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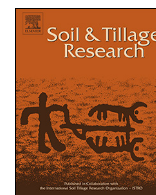

Bacterial and archaeal ammonia oxidizers respond differently to long-term tillage and fertilizer management at a continuous maize site

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ABSTRACT

Fertilizer use and tillage affect both the general soil microbial community and specific N-utilizing microbial groups, but likely to varying degrees. To assess these impacts, soil was collected on three key dates from a long-term (26+ years), rainfed, continuous maize site where tillage (no-till and disk) and N fertilizer rates (0, 40, 80, 120, and 160 kg ha^{-1} yr^{-1}) were applied. Microbial community changes were assessed using fatty acid methyl esters (FAME), and ammonia oxidizer (AO) changes were followed using quantitative PCR. Ammonia-oxidizing archaea (AOA) greatly outnumbered ammonia-oxidizing bacteria (AOB) based upon *amoA* gene copy (10^8 versus 10^4 g^{-1} soil, respectively), and both populations were dynamic across sampling dates. Over the long term, tillage had a differential effect; AOB were almost 10-fold lower in disk tillage plots, but AOA did not differ. N rate had no effect on ammonia-oxidizers abundance, but a moderate correlation (r = 0.423) between AOB and N rate was observed. AOA correlated moderately (r = 0.552) with water-filled pore space. In contrast, the biomass of various microbial groups was highly responsive to sample date, tillage, and N rate. This study found that long-term agronomic practices affected the overall microbial community more than the AO community, while short-term changes over a growing season were surprisingly dynamic for both AO and select groups within the general soil community.

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1. Introduction

In response to economic forces, management innovations, and a greater awareness of potential negative environmental consequences, modern crop production has evolved from intensive tillage (plowing and disking) and fertilization practices to reduced tillage and highly-conserved fertilizer use (Govaerts et al., 2009; Palm et al., 2014). The benefits include lower costs for producers and better natural resource management. This shift in management paradigm towards reduced tillage has led to changes in soil physicochemical and microbiological properties including a redistribution of soil organic matter and associated microorganisms towards the soil surface as well as positive changes in mean aggregate size and stability (Blanco-Canqui et al., 2014; Drijber et al., 2000; Six et al., 2006; Varvel and Wilhelm, 2010). Nitrogen fertilization is a key input for modern agriculture and has significant direct (e.g. pH, osmotic potential, soil solution chemistry), and indirect (e.g. plant diversity and productivity, carbon cycling) effects on microbial community structure and function (Börjesson et al., 2012; Geisseler and Scow, 2014; Wakelin et al., 2008). These agronomic practices may also affect microbial processes directly involved in nitrogen cycling, such as nitrification and denitrification (Groffman, 1985; Staley et al., 1990; Wessén et al., 2010), but limited information is available on how changes in these N-utilizing communities relate to changes within the larger soil community.

A variety of agronomic practices including, reduced N fertilization, minimal tillage, and the use of nitrification inhibitors, help to reduce N loss from cropping systems. Most N_2O losses result from the conversion of ammonia to nitrate, controlled by nitrifying microorganisms, or through incomplete denitrification (Baggs, 2011). In the initial nitrification step, ammonia oxidizing bacteria and archaea (AOB and AOA, respectively) in the soil

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convert ammonia to nitrite via the ammonia monooxygenase gene (*amoA*). Thus, the *amoA* gene as a molecular marker for archaeal and bacterial ammonia oxidizers has been developed (Treusch et al., 2005; Venter et al., 2004) and applied widely to investigate ammonia oxidation in a variety of systems. Molecular ecological studies quantifying AOB and AOA *amoA* abundance found that AOA usually dominate AOB in soils (He et al., 2007; Leininger et al., 2006; Nicol et al., 2004), with many factors influencing AOA and AOB abundance including soil pH, temperature, NH_3 concentration, and vegetation type (Avrahami and Bohannan, 2007; Di et al., 2009; Gubry-Rangin et al., 2010; Wertz et al., 2012). Varying relationships have been observed between AOB and AOA abundance and soil pH (He et al., 2007; Nicol et al., 2008; Shen et al., 2008; Yao et al., 2013), which is strongly influenced by ammonium-based fertilizer application (Bolan et al., 1991). However, N fertilization affects many other plant-soil characteristics, such as plant productivity, nutrient availability, soil aggregation and carbon sequestration, all important determinants of the soil microbial habitat (Blanco-Canqui et al., 2014; Wakelin et al., 2008; Young and Ritz, 2000). In a recent study assessing AOA and AOB abundance in over 700 soils of Scotland, Yao et al. (2013) found abundances were highly related to land use, suggesting multiple habitat drivers. Only 13–16% of the total variance was explained by pH and substrate concentration, with higher positive correlations between AOB abundance and pH than for AOA (Yao et al., 2013). Relationships between ammonia oxidizer (AO) groups and tillage are not as clear; however, a recent study found a strong tendency ($P < 0.1$) for higher AO abundance in no-till plots compared to conventional tillage (Munroe et al., 2016).

This research study examines the effect of tillage (no-tillage and disk tillage) and N fertilizer application rates (0–160 kg N ha^{-1} yr^{-1}) on both specific AO groups and the larger soil microbial community at a long-term field site continually cropped to maize since 1986. We used fatty acid methyl ester analysis (FAME) analysis to provide insight into microbial community composition (Drijber et al., 2000; Wortman et al., 2013) and quantitative PCR of the *amoA* gene to quantify the abundance of both AOA and AOB over the growing season. We hypothesized that AO microorganisms would be more responsive to long term N fertilization (though its effect on soil pH and AO substrate availability) and show less seasonal dynamics than the general soil microbial community. Tillage, through impacts on microbial habitat, mineralization processes and fertilizer distribution would be more pronounced for specific groups (e.g. fungi) within the general soil microbial community compared to the AO community.

