Fertility practices and rhizosphere effects alter ammonia oxidizer community structure and potential nitrification activity in pepper production soils

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ABSTRACT

Increasing nitrogen use efficiency is critical to the productivity and long-term sustainability of vegetable production systems in the US Midwest. Understanding the impact of alternative fertility sources on the ecology of the soil nitrogen cycle has potential to allow for management that will increase crop N uptake and reduce loss. We determined how repeated applications of urea, composted chicken litter, hairy vetch green manure with alfalfa meal, and an unfertilized control affected the structure and potential nitrification activity (PNA) of ammonia oxidizers in bulk and rhizosphere soil when N needs were expected to be high. PNA was greater in animal and green manure treatments relative to urea and the unfertilized control in bulk soil, and greater in the rhizosphere relative to bulk soil regardless of fertility treatment. A strong correlation was observed between PNA and ammonia oxidizing bacteria (AOB) abundance, suggesting that AOB rather than ammonia oxidizing archaea (AOA) controlled nitrification in this system. However, AOB abundance did not differ between bulk and rhizosphere soil, and PNA was lower in the urea treatment despite greater AOB abundance indicating that other factors could have affected PNA. For example, greater availability of labile carbon (C) could have stimulated PNA through various mechanisms, and lower pH and/or specific nitrification potential per AOB cell could have reduced PNA in the urea treatment. AOB community structure was more diverse in all fertility treatments relative to the unfertilized control in bulk soil, and community structure differed between bulk and rhizosphere soil indicating niche differentiation. However, differences in AOB community structure and PNA were only observed in rhizosphere relative to bulk soil indicating that the rhizosphere had a greater effect on nitrification dynamics than fertility practices. These findings indicate that organic fertility amendments stimulate PNA, but they could also increase N loss and should be investigated further. The rhizosphere appears to play a greater role in nitrification dynamics than fertility practices, and more detailed investigations at this key plant-soil interface are warranted.

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Introduction

Demand for fresh, locally sourced vegetables is growing rapidly in the US (Timmons and Wang, 2010), resulting in increased production in non-traditional areas like the Midwest which is currently dominated by cereal crops. Nitrogen (N) is the most limiting nutrient in vegetable crops and growers apply substantial amounts of fertilizer to meet plant needs. Only 50% of fertilizer N is generally utilized by most crops (Smil, 1999), however, and this could be even lower in vegetables because they have less extensive root systems and are more intensively managed than cereals. For example, Zhu et al. (2005) found that only 10% of fertilizer N was recovered in aboveground pepper biomass, and 52% was lost from the soil–plant system. Fertilizer N not recovered by crops is subject to loss via nitrate (NO_3^-) leaching and evolution of nitrous oxide (N_2O), a potent greenhouse gas. Vegetable growers often utilize organic fertility sources because of positive impacts on soil quality (Hoagland et al., 2008; Rudisill et al., 2015), and potential to reduce NO_3^- leaching and N_2O emissions relative to inorganic fertilizers (Drinkwater et al., 1998; Kramer et al., 2006). However, unlike inorganic N fertilizers, organic fertility amendments must be

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mineralized before N is available for crop uptake. Consequently, synchronizing N availability with critical periods of crop uptake is challenging. Greater understanding of ecological factors that regulate the soil N cycle is needed to increase N uptake and reduce loss in emerging vegetable systems.

Nitrification is one of the first steps in the soil N cycle ultimately directing the pathway of N in agricultural systems. During nitrification ammonium (NH_4^+) is oxidized to nitrite (NO_2^-) and then NO_3^- in a two-step process. Nitrate is a highly water soluble compound that can be taken up by plant roots, immobilized in microbial biomass, leached to aquatic systems, or emitted as N_2O via nitrification or subsequent denitrification processes (Cameron et al., 2013). It is now clear that both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) contribute to the first and rate limiting step in nitrification, but there is still much debate about the relative contribution of each group as well as factors that influence their activity in agricultural soils. For example, potential nitrification rates have been correlated with AOA population size in some studies (Hallin et al., 2009; He et al., 2007), while in others rates are correlated with AOB population size (Ai et al., 2013; Glaser et al., 2010; Jia and Conrad, 2009). AOB and AOA belong to separate phylogenetic domains with different cell metabolic and biochemical processes which could lead to niche differentiation and specialization in response to various soil biotic and abiotic factors (Prosser and Nicol, 2012; Wessen et al., 2010). Alternatively, wide ecophysiological diversity within each group could contribute to functional redundancy (Schauss et al., 2009).

