1}$)----------			
2006	6.57	73.75	1.34	1.47	0.09	0.95	34	170

[a]EC is electrical conductivity; OM is organic matter; N is nitrogen; C is carbon; P is phosphorous; K is potassium

Table 2. Nutrient contents of ground cover and fertilizer treatments applied in an organically managed apple orchard, Fayetteville, AR. (n=6).

Treatment	C[a]	N	C:N	P	K
	------ (%)------			------ (%)------	
Compost	20.5	1.6	13.5	0.2	0.5
Wood Chips	29.7	0.7	39.2	0.1	0.3
Paper	36.8	0.2	205.9	0.0	0.0
Mow-and-Blow	40.0	2.2	15.8	0.3	1.5
Commercial Fertilizer	31.3	4.4	7.8	1.4	2.6
Poultry Litter	29.5	1.7	19.4	1.3	1.4

Data for each ground cover and fertilizer are averaged across 2006-2011.
[a]C is carbon; N is nitrogen; P is phosphorous; K is potassium

Table 3. Analysis of variance (ANOVA) summary of the effects of ground cover and fertilizer treatments over time and their interactions on soil properties at the 0-10 cm depth of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007 and 2013.

Soil Property	GC[b]	Fert	GC*Fert	Year	GC*Year	Fert*Year	GC*Fert*Year
DOC[a]	<0.0001*	0.2663	0.0351*	<0.0001*	<0.0001*	0.7543	0.2842
DON	0.0026*	0.0036*	0.0820	<0.0001*	0.0206*	0.5338	0.8424
NO_3^--N	0.0256*	0.0350	0.1083	<0.0001*	0.1642	0.1240	0.2563
NH_4^+-N	0.5325	0.1168	0.1440	<0.0001*	0.2194	0.2199	0.9857
Bio C	0.6768	0.2421	0.0395*	0.0021*	0.0396*	0.0545	0.1684
Bio N	0.7494	0.0899	0.0152*	0.0010*	0.3121	0.3170	0.1243
pH	0.0007*	0.0711	0.1981	0.4248	0.0745	0.6749	0.9856
EC	0.3043	0.4268	0.3492	0.0001*	0.3055	0.3952	0.4011
OM	0.0036*	0.0975	0.0026*	<0.0001*	0.0196*	0.1895	0.0513
DNA	0.0325*	0.3706	0.7745	<0.0001*	0.7866	0.9284	0.5287
Temp	0.0163*	0.9859	0.0046*	<0.0001*	0.0148*	0.0254*	0.0531
H_2O	0.2032	0.9727	0.6848	0.0001*	0.4323	0.5941	0.4878

[a]DOC is dissolved organic carbon, DON is dissolved organic nitrogen, NO_3^--N is nitrate-N, NH_4^+-N is ammonium-N, Bio C is microbial biomass carbon, Bio N is microbial biomass nitrogen, EC is electrical conductivity, OM is organic matter, temp is temperature, and H_2O is soil-water content. [b]GC is ground cover and Fert is fertilizer source.
*$P < 0.05$

Table 4. The effects of ground cover management treatments on the soil properties at the 0-10 cm sample depth in an organically managed apple orchard, Fayetteville, AR (n=18).

Ground Cover	pH	NO_3^--N ($\mu g\ N\ g^{-1}$)	DNA ($ng\ \mu L^{-1}$)
Compost	7.01b	4.61a	35.26a
Mow-and-Blow	6.83c	2.53bc	32.24a
Paper	7.32a	1.17c	25.32b
Wood Chips	6.84c	3.54ab	31.28ab

Means followed by different letters within a column are significantly different ($P < 0.05$). LSD's to compare means at $\alpha = 0.05$, pH (0.15), NO_3^--N (1.98), DNA (6.20)

Table 5. The effects of sample year on soil properties at a sample depth of 0-10 cm in an organically managed apple orchard, Fayetteville, AR, 2007 and 2013 (n=36).

Year	H_2O[a]	EC (μmhos cm^{-1})	Bio N ($\mu g\ N\ g^{-1}$)	NH_4^+-N ($\mu g\ N\ g^{-1}$)	NO_3^--N ($\mu g\ N\ g^{-1}$)	DNA ($ng\ \mu L^{-1}$)
2007	0.17b	46.63b	12.19b	1.78b	0.92b	22.78b
2013	0.22a	333.0a	16.68a	7.93a	5.01a	39.27a

[a]H_2O is soil-water content; EC is electrical conductivity; Bio N is microbial biomass nitrogen; NH_4^+-N is ammonium-N; NO_3^--N is nitrate-N

Means followed by different letters within a column are significantly different ($P < 0.05$). LSD's to compare means at $\alpha = 0.05$, H_2O (0.02), EC (126.9), Bio N (2.48), NH_4^+-N (0.77), NO_3^--N (0.98), DNA (6.15)

Table 6. Soil chemical and biological properties affected by ground cover and fertilizers treatments at the 0-10 cm depth of an organically managed apple orchard, Fayetteville, AR, 2007 and 2013 (n=6).

Ground Cover	Fertilizer	DOC[a] (μg C g^{-1})	Bio C (μg C g^{-1})	Bio N (μg N g^{-1})	OM (%)	Temp (°C)
Compost	None	157.5a	74.0ab	17.7a	3.98a	10.9de
	Poultry litter	140.6ab	47.7cd	12.6bc	2.87bc	10.3e
	Commercial	130.8b	63.2abcd	16.2ab	3.85a	11.0cde
Mow-and-Blow	None	36.0e	81.2a	14.5abc	1.96e	10.9de
	Poultry litter	50.2de	66.3abc	12.7bc	2.24de	11.5abcd
	Commercial	38.9e	66.6abc	13.3bc	2.11de	11.5abcd
Paper	None	81.4c	39.6d	10.7c	2.27de	11.5abcd
	Poultry litter	86.5c	64.8abcd	15.6ab	2.55cd	11.1bcde
	Commercial	83.4c	64.3abcd	15.5ab	2.34cde	10.9de
Wood Chips	None	60.6d	48.3bcd	10.6c	2.21de	11.7abc
	Poultry litter	89.4c	59.3abcd	15.6ab	2.84bc	12.1a
	Commercial	88.0c	83.7a	18.1a	3.20b	11.7abc

[a]DOC is dissolved organic carbon; Bio C is microbial biomass carbon, Bio N is microbial biomass nitrogen, and OM is organic matter.

Means within columns followed by similar letters are not significantly different ($P < 0.05$).

LSD's to compare means at $\alpha = 0.05$, DOC (20.8), Bio C (26.1), Bio N (4.4), OM (0.55), Temp (0.90)

Table 7. Analysis of variance (ANOVA) summary of the effects of ground cover and fertilizer treatments over time and their interactions on soil ecological indices at the 0-10 cm depth of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007 and 2013.

Diversity Index	GC	Fert	GC*Fert	Year	GC*Year	Fert*Year	GC*Fert*Year
R[a]	0.0024*	0.0371*	0.0028*	<0.0001*	<0.0001*	0.0473*	0.1271
H	0.0128*	0.0442*	0.0146*	<0.0001*	0.0015*	0.1550	0.6922
J	0.0461*	0.2158	0.0081*	0.0423*	0.0094*	0.2546	0.0903
D	0.0026*	0.1793	0.2499	0.0005*	0.0099*	0.4776	0.4940
E	0.1908	0.9431	0.0953	0.0480*	0.0294*	0.3365	0.4767
Rr	0.0004*	0.1327	0.0019*	<0.0001*	<0.0001*	0.0393*	0.0069*
Fo	0.2323	0.0225*	0.0142*	0.0003*	<0.0001*	0.5431	0.1110

[a]R is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; Rr is range weighted richness; and Fo is functional organization.

* $P < 0.05$

Table 8. Ecological diversity indices calculated from DGGE profiles of *nirK* in the 0-10 cm soil depth affected by ground cover and fertilizer combinations, in an organically managed orchard in Fayetteville, AR

Ground Cover	Fertilizer	R[a]	H	J	Fo
Compost	None	41.0a	3.46a	0.93ab	0.41bc
	Poultry litter	36.1abc	3.37ab	0.94a	0.43abc
	Commercial	35.1bc	3.37ab	0.95a	0.41bc
Mow-and-Blow	None	25.2f	2.95de	0.92ab	0.44ab
	Poultry litter	26.8ef	3.00cde	0.92ab	0.45a
	Commercial	34.3bcd	3.02cde	0.85c	0.45a
Paper	None	29.5ef	3.14bcd	0.94a	0.42abc
	Poultry litter	31.2cde	3.18bcd	0.93ab	0.40c
	Commercial	31.3cde	3.19bcd	0.93ab	0.46a
Wood Chips	None	27.2ef	2.83e	0.89bc	0.42abc
	Poultry litter	36.7abc	3.26abc	0.92ab	0.45a
	Commercial	37.0ab	3.31ab	0.93ab	0.45a

[a]R is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; and Fo is functional organization.
Sample sizes listed in Appendix A, Table 3.
Means within columns followed by similar letters are not significantly different ($P < 0.05$).
LSD's to compare means at $\alpha = 0.05$, R (5.8), H (0.23), J (0.4), Fo (0.04)

Table 9. The effect of fertilizer treatments and year on species richness (R) in the 0-10 cm depth of a silt loam soil in an organically managed orchard in Fayetteville, AR, 2007 and 2013

Year	None	Poultry litter	Commercial
2007	25.5c	26.6c	31.0b
2013	35.9a	38.8a	37.9a

Sample sizes listed in Appendix A, Table 3.
Means followed by different letters within a column are significantly different ($P < 0.05$)
LSD's to compare means at $\alpha = 0.05$, Whole plot (3.77), Split plot (3.56)

Table 10. The effect of ground cover treatment and year interaction on soil properties measured at 0-10 cm in an organically managed apple orchard in Fayetteville, AR, 2007 and 2013 (n=9).

Ground Cover	Year	Bio C[a] (μg C g^{-1})	DOC (μg C g^{-1})	DON (μg N g^{-1})	OM (%)	Temp (°C)
Compost	2007	64.5abc	73.9c	9.20a	1.84d	14.4b
	2013	58.8abc	212.0a	9.06a	5.29a	7.1d
Mow-and-Blow	2007	93.4a	32.5d	4.72b	1.12e	15.9a
	2013	49.3bc	50.9cd	0.55d	3.08c	6.8d
Paper	2007	75.1ab	38.3d	5.01b	1.19de	15.3a
	2013	37.3c	129.3b	1.45c	3.58bc	7.0d
Wood Chips	2007	63.2abc	44.2d	5.41b	1.23de	15.6a
	2013	64.3abc	114.4b	0.61d	4.26b	8.0c

[a]Bio C is microbial biomass carbon, DOC is dissolved organic carbon, DON is dissolved organic nitrogen, and OM is organic matter.

Means within columns followed by similar letters are not significantly different ($P < 0.05$).

LSD's to compare means at $\alpha = 0.05$, Bio C- Whole plot (36.8), Split plot (25.9), DOC- Whole plot (26.9), Split plot (22.9), DON- Whole plot (3.2), Split plot (2.2), OM- Whole plot (0.69), Split plot (0.69), Temp-Whole plot (0.90), Split plot(0.80)

Table 11. Ecological diversity indices calculated from DGGE profiles of *nirK* in the 0-10 cm soil depth affected by ground cover in 2007 and 2013, in an organically managed orchard in Fayetteville, AR

Ground Cover	Year	R[a]	H	J	D	E	Fo
Compost	2007	31.9b	3.30abc	0.95a	23.34ab	0.74a	0.37e
	2013	43.0a	3.50a	0.93ab	25.21a	0.60d	0.46ab
Mow-and-Blow	2007	26.7c	2.95ef	0.90bc	16.59cd	0.62cd	0.43bcd
	2013	30.9b	3.04def	0.89c	19.32c	0.62cd	0.47a
Paper	2007	30.0bc	3.13cde	0.92abc	18.99cd	0.63cd	0.45abc
	2013	31.3b	3.21bcd	0.94a	21.21bc	0.67bc	0.40de
Wood Chips	2007	22.2d	2.89f	0.94a	15.43d	0.70ab	0.42cd
	2013	45.0a	3.37ab	0.89c	27.06a	0.60d	0.46ab

[a]R is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; and Fo is functional organization. Sample sizes listed in Appendix A, Table 3.

Means followed by similar letters are not significantly different ($P < 0.05$).

LSD's to compare means at $\alpha = 0.05$, R- Whole plot (4.2), Split plot (3.9), H- Whole plot (0.20), Split plot (0.17), J- Whole plot (0.04), Split plot (0.03), D- Whole plot (3.8), Split plot (4.6), E- Whole plot (0.73), Split plot (0.82), Fo-Whole plot (0.04), Split plot (0.03)

Table 12. Range weighted richness (Rr) calculated from DGGE profiles of communities affected by ground cover and fertilizer treatment interactions in the 0-10 cm soil depth of an organically managed orchard in Fayetteville, AR, 2007 and 2013

	2007			2013		
Ground Cover	None	Poultry litter	Commercial	None	Poultry litter	Commercial
Compost	223.7defg	223.1defg	194.0efgh	587.7a	381.4bc	360.8bc
Mow-and-Blow	126.2ghi	103.6ghi	214.4defgh	120.1ghi	197.0defgh	293.3cde
Paper	139.9fghi	166.6efghi	230.9defg	191.1efgh	257.5cdef	203.9defgh
Wood Chips	41.2i	90.9hi	151.3fghi	322.3bcd	593.9a	450.8b

Sample sizes listed in Appendix A, Table 3.
Means within columns followed by similar letters are not significantly different ($P < 0.05$).
LSD's to compare means at $\alpha = 0.05$, Whole plot (134.4), Split plot (127.1)

Table 13. Analysis of variance (ANOVA) summary of the effects of ground cover and fertilizer treatments over time and their interactions on soil properties at the 10-30 cm depth of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007 and 2013.

Soil Property	GC[a]	Fert	GC*Fert	Year	GC*Year	Fert*Year	GC*Fert*Year
DOC[a]	0.0017*	0.0747	0.5700	0.0860	0.0002*	0.1191	0.9817
DON	0.0179*	<0.0001*	0.0042*	<0.0001*	0.0010*	<0.0001*	0.0778
NO_3^--N	0.0007*	0.0066*	0.1783	<0.0001*	<0.0001*	0.0478*	0.4249
NH_4^+-N	0.2855	0.6620	0.9722	<0.0001*	0.1003	0.5463	0.9877
Bio C	0.2057	0.3396	0.4185	<0.0001*	0.2010	0.5933	0.8801
Bio N	0.7795	0.1091	0.0552	<0.0001*	0.2990	0.7842	0.9561
pH	0.1163	0.1511	0.0027*	<0.0001*	<0.0001*	0.9331	0.8619
EC	0.0011*	0.0558	0.9007	<0.0001*	<0.0001*	0.0693	0.9133
H_2O	0.0716	0.7834	0.5187	0.0001*	0.0679	0.9474	0.6543
OM	0.0445*	0.8291	0.4186	<0.0001*	0.2399	0.9043	0.5339
DNA	0.0100*	0.5079	0.1216	0.0170*	0.7301	0.7941	0.5285

[a]DOC is dissolved organic carbon; DON is dissolved organic nitrogen; is nitrate-N; NH_4^+-N is ammonium-N; Bio C is microbial biomass carbon; Bio N is microbial biomass nitrogen; EC is electrical conductivity; OM is organic matter; H_2O is soil-water content.

* $P < 0.05$

Table 14. The effects of sample year on soil properties at a sample depth of 10-30 cm in an organically managed apple orchard, Fayetteville, AR, 2007 and 2013 (n=36).

