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2 Nutrient Management in an Organic Fruit Tree Orchard Soil
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Abstract

Mechanisms through which microorganisms are impacted by surface application of ground cover and nutrient source in organic orchard management are not fully understood. Twelve ground cover (compost, wood chips, paper mulch, or mow-and-blow) by fertilizer (poultry litter, organic commercial, or a no-fertilizer control) treatment combinations were applied annually in April beginning in 2006, with soil collected in March 2007 and 2013 to determine the 1-year and 7-year effects of treatments on soil chemical and biological properties. Organic matter (OM) increased through time regardless of ground cover treatment averaged across fertilizers, with the greatest increase occurring with compost addition. Soil water content, microbial biomass N, ammonium-N, and nitrate-N were greater in 2013 than in 2007 at the 0-10 cm depth. Denaturing gradient gel electrophoresis analysis of denitrifying (*nirK*) communities revealed that fertilizers did not have a significant main effect or interaction with ground covers. Greater *nirK* richness and diversity coincided with clustering of the *nirK* community composition within compost treatments, and greater dissolved organic carbon concentrations with compost compared to wood chip or mow-and-blow treatments in 2007. By 2013 *nirK* communities in wood chip and compost ground covers tended to cluster together. Furthermore, soil *nirK* richness increased significantly from 2007 to 2013 in compost and wood chip treatments and was greatest in 2013 under those two ground covers. Diversity with wood chip addition increased from among the least in 2007 to among the greatest in 2013. Greater richness and diversity and community composition in the *nirK* community within a year of compost additions and gradually over time with wood chip addition indicate potential for enhanced denitrification in low OM, organic apple orchard soil receiving compost and wood chips mulches.

1.0. Introduction

Orchards are perennial systems requiring long-term management strategies to ensure their success. Many factors affect orchard management decisions. Characteristics of site location are crucial in making initial management decisions. Apple orchards situated on low organic matter soils characteristic of the southeastern U.S. or many of China's orchards pose challenges that may be at least partially addressed through the use of organic soil amendments where one apparent benefit is the addition of organic matter (OM) (Mays et al., 2014, 2015), but another may be the alteration of microbial communities (Qian et al., 2014; Sun et al., 2014). Soil organic matter consists of the decomposing and humified plant and animal residues, products and byproducts, along with the microbial biomass performing the relevant biochemical processes (Lal, 2007). Increased soil OM and microbial activity are among the benefits observed in organically managed orchards. 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 clay loam. Greater OM (80%), cation exchange capacity (CEC), calcium (Ca) and phosphorus (P) availability, pH and respiration were observed in the mulch treatment.

Ground covers and nutrient sources applied to the soil surface have both immediate and lasting effects. In terms of N management, the goal is to facilitate efficient internal terrestrial N cycling without promoting processes that result in atmospheric and aquatic pollution from excess N. Organic systems have led to similar or increased (Pimentel et al., 2005) and decreased (Hansen et al., 2001; Kramer and Gleixner, 2006) nitrate leaching compared to conventional systems. Kramer and Gleixner (2006) reported organically managed apple orchard soil had greater OM, microbial activity, increased denitrification potentials, denitrification rates, and denitrification efficiency and decreased nitrate pollution. Nitrate leaching was greater in the

conventionally managed soil, and the greatest N₂ emissions were observed in the organically managed soils while the N₂O emissions were not significantly different (Kramer and Gleixner, 2006). Denitrification has wide-reaching economic and environmental impacts because it is a mechanism of soil N loss and because of the potential release of the greenhouse gas nitrous oxide.

Denitrification is a step-by-step pathway that ultimately reduces nitrate (NO₃⁻) to N₂, by nitrate, nitrite, nitric oxide, and nitrous oxide reductases (Zumft, 1997). There are two functionally equivalent nitrite reductase genes, copper containing (*nirK*) and cytochrome cd1 containing (*nirS*), each found in a large number of diverse organisms in soils (Coyne et al., 1989; Braker et al., 1998; Heylen et al., 2006). The *nirK* harboring organisms may be more important in agricultural ecosystems compared to their *nirS* counterparts (Attard et al., 2011; Dandie et al., 2011). Denitrification has been well studied in conventionally and organically managed systems; however, impacts of organic ground cover and nutrient source amendments on denitrification from perennial horticultural systems of 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.

The objective of this study was to determine if twelve ground cover (compost, shredded paper, wood chips, and mow-and-blow as an informal control) and fertilizer (poultry litter, commercial, and no fertilizer) treatment combinations applied annually to an organically managed, apple orchard soil changed the soil denitrifying community or the potential for denitrification after one or seven years. The research objective was achieved by analyzing the diversity of the copper containing (*nirK*) soil nitrite reductase gene using denaturant gradient gel electrophoresis (DGGE) and measuring soil properties to determine if available nutrients and soil

conditions conducive for denitrification had changed over time. The *nirK* gene community richness, diversity, and community composition were expected to be greatest and most similar in treatments where substrate availability (OM, dissolved organic carbon (DOC), NO_3^-) and soil conditions such as pH were most conducive to denitrification.

