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Impacts of different N management regimes on nitrifier and denitrifier communities and N cycling in soil microenvironments

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ABSTRACT

Real-time quantitative PCR assays, targeting part of the ammonia monooxygenase (*amoA*), nitrous oxide reductase (*nosZ*), and 16S rRNA genes were coupled with ^{15}N pool dilution techniques to investigate the effects of long-term agricultural management practices on potential gross N mineralization and nitrification rates, as well as ammonia-oxidizing bacteria (AOB), denitrifier, and total bacterial community sizes within different soil microenvironments. Three soil microenvironments [coarse particulate organic matter (cPOM; >250 μm), microaggregate (53–250 μm), and silt-and-clay fraction (<53 μm)] were physically isolated from soil samples collected across the cropping season from conventional, low-input, and organic maize–tomato systems (*Zea mays* L.–*Lycopersicum esculentum* L.). We hypothesized that (i) the higher N inputs and soil N content of the organic system foster larger AOB and denitrifier communities than in the conventional and low-input systems, (ii) differences in potential gross N mineralization and nitrification rates across the systems correspond with AOB and denitrifier abundances, and (iii) *amoA*, *nosZ*, and 16S rRNA gene abundances are higher in the microaggregates than in the cPOM and silt-and-clay microenvironments. Despite 13 years of different soil management and greater soil C and N content in the organic compared to the conventional and low-input systems, total bacterial communities within the whole soil were similar in size across the three systems ($\sim 5.15 \times 10^8$ copies g^{-1} soil). However, *amoA* gene densities were ~2 times higher in the organic (1.75×10^8 copies g^{-1} soil) than the other systems at the start of the season and *nosZ* gene abundances were ~2 times greater in the conventional (7.65×10^7 copies g^{-1} soil) than in the other systems by the end of the season. Because organic management did not consistently lead to larger AOB and denitrifier communities than the other two systems, our first hypothesis was not corroborated. Our second hypothesis was also not corroborated because canonical correspondence analyses revealed that AOB and denitrifier abundances were decoupled from potential gross N mineralization and nitrification rates and from inorganic N concentrations. Our third hypothesis was supported by the overall larger nitrifier, denitrifier, and total bacterial communities measured in the soil microaggregates compared to the cPOM and silt-and-clay. These results suggest that the microaggregates are microenvironments that preferentially stabilize C, and concomitantly promote the growth of nitrifier and denitrifier communities, thereby serving as potential hotspots for N_2O losses.

1. Introduction

Managed ecosystems have received much attention for their role in nitrogen (N) cycling as the increased rate of mineral N fertilizer application to crop production systems significantly contributes to the global increase of nitrous oxide (N_2O) emissions (Mosier et al., 1998) and N leaching (Foster et al., 1986). A better mechanistic understanding of nutrient cycling within cropping systems – both conventional and alternative – is necessary to promote long-term crop management practices that enhance agroecosystem services, such as, carbon (C) storage, N retention, and mitigation of greenhouse gas emissions.

Agricultural management practices (e.g., tillage, organic amendment addition, cropping rotation, and irrigation) can strongly influence the size, composition, and activity of the microbial community in soils (Beare et al., 1992; Bossio et al., 1998; Frey et al., 1999). For example, agricultural practices, such as mineral N fertilizer application, significantly alter ammonia-oxidizing bacteria (AOB) populations, which subsequently impact nitrification and production rates of nitrous oxide (N_2O), a potent greenhouse gas, in crop production systems (e.g., Kowalchuk and Stephen, 2001). Cropping

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systems that receive high organic matter inputs have been characterized by greater microbial activity as well as greater N mineralization compared to systems receiving only mineral fertilizer-N (Gunapala and Scow, 1998; Kramer et al., 2002). However, if organic matter-C additions occur prior to the peak in N demand by the crops, then this can lead to increased microbial immobilization of NO_3^- (Recous et al., 1990; Sakala et al., 2000), which can reduce N losses from the system via leaching and/or denitrification (Robertson, 1997; Scow, 1997). Consequently, the interactions between nutrient management practices and microbial-mediated N dynamics need to be better understood in order to optimize trade-offs between greater soil organic matter levels and potential N losses in agroecosystems.

Characterizing microbial communities associated with physico-chemically and spatially heterogeneous soil microenvironments may reveal the microbial communities responsible for important ecosystem processes and elucidate how microbial processes scale up from the soil to the ecosystem level. In a comparison between aggregated and unaggregated soils, Sexstone et al. (1988) suggested that the spatial separation of available C, NO_3^-, and denitrifying bacteria was the major factor limiting denitrification in macroaggregated soil. Additionally, Seech and Beauchamp (1988) have shown that denitrifier population sizes and activity increase as the size of aggregates decrease. Contrastingly, others have shown that denitrification (i.e., N_2O production) is positively correlated with intact aggregate size (Drury et al., 2004; Miller et al., 2009). Molecular analyses have shown that the microaggregate environment selects for a microbial community dominated by *Actinobacteria* and higher bacterial abundance than the communities found on the outside of the microaggregate and those associated with macroaggregates (Ranjard et al., 2000; Mummey et al., 2006).