Year	H_2O[a]	OM (%)	Bio C ($\mu g\ C\ g^{-1}$)	Bio N ($\mu g\ N\ g^{-1}$)	NH_4^+ ($\mu g\ N\ g^{-1}$)	DNA ($ng\ \mu L^{-1}$)
2007	0.24a	0.96b	74.06a	3.76a	0.05b	4.32a
2013	0.17b	2.24a	17.42b	1.99b	0.52a	2.88b

[a]H_2O is soil water content; OM is organic matter; Bio C is microbial biomass carbon; Bio N is microbial biomass nitrogen (Bio N); (NH_4^+-N) is ammonium-N.
Means followed by different letters within a column are significantly different ($P < 0.05$).
LSD's to compare means at $\alpha = 0.05$, H_2O (0.03), Bio C (12.5), Bio N (0.51), NH_4^+ (0.16), DNA (1.19)

Table 15. Soil properties in the 10-30 cm depth affected by ground cover treatment and year, in an organically managed apple orchard in Fayetteville, AR, 2007 and 2013 (n=9).

Ground Cover	Year	EC[a] (μmhos cm $^{-1}$)	pH	DOC (μg C g $^{-1}$)	DON (μg N g $^{-1}$)	NO_3^- (μg N g $^{-1}$)
Compost	2007	26.80e	6.64bc*	35.57b	4.39d	1.77c
	2013	215.02a	6.96ab*	51.10a	23.20a	8.59a
Mow-and-Blow	2007	16.10e	6.38c	27.24cd*	3.76d	0.64c
	2013	128.71d	6.44c	19.94d*	7.96cd	1.44c
Paper	2007	16.42e	6.56bc	26.64cd	3.10d	0.36c
	2013	158.09c	7.13a	29.52bc	15.05b	1.01c
Wood Chips	2007	16.18e	6.30c	30.94bc	4.16d	0.89c
	2013	182.06b	6.95ab	30.71bc	12.38bc	4.61b

[a] EC is electrical conductivity; DOC is Dissolved organic carbon; DON is dissolved organic nitrogen; (NO_3^-) is nitrate-N

Means within columns followed by similar letters are not significantly different $P < 0.05$.

* Significantly different on the split plot level.

LSD's to compare means at $\alpha = 0.05$, EC- Whole plot (22.3), Split plot (18.8), pH-Whole plot (0.42), Split plot (0.17) DOC- Whole plot (8.43), Split plot (6.27), DON-Whole plot (4.80), Split plot (4.64), NO_3^- -Whole plot (1.79), Split plot (1.40)

Table 16. Soil chemical and biological properties affected by ground cover and fertilizer treatments at the 10-30 cm depth of a silt loam soil, in an organically managed apple orchard in Fayetteville, AR (n=6).

Ground Cover	Fertilizer	DON[a] ($\mu g\ N\ g^{-1}$)	pH
Compost	None	25.13a	6.81c
	Poultry litter	9.27d	6.75d
	Commercial	6.99def	6.84bc
Mow-and-Blow	None	9.74cd	6.35i
	Poultry litter	4.28ef	6.47g
	Commercial	3.56f	6.41h
Paper	None	14.73b	6.97a
	Poultry litter	7.17def	6.70e
	Commercial	5.33ef	6.88b
Wood Chips	None	13.52bc	6.63f
	Poultry litter	7.66de	6.64f
	Commercial	3.63f	6.60f

[a]DON is dissolved organic nitrogen.
Means within columns followed by similar letters are not significantly different ($P < 0.05$).
LSD's to compare means at $\alpha = 0.05$, DON (3.90), pH (0.05)

Table 17. The effect of fertilizer on soil properties at the 10-30 cm soil depth of a silt loam soil over time in an organically managed orchard in Fayetteville, AR, 2007 and 2013. (n=4).

Year	Fertilizer	DON[a] (μg N g^{-1})	NO_3^- (μg N g^{-1})
2007	None	3.93c	0.75c
	Poultry litter	3.86c	1.25c
	Commercial	3.77c	0.74c
2013	None	27.63a	2.53b
	Poultry litter	10.33b	5.13a
	Commercial	5.98c	4.08a

[a]DON is dissolved organic nitrogen; (NO_3^-) is nitrate-N

Means within columns followed by similar letters are not significantly different ($P < 0.05$)

LSD's to compare means at $\alpha = 0.05$, DON- Whole plot (4.13), Split plot (3.42), NO_3^- - (1.24), Split plot (1.21)

Table 18. P-values from analysis of variance (ANOVA) for ecological indices from DGGE of 10-30 cm depth.

Diversity Index	GC	Year	GC*Year
R[a]	0.8360	0.1445	0.5507
H	0.4464	0.4689	0.3044
J	0.5698	0.2510	0.2610
D	0.8108	0.2316	0.3967
E	0.9127	0.2058	0.2608
Rr	0.8778	0.1593	0.6957

[a]R is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; Rr is range weighted richness.

Table 19. Ecological diversity indices calculated from DGGE profiles of *nirK* in the 10-30 cm soil depth affected by ground cover and year 2007 and 2013, in an organically managed orchard in Fayetteville, AR (n=9).

Ground Cover	Year	R	H	J	D	E	Rr
Compost	2007	32.2	3.13	0.91	17.92	0.59	251.02
	2013	32.0	3.14	0.90	17.12	0.54	257.34
Mow-and-Blow	2007	32.3	3.05	0.90	18.25	0.57	319.66
	2013	29.7	3.03	0.91	17.62	0.59	258.20
Paper	2007	30.0	2.69	0.83	16.13	0.53	269.11
	2013	27.0	2.79	0.92	16.43	0.67	217.07
Wood Chips	2007	35.2	3.21	0.91	19.46	0.56	328.69
	2013	24.4	2.74	0.91	13.43	0.61	186.81

[a]R is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; and Fo is functional organization. Sample sizes listed in Appendix A, Table 4.
There are no differences in means or treatment effects on indices; table is for reference only.

4. Soil Microbial and Nutrient Responses to Seven Years of Ground Cover Management in an Organic Apple Orchard

Keywords: Ground covers, long-term, nutrient cycling, microorganisms

4.1. Abstract

Organically managed orchards require sources of nutrition acquired from alternatives, typically organic fertilizers or ground covers, to synthetic, inorganic fertilizers used in conventionally managed systems. Through the addition and subsequent decomposition of organic matter, microbial activity is stimulated. The goal of organic management is to facilitate efficient internal terrestrial N cycling without promotion of N losses, especially as those can result in atmospheric and aquatic pollution. The objective of this study was to determine the effects of seven years of annual additions of four ground covers (compost, wood chips, paper mulch, and mow-and-blow) and three organic fertilizers (poultry litter, organic commercial fertilizer, and a no-fertilizer control) in a total of twelve treatment combinations on nutrient cycling potential in an organic apple orchard soil established in 2006. Organic matter, microbial biomass, enzyme activity, dissolved organic carbon and nitrogen, and nitrate concentrations were measured in March and May annually from 2007-2013 at the 0-10 cm soil depth to evaluate the impacts of treatment combinations on decomposition and microbial activity. Organic matter content increased in all treatment combinations, 1.83% to 3.75% in the 0-10 cm depth, from 2007 to 2013. Dissolved organic carbon concentrations increased gradually over time across fertilizers in the wood chip and paper ground covers, dramatically increased in the compost ground cover from an average of 76 μg C g^{-1} in 2007 to highs of 287, 355, and 399 μg C g^{-1} (commercial, poultry litter, control, respectively) in 2010. Microbial biomass nitrogen was variable over time, with concentrations across treatments showing a trend of increasing from 2007-2011 during the transition and early production years, but then decreasing thereafter such

that concentrations in 2013 were not significantly different from 2007. There was an elevated response early with mow-and-blow additions in both dehydrogenase and β-glucosaminidase activities. The trends over time in dissolved nutrients and microbial biomass suggests that the community is not growing continually over time, although it could be shifting in composition and diversity.

4.2. Introduction

As public concern over environmental and health impacts of synthetic fertilizer and pesticide use in crop production grows, organic fruit production is increasing. Sales of organic crops increased 69% from 2008-2014, with apples topping the list of sales of organic fruit with $250 million in sales in 2014 in the United States (NASS, 2015). Large numbers of organic farms per capita are concentrated in the northeast, north central and western U.S. (NASS, 2015). Regional climate in these areas is well suited for fruit production and also for organic management. Consequently, the high density of apple orchards and research programs in place in the northeast and northwest are the major source of organic management data collected in the US to date. In the southeast, naturally occurring soil organic matter content is generally low in Ultisols, the predominant soil order of the region (Eswaran et al., 2002). In addition, humid and warm temperatures promote insect, disease, and weed growth (Harvell et al., 2002). Arkansas is one of several states in the southeast region that has less than 50 organic farms (NASS, 2015a), and research investigating organic management strategies for apple and other fruit tree production in this area has been limited. However, consumer demand for locally produced food has also been increasing, the National Agricultural Statistics Service (2015b) reported nationwide increases in the number of farmers' markets (180%), regional food hubs (288%), and farm-to-table programs at schools (430%) since 2006, indicating a growing need for locally appropriate research to meet challenges. The apple orchard used in the present study, was established in 2006 in Fayetteville, AR.

One commonly implemented management strategy in organic orchards is the application of ground covers or organic amendments as a mulch. Benefits have included increased organic matter (OM), cation exchange capacity (CEC), soil microbial activity, and reduced nitrate

concentrations (Marriot and Wander, 2006; Hansen et al., 2001; Yao et al., 2005). Selection of ground covers is dependent upon orchard management goals, and also on regional availability. The ground covers used in this study were obtained locally (compost, shredded paper, wood chips, and a mow-and-blow control) and applied in combination with organic fertilizers (composted poultry litter, commercial fertilizer, and no-fertilizer control) to supply necessary nutrients, such as nitrogen, to the crop. In order for the nutrients in the ground covers and fertilizers to be released and become available for uptake by the trees, they must first be decomposed.

Soil microorganisms perform key ecosystem services that are necessary for healthy soil functioning, including litter decomposition and nutrient cycling. It is imperative to understand how and to what extent additions affect the microbial community and soil nutrient content throughout the life of the orchard, so nutrient management strategies can be implemented to avoid atmospheric or aquatic pollution by limiting nitrogen loss through greenhouse gases or leaching. This is a challenge of organic management but there are also many benefits of organic management that have been reported. Evidence supporting organic or integrated management over conventional management is widespread and includes different cropping systems and a range of soil amendments in various soil types. Kramer et al. (2006) supplied synthetic fertilizers in conventional treatments, alfalfa or compost in organic treatments, and integrated combinations to an apple orchard in Yakima, Washington, and observed organic systems decreased nitrate pollution, increased denitrification potential, rate, and efficiency while increasing the OM and microbial biomass and activity when compared to conventional treatments. Goyal et al. (1999) also reported increases in soil C and N and microbial biomass in integrated systems (combinations of inorganic fertilizer and wheat straw, farmyard manure, or green manure)

compared to synthetic fertilizer only in a pearl millet/wheat rotation on a sandy loam soil. Baldi et al. (2010) reported that compost applied at 10 t ha $^{-1}$ yr $^{-1}$ to a silty loam in an Italian peach orchard positively affected fertility by increasing soil organic matter and supporting fruit production and concluded this rate could effectively replace mineral fertilizers but noted nitrate levels could be a concern with large amounts of organic inputs.

Positive effects reported with ground cover and fertilizer addition and specific rates of application may change as soil type or organic amendment source are changed. Regionally available materials need to be tested to confirm expected treatment responses because composition of amendments can vary greatly depending on nutrient content of initial substrate and for some materials, manufacturing process, such as composting. For example, Canali et al. (2004) observed the effects of two compost additions, poultry manure and mineral fertilizer, in an Italian citrus orchard soil, and after 6 years of annual additions, the only significant treatment differences out of twenty measured properties were greater potentially mineralizable C and N pools and a higher basal respiration rate in the compost treatments. Hoagland et al. (2008) reported a lack of N supply from wood chips compared to legume based ground covers. Wood chips addition caused short-term N immobilization, and retained soil moisture which increased tree growth but also encouraged N loss through denitrification. Although there have been studies examining effects of organic amendments on soil biological and chemical properties in other climates, none have measured immediate and long-term effects of annual organic ground cover and fertilizer interactions in the southeast region of the U.S.

The objective of this study was to determine how seven years of annual ground cover (compost, shredded paper, wood chips, and mow-and-blow as an informal control) and fertilizer (poultry litter, commercial organic, and a no-fertilizer control) treatment combinations have

affected soil carbon and nitrogen pools and microbial biomass and activity over time in the surface soil. Soil biological and chemical property measurements were analyzed before (March) and after (May) yearly ground cover applications (April) to determine how nutrient contents and microbial populations responded to additions immediately (May) and long-term (March) and if responses were the same each year or changed throughout the life of the orchard. It was hypothesized that treatments which add more OM will have larger and more active soil communities in response to substrate availability and treatments with low C: N ratios will result in more mineralization and greater amounts of inorganic N.

4.3. Materials and Methods

4.3.1. Experimental Design

Enterprise apple trees (*Malus domestica* Borkh.) with M26 rootstock were planted on a pH and organic matter adjusted 0.30-ha plot at the University of Arkansas Agriculture Experiment and Extension Center in Fayetteville, Arkansas (36°N, 94°W) that was tilled, limed, and leveled in 2005 in preparation for organic orchard management which began in 2006 (Table 1). The orchard is located on two silt loam soils, a Captina (Fine-silty, siliceous, active, mesic Typic Fragiudult) and a Pickwick (Fine-silty, mixed, semiactive, thermic Typic Hapludult) (NRCS, 2014). The orchard was managed following the National Organic Program Standards (AMS, 2012). Treatments were applied every April to the surrounding tree area (2 x 2 m^2) and two guard trees. Twelve treatments included ground covers of compost (C), white shredded paper (P), wood chips (W), or a mow-and-blow control (M) applied at a depth of 7.5-12 cm in combination with, a no fertilizer control, locally available poultry litter, or a commercial pelletized fertilizer, a pelletized poultry manure (Perdue AgriRecycle, Seaford, DE) was used

through 2011 and was discontinued and replaced with an alfalfa (*Medicago sativa*) based commercial organic product (Bradfield Organics Feed Solutions, St. Louis, MO) in 2012. Fertilizers were applied as a nutrient source at a rate of 50 g of N per tree per year of tree age to a maximum of 450 g N per tree, or without an added fertilizer in a no-fertilizer control. From 2006-2011, the compost substrate was vegetative waste (i.e., grass clippings, wood pruning, and yard waste), composted 90 to 120 d, obtained from the City of Fayetteville, AR, and changed in 2012 to compost from PC Turnkey in Springdale, AR, composted using an active-pile process. Wood chips were sourced from the City of Fayetteville, AR and consisted of mainly hardwood species. Shredded paper was acquired from the University of Arkansas, Fayetteville, AR. The mow-and-blow treatments consisted of tall fescue (*Festuca arundinacea* Schreb. 'KY 31') planted between rows and other naturally occurring native, herbaceous species. Each ground cover treatment was applied to the surrounding tree area (2 x 2 m^2) and two guard trees every April at a depth of 7.5-12 cm. Average carbon (C), nitrogen (N), phosphorus (P), and potassium (K) concentrations for ground covers and fertilizers are shown in Table 2. Ground cover and fertilizer average nutrient (carbon, nitrogen, phosphorus and potassium) concentrations are shown in Table 2. Compost was not applied in 2010 and the orchard was tilled within rows in July 2008, samples in 2009 and 2010 were taken from outside the tillage area as described below to avoid any potential effects of tillage on measured soil properties. All details describing the orchard preparation and management and the sources and characterization of the ground cover and fertilizers are described in Choi et al. (2011a), Choi et al. (2011b), Mays et al. (2014), and Mays et al. (2015). Weather data were collected at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, AR and the means reported in Table 3 were

obtained from a report generated from National Oceanic and Atmospheric Association (NOAA) data (2015).

4.3.2. Soil Collection and Characterization

Samples were collected using a sterilized soil probe from the 0 - 10 cm soil depth in March and May (before and after annual April treatment applications) from 2007 to 2013 and stored in sterile bags. Composite samples were obtained by randomly collecting cores with a sterilized probe in an area 15 cm or more from the trunk of the tree, 60 cm between trees in a row and 45 cm between rows. Soil temperature at 10 cm depth was measured at each tree. Soils were immediately stored at 4 $^{\circ}$C until sieved through a sterilized 2-mm sieve, and returned to storage, moist at 4 $^{\circ}$C.