2.0. Materials and Methods

2.1. Experimental Design

The 0.30-ha organic apple orchard is located on a historically horticulturally managed site at the University of Arkansas Main Agriculture Experiment and Extension Center in Fayetteville, Arkansas (36°N, 94°W). The site is mapped to two soils: Captina (Fine-silty, siliceous, active, mesic Typic Fragiudult) and Pickwick (Fine-silty, mixed, semiactive, thermic Typic Hapludult) silt loams (NRCS, 2014). Annual precipitation for years 2006-2008 was 126.9 cm, and average annual temperature was 14°C (Choi and Rom, 2011a and b). Before planting, the area was tilled and leveled in 2005. Lime to adjust pH and 5 t ha⁻¹ of composted horse manure to add organic matter were mixed into soil, and a grass and winter wheat cover crop were planted (Choi and Rom, 2011a). Enterprise apple trees (*Malus domestica* Borkh.) on M26 rootstock were planted in 2006 in plots of three trees with 2 m between trees and 4 m between rows to achieve an approximate tree density of 1485 trees ha⁻¹ with guard trees lining the outside rows and orchard ends. Trees were trained on a 3.5-m tall vertical axis with a 2-wire trellis support system. Drive-row alleys between tree rows were perennially managed with established ‘Kentucky-31’ tall fescue (*Festuca arundinacea* Schreb.) and naturally growing herbaceous plants. Full description of orchard establishment and management is in Choi and Rom (2011a

and b). The orchard was managed following the National Organic Program Standards for the duration of the research (AMS, 2012).

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. Surface applied ground covers included compost, shredded paper, wood chips, and a mow-and-blow control. The compost obtained from the City of Fayetteville, AR until 2011 was vegetative waste (i.e., grass clippings, wood pruning, and yard waste) that was composted for 90 to 120 d. Beginning in 2012, compost was vegetative waste composted using an active-pile process obtained from PC Turnkey in Springdale, AR. Wood chips were obtained from the City of Fayetteville, AR and consisted of mainly chipped yard waste of leaves and branches of hardwood species. White 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, mowed and simultaneously blown under tree canopies with a side-discharge mower after seed head formation each spring and periodically throughout the growing season. 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 one of three nutrient treatments: locally available poultry litter, commercial organic fertilizer, or a no-fertilizer control. For the commercial fertilizer, a pelletized poultry manure (either 10N-2P₂O₅-8K₂O Nature Safe, Irving TX, or Perdue AgriRecycle, Seaford, DE) was used through 2011 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 to

a 450 g N per tree maximum (i.e. 100 g N in 2007 and 400 g N in 2013 per tree). The nutrient source treatments were applied uniformly under the tree canopy in a 2-m width band extending the entire three-tree plot. Average carbon (C), nitrogen (N), phosphorus (P), and potassium (K) concentrations for ground covers and fertilizers from 2006-2011 are shown in Table 1. Approximate amounts of biomass applied per square meter for each ground cover and nutrient source were calculated for years 2006 - 2008 from samples dried at 70 °C for three days. Amounts were multiplied by the fraction of C and N in the biomass after grinding to pass through a 20 mesh screen and analysis for % C and % N by dry combustion with LECO FP 428 and with LECO CN 2000 and the micro-Kjeldahl technique, respectively (Table 1).

2.2 Soil Sampling, Storage and Characterization

Soil properties averaged across the entire site at the beginning of the experiment in 2006 (n = 36) were pH equal to 6.6, an electrical conductivity (EC) of 73.8 $\mu\text{mhos cm}^{-1}$, bulk density of 1.34 g cm^{-3} , 1.5 % organic matter, 0.95 % C, 0.09 % N, 34 mg P kg^{-1} , and 170 mg K kg^{-1} . Soil temperature at 10 cm depth was measured around each tree at the time of sampling. Samples were collected in March 2007 and 2013 from 0-10 and 10-30 cm depths. Composite samples were obtained by sterilized soil probe from the center tree in a three-tree plot, randomly collecting eight cores at least 15 cm away from the trunk and within 60 cm between trees in a row and 45 cm between rows. Soil samples were stored in sterile bags on ice in the field and at 4 °C upon return to the laboratory, sieved aseptically through a 2-mm sieve, and stored moist at 4 °C with a subsample frozen at -76 °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 and pH of the soil were measured potentiometrically using 1:2 soil-to-water ratio. Organic matter (OM) content was determined using loss-on-ignition (6 hr at 550°C).

2.3 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 K₂SO₄, 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 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 (NO₃⁻-N), and ammonium-N (NH₄⁺-N) per g dry soil from unfumigated soil samples. Concentrations of NH₄⁺-N and NO₃⁻-N were determined colorimetrically using a Skalar segmented-flow autoanalyzer (Skalar Inc., Norcross, GA). The salicylate hypochlorite procedure was used to measure NH₄⁺-N (Mulvaney, 1996). Using a modified Greiss-Illosvay procedure, NO₃⁻-N was determined by utilizing Cd/Cu reduction of NO₃⁻ to NO₂⁻ (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).

2.4 PCR-DGGE

DNA was extracted from soil (~500 mg) using the NucleoSpin Soil DNA Extraction Kit (Clontech Inc., Mountain View, CA) according to the manufacturer's protocol. Extracted DNA was quantified using nano-drop spectrophotometry (ND2000; Thermo Fisher Scientific Inc., Waltham, MA), and all samples were normalized 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 GCG CCG CCC GCC CCG CCC CCG TCG CCC 3') (Throbäck et al., 2004). Reactions (25 μ L) contained a final concentration of 1 $\times$ PCR buffer (10 mM tris- HCl, 50 mM KCl, 0.01% (wt:vol) gelatin), 1.5 mM MgCl₂, 200 μ M each dNTP, 600 ng/ μ L BSA, 0.5 μ M of each primer, 1.25 units of Taq polymerase (GoTaq®; Promega, Madison, WI) and 1 μ L of 5 ng μ L⁻¹ template DNA for 0-10 cm samples, and 2 μ L of 5 ng μ L⁻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.

Amplification was confirmed by gel electrophoresis in 1.5% agarose gels stained with ethidium bromide and 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.