2. Materials and methods

2.1. Field site

Soil samples were collected at the University of Nebraska Haskell Agricultural Laboratory near Concord, NE (latitude 42° 23″ N and longitude 96° 59″ W). The study site has a mean seasonal temperature of 17.5 °C and average annual rainfall of 670 mm. The soil series is Coleridge silty clay loam (fine-silty, mixed, superactive, mesic Cumulic Haplustolls) with some Baltic silty clay (fine, smectitic, calcareous, mesic Cumulic Vertic Endoaquolls) and Maskell loam (fine-loamy, mixed, superactive, mesic Cumulic Haplustolls). This rainfed cropping study was established in 1985 with a randomized block, split–split plot design with blocks replicated four times. Tillage (no-tillage, disk tillage and moldboard plow) was the main plot, crop rotation (continuous maize [*Zea mays* L.] vs maize soybean [*Glycine max* (L) Merr.] rotation) was the sub-plot, and N-rate (0, 40, 80, 120, and 160 kg ha^{-1} yr^{-1}) was the sub-sub-plot. For this study, we sampled two tillage treatments (no-tillage and disk tillage) under monoculture maize and all five N-rates. Nitrogen fertilizer was applied to maize as ammonium nitrate, which was broadcast before planting. The disk tillage treatment consisted of disking in spring to a depth of 10 cm before and after fertilizer application. Plot treatments (fertilization and tillage) occurred on May 2, 2012, and maize crops were planted one week later on May 9, 2012. Soil organic C ranged between 22 and 28 g kg^{-1}, and increased with N rate only in the surface 0–7.5 cm depth and was not affected by disk tillage (Blanco-Canqui et al., 2014). Maize residue yields increased from 5.4 to 7.7 Mg ha^{-1} as N rate increased from 0 to 160 kg ha^{-1} yr^{-1}. Additional site and management details are given in Blanco-Canqui et al. (2014).

2.2. Soil sampling

Soil samples (n = 120) were collected from continuous maize plots in 2012 over three key dates during the growing season; preplanting (May 1, 2012), directly after planting (May 14, 2012) and mid-season (July 6, 2012). Growth stage measured on July 11, 2012 showed maize plants at V12. Six soil cores (5 cm diameter) were collected in each plot to a depth of 15 cm and combined to form one plot composite sample (40 plot composite samples were collected on each sample date). Samples were transported back to the lab and refrigerated at 4 °C until processed. Samples were sieved (4 mm) and separated into air-dried samples (for pH and electrical conductivity (EC), analysis) and frozen samples stored at −20 °C (for FAME analysis and DNA analysis). Frozen soil samples for DNA analysis were subsequently freeze-dried overnight and stored at −20 °C prior to extraction. Soil pH and EC were analyzed on 10 g of air-dried soil using a 1:1 soil-water slurry, and measured using a SympHony pH/EC meter. Water-filled pore space (WFPS) was calculated from a mean bulk density of 1.30 Mg m^{-3} for the 0–15 cm depth (Martellotto, 2010), a particle density 2.65 Mg m^{-3} and gravimetric water content at time of sampling. Bulk density at this site was unaffected by tillage and N rate except for a slight decrease at the highest N rate (Martellotto, 2010).

2.3. DNA extraction and quantitative PCR

DNA was extracted from approximately 500 mg of freeze-dried soil using MoBio Ultra Clean® soil DNA isolation kits (San Diego, CA) according to the manufacturer's 'Alternative Protocol for maximum yields' using the MoBio Vortex Adapter tube holder (MoBio Catalog No. 13000-V1) and the suggested vortex mixing and incubation protocol to reduce DNA shearing. Briefly, shearing in this protocol was reduced by vortex mixing of repeated 3–4 s of vortex mixing interspersed with 5 min incubations at 70 °C compared to a single 10 min vortex mixing step. Purified DNA extracts (50 μL) were stored at −20 °C.

The *amoA* genes of AOB and AOA were amplified in all 120 soil DNA extracts using quantitative real-time polymerase chain reaction (qPCR) assays. Prior to qPCR, each extract was diluted 1:10 in sterile H_2O to overcome any residual PCR inhibition. A 491-bp fragment of AOB *amoA* was amplified using forward primer amoA-1F (5′-GGGGTTTCTACTGGTGGT-3′) and reverse primer amoA-2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′) (Rotthauwe et al., 1997). A 635 bp fragment of AOA *amoA* was amplified using forward primer amoA19F (5′-ATGGTCTGGCTWAGACG-3′) (Leininger et al., 2006) and reverse primer amoA643R (5′-TCCCACTTWGACGCGGCCATCCA-3′) (Treusch et al., 2005). All samples for qPCR were quantified in triplicate with Sybr Green I fluorescent dye to quantify amplification in each reaction. Reactions were carried out in 20 μL reactions with 5 μL of DNA template (1–10 ng), 0.2 μL of each forward and reverse primer, 4.6 μL of H_2O and 10 μL of Qiagen QuantiTect SYBR Green PCR Master Mix (Venlo, Limburg, The Netherlands). All qPCR were carried out using an Eppendorf Mastercycler realplex[4] (Hamburg,

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