Gravimetric soil water content was determined by measuring soil (10 g) weight change after drying at 105 $^{\circ}$C for at least 24 hrs, and used to express soil properties as per gram of oven-dry soil. Soil pH and electric conductivity (EC) were measured with a potentiometer using 1:2 soil-to-water (wt:vol) ratio. Organic matter content was determined using loss on ignition (6 hr at 550 $^{\circ}$C).

4.3.3. Extractable C and N

Dissolved organic carbon (DOC), dissolved total nitrogen (DTN), nitrate (NO_3^--N), and ammonium (NH_4^+-N) were measured from moist (unfumigated) soil (8.0 g) using the single-extraction approach described by Jones and Willet (2006). Extraction of C and N from moist soil was completed by adding 1:5 (wt:vol) 0.5 *M* K_2SO_4 , shaking on a reciprocating shaker for 30 minutes, and filtering through Whatman #42 filters. The DOC and DTN concentrations per g dry

soil were calculated after measuring C and N in extracts using a Shimadzu TOC-V PC-controlled total organic carbon and attached total N analyzer (Shimadzu, Columbia, MD). The chloroform-fumigation extraction method (Vance et al., 1987) was used to determine microbial biomass C and N. Soils were fumigated for 24 hrs with ethanol-free chloroform and were extracted to measure DOC and DTN as previously described. Microbial biomass C and N were calculated as the difference in DOC and DTN concentrations between fumigated and unfumigated samples.

Soil ammonium-N (NH_4^+-N) and nitrate-N (NO^-_3-N) concentrations were measured using a Skalar segmented-flow autoanalyzer (Skalar Inc, Norcross, GA). Ammonium concentrations were measured using a salicylate hypochlorite procedure and nitrate was measured using the Greiss-Illosvay procedure, utilizing Cd/Cu reduction of NO_3^- to NO_2^- (Mulvaney, 1996). Dissolved organic nitrogen (DON) was calculated by subtracting inorganic N (sum of NO_3^--N and NH_4^+-N concentrations) from dissolved total nitrogen concentrations (DTN) (Jones and Willet, 2006).

4.3.4. Potentially Mineralizable N

Potentially mineralizable nitrogen (PMN) was determined by incubating soil (8 g) in anaerobic conditions for 7 days at 40 $^\circ$C (Bundy and Meisinger, 1994). Ammonium concentrations after the 7-day incubation were measured as described above, but using 1M K_2SO_4 (20 mL) to displace N on exchange sites. Ammonium concentrations of unfumigated microbial biomass extracts served as initial (day 0) concentrations and were subtracted from post incubation (day 7) concentrations to calculate PMN.

4.3.5. Enzyme Activities - Dehydrogenase, β-Glucosaminidase

Dehydrogenase enzyme activities (μg triphenyl formazan (TPF) g^{-1} soil for 24 hr^{-1}) were determined using colorimetric analysis (Casida et al., 1964, Tabatabai, 1994). Moist soil (3 g), calcium carbonate (30 mg $CaCO_3$), 3% 2, 3, 5, - triphenyltetrazolium chloride (0.5 mL) solution and Milli-Q water (1.25 mL) were incubated for 24 hrs at 37 °C. Soil was washed quantitatively with methanol (50 mL total) and filtered after incubation, and absorbance of the filtrate was measured by spectrophotometry at 485 nm.

β-Glucosaminidase (NAGase) enzyme activities were determined colorimetrically using the method described by Parham and Deng (2000), and measured with a spectrophotometer (Cary 50, Varian, Inc., Palo Alto, CA) at 405 nm. Moist soil subsamples (1 g) were incubated with (4 mL) acetate buffer (pH 5.5) and *p*-nitrophenyl-N-actetyl-B-D glucosaminidase (10 m*M* pNNAG) substrate at 37 °C for 1 hr. Reactions were stopped with sodium hydroxide (4 mL of 0.5 *M* NaOH) and calcium chloride (0.5 *M* $CaCl_2$), and filtered through Whatman #40 filter paper.

4.3.6. Statistical Analyses

The design was a split-split plot. The whole plot was 4 x 3 randomized complete block (ground cover x fertilizer), and the split plot was year (2007-2013) and the split-split plot was month (March and May). Ground cover, fertilizer, year and month effects on measured soil parameters were evaluated by analysis of variance (ANOVA) using SAS (version 9.4, SAS Institute Inc., Cary, NC). Least significant differences (LSD) were used to separate means at $\alpha = 0.05$, with up to three LSD's used when significant interactions involved all possible factor levels. Only the highest order interaction that was significant is depicted graphically. To narrow

the results to the scope of the current study, only ground cover main effects or significant interactions involving ground cover are presented. A full set of significant interactions is presented in Appendix A, Tables 1 and 2.

4.4. Results

4.4.1. Soil Characteristics

There was a significant ground cover by fertilizer by year by month effect on soil temperature ($P < 0.0001$, Table 4). As expected, soil temperature fluctuated most as a result of sampling time and May was always higher than March (Figure 1). Few differences stand out between ground cover and fertilizer treatments except for March 2010 and March 2012. Soil temperature data are missing for March 2008, which accentuates differences in temperature with ground cover application from May 2007 to May 2008, where compost and wood chip were similar and paper and mow-and-blow were similar (Figure 1).

Water content was significantly affected by ground cover and year interaction ($P < 0.0001$, Table 4). In 2007, water content was lower than any other year regardless of ground cover application (Figure 2). All treatments had similar effects on water content over time, increasing yearly until 2010 or 2011 (mow-and-blow) and then decreasing and becoming stable in following years. Water content in mow-and-blow control treatment soil was less than other treatments until 2011, while soils with compost addition had greater water content than others until 2011. After 2011, there were no significant treatment differences in soil water content.

4.4.2. Nutrient supply and change in nutrient status over time

Ground cover and fertilizer interaction effects on organic matter content differ significantly with month and between years ($P < 0.0001$, Table 4). Organic matter content

increased in all treatment combinations, 1.83% to 3.75%, from 2007 to 2013 (Figure 3). The trend over time in paper, wood chips and the mow-and-blow control was a gradual and steady increase regardless of fertilizer, and final organic matter amounts were similar in all treatment combinations (although mow-and-blow with no fertilizer and paper with commercial fertilizer were significantly different). Compost addition had more variable and dramatic effects on organic matter content. There was a rapid increase from May 2008 to March 2010, followed by a relatively stable period and OM was continually greater compared to other treatments. Fertilizer type did not vary the organic matter content at most times in the compost treatment; however, there was a negative effect of poultry litter addition with compost compared to the other two nutrient sources measured in 2012 and 2013.

There was a significant effect of ground cover and fertilizer interaction through the years on DOC concentrations (Table 4, $P = 0.043$). Paper, wood chips, and mow-and-blow additions did not alter DOC concentrations greatly over years, although concentrations were highest across the three ground covers in 2011 (Figure 4). Fertilizer significantly increased DOC in these three ground covers, but only in some years. Dissolved organic carbon increased with poultry litter addition compared to the control in mow-and-blow in 2009 and 2013, in paper in 2008, 2009 and 2013, and in wood chips in 2013. The DOC was increased by poultry litter more often than from the commercial fertilizer, which increased DOC in paper in 2008, and wood chips in 2013. The only difference in DOC response between the poultry litter and commercial fertilizer in these ground covers was more DOC with poultry litter in the mow-and-blow treatment in 2009.
In contrast, DOC response to compost application was lower with poultry litter in 2009 and 2012, but more frequently with commercial fertilizer (2009-2011, and 2013) compared to without fertilizer. Regardless of fertilizer, DOC soil concentrations under the compost ground

cover increased rapidly until 2010 to concentrations double that of soil receiving other ground covers. The DOC concentrations then decreased through the remaining years but were consistently higher compared to other ground cover treatments.

Dissolved organic nitrogen (DON) concentration was significantly affected by ground cover, year and month (Table 4, $P = 0.0131$). Trends were similar in paper, wood chips and mow-and-blow over time (Figure 5). Concentrations were similar initially in these treatments, steadily increased in 2009 and 2010 then steadily decreased in 2012 and 2013 with only slight differences between adjacent measurements. Paper treatments had more DON than mow-and-blow treatments in March 2009, 2010, 2011 and May 2010, 2011, 2013, and wood chip treatments were greater than mow-and-blow in March 2010 and May 2011. Paper treatments had more DON than wood chip treatments in May 2008, 2009, 2010, and 2013, but DON concentrations were never different in March. The least amount of DON was measured in 2013; in March concentrations were less than 2 $\mu g\ N\ g^{-1}$, and in May there was close to 0 $\mu g\ N\ g^{-1}$ with wood chip addition. Compost addition increased DON at a faster rate and to a larger magnitude than other ground cover additions. Although DON in compost was similar to initial (2007) and final (2013) concentrations of some other ground covers, DON was greater than in soil from the other ground covers from 2008-2012.

There was a significant ground cover by fertilizer by year by month effect on potentially mineralizable nitrogen (PMN) concentrations ($P < 0.0001$, Table 4). Treatment effects on PMN were highly variable across time, with few trends spanning treatment combinations (Figure 6). However, PMN was not measured until 2009, potentially affecting the observed long-term trends. On average, there was less PMN in compost and wood chip treatments than paper and mow-and-blow treatments. The response of PMN to compost addition was mostly stable across

fertilizers through time, decreasing in 2012 and 2013 to less than initial (2007) concentrations. Fertilizer addition did not change PMN in soil receiving wood chips, except in May 2011, when PMN was markedly higher with wood chip addition in the no-fertilizer control treatment. In 2013 there was more PMN in fertilized soils receiving wood chips compared to the no-fertilizer control-wood chip treatment combination. Potentially mineralizable nitrogen in the paper treatments did not vary much over time besides an increasing trend until 2012. Of all the ground covers, the fertilizer effect was most apparent in paper treatments, where the positive effect of fertilizer was not always significant (poultry litter > no fertilizer in March 2009, 2011, 2013 and May 2010, 2012; commercial fertilizer > no fertilizer in March 2009 and May 2010) but the trend was consistent over time. The response in PMN to the mow-and-blow control ground cover was variable with fertilizer and where there were short-term differences between adjacent sampling dates. Potentially mineralizable nitrogen concentrations spiked with poultry litter addition in the mow-and blow treatment every year in May except in 2012; whereas high PMN concentrations in commercial fertilizer treatment were also measured May but were less frequent (not 2010 or 2011). In contrast, mow-and-blow with the no-fertilizer control had almost no effect on PMN concentrations over time.

There was a significant ground cover by year by month effect on ammonium-N concentrations ($P = 0.0157$, Table 4). Ammonium-N concentrations fluctuated month to month within years and were different depending on year (Figure 7). May concentrations were usually greater than March but there were exceptions in 2008 and 2012. High NH_4^+-N concentrations were measured in May 2009 and 2010 in all treatments, except with compost addition which had less effect on concentrations at these and other time points. The response in early years in NH_4^+-N to mow-and-blow was different than other ground covers. By May 2007 concentrations were

higher than other treatments and NH_4^+-N was continuously elevated with no drastic decrease until March 2010. Ammonium concentrations did not differ in mow-and-blow from other treatments past this date.

There was a significant ground cover by fertilizer by year effect on soil nitrate concentrations (P = 0.0422, Table 4). Nitrate concentrations were low and similar in 2007 and similar across treatments (except wood chips with poultry litter) but responded differently through time depending on ground cover and fertilizer interaction (Figure 8). There are two prominent trends across ground covers: 1) increasing NO_3^--N over time peaking in 2013, and 2) poultry litter addition resulting in significantly more NO_3^--N than commercial fertilizer or the no-fertilizer control. In all compost treatments, NO_3^--N concentrations spiked in 2009. There was a similar response of the same magnitude measured in NO_3^--N concentration in the mow-and-blow control treatment with poultry litter addition. Effects on NO_3^--N concentrations from other ground cover and fertilizer treatments were not as marked until 2013. After 2008, there was a steady but slow increase in NO_3^--N in all paper, wood chips and the mow-and-blow treatments; however, poultry litter addition resulted in a greater increase in NO_3^--N concentrations in some years. The paper and poultry litter combination tended to have a greater NO^-_3-N concentration than in the commercial fertilizer or control with paper ground cover, but concentration was not significantly greater until 2012, and in 2013 the greatest amount of NO_3^--N measured during the duration of the study was in this treatment.

4.4.3. Microbial community size and activity

There was a significant ground cover by fertilizer by year by month interaction effect on microbial biomass carbon (Bio C) and nitrogen concentrations (Table 4) which varied differently

from March to May depending on the year (Figure 9). Generally, Bio C in compost, paper and wood chip treatment combinations increased initially (2007-2008), temporarily decreased in March 2009, and then continued to increase, peaking between 2010 and 2011, before finally decreasing to concentrations similar to 2007. In all compost treatments, there was a large spike in Bio C concentrations in March 2010, (near 200 μg C g^{-1} of dry soil), at least double and up to quadruple flanking measurements. There was a spike of a smaller magnitude in the wood chip treatments in March 2010, followed by another in 2011, with eventual decline through the final years to concentrations similar to those measured in 2007. Overall, trends in Bio C in wood chips and paper treatments were similar, but concentrations in the paper treatments were of a greater magnitude and the decrease in concentrations in May 2010 was not as drastic, creating an extended peak from 2010 to 2011 instead of two distinct peaks in biomass concentration. Microbial biomass C in the mow-and-blow treatments was very consistent in 2007 and 2008, and similar to other treatments exhibiting a temporary decrease in March 2009 before a slow and steady increase from 2009 to 2011. Unlike other treatments, in mow-and-blow the no-fertilizer control never spiked, but changed gradually through time, although poultry litter (2011 and 2012) and commercial fertilizer (2012 and 2013) did result in marked increases in Bio C in May samplings.

Microbial biomass nitrogen (Bio N) concentrations were very responsive to treatments with short and long-term fluctuations (Figure 10). Overall, initial concentrations were similar across treatments and then increased to varying magnitudes and for different durations before decreasing in 2013 to concentrations similar to those measured in 2007. There was a trend of steady increase in Bio N with compost addition from May 2007 until March 2010 (except commercial fertilizer in May 2009), concentrations then remained fairly stable until decreasing

in 2013. Microbial biomass N with wood chip addition fluctuated within years, peaking in March from 2008 to 2011. Concentrations were similar across fertilizers in the wood chip ground cover except that poultry litter resulted in higher Bio N in 2009 and 2010 compared to the other two fertilizers. The most dramatic response in Bio N was with the addition of paper; Bio N increased from 2007 to 2010, and included the greatest concentration of Bio N measured during the study in the poultry litter treatment combination. However, concentrations tended to decrease in 2012 and 2013.

There was a significant ground cover by year by month effect on dehydrogenase activities (P = 0.0383, Table 4). Dehydrogenase activities varied month to month and through years but was similar across ground covers in 2007 (Figure 11). Early on (2007-2008) more activity was measured in March than in May, while in following years May was usually more active but activities varied with ground cover. There were higher activities in mow-and-blow in 2007-2008 compared to other ground covers, and activities peaked in March 2008. From 2009 through 2012, dehydrogenase activities were steady in mow-and-blow. Compost, paper and wood chip additions increased activities as well, but effects were highly variable through time and activities did not peak until May 2010. There was a large difference in activities in response to wood chip treatments, increasing from among the lowest to the highest dehydrogenase activities measured from May 2009 to May 2010. Dehydrogenase activities were similar in all treatments in March 2013 and lower than any other time point.

There was also significant ground cover by year by month effect on β-Glucosaminidase activities (NAGase) (P=0.0003, Table 4). β-Glucosaminidase activities also fluctuated over the duration of the study; however, March to May differences within year did not vary greatly (Figure 12). There was an elevated response in the early years of the study with mow-and-blow

additions until 2010, and again in May 2012 and May 2013, which was the greatest amount of NAGase activity measured throughout the study. In 2011, NAGase activities were high in mow-and-blow, paper, and wood chips. Compost addition did not produce large changes in NAGase activities through time and was consistently lower than other ground covers through time.