The PCR amplified products (~ 20 μ L) were loaded onto polyacrylamide DGGE gels for community profile creation. Protocols were modified for F1aCu:R3Cu from Throbäck et al. (2004). Vertical gels 1.0-mm thick containing 7% polyacrylamide (acrylamide:bisacrylamide ratio of 37.5:1) were electrophoresed in 1.5X TAE buffer (40 mM Tris-acetate and 1 mM 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 1:10,000 diluted SYBR Green solution and visualized using a Kodak EDAS 290 system and ID software package (Kodak, New Haven, CT).

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.

2.5 Data Analysis

Total band number per lane in DGGE gels represents *nirK* gene 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. Shannon-Weaver index of diversity (H) was calculated as

$$H = -\sum p_i \ln p_i \quad (1)$$

where p_i is the proportion of the taxon i in the community as represented by a DNA band at a given height (intensity) divided by the sum of all peak heights in the densitometric curve (i.e. peak heights for all the DNA bands in a gel lane per sample) as described in Wakil et al. (2008)

and Eichner et al. (1999). Shannon-Weaver index of equitability or evenness (J) was calculated as

$$J = H / \ln R \quad (2)$$

Range-weighted richness (Rr) was calculated according to Marzorati et al. (2008) using the equation

$$Rr = (R^2 \times D_g) \quad (3)$$

where R equals the total number of bands and D_g is the denaturing gradient between the first and the last band for a given sample (i.e. within a gel lane). Range-weighted richness 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 presence of DGGE bands within a gel lane was also used to create a similarity matrix containing pairwise similarity values for 0-10 cm depth samples for both 2007 and 2013 from Dice similarity coefficients (S) according to the equation

$$S = 2a / 2a + b + c \quad (4)$$

where a represents the number of bands in both lanes (samples) being compared (A + B), b represents the number of bands in sample A and c represents the number of bands in sample B.

From the similarity matrix, unweighted pair-group method with arithmetic means (UPGMA) was used to create a dendrogram with branch quality tested by cophenetic correlation in Gel Compar II of the *nirK* communities in order to visualize relatedness among samples (replicates, treatments and years; Applied Maths, Inc, Austin, TX).

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

diversity and soil variables. The 0-10 cm and 10-30 cm depths were analyzed separately. Interactions are presented only when they are significant ($P < 0.05$); otherwise, significant main effects only are presented. During the DGGE analysis, some replications in the 0-10 cm depth were excluded because of low quality results, resulting in unequal 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 (i.e. largest) LSD for each factor level was chosen to perform mean separations. 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. Thus, at the 10-30 cm depth, the effects of ground cover averaged across fertilizer and year on diversity are evaluated qualitatively.

3.0. Results

3.1.0. 0-10 cm Soil Depth

3.1.1. Ground Cover by Fertilizer Interactions

Range-weighted richness of the denitrifier (*nirK*) community (Tables 2, 3) was the only diversity parameter significantly affected by the three-way interaction of ground cover by fertilizer by time. In 2007, range-weighted richness in soils receiving compost alone or compost + poultry litter was significantly greater than wood chips alone or wood chips + poultry litter (Table 3). The wood chips + poultry litter and compost alone treatments were similar with the greatest range-weighted richness in 2013. Regardless of fertilizer, both compost and wood chip communities had greater range-weighted richness in 2013 than in 2007. In contrast, range-weighted richness did not significantly change in mow-and-blow or paper within a fertilizer treatment between 2007 and 2013.

Constructing a dendrogram from a similarity matrix allows visualization of changes in *nirK* communities that also depict the distinction over time. Communities in all treatments changed over time, but by 2013 *nirK* communities in wood chip and compost ground covers tended to cluster together with less consistent groupings by fertilizer (Fig. 1). These communities were then clustered with some of the samples from the paper followed by mow-and-blow. Except for compost in 2007, there was less distinct clustering in 2007.

The *nirK* richness, diversity, and evenness (Tables 2, 4), and DOC, microbial biomass C and N, OM, and temperature (Tables 4, 5) were all significantly affected by the two-way interaction of ground cover by fertilizer independent of (i.e. averaged across) sampling year. Regardless of fertilizer, compost increased *nirK* richness compared to the other three ground covers alone (Table 4). Commercial fertilizer increased richness in soil receiving wood chips and mow-and-blow, but decreased *nirK* richness in soil receiving compost. Poultry litter and commercial fertilizer increased *nirK* diversity (i.e. H) in wood chips, but not for other ground covers. The *nirK* evenness was similar among fertilizer treatments within a ground cover except for mow-and-blow where, although *nirK* richness increased, commercial fertilizer reduced *nirK* evenness.

Compost + poultry litter or compost + commercial fertilizer resulted in more DOC than any other treatment (Tables 4, 5). However, with compost, poultry litter had a negative effect on microbial biomass C concentration and soil OM, with smaller measurements than compost alone. Compared to the wood chips alone, wood chips + commercial fertilizer significantly increased soil DOC, microbial biomass C and N, and OM, and wood chips + poultry litter increased soil DOC, microbial biomass N, and OM, but not microbial biomass C. There were no differences in mow-and-blow or paper treatments in response to fertilizers with the exception of increased

microbial biomass N in paper + poultry litter and paper + commercial fertilizer compared to paper alone. 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 difference in soil temperature with fertilizer addition within a ground cover, and no consistent trend across ground covers.

3.1.2. Ground Cover Effects Averaged Across Fertilizer Treatments

All diversity parameters at the 0-10 cm depth were also significantly affected by the two-way interaction of ground cover and year regardless of fertilizer (i.e. averaged across the fertilizer treatments) (Table 2). The *nirK* richness increased significantly between 2007 and 2013 and was greatest in compost and wood chips (Table 6). Compost increased *nirK* community diversity compared to mow-and-blow and wood chips in 2007 and mow-and-blow and paper in 2013. Diversity in 2013 progressed from least to greatest in the order of mow-and-blow control $\leq$ paper $\leq$ wood chips $\leq$ compost, with diversity in wood chips significantly increasing from among the least diverse in 2007 to among the greatest in 2013. Diversity did not change from 2007 to 2013 in either paper or mow-and-blow treatments. The *nirK* community was more even in 2007 than 2013 with wood chips. Evenness did not change through time with other ground covers but compost and paper were more even than mow-and-blow and wood chips in 2013.

