Soil Biology & Biochemistry 68 (2014) 410–418

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

The response of ammonia-oxidizer activity and community structure to fertilizer amendment of orchard soils

S.L. Strauss [a], C.L. Reardon [b], M. Mazzola [a,*]

[a] Tree Fruit Research Laboratory, USDA Agricultural Research Service, 1104 N. Western Ave, Wenatchee, WA 98801, USA
[b] Columbia Plateau Conservation Research Center, USDA Agricultural Research Service, Adams, OR 97810, USA

ARTICLE INFO

Article history:
Received 2 January 2013
Received in revised form
10 October 2013
Accepted 11 October 2013
Available online 25 October 2013

Keywords:
Ammonia-oxidizing bacteria
Ammonia-oxidizing archaea
Orchards
Agricultural soil
Fertility management
N cycling

ABSTRACT

Soil microorganisms have a significant role in determining the relative loss and retention of plant available nitrogen (N) and thus relative efficiency in the use of fertility inputs to agricultural production systems. Although the effect of management system on activity of the N-cycling microbial communities has been evaluated in certain annual cropping systems, results have been variable and there have been few studies conducted in perennial crops, such as apple. We examined the effect of organic and mineral fertility inputs to organic and conventional orchard soils on the overall activity, abundance and diversity of ammonia-oxidizing bacteria and archaea. Apple rootstocks were cultivated in orchard soils receiving one of five fertility treatments: *Brassica napus* seed meal, plant-based compost, urea, urea with plant-based compost, and a no-treatment control. Based on analysis of the ammonia monooxygenase gene (*amoA*), ammonia-oxidizing archaea (AOA) were more abundant than ammonia-oxidizing bacteria (AOB) in both untreated conventionally and organically managed orchard soils. However, AOB abundance was significantly different in both organically and conventionally managed soils with fertilizer amendments. The microbial community of the conventional orchard soil appeared to be limited by inorganic N since a response in potential activity to N input was only observed in treatments with urea. In the organic orchard soil, an increase in AOB gene abundance was detected only in response to the urea plus compost fertility treatment. Soil management and fertilizer additions had little effect on AOA gene abundance compared to the no-treatment control. Although composition of the AOB community was similar between the conventional and organically managed soils, AOA communities were significantly different. The different responses of the bacterial and archaeal ammonia-oxidizer communities to organic and conventional management and fertilizer amendments highlight the need for an increased focus in agricultural research to understand and improve the specificity of fertilizer application for orchard production systems.

Published by Elsevier Ltd.

1. Introduction

The effect of alternative fertilizers and organic management on nitrogen (N) cycling have focused on ammonia-oxidation, the first and rate-limiting step in nitrification, and the primary process controlling soil nitrate (NO_3^-) concentrations. Both bacterial and archaeal ammonia-oxidizers are ubiquitous in agricultural soils (Leininger et al., 2006); however, the ratio and contributions of each group to the process are still unclear. Initially, it appeared that the recently discovered ammonia-oxidizing archaea (AOA; Treusch et al., 2005) were more abundant than ammonia-oxidizing bacteria (AOB) in all soil types (Chen et al., 2008; Leininger et al., 2006). However, further examination of soil treated with mineral and organic fertilizers concluded that the abundance of AOA was influenced by differences in soil pH (Hallin et al., 2009; Nicol et al., 2008), ammonium (NH_4^+) concentration (Di et al., 2010; Jia and Conrad, 2009; Zhong et al., 2010), and labile carbon (C; Wessén et al., 2010). Discrepancies in the distribution and relative contribution of AOA and AOB to the measured rates of ammonia-oxidation have been observed under different crops. For instance, AOB were suspected as the primary contributor to ammonia-oxidation in rice paddy soils due to their positive correlation with potential ammonia-oxidation rates (Wu et al., 2011). Conversely, the AOA abundance was positively correlated with potential ammonia-oxidation rates in Swedish agricultural soils planted to maize (Hallin et al., 2009) which had greater organic matter content than the rice paddy soils. Although several studies (Di et al., 2010; Hallin et al., 2009; Wessén et al., 2010) have investigated

* Corresponding author. Tel.: +1 509 664 2280; fax: +1 509 664 2287.
E-mail address: mark.mazzola@ars.usda.gov (M. Mazzola).

0038-0717/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.soilbio.2013.10.016

the response of AOA and AOB to different fertilizers in cereal crops, little has been done to investigate these responses in perennial cropping systems.

Physiological differences of the perennial root system compared to cereal crops may result in dramatic differences to the soil microbial community, and thus response to organic management and alternative fertilizers. When apple (*Malus domestica* Borkh.) orchard soils in central Washington were cultivated to wheat the resulting bacterial and fungal community structures were altered significantly from that originally resident to the orchard soil (Mazzola and Gu, 2000). Additional examination found that cropping of successive cycles of specific wheat varieties in the soil from the same orchard (Mazzola and Gu, 2002) altered the fluorescent pseudomonad population to a composition that approximated the original community resident under apple cultivation (Mazzola, 1999). As such, it is plausible that the underlying functional microbiology operating in N cycling in a perennial system could differ significantly from that typical of an annual cropping system.