4.5. Discussion

Effects of organic ground covers and fertilizers to soil nutrients and microbial processes can be linked to environmental conditions (temperature, moisture and pH) and the quality of the substrate. Microorganisms have a low C:N ratio (5:1-15:1) and will immobilize N when decomposing organic materials with limited N contents; therefore, lower C:N residues (< 20:1) are considered higher quality resources. In addition, decomposition rate varies with the litter composition; sugars, proteins and starches are easily decomposed, while hemi-cellulose, cellulose and lignin are increasingly difficult to decompose (Tisdall and Oades, 1982). Ground covers may positively affect soil physical properties. Increased water holding capacity (Neilsen et al., 2003) is one frequently reported as benefit of mulch. Granatstein and Mullinix (2008) compared a variety of inert and living mulches with the goals of weed control, increasing water retention and providing N to organic fruit trees in the Pacific Northwest. Wood chips, shredded paper, and clover provided the best weed control and mulched plots retained 15-20% more moisture than unmulched plots. Alfalfa hay, wood chips, and paper contributed to the highest infiltration rates. In this study, all ground covers increased moisture likely because of protection of the soil surface and increasing the soil organic matter and ground covers also affected soil

temperature but ground cover and fertilizer effects had impacts beyond moisture and temperature. In the current study, focus was on effects of treatments on microbial responses and changes in nutrient availability.

Soil organic matter increase was expected with annual additions of ground covers and fertilizers (Yao et al., 2005; Kramer et al., 2006); however, rate of increase and total accumulation in the compost treatment exceeded expectations. Previous studies have shown that significant increases in OM can occur with short-term compost applications. Soil OM increases of 75%, 145%, and 185% relative to a control were reported by Cayuela et al. (2004) following single applications of compost with various initial ratios of olive mill waste to sheep manure that were made to a loam soil with an initial 1.8% OM. Yearly application of three municipal biosolids and two animal biowastes and peat moss significantly increased soil OM content up to 2% after four years, and all treatments had more OM than the control by the second year of application to a loamy sand (Zebarth et al., 1999). Baldi et al. 2010 reported OM increases of 169% after 9 years with the highest rate of compost applied, 10 t ha $^{-1}$ yr $^{-1}$, in a peach orchard. In contrast, smaller increases in soil C and N were reported by Neilsen et al. (2003), after applying two biosolids, shredded paper, an alfalfa mulch, and black plastic mulch for seven years to an apple orchard soil, the most total C and N was in a biosolid treatment (19 g C kg $^{-1}$, 1.8 g N kg $^{-1}$) compared to the control (10 g C kg $^{-1}$, 1.0 g N kg $^{-1}$). Increases in OM with compost addition in the present study were over 6% after four years of applications (2006-2009). A horticulture decision to not apply compost in 2010 was made to protect the trees in that treatment, but was the only skipped treatment application in the study. Organic matter in compost treatments decreased from 2010 and was more comparable to the other ground covers. Ground cover and nutrient source application rates were not investigated as part of this study and should be

investigated in the future for optimization in organically managed, perennial systems in southeastern U.S. Organic matter content increased similarly in other treatments, which is surprising considering that the average nutrient content (N, P, K) and C:N ratio of the three materials was very different.

Fertilizer addition rarely affected the response in organic matter content, even in treatments receiving paper which had the highest C:N ratio. This suggests that the C:N ratio is not the only factor controlling decomposition rates. In addition to the C:N ratio, the substrate quality of the materials affects nutrient cycling (Bengtsson et al., 2003; Mungai and Motavalli, 2005). The lignin content of compost will vary with initial substrate, but a compost of similar initial substrates was 39% (Francou et al., 2008). The lignin content of the other three ground covers was likely lower which could explain in part why these ground covers accumulated less organic matter (Francou et al., 2008). Wood chips contain approximately 28% lignin (Holland et al., 1990). Lignin content of tall fescue has been found to be less than 12% (Chen et al. 2002), and office paper lignin content is 6.5% or less (Komilis and Ham, 2003).

Dissolved organic carbon and nitrogen concentrations, including the temporal trends in concentrations, appear to be strongly influenced by the soil organic matter content. Dissolved organic carbon and nitrogen are the soluble fraction of organic matter that is physically available for use by microorganisms containing both labile and recalcitrant fractions but may not directly be related to mineralization rates (Cook and Allan, 1992; Aiken and Costaris, 1995; Chantigny, 2002). Gonet and Debska (2006) reported DOC and DON concentrations that were still elevated above the control 10 years after varying rates of cattle slurry were applied to soil. Moisture and temperature are also positively correlated with DOC production (Christ and David, 1996), decomposition and mineralization of soil organic matter (Kirschbaum, 1995; Leiros et al., 1999).

Chow et al. (2006) reported DOC production ranging from 150 μg g^{-1} to 400 μg g^{-1} during a 60-day incubation in a California delta peat soil at water contents of 0.3 g g^{-1}, and reported DOC in the same soil was more than double those concentrations when the soil water content was increased. Interestingly, in this study, the trend in soil water content over time in the ground cover treatments is almost identical to the trend observed in DOC with compost additions. Dissolved organic nitrogen concentrations in the compost treatments follow the same overall trend as DOC and OM except initial and final DON concentrations are the same across treatments.

Potentially mineralizable nitrogen is a measure of ammonium N that is easily decomposable and available for microorganisms to use. In contrast to other pools of nutrients measured in the study, PMN in compost treatments is lower than other treatments, which is interesting because amount of substrate or C:N ratio is not N-supplying in this case, suggesting that organic N present in compost treatments is tied up in complex molecules, such as lignin, as previously discussed. Generally PMN was highest in mow-and-blow treatments which has a low C:N, a low lignin content, and fertilizer addition increased the amount of easily decomposed compounds present. Burger and Jackson (2003) reported more PMN with organic management (legume cover crop, composted poultry litter, harvest residue) of a tomato-corn rotation than in conventional (harvest residue, fertilizer) tomato-wheat rotation. The greatest amount of PMN measured was ~ 40 μg N g^{-1}, measured in June, which is consistent with the range of PMN measured in the current study, with paper and wood chip additions (36 to 48 μg N g^{-1}) (Burger and Jackson, 2003).

Ammonium concentrations in this study fluctuated greatly. In comparison, concentrations were greater than soil from either conventional or organic cropping systems at any of the five

sample dates (< 2 μg NH_4^+ g^{-1}) measured by Burger and Jackson (2003). Continually elevated NH_4^+-N in mow-and-blow compared to other ground covers from 2007-2009 supports the idea that mineralization was greatest in mow-and-blow compared to other ground covers early in the life of the organic apple orchard because easily broken down substrates stimulated microbial activity. Overall, the trend in NH_4^+-N is similar across ground covers, although at times NH_4^+-N concentrations differ in magnitude and were different from trends in other measured variables, suggesting that environmental factors influence both the potential availability and the production of NH_4^+-N.

Overall, NO_3^--N was low with a few exceptions in 2009 and 2013. Less NO_3^--N has been reported with organic amendments vs. integrated or conventional fertilization in a Macadamia nut orchard in Honolulu, Hawaii (Bittenbender et al., 1998) and apple orchard in Yakima, Washington (Kramer et al., 2006). In 2013, NO_3^--N was very high across ground covers with fertilizer application, possibly a result of increased N mineralization rates stimulated by labile N from dead microbial biomass after a very cold winter (Table 3; Figure 1 and 8) (DeLuca et al., 1992; Herrmann and Witter, 2002). High NO_3^--N in 2013 corresponds with low NH_4^+-N in May 2013, which is not surprising because nitrification increases rapidly with temperature (Morrill and Dawson, 1967; Avrahami et al., 2003). Evanylo et al. (2008) compared an uncomposted poultry litter, compost, fertilizer and a control and reported the greatest concentration of nitrate (5.49 mg L^{-1}) in runoff during a rainfall simulation with the addition of an uncomposted poultry litter manure. Similarly, in the current study, the poultry litter fertilizer resulted in the greatest response in NO_3^- in all ground covers. Although the C:N of poultry litter is higher than the commercial fertilizer and they were applied at the same rate of N, the data suggest ammonium in poultry litter is readily nitrified (Preush et al., 2002).

Some of the most unexpected results of the study were the microbial biomass responses to treatments, including few changes in initial and final concentrations, paper additions having the greatest response and more microbial biomass measured in March rather than May in some years. A larger, more active microbial community with organic amendments is one of the most commonly published benefits of organic management. Farmyard manure, green manure (legume cover crop), and wheat straw applied with fertilizer had positive effects on microbial biomass C ranging from 273 mg C kg^{-1} to 423 mg C kg^{-1} in a pearl millet-wheat rotation on a sandy loam in a tropical climate (Goyal et al., 1999). In comparison, the most microbial biomass C in the current study was 214 mg C kg^{-1}, measured in March 2011, with paper and poultry litter addition. Paper had the largest positive impact on Bio C concentrations, and was increased by fertilizer. Residue quality may be controlling the fluctuations in microbial biomass, as paper with the lowest lignin content treatment had the greatest effect on biomass, followed by the two ground covers with intermediate lignin content, wood chips and mow-and-blow.

Interestingly, large responses in Bio C were measured in March 2010, a peak in Bio C concentrations in compost treatments and more Bio C were also measured in paper and wood chip treatments at this date, which corresponded to one of the coldest March temperatures. While similar temperatures were measured in March 2013, microbial biomass C was very low across treatments, which is more consistent with expectations of microbial biomass and processes correlating with temperatures (Myers, 1975; Hassan et al., 2015). One possible explanation for the difference in microbial biomass concentrations between years may be attributed to differences in timing in response to cold temperatures. DeLuca et al. (1992) and Herrmann and Witter (2002) both reported increased mineralization rates in response to freeze thaw cycles resulting from labile nutrients contained within dead microbial biomass. In

March 2010, the high biomass could be a result of growth from microorganisms using the labile nutrients from the dead biomass while in 2013, biomass may have died and the labile nutrients are not being taken up by the new biomass yet.

Microbial biomass nitrogen responded to treatments similarly as microbial biomass C. One interesting response that stands out was very low microbial biomass N measured in May 2009 in compost with commercial fertilizer addition, which corresponds to very low PMN and very high nitrate in this treatment in 2009. Nitrate-N was also high in 2009 in the other fertilizer treatments receiving compost and while microbial biomass N and PMN, were different, nitrification is apparently high across fertilizers. Nitrifiers are a physiologically specialized group (Head et al., 1993) that are present in low amounts compared to the total microbial population (Hermansson and Lindgren, 2001) and differences in quantities, or activities regardless of cell numbers, of nitrifiers may not be reflected in the total microbial biomass.

Dehydrogenase activities are an indicator of overall aerobic activity and are variable across ground covers through time. Tejada et al. (2006) concluded compost and poultry litter treatments increased dehydrogenase activity over time, but more so in poultry litter, because higher amounts of labile carbon in these treatments stimulated activity. In this study, DHase, NAGase activities, and NH_4^+-N concentrations were initially greatest in the mow-and-blow suggesting decomposition and mineralization of N of labile compounds was initially greatest in the treatment not receiving an added ground cover layer, only clippings of plant materials growing within that system. Soil receiving the other ground covers eventually surpassed mow-and-blow in DHase activities and peaked in 2010; however, NAGase activities were never greater than in the mow-and-blow treatments, but paper and wood chips were similar in 2011. Goyal et al. (1999) reported the wheat straw application promoted more dehydrogenase activities

and microbial biomass, but rates of less than 67 μg triphenyl formazan (TPF) g^{-1} soil for 24 hr^{-1} were much less than those measured in the current study, which were typically over 100 μg TPF g^{-1} soil. Similarly, more NAGase and DHase activities were measured with the additions of straw and alfalfa compared to poultry manure or sewage sludge incorporated four times within a two-year period to a coarse loamy Alfisol in California (Martens et al., 1992).

4.6. Conclusion

Although compost addition had the largest impact on the soil organic matter content as well as DOC and nitrogen concentrations, microbial activities (DHase and NAGase) and Bio C and N were not stimulated to a greater extent compared to the other treatments through seven years of annual ground cover and nutrient applications. However, the ability of the community to nitrify was not negatively impacted by compost amendments, as greater or similar amounts of soil nitrate-N were measured in 2009 and 2013 without larger total microbial biomass. In the other ground cover treatments, greater amounts of PMN, NH_4^+-N, microbial biomass, DHase and NAGase activities throughout the study indicate that these residues are being cycled more readily than compost. Substrate quality of ground cover and nutrient combinations seem to impact soil microbial biomass, enzyme activities and resultant nutrient availability to a greater extent than the total C and N added or the C:N ratio.

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4.8. Tables

Table 1. Initial properties of an organically managed apple orchard silt loam soil, Fayetteville, AR, 2006 (n=36).

Year	pH	EC[a] (μmhos cm $^{-1}$)	Bulk Density (g cm$^{3\ -1}$)	OM (%)	N	C	P	K
					----------(mg kg $^{-1}$)----------			
2006	6.57	73.75	1.34	1.47	0.09	0.95	34	170

[a]EC is electrical conductivity; OM is organic matter; N is nitrogen; C is carbon; P is phosphorous; K is potassium

Table 2. Nutrient contents of ground cover and fertilizer treatments applied in an organically managed apple orchard, Fayetteville, AR (n=6).

Treatment	C[a]	N	C:N	P	K
	------ (%)------			------ (%)------	
Compost	20.5	1.6	13.5	0.2	0.5
Wood Chips	29.7	0.7	39.2	0.1	0.3
Paper	36.8	0.2	205.9	0.0	0.0
Mow-and-Blow	40.0	2.2	15.8	0.3	1.5
Commercial Fertilizer	31.3	4.4	7.8	1.4	2.6
Poultry Litter	29.5	1.7	19.4	1.3	1.4

Data for each ground cover and fertilizer are averaged across 2006-2011
[a]C is carbon; N is nitrogen; P is phosphorous; K is potassium

Table 3. Monthly mean air temperatures and precipitation measured at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, AR from 2007 to 2013 (NOAA, 2015).