Similar to the responses measured using DGGE, ground cover treatments averaged across fertilizer had varying effects on DOC, DON, microbial biomass C, and OM depending on year (Table 5). Dissolved organic carbon concentration was about twice as great in compost in 2007 compared to the other ground covers (Table 6). By 2013, ground covers, with the exception of the mow-and-blow, increased DOC from the 2007 values, with the largest concentrations

continuing to be measured in the compost treatments. In contrast to DOC, DON decreased significantly through time from all ground covers regardless of fertilizer, except compost which resulted in greater DON than other ground covers in both years.

Organic matter increased over time in all treatments. Soil OM increased from 1.84 % in 2007 to the greatest overall OM of 5.29 % with compost. Conversely, OM in mow-and-blow treatments increased to a lesser extent and was significantly lower in OM in 2013 than soil receiving wood chips and compost. Microbial biomass C was smaller by about half in 2013 than 2007 in mow-and-blow and paper treatments, but did not vary by year with the addition of compost or wood chips.

There was a main effect of ground cover, but no significant interactions, on soil pH (Table 5), which ranged from 7.3 in the paper treatment, followed by 7.0 in compost, with lower and similar pH of 6.8 in soil under wood chips and the mow-and-blow treatments. Nitrate-N (NO_3^- -N) concentrations were also affected by the main effect of ground cover with concentrations greatest in compost ($4.6 \mu\text{g g}^{-1}$) and wood chips ($3.5 \mu\text{g g}^{-1}$). Nitrate-N concentration in the mow-and-blow soil ($2.5 \mu\text{g g}^{-1}$) was not different from soil receiving wood chips ($3.5 \mu\text{g g}^{-1}$), while nitrate-N concentration with paper ($1.2 \mu\text{g g}^{-1}$) was significantly less than with wood chips. The soil DNA concentration was significantly greater in compost ($8.57 \mu\text{g g}^{-1}$), mow-and-blow ($8.07 \mu\text{g g}^{-1}$), and wood chip ($7.54 \mu\text{g g}^{-1}$) ground covers compared to paper ($6.11 \mu\text{g g}^{-1}$). A main effect of year (Table 5) was observed for EC, water content, microbial biomass N, NH_4^+ -N, NO_3^- -N, and DNA concentration, which were all greater in 2013 than in 2007 (data not shown).

3.1.3. Fertilizer Impacts by Year

Fertilizer, averaged across ground cover treatments, had an enriching effect on the denitrifier (*nirK*) community that differed over time (Table 2). One application of commercial fertilizer increased *nirK* richness in 2007 compared to no fertilizer and poultry litter with values of 31 compared to 26 and 27, respectively. Richness increased with fertilizer over time, and in 2013 there were no differences among fertilizer treatments (ranging from 36 to 39).

3.2.0. 10-30 cm Soil Depth

3.2.1. Ground Cover by Fertilizer Interactions

Ground cover by fertilizer interactions had significant effects on DON and pH regardless of year (Table 5). Soil DON concentration averaged across years was greater without the addition of fertilizer within all four ground cover treatments (Table 7). Wood chips + poultry litter produced a greater DON concentration than wood chips + commercial fertilizer. A similar, although not significant, pattern of fertilizer effects on DON concentration occurred in the other ground covers. Soil pH was greatest in paper alone directly followed by paper + commercial fertilizer. Compost and paper applied with any fertilizer had greater soil pH than all wood chips and mow-and-blow treatments.

3.2.2. Ground Cover Effects Averaged Across Fertilizer Treatments

Although only 25 and 23 measurements were useable in 2007 and 2013, respectively, out of 36, limiting ability to detect significance in treatment effects, there were no apparent effects of ground cover or year on the diversity parameters of the *nirK* community calculated from the DGGE profiles (data not shown). Richness ranged from 24 to 35 in the 10-30 cm depth (data not shown); in comparison, *nirK* richness ranged from 25 to 45 in the 0-10 cm depth (Table 4). On

average, lower but not drastically different diversity index values were observed in the 10-30 cm depths. 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.

There was a two-way ground cover by year interaction on electrical conductivity (EC), pH, DOC, DON, and NO_3^- -N (Table 5). There were no differences in EC among ground cover treatments in 2007 (Table 8). Electrical conductivity increased over time in all ground covers and in 2013 EC was greatest in compost followed by wood chips, then paper, and finally the mow-and-blow control. The soil pH was similar across ground covers in 2007 and increased in compost, paper and wood chips treatments over time. Paper resulted in the highest pH in 2013, followed by compost and wood chips which were similar and greater in pH than the mow-and-blow.

Compost resulted in the only significant increase in DOC over time within a ground cover. There was both a 1-year and 7-year increase in DOC with compost compared to mow-and-blow and paper. In 2007, there were no differences in DON or NO_3^- -N concentrations. Compost, paper and wood chips all increased soil DON concentrations over time, but DON was greatest with compost in 2013. Compost followed by wood chips increased NO_3^- -N most through time and, in 2013, NO_3^- -N was greater than compared to mow-and-blow and paper.