Recent advances in environmental molecular biology techniques have enabled researchers to investigate the relationship between soil management, microbial communities, and ecosystem processes, especially pertaining to the N cycle. For example, by comparing the relative contributions of nitrification and denitrification to total N_2O emissions measured from a ^{15}N incubation experiment with assessments of nitrifier and denitrifier community composition and abundance, Ma et al. (2008) found nitrification was the primary source of N_2O emissions in wetland soils and that cultivation increased nitrifier but not denitrifier abundance. Avrahami et al. (2002) combined N_2O flux measurements with community assays of the *amoA* and *nirK* genes and found, while greater ammonium additions increased nitrification and subsequent N_2O release rates, that the community structure of the ammonia oxidizers was not changed. Relative abundances of terminal-restriction fragments (TRFs) of amplified *nirK* fragments increased, however, with increasing ammonium additions.

Agricultural soils receiving different C and N input, in terms of quality and quantity, are expected to differ in rates of microbial-mediated N transformations and C cycling. The aims of this study were to (i) examine the responses of AOB, denitrifier, and total bacterial abundances to differences in long-term N management in *conventional* (annual mineral fertilizer applications), *low-input* (mineral fertilizer and cover crop applied in alternating years), and *organic* (annual manure and cover crop additions) cropping systems across one growing season; and (ii) determine whether AOB and denitrifier densities are related to short-term N mineralization and nitrification rates across different soil microenvironments. We addressed these objectives in a long-term agricultural site where Kong et al. (2005) found significantly higher soil C stocks and greater soil aggregation in the organic compared to the conventional and low-input maize–tomato systems (*Zea mays* L.–*Lycopersicum esculentum* L.)]. We hypothesized that (i) the higher N inputs and soil N content of the organic system foster larger AOB and denitrifier communities than in the conventional and low-input systems, (ii) differences in potential gross N mineralization and nitrification rates across the systems correspond with AOB and denitrifier abundances, and (iii) *amoA*, *nosZ*, and 16S rRNA gene abundances are higher in the microaggregates than in the cPOM and silt-and-clay microenvironments. Real-time quantitative polymerase chain reactions (qPCR) were combined with ^{15}N isotopic pool dilution assays to test our hypotheses.

2. Materials and methods

2.1. Study site, experimental design, and field operations

The field study took place on the Long Term Research in Agricultural Sustainability (LTRAS) plots at the Russell Ranch experimental site (Davis, CA, USA; 38°32′24″ N 121°52′12″ W), which is located in a region characterized by wet winters and hot, dry summers. Two soil types dominate the site: i) Yolo silt loam (fine-silty, mixed, nonacid, thermic Typic Xerorthent) and ii) Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralf). The field study was conducted during the 2006 maize growing season in three maize–tomato (*Z. mays* L.–*L. esculentum* L.) cropping systems ($n = 3$): *conventional* (annual synthetic fertilizer applications), *low-input* (synthetic N fertilizer applied in alternate years with cover crop-N incorporated the years without synthetic N fertilization) and *organic* (annual composted manure and cover crop additions) (see description in Table 1). Since 1993, these maize–tomato cropping systems have been arranged in a completely randomized design with three 0.4 ha replicates under furrow irrigation and conventional tillage.

During the winter cover crop (hairy vetch: *Vicia dasycarpa*) growing season, microplots (1.0 × 1.0 m) were established in each cropping system replicate (3 treatments × 3 replicates = 9 microplots). In the final week of April 2006, the winter cover crop biomass in the microplots was mowed and roto-tilled into the microplots to a depth of 15 cm. Maize was direct-seeded into the conventional and then into the low-input and organic plots in the second and final weeks of May, respectively. At the start of May, composted manure was incorporated into the organic cropping system at a rate of 373 kg N ha^{-1}, while in the first week of June, the conventional system received 51 kg N ha^{-1} as N–P–K starter and 170 kg N ha^{-1} as

Table 1
Distribution of soil microenvironments and total soil nitrogen (N) levels after cover crop incorporation (T_i sampling event) and descriptions of N inputs to the conventional, low-input, and organic maize-tomato systems. Lower-case letters indicate significant differences associated with a soil microenvironment effect on the proportions of soil microenvironments across the cropping systems ($p < 0.05$). Total soil N assigned with different upper-case letters are significantly different at the $p < 0.05$ level.

Cropping System	Distribution of Soil Microenvironments			Total Soil N (Mg ha^{-1})	N inputs	
	cPOM (>250 μm)	Microaggregate (53–250 μm) (%)	Silt-and-clay (<53 μm)		Even Years of Cropping	Odd Years of Cropping
Conventional	1.3[c]	40.7[b]	58.0[a]	1.41[B]	NH_4–NO_3 fertilizer	NH_4–NO_3 fertilizer
Low-input	2.0[c]	33.0[b]	64.7[a]	1.33[B]	winter legume cover crop	NH_4–NO_3 fertilizer
Organic	2.3[c]	36.0[b]	61.7[a]	1.85[A]	winter legume cover crop and composted manure	winter legume cover crop and composted manure