	2007		2008		2009		2010		2011		2012		2013	
Month	T[a]	P	T	P	T	P	T	P	T	P	T	P	T	P
	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm
Jan.[b]	16.7	1.3	20.7	30.2	14.6	0.3	20.9	0.0	11.3	7.6	21.0	56.6	18.9	65.5
Feb.	20.5	0.5	21.9	123.4	25.2	38.1	11.9	6.1	26.8	63.2	25.2	48.8	23.2	55.1
Mar.	40.5	5.1	32.6	108.7	35.6	15.7	29.3	29.7	32.2	32.8	40.7	119.1	26.4	108.5
Apr.	38.1	50.5	37.6	153.2	38.3	27.7	44.4	22.4	40.5	267.5	45.3	53.1	38.0	144.5
May	48.8	47.0	48.2	129.0	45.7	38.1	49.8	65.5	44.6	138.7	53.3	45.7	46.8	266.7
June	56.3	48.0	55.1	61.5	58.7	60.7	60.1	3.0	61.9	16.5	59.9	64.5	57.6	24.9
July	59.0	15.0	60.3	114.3	59.6	31.8	61.7	240.3	68.0	9.1	65.3	36.1	59.7	63.8
Aug.	64.2	0.0	59.2	0.0	58.8	55.9	65.1	0.0	66.2	52.1	60.1	88.4	58.1	154.7
Sept.	53.6	22.1	49.8	158.0	48.8	78.5	56.5	215.6	48.4	94.7	54.3	73.7	54.9	101.9
Oct.	43.9	20.1	41.7	81.0	36.7	214.4	40.8	17.0	43.0	50.0	41.9	4.6	41.9	31.5
Nov.	36.3	4.3	32.9	19.1	33.6	11.2	36.5	7.9	32.6	151.4	31.7	22.6	27.7	64.3
Dec.	22.1	33.3	16.9	1.8	20.7	0.0	14.9	0.0	16.0	1.0	29.0	43.2	17.3	88.6

[a]T is Temperature; P is Precipitation; [b]Jan. is January; Feb. is February; Mar. is March; Apr.is April; Aug. is August; Sept is September; Oct. is October; Nov. is November; Dec. is December

Table 4. Analysis of variance (ANOVA) summary of the effects of ground cover and fertilizer treatments, month and year, and their interactions on soil properties at the 0-10 cm depth of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007-2013

Soil Property	GC[a]	GC*Fert	GC*Yr	GC*Fert*Yr	GC*Yr*Mo	GC*Fert*Yr*Mo
H_2O[b]	<0.0001*	0.1738	<0.0001*	0.6291	0.0882	0.4875
OM	<0.0001*	0.0459	<0.0001*	0.0022*	<0.0001*	<0.0001*
Temp	<0.0001*	0.0006*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
DOC	<0.0001*	<0.0001*	<0.0001*	0.0433*	0.2105	0.0515
DON	<0.0001*	<0.0001*	<0.0001*	0.1405	0.0131*	0.2956
NO_3^--N	0.0001*	0.6194	<0.0001*	0.0393*	0.0422*	0.0671
NH_4^+-N	<0.0001*	0.0602	0.0031*	0.4753	0.0157*	0.9559
PMN	<0.0001*	0.0133*	<0.0001*	0.0250*	<0.0001*	<0.0001*
BioC	0.0036*	0.0221*	<0.0001*	0.5372	<0.0001*	0.0294*
BioN	<0.0001*	0.0325*	<0.0001*	0.1340	<0.0001*	0.0003*
DHase	0.0002*	0.0160*	<0.0001*	0.1190	0.0383*	0.1704
NAGase	<0.0001*	0.2144	<0.0001*	0.2230	0.0003*	0.1885

[a]GC is ground cover and Fert is fertilizer source; [b]H_2O is water content; OM is organic matter; Temp is soil temperature; DOC is dissolved organic carbon; DON is dissolved organic nitrogen; NO_3^--N is nitrate-N; NH_4^+-N is ammonium-N; Bio C is microbial biomass carbon; Bio N is microbial biomass nitrogen; DHase is dehydrogenase activity; NAGase is β-glucosaminidase activity. Complete ANOVA found in Table 1, Appendix A

* $P < 0.05$

4.9. Figure Captions

Figure 1. Soil temperature (°C) measured at 10 cm in every ground cover and fertilizer treatment combination in March and May from 2007 to 2013, in an organically managed apple orchard in Fayetteville, AR. Soil temperature data for March 2008 are missing. Fertilizer treatments effects are depicted within each ground cover treatment graph, open circles are no fertilizer control, closed triangles are poultry litter, and closed squares are commercial fertilizer (n = 3). LSD's to compare means at $\alpha = 0.05$, Whole plot (1.33), Split plot (1.27), Split-split plot (1.16)

Figure 2. Soil water content (g H_2O g^{-1} of dry soil) affected by ground cover (compost, paper, mow-and-blow, and wood chips) and year averaged across all three fertilizers for both March and May samplings for each year from 2007 to 2013 in an organic apple orchard in Fayetteville, AR (n = 18). LSD's to compare means at $\alpha = 0.05$, Whole plot (0.021), Split plot (0.019)

Figure 3. Organic matter content (%) in each ground cover and fertilizer treatment combination measured in March and May from 2007 to 2013 in an organic apple orchard soil in Fayetteville, AR. Fertilizer treatments effects are depicted within each ground cover treatment graph, open circles are no fertilizer control, closed triangles are poultry litter, and closed squares are commercial fertilizer (n = 3). LSD's to compare means at $\alpha = 0.05$, Whole plot (1.22), Split plot (1.14), Split- split plot (1.07)

Figure 4. Dissolved organic carbon (DOC) concentrations (μg C g^{-1} of dry soil) as affected by ground cover and fertilizer treatment combinations from 2007 to 2013 in an organic apple orchard soil in Fayetteville, AR. Fertilizer treatments effects are depicted within each ground cover treatment graph, open circles are no fertilizer control, closed triangles are poultry litter, and closed squares are commercial fertilizer (n = 3). LSD's to compare means at $\alpha = 0.05$, Whole plot (48.32), Split plot (44.65)

Figure 5. Dissolved organic nitrogen (DON) concentrations (μg N g^{-1} of dry soil) affected by ground cover (compost, paper, mow-and-blow, and wood chips) averaged across all three fertilizers (no fertilizer control, poultry litter, and commercial) in March and May from 2007 to 2013 in an organic apple orchard in Fayetteville, AR (n = 9). LSD's to compare means at $\alpha = 0.05$, Whole plot (4.7), Split plot (4.4), Split- split plot (4.3)

Figure 6. Potentially mineralizable nitrogen (PMN) (μg NH_4^+-N g^{-1} of dry soil) affected by ground cover and fertilizer combinations in March and May from 2007 to 2013 in an organic apple orchard soil in Fayetteville, AR. Fertilizer treatments effects are depicted within each ground cover treatment graph, open circles are no fertilizer control, closed triangles are poultry litter, and closed squares are commercial fertilizer (n = 3). LSD's to compare means at $\alpha = 0.05$, Whole plot (16.28), Split plot (15.09), Split- split plot (14.93)

Figure 7. Ammonium concentrations (μg NH_4^+-N g^{-1} of dry soil) ground cover (compost, paper, mow-and-blow, and wood chips) averaged across all three fertilizers (no fertilizer control, poultry litter, and commercial) measured in March and May from 2007 to 2013 in an organic apple orchard in Fayetteville, AR (n = 9). LSD's to compare means at $\alpha = 0.05$, Whole plot (1.98), Split plot (1.88), Split- split plot (1.82)

Figure 8. Nitrate concentrations (μg NO_3^--N g^{-1} of dry soil) affected by affected by ground cover (compost, paper, mow-and-blow, and wood chips) and fertilizer (no fertilizer control, poultry litter, and commercial) combinations from 2007-2013 in an organic apple orchard in Fayetteville, AR (n = 3). LSD's to compare means at $\alpha = 0.05$, Whole plot (10.04), Split plot (9.45), Split-split plot (9.44)

Figure 9. Biological carbon (μg C g^{-1} of dry soil) as affected by ground cover and fertilizer treatment combinations from 2007 to 2013 in an organic apple orchard soil in Fayetteville, AR. Fertilizer treatments effects are depicted within each ground cover treatment graph, open circles are no fertilizer control, closed triangles are poultry litter, and closed squares are commercial (n = 3). LSD's to compare means at $\alpha = 0.05$, Whole plot (51.50), Split plot (48.46), Split- split plot (44.35)

Figure 10. Biological nitrogen (μg N g^{-1} of dry soil)) as affected by ground cover and fertilizer treatment combinations from 2007 to 2013 in an organic apple orchard soil in Fayetteville, AR. Fertilizer treatments effects are depicted within each ground cover treatment graph, open circles are no fertilizer control, closed triangles are poultry litter, and closed squares are commercial (n = 3). LSD's to compare means at $\alpha = 0.05$, Whole plot (10.23), Split plot (9.66), Split- split plot (9.34)

Figure 11. Dehydrogenase activities (μg triphenyl formazan (TPF) g^{-1} soil for 24 hr^{-1}) affected by ground cover(compost, paper, mow-and-blow, and wood chips) addition averaged across all three fertilizers (no fertilizer control, poultry litter, and commercial) in March and May from 2007-2013 in an organic apple orchard in Fayetteville, AR (n = 9). LSD's to compare means at $\alpha = 0.05$, Whole plot (55.73), Split plot (52.26), Split- split plot (51.40)

Figure 12. β-Glucosaminidase (NAGase) (μg g^{-1} hr^{-1}) activity affected by ground cover(compost, paper, mow-and-blow, and wood chips) addition averaged across all three fertilizers (no fertilizer control, poultry litter, and commercial) in March and May from 2007-2013 in an organic apple orchard in Fayetteville, AR (n = 9). LSD's to compare means at $\alpha = 0.05$, Whole plot (15.60), Split plot (14.30), Split- split plot (14.30)

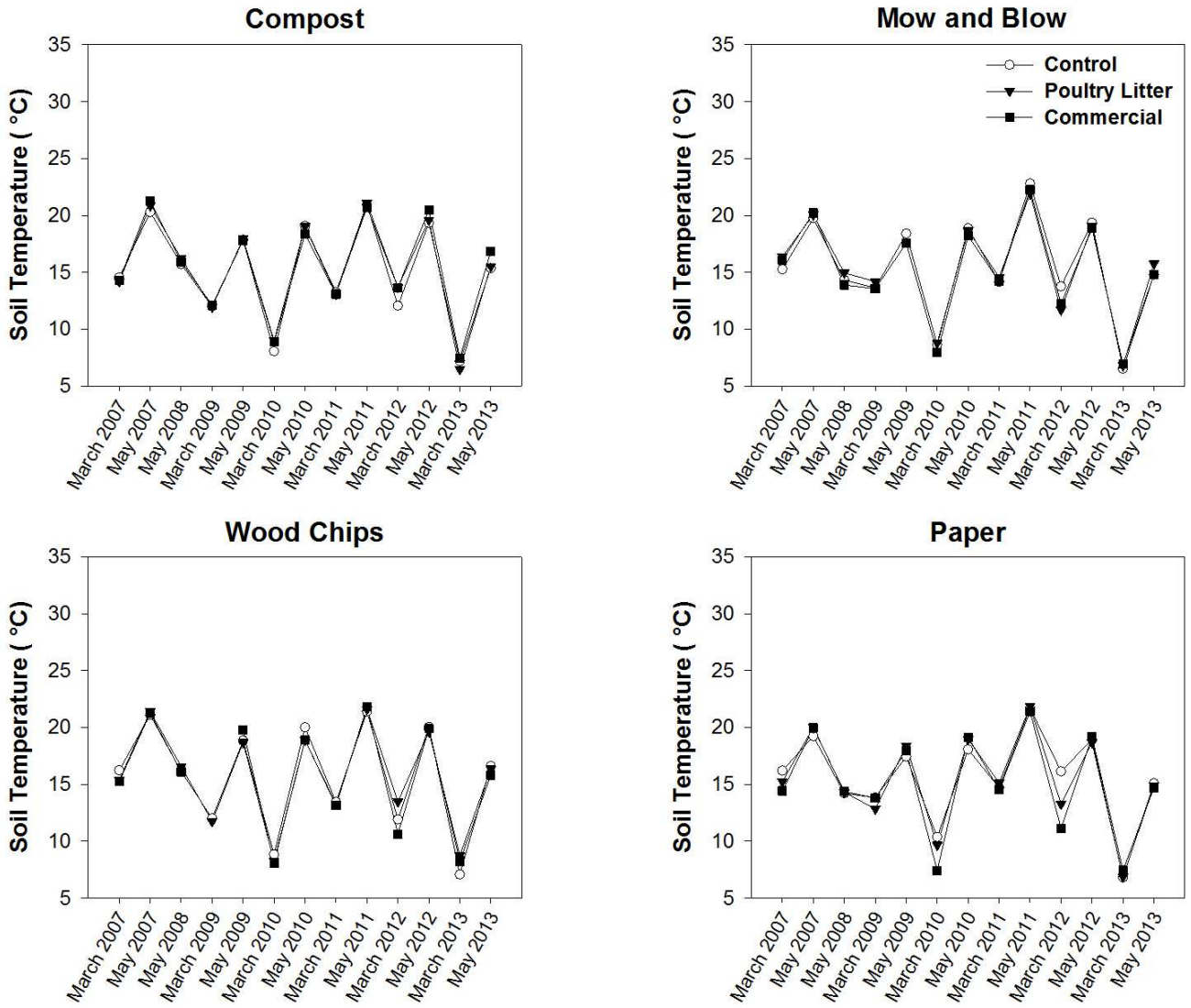
Compost
Mow and Blow
Wood Chips
Paper
Soil Temperature (°C)
Control
Poultry Litter
Commercial
35
30
25
20
15
10
5
March 2007
May 2007
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 1

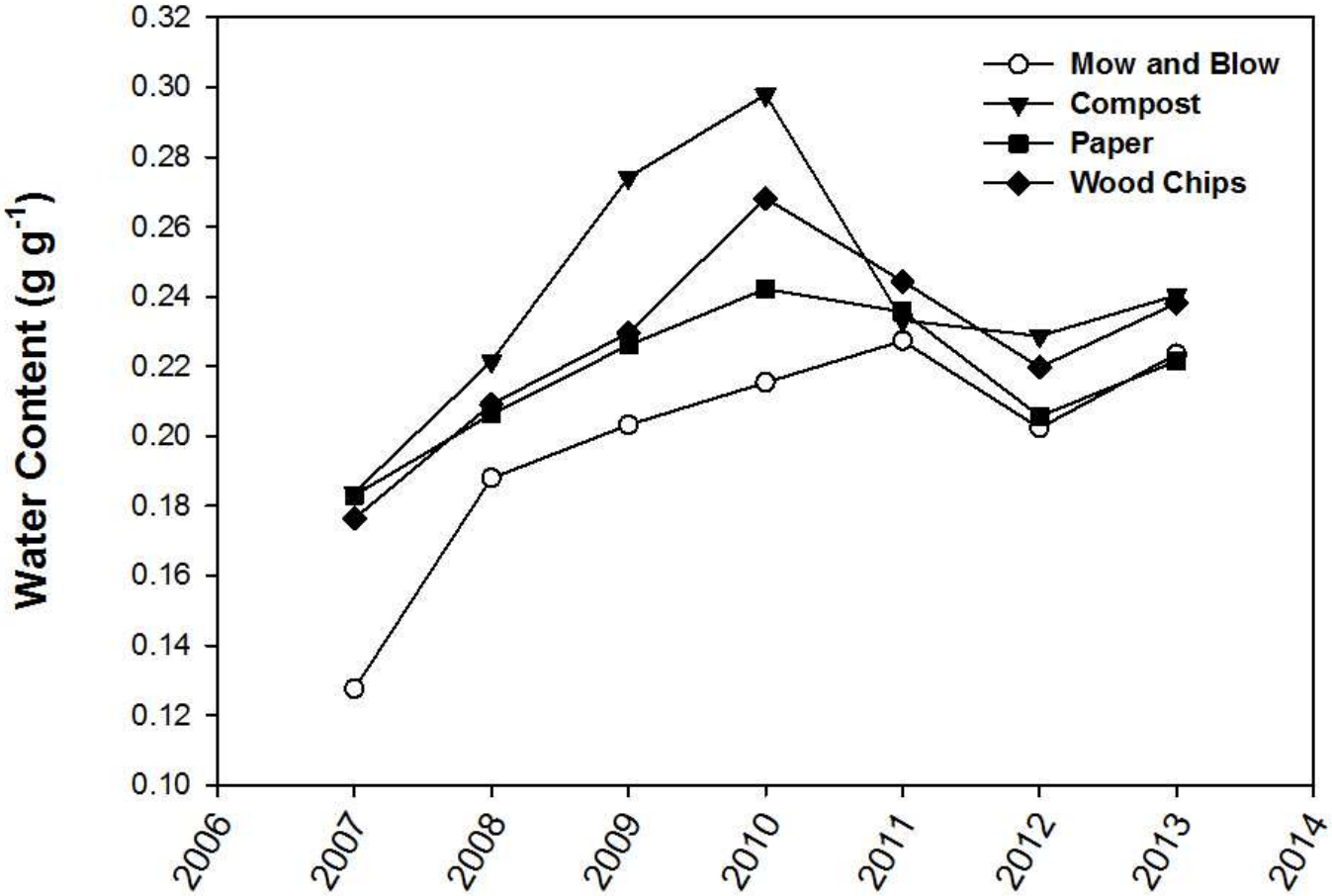
Water Content (g g-1)
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
2006
2007
2008
2009
2010
2011
2012
2013
2014
Mow and Blow
Compost
Paper
Wood Chips