There was a main effect of ground cover on OM content (Table 5) ranging in order from compost (1.78 %) $\geq$ wood chips (1.61 %) $>$ paper (1.56 %) $\geq$ mow-and-blow control (1.46 %). A main effect of year was observed for multiple soil properties. However, soil OM and ammonium-N (NH_4^+ -N) concentrations increased through time while water content, microbial biomass C and

N concentrations decreased, with microbial biomass C decreasing by $56.6 \mu\text{g C g}^{-1}$ between 2007 and 2013 (data not shown).

3.2.3. Fertilizer Impacts by Year

Fertilizer by time (independent of ground cover) had significant two-way interactions on DON and NO_3^- -N concentrations at the 10-30 cm soil depth (Table 5). In 2007 there were no differences among fertilizers in DON, which ranged from 3.77 to $3.93 \mu\text{g N g}^{-1}$, or NO_3^- -N, which ranged from 0.74 to $1.25 \mu\text{g N g}^{-1}$. In 2013, DON concentration was different with each fertilizer and was greatest in soil with no fertilizer at $27.63 \mu\text{g N g}^{-1}$, compared to 10.33 and $5.98 \mu\text{g N g}^{-1}$ averaged across poultry litter and commercially fertilizer, respectively. Nitrate-N trends were opposite of DON and increased with fertilizer in 2013; the no-fertilizer soil had a concentration of $2.53 \mu\text{g N g}^{-1}$ which was significantly lower than 5.03 and $4.08 \mu\text{g N g}^{-1}$ averaged across poultry litter and commercially fertilized treatments, respectively.

4.0. Discussion

Investigating the *nirK* community and soil conditions affecting denitrification offers insight into how repeated annual ground covers and nutrient source surface applications impacted the potential for denitrification in this organically managed orchard. Although there were several significant interactions, the strongest effects on soil properties were generally measured in the compost at the 0-10 cm depth in 2007 and compost and wood chips treatments at the 0-10 and 10-30 cm depths by 2013. Because of the limitations in amplification and separation of bands on a DGGE gel, care must be taken in interpretation of diversity indices as true representation of all microbial community members. There are limitations in thresholds of what

is amplified and detected on a DGGE gel; there is also the assumption that a band at each migration distance represents a unique sequence and thus a unique taxon within the community. Breakdown of representation of diversity was investigated using T-RFLP with various levels of community composition thresholds (Blackwood et al., 2007). However, non-parametric methods to analyze microbial communities can reveal relationships that may or may not complement results obtained using diversity indices. Clustering of *nirK* communities supported that compost was already differentiating the soil microbial community in 2007, after one year of additions, and that the two ground covers adding the most OM, compost and wood chips, were most similar by 2013 followed by paper, which was also showing several distinctions from mow-and-blow in other soil properties after seven years of ground cover and nutrient additions.

Effects of surface applied ground cover and nutrient amendments are expected to take time before being measured at depth. Impacts were limited below the 10 cm depth of incorporation of surface applied organic amendments to reclaim loss of topsoil (Larney et al., 2005). The exception of a transient increase in nitrate from alfalfa amendment at the 15-30 cm depth one year after application was not detected when sampled two years later (Larney et al., 2005). In a four-year study investigating the differences in integrated versus organic fruit management in the Northeastern U.S., differences in soil physical, chemical, and biological properties were mainly limited to the upper 15 cm (Peck et al., 2011).

The strong effects of wood chips and compost are consistent with previous studies in this orchard and with other ground cover management studies in orchards. Between 2006-2008, during orchard establishment, compost and wood chips added twice the organic C and more than twenty-six and ten times the amount of N to the systems as paper ground cover resulting in measurable increases in soil OM, and in early tree growth and N uptake (Choi and Rom, 2011a

and b). Tree growth in paper and mow-and-blow treatments was initially slower with a greater increase in leaf N use efficiency from year 2007 to 2008, although trees from those two ground covers did not bear fruit in year 2008 (Choi and Rom, 2011a and b).

Compost treatments, resulted in greater excess N compared to wood chip treatments (Choi and Rom, 2011b). TerAvest et al. (2010) applied ¹⁵N labeled composted poultry litter in combination with a wood chip mulch, legume cover crop, and tillage treatments in a sandy loam apple orchard soil in central Washington and observed the greatest benefits in N accumulation and uptake efficiency from wood chip additions. Wood chips provided better conditions for apple trees than legume cover crops, which outcompeted the new trees for nutrients (TerAvest et al., 2010). However, in a similar study on organic apple orchard management strategies, 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 retained moisture and caused short-term N immobilization, but also encouraged N loss through denitrification (Hoagland et al., 2008). Overall, there was increased tree growth, but not enough N uptake by apple trees as determined by foliar samples (Hoagland et al., 2008).

Care must be taken in extrapolating results and conclusions from this environment as there is diversity in the genes harbored and completeness of the denitrification pathway among denitrifying organisms. Each reduction in the denitrification pathway may be accomplished by a variety of organisms only if they carry the genes that code for the enzymes responsible for reductions, and different organisms do not respond to the environment similarly (e.g. Kandeler et al., 2006). Thus, the results for *nirK* harboring organisms may not represent the full community of denitrifiers (e.g. the *narG*, *nirS*, *nosZ* communities, Kandeler et al., 2006). However, the *nirK* community diversity and composition has not been studied extensively with DGGE in orchard

soil, although it has been investigated in agricultural row crop soil. Richness of *nirK* communities in potato field soil from a potato-spring wheat rotation in Canada was less than the *nirK* richness in the current study at the 0-10 cm depth; although, different primers were used during PCR which could affect *nirK* gene richness (Wertz et al., 2009). Diversity overlapped while richness was greater in this study compared to that in soil under a Broadbalk wheat experiment investigating the impacts of N management which was established 160 years ago (Clark et al., 2012). Thus, the organic apple orchard soil *nirK* community in this study appears to be relatively rich compared to row crop agricultural soil.

