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Microbial communities in soil profile are more responsive to legacy effects of wheat-cover crop rotations than tillage systems

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ABSTRACT

Declining trends in soil health under continuous monoculture systems of winter wheat are a concern for sustainable production in the Southern Great Plains of the US. This study was conducted to evaluate the long-term implementation of conservation tillage in combination with nitrogen treatments and summer cover crop (cowpeas) rotations with winter wheat, for their legacy effects on soil health attributes of microbial communities and soil organic carbon (SOC). Microbial biomass and composition were estimated, along with soil physicochemical parameters in the soil profile during the annual rotation cycle of wheat and cover crops. Positive legacy effects of cover crop rotations were evident, as arbuscular mycorrhizal fungi (AMF) biomass during the wheat-growing season was significantly higher in cover crop treatments (by around 30-70%) compared to summer fallow treatment. Some dominant taxons such as *Acidobacteria, Actinobacteria*, *Proteobacteria* (> 70% of prokaryotic relative abundance) and *Ascomycota* (> 50% of fungal relative abundance) were detected in all experimental treatments. Microbial composition did not significantly change at phylum level, although some reorganization at OTU level was evident throughout the soil profile, mostly because of nitrogen treatments. Several *Glomeromycota* OTUs were significantly altered by soil depth and by nitrogen fertilization suggest distinct mycorhizosphere interactions in subsurface soil than the surface soil. Tillage treatment did not significantly alter the microbial abundance and their diversity. Differences in microbial biomass-C concentration among experimental treatments did not result in a change in SOC concentrations within the soil profile. Results of this study demonstrated that summer cowpea appeared to be an effective cover crop for enhancing beneficial microbial biomass and expansion of the mycorrhizosphere to deeper soil layers. Cover crop rotations appeared to be a suitable option for rapidly enhancing soil health in winter wheat production systems.

1. Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop cultivated around the world, and in some regions on more land than any other crops. Almost 8.2 million hectares were planted to winter wheat in the Southern Great Plains (SGP) of the US in 2015. Most wheat agriculture in the SGP, and around the globe, is a mono-crop production system, and relies on inorganic fertilizer inputs for nitrogen (N), using up almost 30% of all N fertilizers produced globally (Raun and Johnson, 1999). N use efficiency (NUE) in wheat is around 40-55% of applied N. Thus N fertilization has many environmental impacts as a substantial amount of applied N could be lost through leaching, ammonia volatilization, and NOx gas emissions (Cassman et al., 2002; Barraclough et al., 2010). N fertilization and repeated tillage in mono-cropping wheat systems have also depleted soil organic carbon (SOC), with subsequent reductions in soil health attributes, such as microbial diversity (Nielsen and Calderón, 2011). Repeated tillage facilitates oxidation through physical disruption, which increases mineralization and depletion of soil organic matter (Conant et al., 2007), and led to larger CO_2 emissions (Reicosky and Archer, 2007), whereas no-till systems are anticipated to increase organic matter and enhance microbial communities (Campbell et al., 1998; Peterson et al., 1998). No-till systems have also been noted to increase fungal biomass, as less disturbance minimizes hyphal breakage and damage to AMF spores (Frey et al., 1999; Helgason et al., 2010; Sharma-Poudyal et al., 2017). Similarly, cover crops instead of fallowing in summer can substantially add soil organic matter and also increase soil microbial communities (Black et al., 1981; Janzen, 1987; Wienhold et al., 2006). Additionally, including a legume

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cover crop such as cowpea as a green manure can increase soil N and nutrient use efficiency (Drinkwater et al., 1998; Tonitto et al., 2006). Legumes can also form symbiotic relationships with mycorrhizal fungi (Snapp et al., 2005), and in some studies increased abundance of specific group of mycorrhizal fungi and bacteria were observed in a maize-legume rotation (Bünemann et al., 2004; Mathimaran et al., 2007). Mycorrhizal infection increased in cereal grasses by integrating with a legume (Bagayoko et al., 2000; Horst et al., 2001). These sorts of below ground effects in continuous rotations are collectively called legacy effects. The positive legacy effects such as enhancing competent AMF diversity, root colonization, and improving microbial diversity could be beneficial for soil health and attaining higher agronomic yields. However, some legacy effects may not be desirable, such as replacing competent AMF by non-host cover crops and increasing nutrient immobilization and mineralization potential in subsurface soil.

Wheat producers are aware of sustainable practices for conserving soil health and biological diversity and the need for reversing organic matter loss (Drinkwater et al., 1998; McDaniel et al., 2014). If a wheat producer has to choose between one or a combination of sustainable practices, there are still knowledge gaps on comparable legacy effects. For example, it is not clear whether wheat-legume crop rotations will increase diversity of competent AMF diversity and biomass. It is not clear if increasing microbial diversity and biomass will impact residual soil nutrient pools, as some functional groups such as denitrifiers can significantly reduce N use efficiency. It is also critical to understand how changes in microbial communities will impact the SOC levels at the whole profile level as cover crops with their deep root systems can increase subsurface microbial activity (Stone et al., 2014), which may enhance or diminish SOC storage depending on microbial community composition and growth strategies (Liang et al., 2017).