Perennial crops, such as apple, require high inputs of N fertilizer to offset microbial mobilization and loss while meeting the nutrient demands of the trees (Hoagland et al., 2008). An ideal management strategy would maximize the amount of soil NH_4^+ and nitrate NO_3^- available to plants and decrease the amount lost through leaching or denitrification. Organic and integrated management practices are thought to approximate this strategy with reports of enhanced soil quality and profitability, and lower environmental impacts compared to conventionally managed orchards (Reganold et al., 2001). However, few assessments exist regarding the effects of management systems on the orchard soil microbiology functional in N cycling, and data from cereal cropping systems have yielded variable results with either an increase (Ge et al., 2010; Wessén et al., 2010) or no difference (Kuramae et al., 2012) in soil N in response to different combinations of fertilizers. Further analysis of soil microbial communities and their responses to fertility inputs may enable the optimization of fertilizer application programs and result in enhanced N use efficiency in apple orchards.

In this study, we examined the effects of organic and inorganic fertilizers on the abundance, potential activity, and community composition of AOA and AOB in both conventionally and organically managed apple orchard soils from the Columbia River Valley in Washington State (USA). Orchard soils located along the Columbia River in Douglas and Chelan counties of central Washington vary dramatically in texture regardless of orchard management (Beieler, 1978). Since soil texture can affect soil microbial community composition as well as C and N cycling (Franzluebbers et al., 1996; Marschner et al., 2004), two conventionally managed orchard soils with different textures were compared. Conventionally managed orchards in this region typically use synthetic fertilizers, such as urea, as a N supplement while organically managed orchards use various N inputs including composted animal manures, blood, feather, or canola (*Brassica napus*) seed meal (SM). *B. napus* SM amendment has been utilized as an organic fertilizer based on a high C:N ratio of approximately 8:1 (Snyder et al., 2009) and the ability to induce suppression of certain apple root pathogens (Cohen et al., 2005). In this study, we tested the effects of canola SM, plant-based compost, urea, and urea with plant-based compost at standard field application rates on the ammonia-oxidizing communities resident to conventional and organic apple orchard soils.

2. Materials and methods

2.1. Orchard soils

Soils used in this study were from three commercial orchards in the Columbia River Valley: conventionally managed orchards in Manson, WA (GC) and Wenatchee, WA (SR) and an organically managed orchard in Chelan, WA (RF). The sampled orchard blocks were planted to Jonagold on G11 rootstock at the RF orchard, Gala on G11 rootstock at the SR orchard, and Golden Delicious on M7 rootstock at the GC orchard. Soil properties of GC are previously published (Mazzola, 1998; Mazzola et al., 2009) and the characteristics of the three soils are summarized in Table 1. Soils were collected to a depth of 20–30 cm within the orchard rows in August (RF and SR) and September (GC) and stored in covered 10-gallon bins at room temperature. All experiments were initiated 28 days after soil collection in which the soils were homogenized prior to treatment allocation.

2.2. Greenhouse experiment

One of five treatments was applied to individual 17 kg samples of soil from the GC and RF orchards: *B. napus* SM, plant-based compost, urea, urea + plant-based compost or a no-treatment control. Application rates of the different fertilizers were based on common orchard practices and were made on the basis of soil dry weight. Seed meal from *B. napus* cv. Athena possessed a total N content of 5.8% and a total C content of 49.2% and was applied at 1.0 g kg^{-1} soil. The plant-based compost (Green Mix Garden Compost, Waupaca Northwoods, LLC, Waupaca, WI), with a total N content of 1% and total C content of 26.4%, was applied at 5.3 g kg^{-1} soil. Urea, with a total N content of 46%, was applied at 0.14 g kg^{-1} soil. Soil was mixed using a cement mixer (Kobalt, Monarch Industries, Inc.), placed in a plastic bag and incubated in the dark at 22 °C for 21 days. After the initial incubation, seven replicates per treatment were established, each containing 2.4 kg of soil in 1-gallon plastic pots and planted with one Gala M9 rootstock (Willamette Nurseries, Canby, OR). Plants were grown in a greenhouse at 22 °C with supplemental lighting to maintain a 16-h photoperiod and watered three times a week. The SR orchard soil was left untreated as a comparison for soil texture of conventionally managed orchards, and was incubated and planted in the same manner as the GC and RF soils.

Subsamples ($n = 5$) of soil from the root zone (approximate depth of 5 cm) were collected 55 days after planting by excavating and shaking soil from the entire root system. These soil samples were used for determination of pH, NO_3^- and NH_4^+ concentrations, potential ammonia-oxidation activity (PAO), and functional gene abundances. Measurements of soil pH, NO_3^- and NH_4^+ concentrations and PAO were made within 24 h of soil collection. Soils for DNA analysis were stored at −20 °C.

2.3. Soil geochemistry

Concentrations of NO_3^- and NH_4^+ were commercially determined using standard flow injection analysis methods on 0.2 M potassium chloride extracts of triplicate 50 g soil subsamples for each treatment by Soil Test Farm Consultants, Inc (Moses Lake, WA). Soil solution pH was measured in deionized water using a 1:2 volume ratio of soil:water. The slurry was mixed and allowed to equilibrate for 30 min prior to analysis with a benchtop pH meter (Beckman, Fullerton, CA). Soil pH was measured on triplicate samples for each soil type. Gravimetric soil moisture was determined according to the methods of Robertson et al. (1999).

2.4. PAO measurements

Potential ammonia-oxidation rates were determined according to the shaken soil-slurry method (Hart et al., 1994). Briefly, 5 g of soil was added to 50 ml of solution (pH 7.2) containing 300 μM KH_2PO_4, 700 μM K_2HPO_4, 750 μM $(NH_4)_2SO_4$, and 50 mM sodium