Figure 2

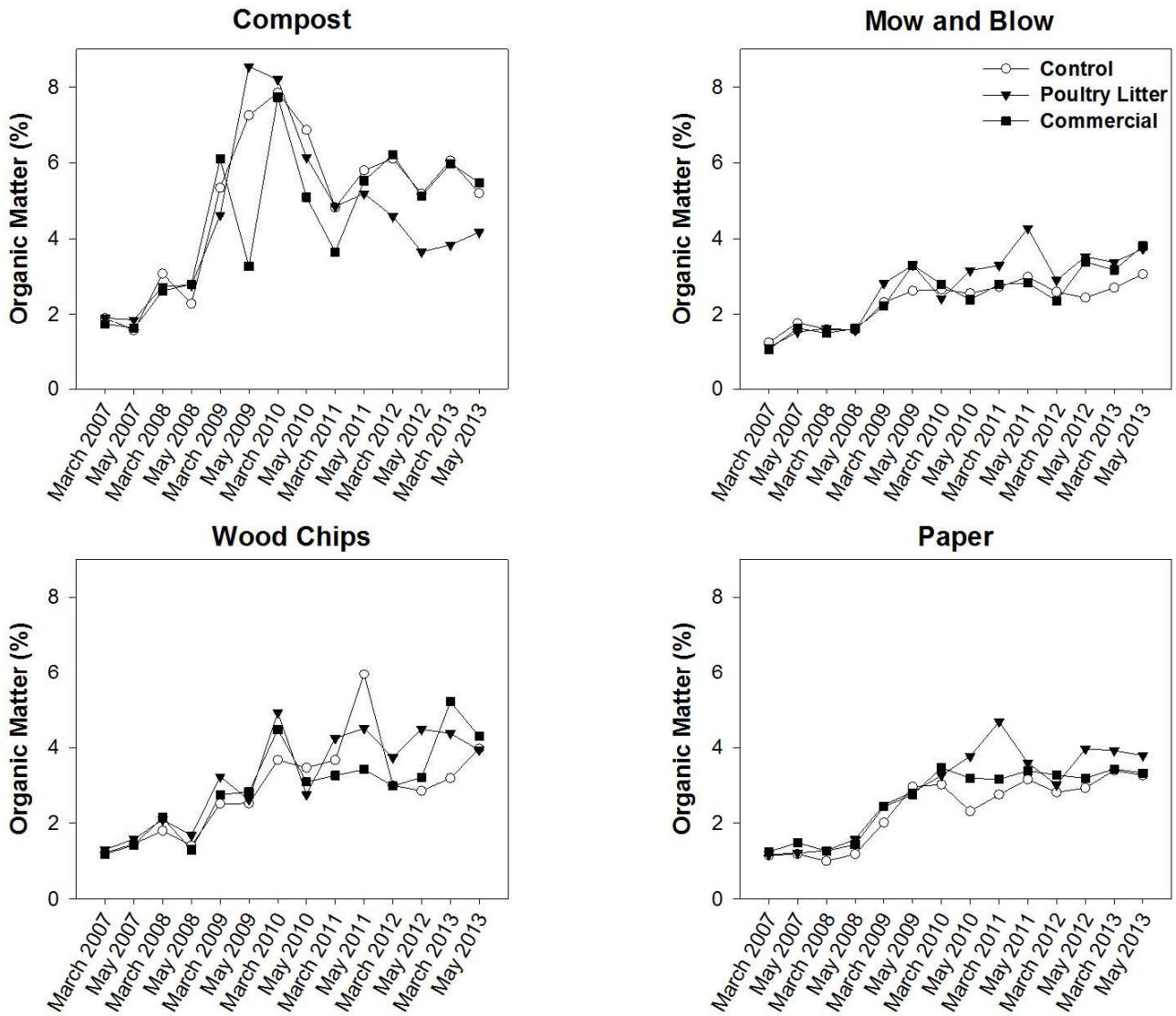
Compost
Mow and Blow
Wood Chips
Paper
Organic Matter (%)
0
2
4
6
8
Control
Poultry Litter
Commercial
March 2007
May 2007
March 2008
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 3

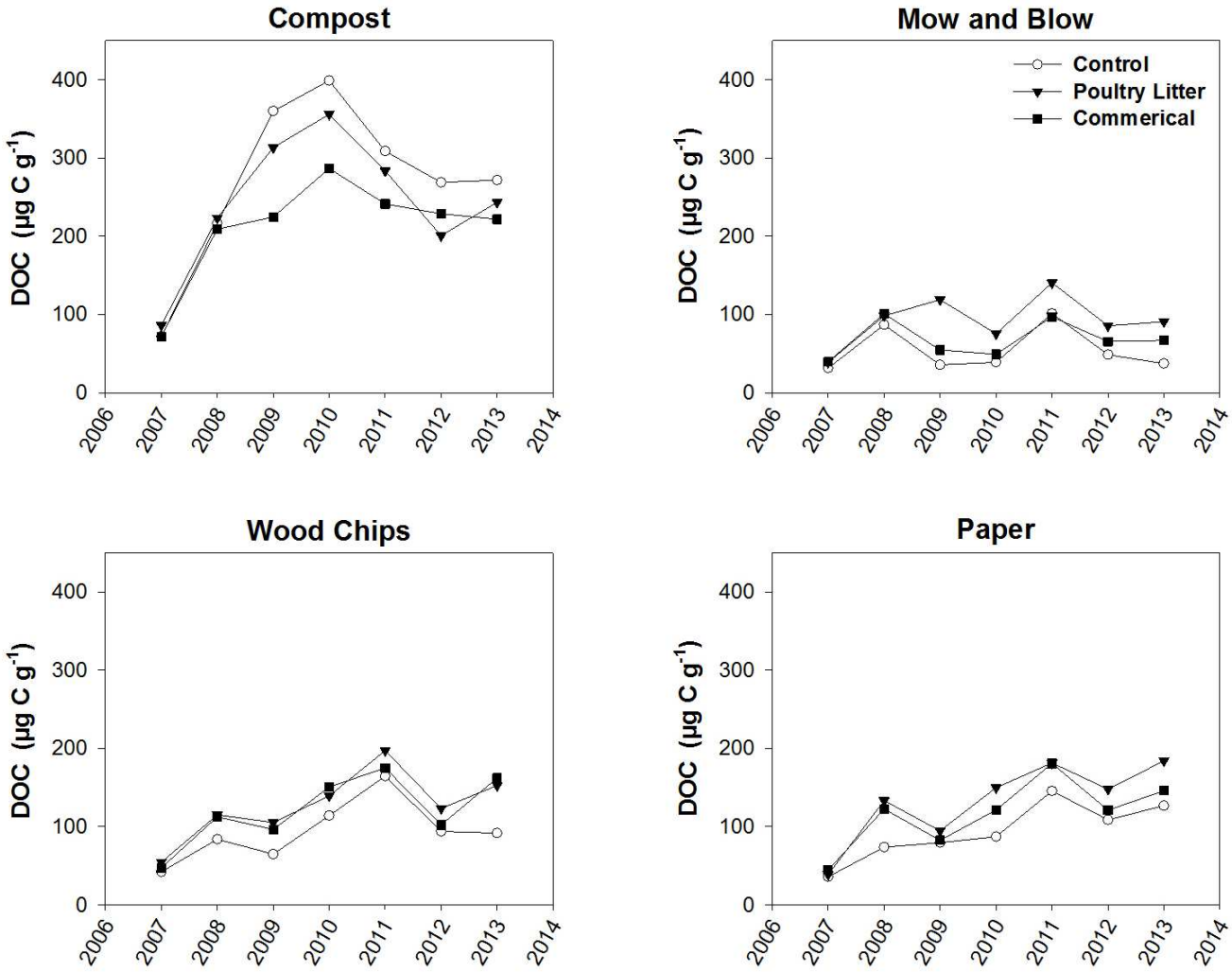
Compost
Mow and Blow
Wood Chips
Paper
Control
Poultry Litter
Commerical
DOC (µg C g-1)
0
100
200
300
400
2006
2007
2008
2009
2010
2011
2012
2013
2014

Figure 4

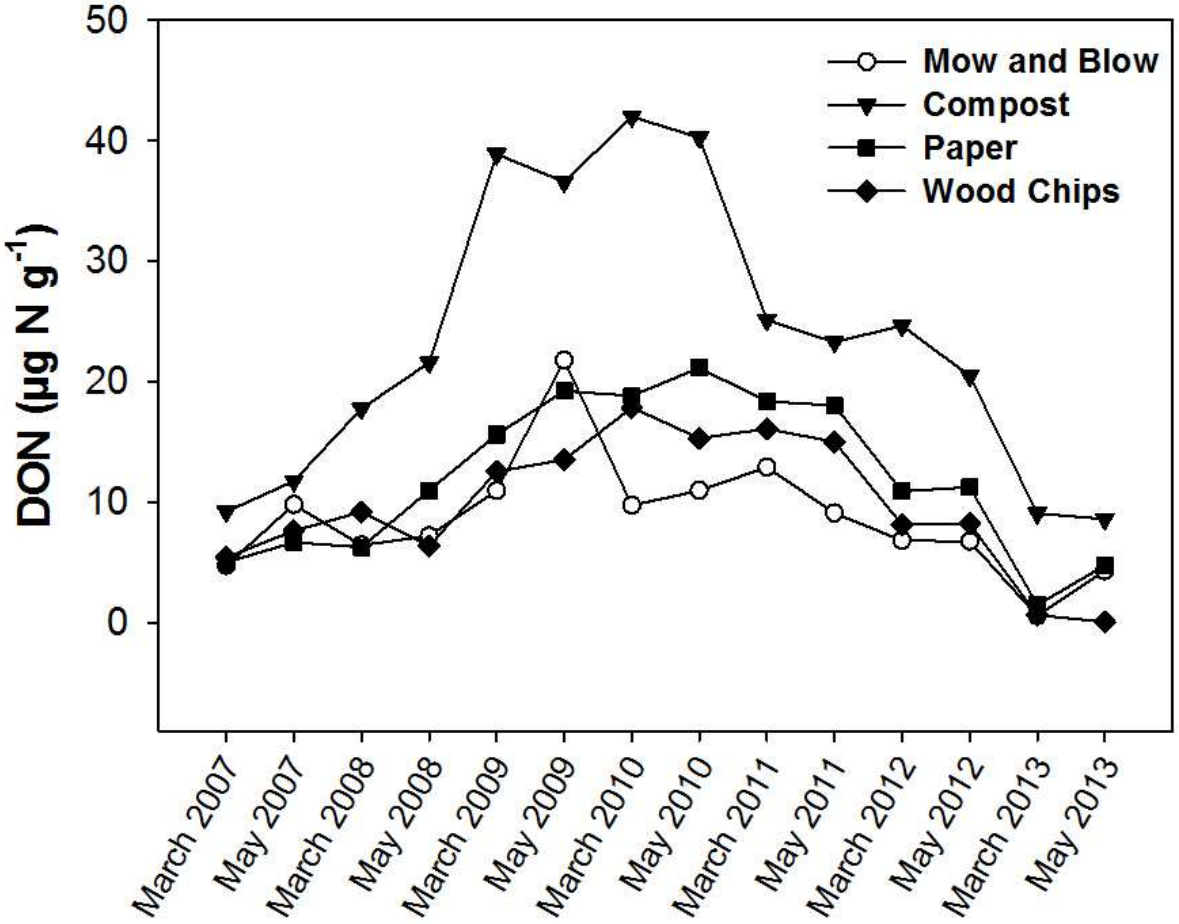
DON (µg N g-1)
50
40
30
20
10
0
Mow and Blow
Compost
Paper
Wood Chips
March 2007
May 2007
March 2008
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 5

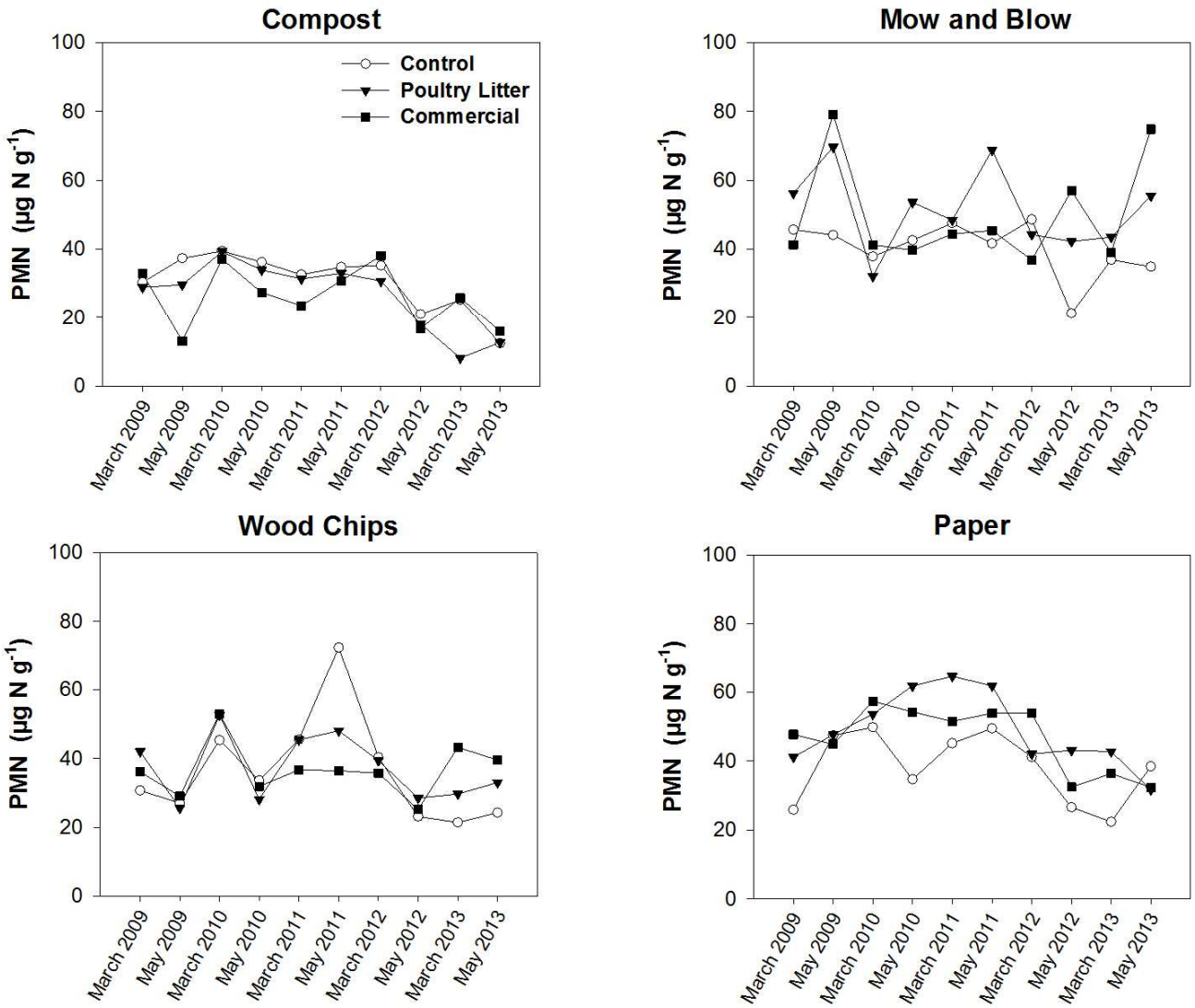
Compost
Mow and Blow
Wood Chips
Paper
Control
Poultry Litter
Commercial
PMN (µg N g-1)
0
20
40
60
80
100
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 6