Studies comparing inorganic fertilizers and raw animal manures have observed changes in ammonia oxidizer populations and potential nitrification activity (PNA) (Ai et al., 2013; Chu et al., 2008; Enwall et al., 2007; Hallin et al., 2009; Wessen et al., 2010). Results varied widely given soils and the type of fertilizer applied, however, and the mechanisms regulating these differences are still unclear. In some studies, AOB populations and nitrification activity were more stimulated by inorganic fertilizers than animal manure (Chu et al., 2008; Strauss et al., 2014), whereas in another incorporation of animal manure in conjunction with an inorganic fertilizer resulted in greater nitrification activity and abundance of AOA and AOB compared to inorganic fertilizer alone (He et al., 2007). Vegetable growers rarely use raw animal manure because of food safety concerns and instead rely on composted materials and leguminous cover crops to supply N. These amendments are generally more stable and have less immediately available NH_4^+ than raw animal manure which is likely to influence the soil N cycle. For example, while raw animal manure often increases N_2O emissions relative to inorganic N fertilizer, solid organic fertilizers have been found to produce an average of 28% fewer emissions than inorganic fertilizer (Aguilera et al., 2013). Few studies have compared composted animal manure or cover crops with inorganic fertilizers on nitrification dynamics, however, particularly in intensively managed vegetable systems.

The plant rhizosphere is a zone of intense microbial activity that can have profound effects on plant nutrient acquisition and health (Berendsen et al., 2012). Nitrifying populations (Chen et al., 2008; Hussain et al., 2011; Kleineidam et al., 2011) and PNA (Ai et al., 2013; Enwall et al., 2007) are often enhanced in the rhizosphere compared to bulk soil. These differences are thought to result from rhizodeposition, a process by which plant roots release organic C stimulating ammonification from soil organic matter (Herman et al., 2006). Such relationships are likely to be critical to enhancing crop N uptake, particularly in the presence of organic fertility amendments. However, limited knowledge exists of how alternative fertilizer sources influence nitrification dynamics in the rhizosphere (Ai et al., 2013), and additional studies are needed to optimize N dynamics at this key plant-soil interface.

The objective of this study was to determine how three years of repeated applications of urea, composted chicken litter, green manure based on cover crop of hairy vetch (*Vicia villosa*) plus alfalfa meal (*Medicago sativa*), and an unfertilized control affected the structure and potential nitrification activity of ammonia oxidizing organisms in bulk soil and the rhizosphere of sweet pepper (*Capsicum annuum*). We hypothesized that (1) PNA activity would be stimulated by organic relative to inorganic fertility amendments, (2) PNA activity would be greater in the rhizosphere vs. bulk soil, and (3) differences in PNA activity would be correlated with increased abundance and diversity of ammonia oxidizing communities.

2. Materials and methods

2.1. Site description, soil treatments, and sampling

The field trial was conducted at Meigs Horticulture Research Farm (40° 17′ 21.051″–86° 53′ 3.12″) in Tippecanoe County, Indiana. Soils were from the Drummer series (fine-silty, mixed, superactive, mesic Typic Endoaquolls). The experiment was conducted for three years with the amendments applied in each growing season (2011–2013) at rates estimated to supply sufficient N for the pepper crop. Four treatments were: urea, partially composted and dehydrated chicken litter, green manure (fall-seeded hairy vetch supplemented with dehydrated alfalfa meal), and an unamended control. The carbon:nitrogen (C:N) ratio of the chicken litter, hairy vetch, and alfalfa meal amendments were 7:1, 11:1, and 15:1, respectively, resulting in 5233 and 10,669 kg ha^{-1} C added over three years to the AM and GM plots respectively. Details of the initial soil characteristics, climate, cropping history, and amendment application rates are explained in Rudisill et al. (2015). Plots were arranged in a randomized complete block design with four replicates. All plots were tilled each spring to incorporate amendments and prepare beds for transplanting. Ten replicate soil cores were collected and pooled within each plot to a depth of 15.0 cm on August 10, 2013, when plant were actively growing and producing fruit and N needs were expected to be high. Rhizosphere samples were obtained by removing two randomly selected plants from each plot, shaking roots, and collecting soil adhering to plant roots. Replicate samples were pooled in polyethylene bags, placed on ice during transport, and stored at -80 °C, 4 °C, or air-dried upon arrival for downstream DNA isolation, potential enzymatic assays, and chemical analyses, respectively.

2.2. Soil geochemistry

Mineral N (ammonium (NH_4^+)) and total NO_x (NO_3^- and NO_2^-) was quantified spectrophotometrically using a SEAL AQ2 discrete analyzer (SEAL Analytical, Mequon, WI) following 1.0 M KCl extraction (1:2.5 soil to solution ratio w/v) of air-dried, 2.0 mm sieved soil samples. Soil solution pH was measured in a slurry containing a 1:2 ratio of air-dried, 2.0 mm sieved soil to deionized water (Kalra, 1995). Total C and N were quantified by combustion on a FlashEA 1112 (Thermo Scientific, Waltham, MA, USA). Gravimetric soil moisture content was determined by drying 25 g of field moist soil at 80 °C.

2.3. Potential nitrification activity

Assays for potential nitrification activity (PNA) were carried out using a microscale method based on ISO 15685 (Hoffmann et al., 2007). Briefly, 2.5 g of fresh soil was placed in a 50 mL flask, and test medium (300 μM KH_2PO_4, 700 μM K_2HPO_4, 10 mM sodium chlorate, and 1.5 mM $(NH_4)_2SO_4$; pH 7.2) was added to create slurry with exact volume of 10 mL. The reaction was incubated at