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. While all ground cover treatments increased organic matter over seven years of applications, compost resulted in greater *nirK* richness and diversity, OM, DOC, and DON compared to the mow-and-blow after only one application (in 2007). Considering the amount and physical density and chemical complexity, it is not surprising that compost was responsible for large increases in OM (Cayuela et al., 2004; Yao et al., 2005). Soil OM content of a peach orchard soil increased 169% after 9 years of annual applications of compost at 10 t ha⁻¹ yr⁻¹ (Baldi et al., 2010). Organic matter can also affect nitrogen cycling processes such as denitrification via the supply of substrates. Soil organic carbon content explained 76% and 53% of the variability of denitrification in annual crop/grassland rotation and till/no till rotation sites, respectively; water-filled pore space and nitrate also contributed to variability but to a lesser extent (Attard et al., 2011).

Dissolved organic C and N comprise the soluble fraction of OM that is physically available for use by microorganisms (Cook and Allan, 1992; Chantigny, 2003). Correlations

between DOC and *nirK* have been reported by others (Jin et al., 2014; Barta et al., 2010). The nitrate response followed the same trend as DOC and was also similar to trends in *nirK* diversity, greatest in compost and wood chip treatments followed by paper and mow-and-blow. The only treatment that did not increase through time in *nirK* richness was paper, possibly because, of the four ground covers, paper contributed the least nitrate-N. 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. Excess nitrate may be a concern when a large amount of compost is land applied, including in this orchard (Choi and Rom, 2011b).

Decomposition, nutrient cycling, and building soil organic matter, depend upon substrate quality in addition to quantity, the microbial community, and abiotic soil conditions. A determining characteristic of substrate quality is the composition of the materials being added (Bengtsson et al., 2003; Mungai and Motavalli, 2006); sugars, proteins, and starches are easily decomposed, while hemi-cellulose, cellulose, and lignin are increasingly difficult to decompose (Tisdall and Oades, 1982). Based on similar materials used by others, compost, wood chips, mow-and-blow, and paper might be expected to contain lignin contents approximately 39%, 26%, 12%, and 6.5%, respectively (Chen et al., 2002; Francou et al., 2008; Komilis and Ham, 2003). A greater lignin content would make compost followed by wood chips more difficult to decompose than mow-and-blow and paper.

The C:N ratios indicate quality of the substrate because microorganisms incorporate C, N, and other nutrients to create proteins and other key building blocks of their biomass during the decomposition process. The C:N ratio of microorganisms is 5:1-15:1; if N contained in the ground-cover amendment is not sufficient in terms of C, N will be immobilized from soil by microbial biomass (Nicolardot et al., 2001). Lower C:N residues (< 20:1) are considered better

quality resources. The highest rates of immobilization in grasslands from Texas to Montana have occurred in soils with greater C:N ratios (Barrett and Burke, 2000). In contrast, Huang et al. (2008) found that compared to no mulch, the addition of mulch to hardwood plantations in Australia increased OM, immobilization rates, and functional diversity. In response to mulch with a C:N of 98:1 in a New York orchard on a silty clay loam over 12 years, OM doubled, less N leached, respiration was greater, and bacterial counts increased; few differences were detected in the grass treatment except greater soil N retention compared to the herbicide treatment (Yao et al., 2005).

Combining a fertilizer with a larger C:N ground cover could increase decomposition by increasing the N available to the microorganisms (Singh and Gupta, 1977). However, less decomposition with N additions to litter have also been reported, resulting from biochemical interactions of N-containing compounds during decomposition (Hobbie, 2008; Fang et al., 2007). Fertilizers increased OM, DOC, microbial biomass C and N, *nirK* richness, and diversity in the soil receiving wood chips compared to wood chips alone. The addition of fertilizer to paper increased microbial biomass C and N when compared to paper alone suggesting that the total microbial community is growing; however, this did not correspond to increases in *nirK* richness or diversity. The total microbial community is not necessarily related to the *nirK* community because of temporal variability and microhabitat conditions (Kong et al., 2010), which may explain the lack of relationship between microbial biomass responses to treatments and *nirK* communities in general in this study.

An unexpected result of compost + poultry litter was the significant negative effect on OM and microbial biomass C and N compared to the compost alone. Trunk cross-sectional area in treatments with compost + poultry litter or compost + commercial fertilizer was also smaller

initially compared to compost alone in this orchard (Choi and Rom, 2011a). Tejada et al. (2006) added crushed cotton gin compost and poultry manure to a degraded soil in a semi-arid region of Spain, and while both treatments increased biological activities of the soil, there was a greater increase in microbial biomass and enzymatic activities with poultry manure compared to compost addition, presumably from more labile organic matter in poultry manure stimulating decomposition. The combination of compost + poultry litter has not been widely studied, so it is possible there is a synergistic effect when mixing the two and applying to soil; however, the mechanisms governing the unexpected results on OM and microbial biomass C and N obtained with compost + poultry litter are unclear from the current study and deserve future investigation.

Denitrification is dependent upon physical conditions and availability of OM, specifically DOC, and nitrate (Parkin, 1987), but denitrification rates and denitrifier abundances cannot always be strongly correlated to abiotic conditions (Dandie et al., 2008). In this study, the *nirK* community was richest and most diverse in the ground cover treatments with the greatest OM, DOC, and dissolved N concentrations. Total bacterial community richness and diversity has been strongly linked to soil pH (Fierer and Jackson, 2006), and denitrifiers are also positively correlated with soil pH (Hallin et al., 2009; Dandie et al., 2011). However, in this study soil pH was close to neutral in all treatments, suggesting pH is not limiting the *nirK* community composition, diversity or abundance in this study (Fierer and Jackson, 2006; Barta et al., 2010), and other factors such as DOC are most likely having a greater impact on *nirK* communities.