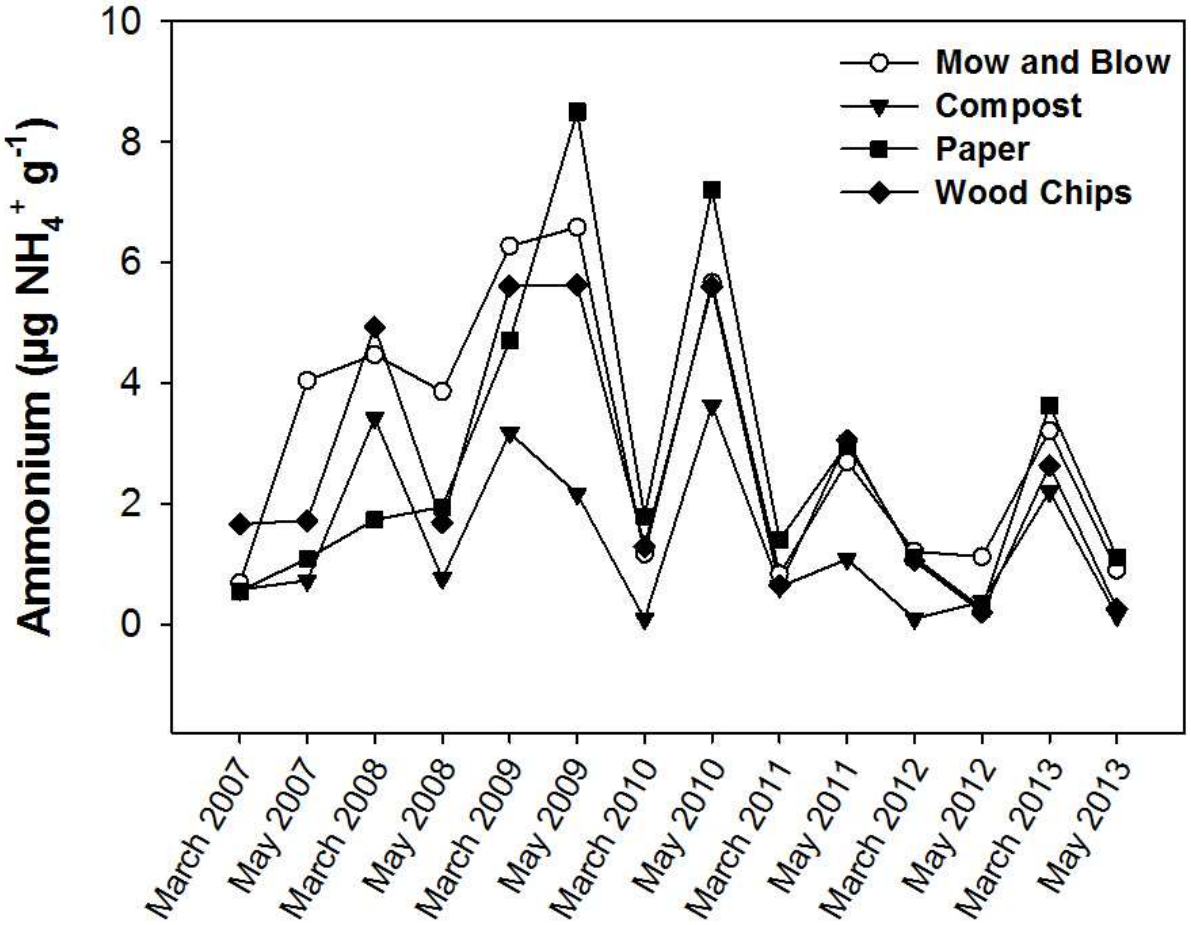
Ammonium (µg NH_4^+ g^{-1})
10
8
6
4
2
0
Mow and Blow
Compost
Paper
Wood Chips
March 2007
May 2007
March 2008
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 7

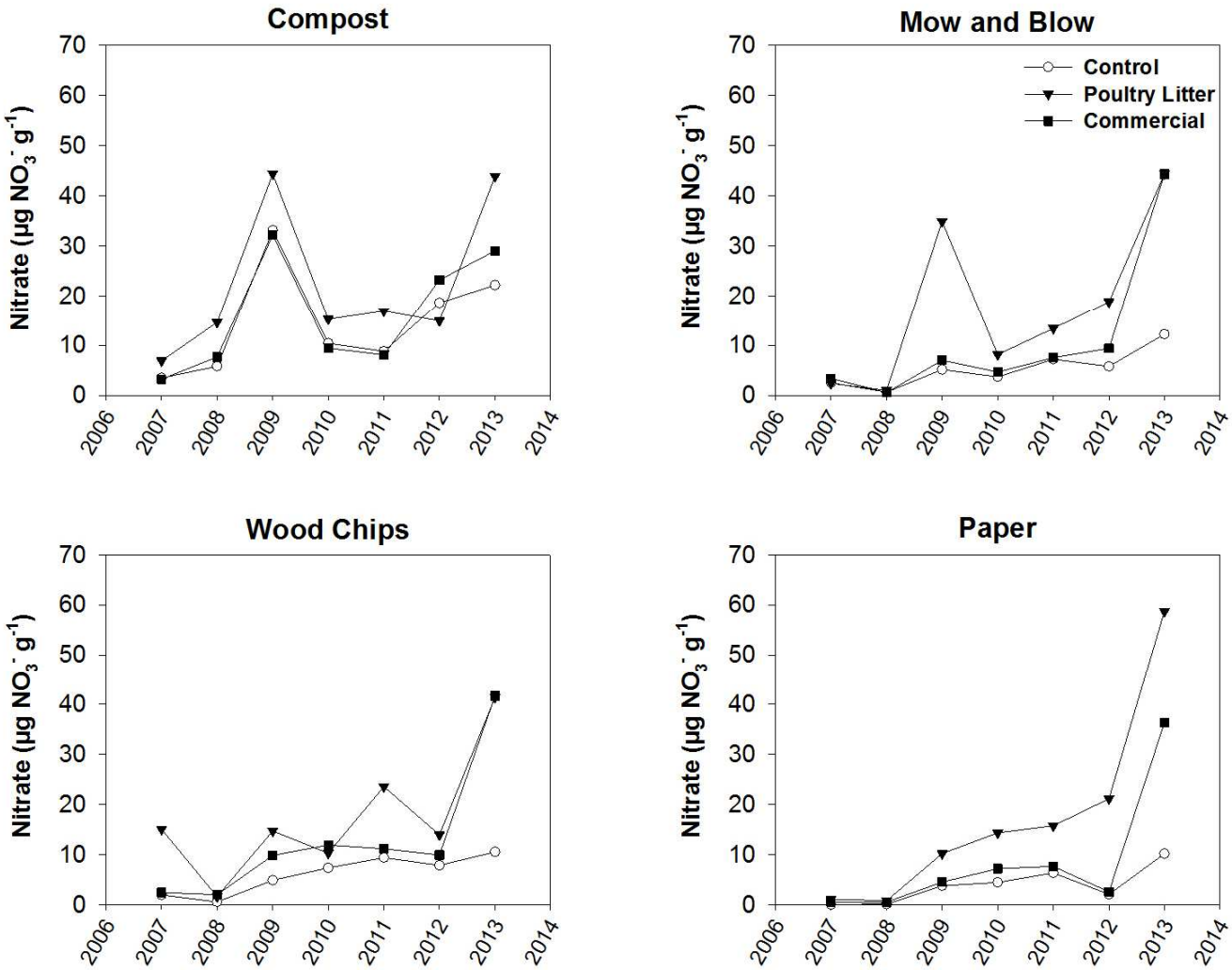
Compost
Mow and Blow
Wood Chips
Paper
Control
Poultry Litter
Commercial
Nitrate (µg NO3- g-1)
0
10
20
30
40
50
60
70
2006
2007
2008
2009
2010
2011
2012
2013
2014

Figure 8

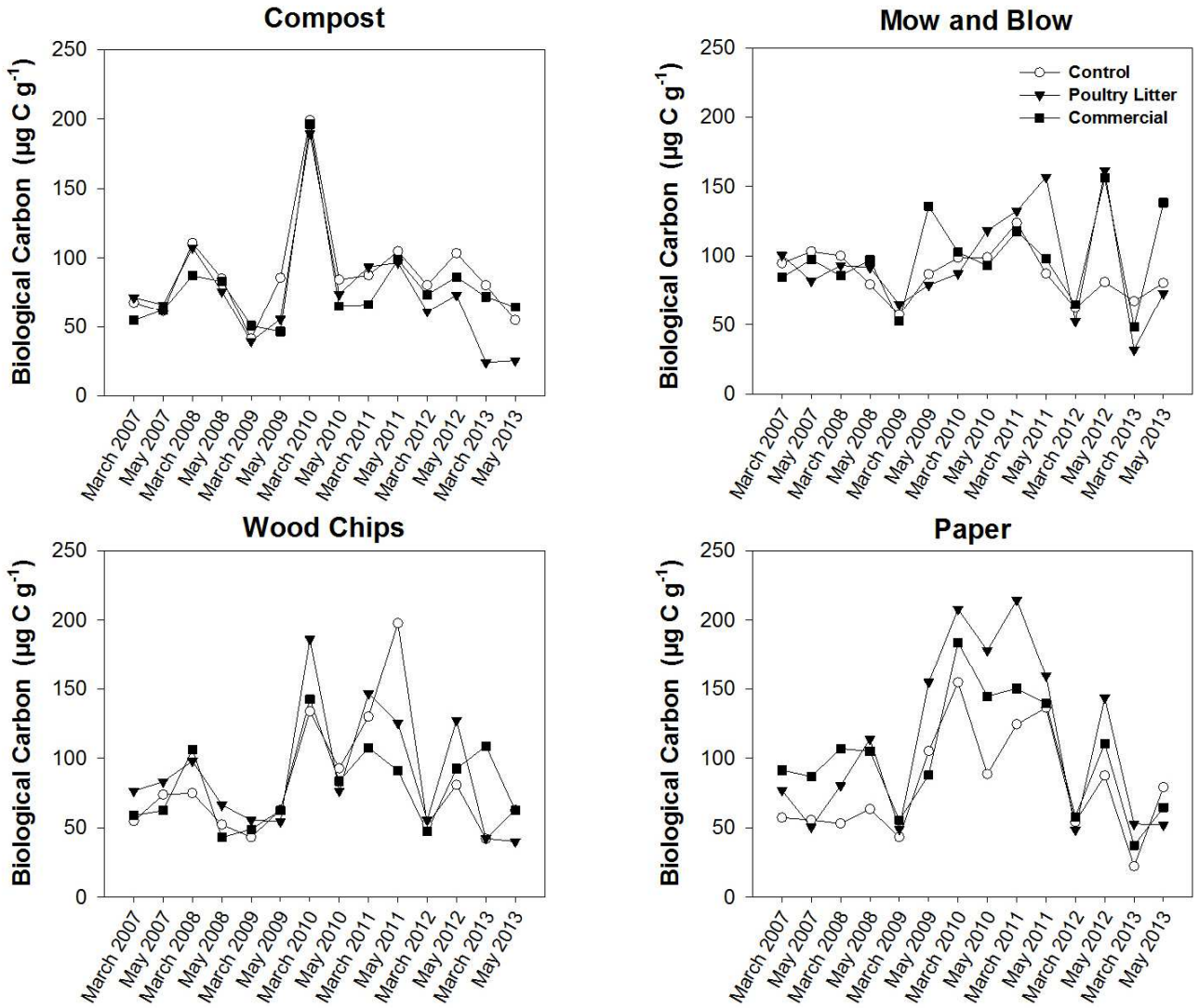
Compost
Mow and Blow
Wood Chips
Paper
Biological Carbon (µg C g-1)
Control
Poultry Litter
Commercial
0
50
100
150
200
250
March 2007
May 2007
March 2008
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 9

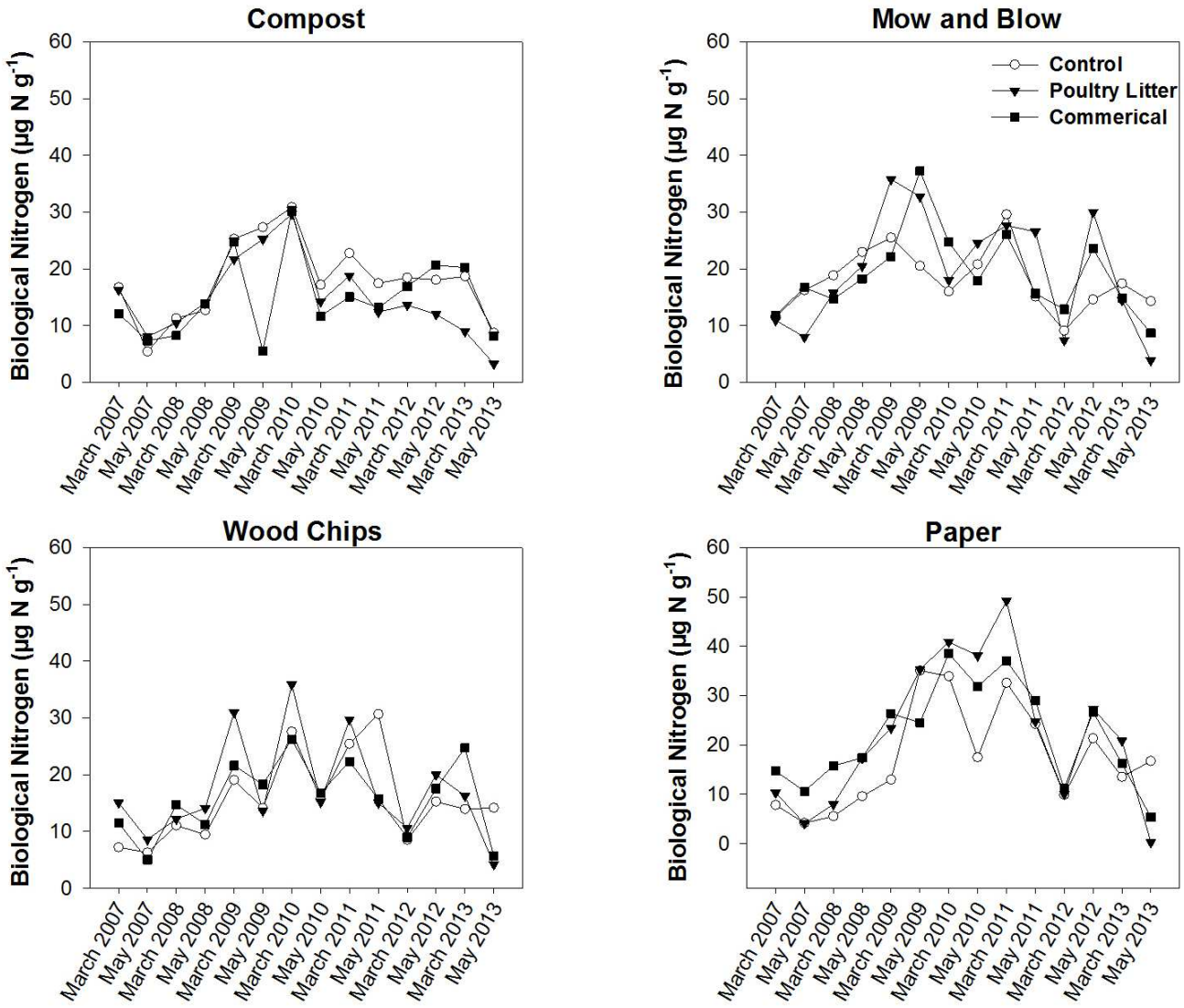
Compost
Mow and Blow
Wood Chips
Paper
Biological Nitrogen (µg N g-1)
Control
Poultry Litter
Commerical
0
10
20
30
40
50
60
March 2007
May 2007
March 2008
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 10

