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Denitrifier community response to seven years of ground cover and 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. 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

Abbreviations: DGGE, denaturant gradient gel electrophoresis; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DTN, dissolved total nitrogen; OM, organic matter.

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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 N_2 emissions were observed in the organically managed soils while the N_2O 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 (NO_3^-) to N_2, 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 (*nir*S), 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 *nir*S 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. 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, 2011b). 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, 2011b). The orchard was managed following the National Organic Program Standards for the duration of the research (AMS, 2012).

The design was a 4×3 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 90–120 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 (2×2 m^2) 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-2P_2O_5–8 K_2O 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 to 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 μmhos 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 to 10 and 10 to 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,