5.0. Conclusion

The *nirK* community richness and diversity as analyzed by DGGE were expected to be greatest and most similar in treatments where substrate availability and pH were most conducive

to denitrification. All ground covers increased organic matter over time, and all ground covers except mow-and-blow increased DOC over time, with compost resulting in the greatest increase in OM and DOC. There was more NO_3^- -N, NH_4^+ -N, and DON in 2013 than in 2007. The *nirK* analysis was impacted most by compost and wood chip ground covers, regardless of fertilizer. Compost was altering *nirK* community composition such that treatments within that ground cover were clustering in 2007 (one year after treatment initiation). All treatments changed over time, but ground cover amendments resulted in closer association of compost and wood chips followed by paper *nirK* communities in 2013 (seven years of treatment applications). These results, along with greater soil *nirK* richness and diversity in response to compost addition in 2007 and gradually to wood chip treatments indicate the potential for enhanced denitrification in organic apple orchards receiving compost and wood chips mulches.

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Fig. 1. Dendrogram to visualize relatedness among *nirK* communities created from similarity matrix calculated using Dice similarity coefficients of DGGE bands and tree topology formed using unweighted pair-group method with arithmetic means (UPGMA). Similarity between two closest relatives is shown by scale along the top of the figure and by number at each node (branch point). Taxa in tree branches represent replicates ($n = 2$ or 3) from 12 treatments of four ground covers by three fertilizers. Taxa names are indicated by ground cover – fertilizer – sampling year. Ground covers include compost, wood chips (WC), shredded paper (paper), and mow-and-blow (mow). Fertilizers include poultry litter (PL), commercial fertilizer (Comm), and a no-fertilizer control (None). Sampling years were 2007 and 2013.

Table 1. Nutrient contents of ground cover and fertilizer treatments applied in an organically managed apple orchard, Fayetteville, AR.

Treatment	C [†] ----- (%)-----	N	C:N	P ----- (%)-----	K ----- (%)-----	C ----- (kg/m ²)-----	N ----- (kg/m ²)-----
Compost	20.5	1.6	13.5	0.2	0.5	44.58	2.83
Wood Chips	29.7	0.7	39.2	0.1	0.3	54.01	1.19
Paper	36.8	0.2	205.9	0.0	0.0	23.02	0.11
Mow-and-Blow	40.0	2.2	15.8	0.3	1.5	1.29	0.08
Commercial Fertilizer	31.3	4.4	7.8	1.4	2.6	1.14	0.16
Poultry Litter	29.5	1.7	19.4	1.3	1.4	3.78	0.19

Data for each ground cover and fertilizer are averaged across 2006-2011 for % and are summed across 2006-2008 for kg/m².

[†]C is carbon; N is nitrogen; P is phosphorus; K is potassium (n = 6 for %; n = 3 for kg/m²).

Table 2. Analysis of variance (ANOVA) *P*-value summary of the effects of ground cover by fertilizer treatments over time and their interactions on soil diversity parameters at the 0-10 depth of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, 2007 and 2013.

Depth (cm)	Diversity Index	GC	Fert	GC*Fert	Year	GC*Year	Fert*Year	GC*Fert*Year
0-10	R [†]	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
	Rr	0.0004*	0.1327	0.0019*	<0.0001*	<0.0001*	0.0393*	0.0069*

[†]R is *nirK* richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; Rr is range-weighted richness (Marzorati et al., 2008).

* *P* < 0.05. Table entry is the actual *P*-value associated with the F-test for that effect.

Table 3. Range-weighted richness (Rr) calculated from DGGE profiles of communities affected by ground cover by fertilizer treatment interactions in the 0-10 cm soil depth of an organically managed orchard in Fayetteville, AR, 2007 and 2013 (n = 2 or 3).

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

[†]Different treatments followed by a similar lower case letter are not significantly different ($P < 0.05$). If the same treatment across years is followed by a different capital letter, it is significantly different ($P < 0.05$).

Table 4. Diversity parameters for the *nirK* community calculated from DGGE profiles in the 0-10 cm soil depth affected by ground cover by fertilizer treatments in an organically managed orchard in Fayetteville, AR (n = 4 - 6 for diversity parameters, 6 for other soil properties).

Ground Cover	Fertilizer	R [†]	H	J	DOC ^a (µg C g ⁻¹)	Bio C (µg C g ⁻¹)	Bio N (µg N g ⁻¹)	OM (%)	Temp (°C)
Compost	None	41a ^{††}	3.46a	0.93ab	157.5a	74.0ab	17.7a	3.98a	10.9de
	Poultry litter	36abc	3.37ab	0.94a	140.6ab	47.7cd	12.6bc	2.87bc	10.3e
	Commercial	35bc	3.37ab	0.95a	130.8b	63.2abcd	16.2ab	3.85a	11.0cde
Mow-and-Blow	None	25f	2.95de	0.92ab	36.0e	81.2a	14.5abc	1.96e	10.9de
	Poultry litter	27ef	3.00cde	0.92ab	50.2de	66.3abc	12.7bc	2.24de	11.5abcd
	Commercial	34bcd	3.02cde	0.85c	38.9e	66.6abc	13.3bc	2.11de	11.5abcd
Paper	None	30ef	3.14bcd	0.94a	81.4c	39.6d	10.7c	2.27de	11.5abcd
	Poultry litter	31cde	3.18bcd	0.93ab	86.5c	64.8abcd	15.6ab	2.55cd	11.1bcde
	Commercial	31cde	3.19bcd	0.93ab	83.4c	64.3abcd	15.5ab	2.34cde	10.9de
Wood Chips	None	27ef	2.83e	0.89bc	60.6d	48.3bcd	10.6c	2.21de	11.7abc
	Poultry litter	37abc	3.26abc	0.92ab	89.4c	59.3abcd	15.6ab	2.84bc	12.1a
	Commercial	37ab	3.31ab	0.93ab	88.0c	83.7a	18.1a	3.20b	11.7abc

[†]R is *nirK* richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; DOC is dissolved organic carbon; Bio C is microbial biomass carbon, Bio N is microbial biomass nitrogen, OM is organic matter, and Temp is temperature.