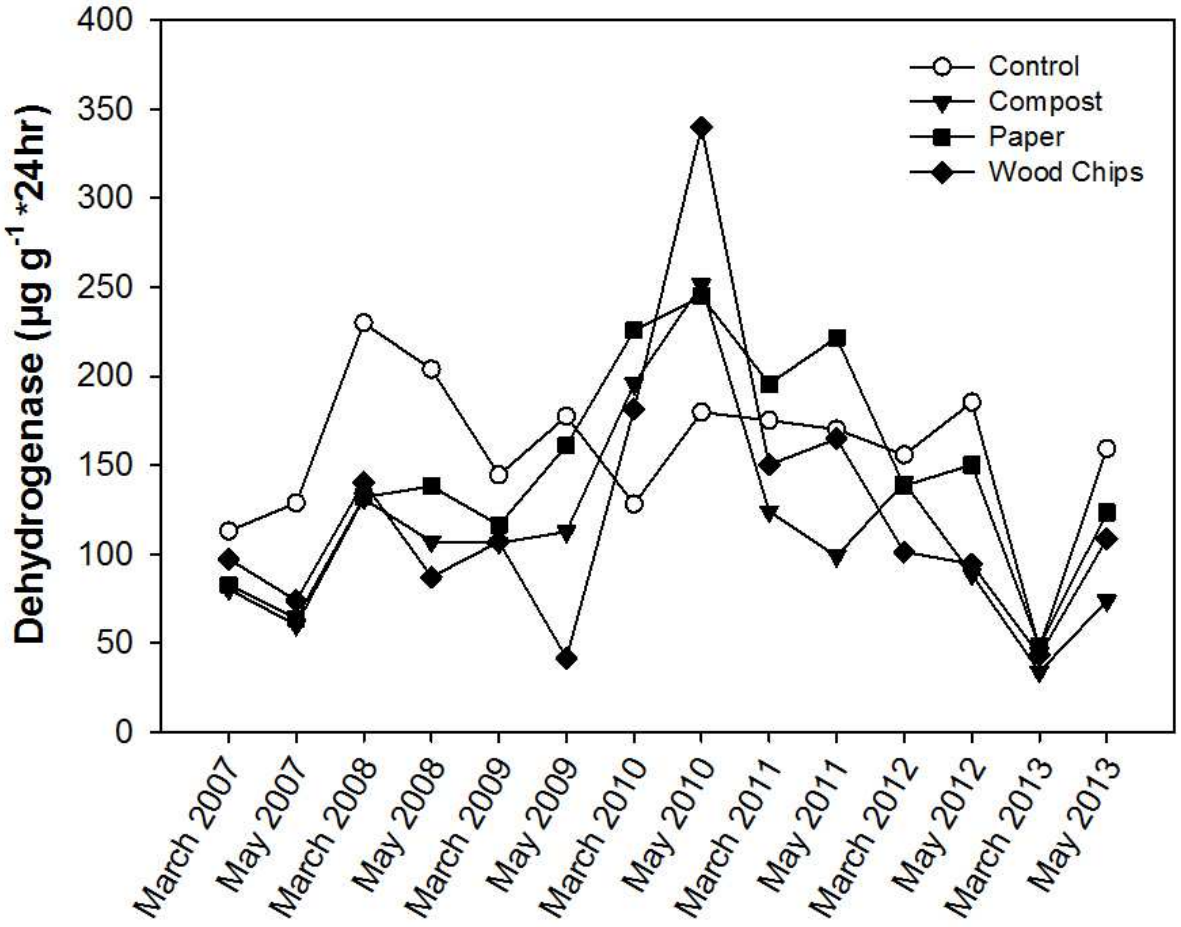
Dehydrogenase (µg g-1 *24hr)
400
350
300
250
200
150
100
50
0
Control
Compost
Paper
Wood Chips
March 2007
May 2007
March 2008
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 11

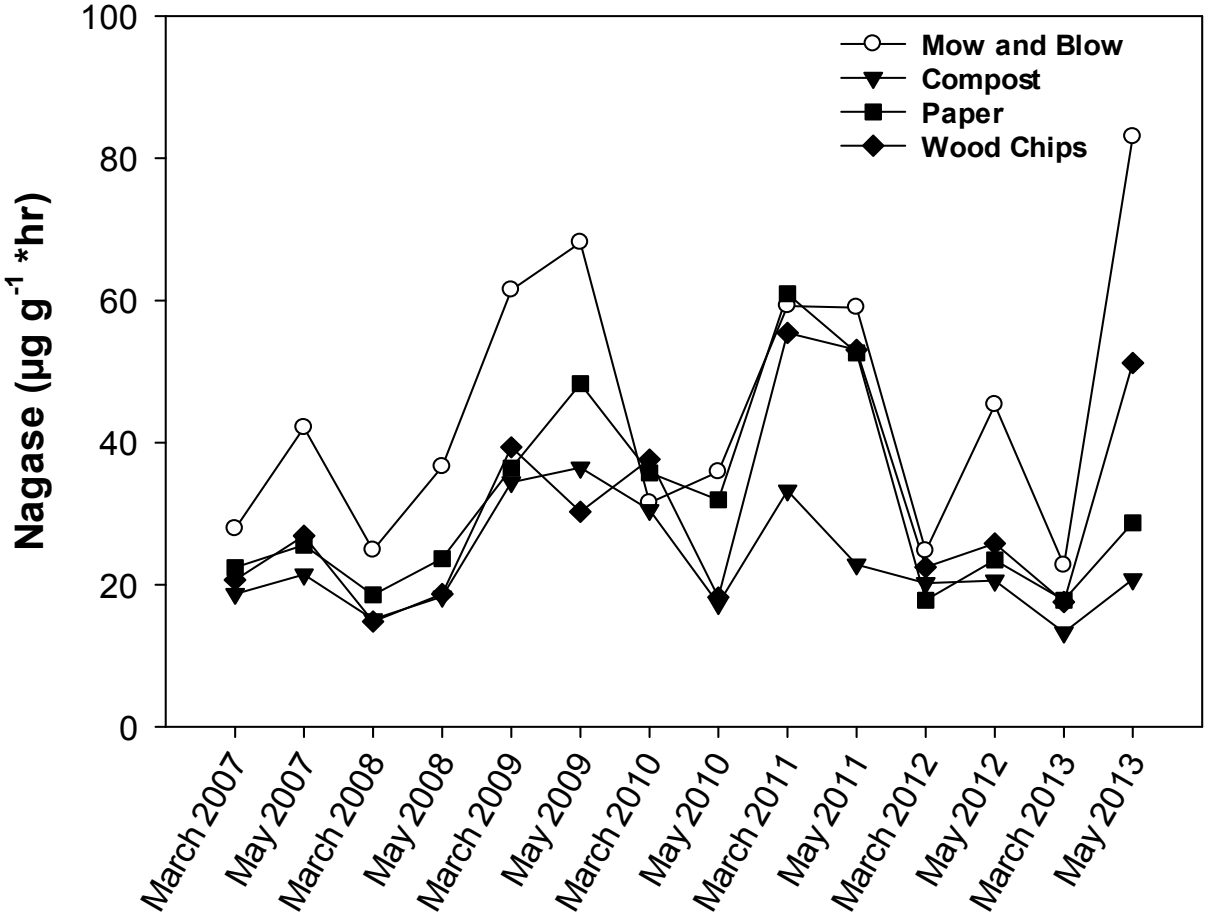
Nagase (µg g-1 *hr)
100
80
60
40
20
0
Mow and Blow
Compost
Paper
Wood Chips
March 2007
May 2007
March 2008
May 2008
March 2009
May 2009
March 2010
May 2010
March 2011
May 2011
March 2012
May 2012
March 2013
May 2013

Figure 1

5. Conclusion

The purpose of this thesis was to determine how locally applicable ground covers (compost, wood chips, paper mulch, and mow-and-blow) combined with one of three organic fertilizers (composted poultry litter, organic commercial fertilizer, and no-fertilizer control) applied annually from 2006-2013 in an organically managed apple orchard system on a highly eroded mineral Ozark highlands soil, affected soil properties and microbial processes in the Southern United States. Results suggest that all treatments were capable of increasing organic matter content and nutrient availability over the seven years of the study. However, the interactions between ground covers and fertilizers, timelines of treatment effects, and microbial community shifts are complex. The long-term nature of the second study in this thesis revealed many treatment differences that were not apparent in the first study when comparing only the beginning and end of the study.

Although compost addition had the largest impact on the soil organic matter content, and dissolved organic carbon and nitrogen concentrations, microbial activities (DHase and NAGase) and microbial biomass C and N were not stimulated to a greater extent compared to the other treatments through seven years of annual ground cover and nutrient applications. However, the ability of the community to nitrify was not negatively impacted, as greater or similar amounts of soil nitrate-N were measured in 2009 and 2013 without an equivalently large amount of total microbial biomass. In the other ground cover treatments, greater amounts of PMN, NH_4^+-N, microbial biomass, DHase and NAGase activities throughout the study indicate that these residues are being cycled more readily than compost. Substrate quality of ground cover and

nutrient combinations seem to impact soil microbial biomass, activity and resultant nutrient availability to a greater extent than the total C and N added or the C:N ratio.

The treatments with the biggest impact on denitrifying *nirK* organisms were compost and wood chips. The change in the compost communities was apparent in 2007 by greater species richness and diversity than the other treatments. Wood chips communities changed greatly over time and in 2013 had similar species richness and diversity when applied in combination with either fertilizer even though DOC concentrations were less than in compost treatments. I hypothesized treatments where substrate availability (OM, DOC, NO_3^--N) and soil conditions (pH, temperature, water content) were most conducive to denitrification, microbial biomass and *nirK* community richness and diversity would be greatest. The hypothesis regarding microbial biomass was not supported by the results, mow-and-blow treatments added less OM and DOC, while compost and wood chip treatments added more OM, DOC, and NO_3^- -N but microbial biomass did not differ across ground covers within a year.

Further investigations into the interaction between compost and fertilizer, especially poultry litter, are needed to fully understand the impacts on the soil biological and chemical properties. More molecular analyses, including qPCR or next generation sequencing, to determine how nitrogen cycling communities are responding would also be beneficial, because they serve as sensitive and dynamic indicators of changes in soil properties, are the agents of decomposition and retain and turnover nutrients in soil.

6. Appendix A

Table 1. Analysis of variance (ANOVA) summary of the effects of ground cover and fertilizer treatments, month and year, and their interactions on soil properties at the 0-10 cm depth of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007-2013

Soil Property	GC	Fert	GC*Fert	Yr	GC*Yr	Fert* Yr	GC*Fert *Yr	Mo	Yr*Mo	GC* Yr*Mo	Fert* Yr*Mo	GC*Fert* Yr*Mo
H_2O[b]	<0.0001*	0.3873	0.1738	<0.0001*	<0.0001*	0.9003	0.6291	0.4937	<0.0001*	0.0882	0.4192	0.4875
OM	<0.0001*	0.0832	0.0459	<0.0001*	<0.0001*	0.0227*	0.0022*	0.5095	<0.0001*	<0.0001*	0.0279*	<0.0001*
Temp	<0.0001*	0.0135*	0.0006*	<0.0001*	<0.0001*	0.0002*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
DOC	<0.0001*	0.0005*	<0.0001*	<0.0001*	<0.0001*	0.1296	0.0433*	<0.0001*	<0.0001*	0.2105	0.0164*	0.0515
DON	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0998	0.1405	0.0280*	0.0062*	0.0131*	0.0250*	0.2956
NO_3^--N	0.0001*	<0.0001*	0.6194	<0.0001*	<0.0001*	<0.0001*	0.0393*	<0.0001*	<0.0001*	0.0422*	<0.0001*	0.0671
NH_4^+-N	<0.0001*	0.8385	0.0602	<0.0001*	0.0031*	0.6165	0.4753	0.0040*	<0.0001*	0.0157*	0.1152	0.9559
PMN	<0.0001*	0.0154	0.0133*	<0.0001*	<0.0001*	0.0002*	0.0250*	0.2663	<0.0001*	<0.0001*	0.3854	<0.0001*
BioC	0.0036*	0.1625	0.0221*	<0.0001*	<0.0001*	0.0066*	0.5372	0.1049	<0.0001*	<0.0001*	0.4769	0.0294*
BioN	<0.0001*	0.2829	0.0325*	<0.0001*	<0.0001*	0.0019*	0.1340	<0.0001*	<0.0001*	<0.0001*	0.0495	0.0003*
Dhase	0.0002*	0.5511	0.0160*	<0.0001*	<0.0001*	0.1785	0.1190	0.0015*	<0.0001*	0.0383*	0.7366	0.1704
Nagase	<0.0001*	0.0016*	0.2144	<0.0001*	<0.0001*	0.0114*	0.2230	<0.0001*	<0.0001*	0.0003*	0.0644	0.1885

[a]DOC is dissolved organic carbon; DON is dissolved organic nitrogen; is nitrate-N; NH_4^+-N is ammonium-N; Bio C is microbial biomass carbon; Bio N is microbial biomass nitrogen; EC is electrical conductivity; OM is organic matter; H_2O is soil-water content.
* $P < 0.05$

Table 2. Analysis of variance (ANOVA) summary of the effects of ground cover and fertilizer treatments, month and year, and their interactions on soil properties at the 10-30 cm depth of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007- 2013

Soil Property	GC	Fert	GC*Fert	Yr	GC*Yr	Fert* Yr	GC*Fert *Yr	Mo	Yr*Mo	GC* Yr*Mo	Fert* Yr*Mo	GC*Fert* Yr*Mo
H_2O[b]	0.0010*	0.6732	0.2984	<0.0001*	0.0012*	0.9969	0.7737	<0.0001*	<0.0001*	0.0013*	0.9994	0.7087
OM	0.0007*	0.9962	0.5770	<0.0001*	0.5476	0.9735	0.7373	<0.0001*	0.0034*	0.2738	0.1063	0.9995
Temp	<0.0001*	0.0004*	0.0295	<0.0001*	<0.0001*	0.0008*	0.0005*	<0.0001*	<0.0001*	0.0136*	0.0581	0.8291
DOC	<0.0001*	<0.0001*	0.0014*	<0.0001*	<0.0001*	<0.0001*	0.0160*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
DON	<0.0001*	<0.0001*	0.1781	<0.0001*	<0.0001*	<0.0001*	0.9580	<0.0001*	<0.0001*	0.0132*	<0.0001*	0.6548
NO_3^--N	0.0584	0.0175*	0.2675	<0.0001*	<0.0001*	<0.0001*	0.5783	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.6808
NH_4^+-N	0.1559	0.0268*	0.1799	<0.0001*	0.2493	0.0464*	0.9442	<0.0001*	0.0012*	0.0001*	0.1642	0.0009*
PMN	0.0100*	0.1080	0.9423	<0.0001*	0.0619	0.9780	0.9087	0.0134*	<0.0001*	0.6276	0.8968	0.9767
BioC	0.0939	0.0739	0.7926	<0.0001*	0.3493	0.1304	0.5875	0.9100	<0.0001*	0.0863	0.1071	0.4246
BioN	0.1207	0.2964	0.9746	0.0014*	0.0730	0.8537	0.7945	<0.0001*	<0.0001*	0.0200*	0.8460	0.9695
Dhase	0.1105	0.0119*	0.3884	<0.0001*	0.1368	0.6561	0.6732	<0.0001*	<0.0001*	0.1956	0.4407	0.5659
Nagase	0.0010*	0.6732	0.2984	<0.0001*	0.0012*	0.9969	0.7737	<0.0001*	<0.0001*	0.0013*	0.9994	0.7087

[a]DOC is dissolved organic carbon; DON is dissolved organic nitrogen; is nitrate-N; NH_4^+-N is ammonium-N; Bio C is microbial biomass carbon; Bio N is microbial biomass nitrogen; EC is electrical conductivity; OM is organic matter; H_2O is soil-water content.
* $P < 0.05$

Table 3. Sample sizes of the DGGE analysis of *nirK* in the 0-10 cm depth in a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007-2013

	2007				2013				Total			
GC[a]	Control	PL	Comm	Total	Control	PL	Comm	Total	Control	PL	Comm	Total
C	3	3	2	8	3	2	2	7	6	5	4	15
MB	3	3	3	9	3	3	3	9	6	6	6	18
P	3	3	3	9	3	3	2	8	6	6	5	17
WC	3	3	3	9	3	3	3	9	6	6	6	18
Total	12	12	11	35	12	11	10	33	24	23	21	68

[a]GC is ground cover; PL is poultry litter; Comm is commercial fertilizer; [b]C is compost; M is mow-and-blow; P is paper; W is wood chips

Table 4. Sample sizes of DGGE analysis of *nirK* in 10-30 cm depth of silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007-2013

	2007				2013				Total			
GC[a]	Control	PL	Comm	Total	Control	PL	Comm	Total	Control	PL	Comm	Total
C[b]	2	1	3	6	2	1	1	4	4	2	4	10
MB	2	3	2	7	2	3	2	7	4	6	4	14
P	2	1	3	6	1	1	3	5	3	2	6	11
WC	1	3	2	6	2	3	2	7	3	6	4	13
Total	7	8	10	25	7	8	8	23	14	16	18	48

[a]GC is ground cover; PL is poultry litter; Comm is commercial fertilizer; [b]C is compost; M is mow-and-blow; P is paper; W is wood chips