Our general hypothesis was that the combination of no-tillage and summer legume crop rotation would enhance microbial biomass and SOC in both surface and subsurface soil profile compared to the individual treatments. We also hypothesized that the legacy effects of these practices would increase the arbuscular mycorrhizal fungi (AMF) biomass during the growing season of winter wheat. The objectives of the study were to quantify microbial groups, their abundance, and composition in relation to soil parameters in the soil profile, as influenced by long term implementation of tillage practices and inorganic and organic-N fertilization through green manure crop rotations.

2. Methods

2.1. Experimental background and layout

The study site was located at the USDA-ARS Grazinglands Research Laboratory near El Reno, OK (35.573326, −98.036166). The growing season of winter-wheat for the current experiment was defined as late-September (planting) through June (grain harvest), combined with a June through August period when either a summer fallow or legume treatment was applied. This experiment consisted of a set of three rows of 8 plots receiving different N treatments (only two used for this study) that were randomly assigned to plots (Figure S4). Each N treatment plot was assigned within a completely randomized design, with a split in each plot related to tillage system. There were 6 N treatments with 4 replicated plots receiving each N treatment (n = 24), with each split based on tillage system (n = 48). For this study, we sampled from three N treatments; summer fallow receiving either no-N or 90 kg N/ha (inorg-N) applied each September and the other treatment is wheat-legume rotation (org-N), cowpea grown for green manure during summer, with no inorganic N applied. The split plots of two tillage regimes were conventional (disked and rototilled) tillage (CT) or no-till (NT) systems.

The predominant soil series of the lowland terrace site was defined as Brewer silty clay loam (Fine, mixed superactive, thermic Udertic argiustolls), 0 to 1% slope, with deep moderately well-drained profiles with loamy surface layers and loamy to clayey subsoil (USDA-NRCS, 1999).

The N source and tillage combinations were initiated in 2011, and maintained on assigned plots and subplots in all years through 2017. After wheat harvest in early-June, no-till sub-plots were sprayed with glyphosate and conventional till plots were disked, to control weeds. Plots planted to legumes were fertilized annually with 26 kg P ha^{-1}. Legumes were inoculated with recommended Rhizobia and sown (2 cm deep at 25 kg ha^{-1}) immediately after wheat harvest, and grown 75 d.

Legume biomass on plots was shredded by flail mower in mid-August (~75 d after planting), left on the soil surface in subplots receiving no-till, and incorporated into conventionally tilled sub-plots to ~4 cm deep by disking. Winter wheat was sown (100 kg seed ha^{-1}) in late-September, and plots without legumes received their assigned inorganic fertilizer treatment. The tillage system - N treatment combinations were maintained on their original assigned plots throughout the 5 years of the experiment. The different applied nitrogen levels of no-N, inorg-N (90 kg ha^{-1}) and org-N (legume green manure) provided the potential for a range of N fertility from background amounts to the amount recommended for the soil of the study site (90 kg ha^{-1}) to achieve optimum grain yield by wheat.

2.2. Soil sampling and preparation

Soil samples were collected during 2016–2017 growing season of wheat-cowpea rotation, which included three times during the growing seasons of wheat: 2 weeks after planting in fall (Sept 23rd), middle of spring regeneration of wheat biomass (March 20th) and 2 weeks before harvest (May 30th). Additional sampling was done in summer 2 weeks before cowpea termination (July 20th). A minimum of three samples were collected from each treatment replicate, randomly from center rows. A hydraulic probe (Giddings Machine Company Inc.) was used to collect soil cores from the 0-60 cm depth, using plastic liners to avoid contamination and transported on ice (4 °C) to the laboratory. Soil cores were split into three depths of approximately (0-20, 20-40 and 40-60 cm), mixed, and subsamples for microbial and biochemical analysis were immediately frozen at −80 °C. A subsample was then freeze-dried and used for C/N and PLFA analysis.

2.3. Plant sample collection & analysis

Wheat biomass was collected (n = 2 × 0.25 m^2 quadrats per subplot) in November (vegetative stage), March (elongation of first hollow stem), late-April (flowering), and June (grain set) to define biomass at key times of growing seasons, and grain production. Whole plant samples were collected (n = 3 × 0.25 m^2 quadrats), and dried to constant weight to define aboveground biomass.

2.4. Soil chemical analysis

Approximately 0.5 g of freeze dried soil was used for determining total soil organic carbon (SOC) and nitrogen (SN), determined by a dry combustion C/N analyzer (Elementar Inc.). Additionally, a separate portion of soil was used for determining water extractable organic carbon (WeOC) and nitrogen (WeN) (labile fractions), determined by wet combustion NPOC/NPN analyzer (Shimadzu Inc.). For WeOC and WeN estimation, 10 g of freeze dried soil was shaken in 50 mL DI H_2O for 1 h, allowed to settle for 5 min, and decanted into a 50 mL centrifuge tube. Samples were centrifuged at 2000 rpm for 5 min to sediment suspended soil particles. Samples were then filtered using Whatman 42 into 20 mL scintillation vials containing 1 drop of 12 M HCl. Filtered samples were capped immediately and refrigerated prior to analysis (within 24 h), to maintain sample integrity. Measurements of bulk density of soil were used to convert percent carbon and nitrogen to an amount per unit area basis. Soil pH was measured in a 1:2 soil:water suspension. Soil moisture was determined in individual soil samples