^{††} For each parameter, treatments followed by a similar letter are not significantly different ($P < 0.05$).

Table 5. Analysis of variance (ANOVA) *P*-value summary of the effects of ground cover and fertilizer treatments over time and their interactions on soil properties at the 0-10 and 10-30 cm depths of a silt-loam soil in an organically managed apple orchard, Fayetteville, AR, in 2007 and 2013.

Depth (cm)	Soil Property	GC [†]	Fert	GC*Fert	Year	GC*Year	Fert*Year	GC*Fert*Year
0-10	DOC ^{††}	<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
	DNA	0.0244*	0.3610	0.6423	<0.0001*	0.8007	0.9599	0.4713
	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
	Temp	0.0163*	0.9859	0.0046*	<0.0001*	0.0148*	0.0254*	0.0531
	H ₂ O	0.2032	0.9727	0.6848	0.0001*	0.4323	0.5941	0.4878
10-30	DOC	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
	DNA	0.0357*	0.5679	0.0826	<0.005*	0.6329	0.6306	0.3469
	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
	OM	0.0445*	0.8291	0.4186	<0.0001*	0.2399	0.9043	0.5339
	H ₂ O	0.0716	0.7834	0.5187	0.0001*	0.0679	0.9474	0.6543

[†]GC is ground cover and Fert is fertilizer source.

^{††}DOC is dissolved organic carbon, DON is dissolved organic nitrogen, NO₃⁻-N is nitrate-N, NH₄⁺-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 H₂O is soil-water content.

**P* < 0.05. Table entry is the actual *P*-value associated with the F-test for that effect.

Table 6. Diversity parameter indices for *nirK* communities calculated from DGGE profiles and soil properties in the 0-10 cm soil depth affected by ground cover in 2007 and 2013, in an organically managed orchard in Fayetteville, AR (n = 7 – 9 for ecological indices, 9 for other soil properties).

Ground Cover	Year	R [†]	H	J	Bio C ^a (μg C g ⁻¹)	DOC (μg C g ⁻¹)	DON (μg N g ⁻¹)	OM (%)	Temp (°C)
Compost	2007	32b B	3.30abc B	0.95a A	64.5abc A	73.9c B	9.20a A	1.84d B	14.4b A
	2013	43a A	3.50a A	0.93abA	58.8abc A	212.0a A	9.06a A	5.29a A	7.1d B
Mow-and- Blow	2007	27c B	2.95ef A	0.90bcA	93.4a A	32.5d A	4.72b A	1.12e B	15.9a A
	2013	31b A	3.04def A	0.89c A	49.3bc B	50.9cdA	0.55d B	3.08c A	6.8d B
Paper	2007	30bcA	3.13cde A	0.92abcA	75.1ab A	38.3d B	5.01b A	1.19de B	15.3a A
	2013	31b A	3.21bcd A	0.94a A	37.3c B	129.3b A	1.45c B	3.58bc A	7.0d B
Wood Chips	2007	22d B	2.89f B	0.94a A	63.2abc A	44.2d B	5.41b A	1.23de B	15.6a A
	2013	45a A	3.37ab A	0.89c B	64.3abc A	114.4b A	0.61d B	4.26b A	8.0c B

[†]R is *nirK* richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; Bio C is microbial biomass carbon, DOC is dissolved organic carbon, DON is dissolved organic nitrogen, and OM is organic matter.

^{††}For each parameter, different treatments followed by a similar lower case letter are not significantly different ($P < 0.05$). For each parameter, if the same treatment across years is followed by a different capital letter, it is significantly different ($P < 0.05$).

Table 7. Soil chemical and biological properties affected by ground cover by 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 [†] ($\mu\text{g N g}^{-1}$)	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

[†]DON is dissolved organic nitrogen.

^{††}Treatments followed by a similar letter for each property are not significantly different ($P < 0.05$).

Table 8. 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 [†] ($\mu\text{mhos cm}^{-1}$)	pH	DOC ($\mu\text{g C g}^{-1}$)	DON ($\mu\text{g N g}^{-1}$)	NO ₃ ⁻ ($\mu\text{g N g}^{-1}$)
Compost	2007	26.80e B	6.64bc B	35.57b B	4.39d B	1.77c B
	2013	215.02a A	6.96ab A	51.10a A	23.20a A	8.59a A
Mow-and-Blow	2007	16.10e B	6.38c A	27.24cd A	3.76d A	0.64c A
	2013	128.71d A	6.44c A	19.94d B	7.96cd A	1.44c A
Paper	2007	16.42e B	6.56bc B	26.64cd A	3.10d B	0.36c A
	2013	158.09c A	7.13a A	29.52bc A	15.05b A	1.01c A
Wood Chips	2007	16.18e B	6.30c B	30.94bc A	4.16d B	0.89c B
	2013	182.06b A	6.95ab A	30.71bc A	12.38bc A	4.61b A

[†] EC is electrical conductivity; DOC is dissolved organic carbon; DON is dissolved organic nitrogen; (NO₃⁻) is nitrate-N.

^{††} For each property, different treatments followed by a similar lower case letter are not significantly different ($P < 0.05$). For each property, if the same treatment across years is followed by a different capital letter, it is significantly different ($P < 0.05$).

Fig. 1.