Soil Biology & Biochemistry 57 (2013) 758–768

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

Comparative diversity and abundance of ammonia monooxygenase genes in mulched and vegetated soils

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ARTICLE INFO

Article history:
Received 25 March 2012
Received in revised form
11 October 2012
Accepted 13 October 2012
Available online 12 November 2012

Keywords:
Ammonia oxidizers
Ammonia monooxygenase
Mulching
Biodiversity

ABSTRACT

Soil microbial habitats are altered by mulching, a common practice in urban areas during which vegetation is removed and soils covered to suppress weeds and retain moisture. Soil microorganisms drive nitrogen-cycling processes in mulched soils, because living plants no longer take up ammonium-N released during decomposition of residual organic matter. Because ammonia oxidizers carry out the first, rate-limiting step of nitrification, we compared ammonia oxidizers in experimental, unfertilized plots of mulched and vegetated soils. We hypothesized that mulched and vegetated soils would support contrasting communities of bacterial and archaeal ammonia oxidizers, as determined by quantitative PCR and primers specific for genes encoding ammonia monooxygenase subunit A (*amo*A). Clone libraries of archaeal *amoA* also were constructed to compare diversity in soil cores, duplicate blocked plots, and treatments (bark-mulched, gravel-mulched, and unmanaged old field vegetation). Gene copies from ammonia-oxidizing bacteria (AOB) ranged from 2.2×10^6 to 2.7×10^7 gene copies per gram dry soil and did not differ across treatments. In contrast, gene copies from ammonia-oxidizing archaea (AOA) ranged from 9.1×10^5 to 1.0×10^8 copies per gram dry soil, with bark-mulched soils having significantly lower abundance. Community structure of AOA in gravel-mulched soils was distinct from the other two treatments. At 97% amino acid similarity, 22 operational taxonomic units, or OTUs, were identified, with only one OTU found in all 18 clone libraries. This ubiquitous OTU-1, which was highly similar to published *amoA* sequences recovered from soils, comprised 55% of all 482 translated sequences. Greater variability in OTU richness was observed among cores from mulched soils than from vegetated soils. Our observations supported our hypothesis that AOA communities differ in mulched and vegetated soils, with mulched soils providing altered and variable microniches for these N cycling microorganisms.

1. Introduction

Land areas affected by urbanization are increasing worldwide. Today more than half of the world's population lives in urban areas, and by the year 2050 urban residents are projected to make up 70% of the world's population (United Nations, 2007). Development and landscaping practices associated with urbanization drastically alter soil habitats (Kaye et al., 2005), thereby affecting resident soil microorganisms. Mulching, a commonplace soil management practice in urban areas, involves removing plant cover and replacing it with more inert materials to suppress weeds, increase moisture retention, and reduce erosion (Borland, 1990; Kemper et al., 1994). In the absence of living plants, mulching results in altered soil conditions and a shift to microbially driven biogeochemical cycling. Mulch composition can be organic (e.g., wood chips, bark) or inorganic (e.g., gravel, sand, plastic sheeting), and varied types of mulch material can provide different substrates for fueling microbial activity in soils.

Among the biogeochemical cycles occurring in soils, nitrogen (N) cycling is altered by mulch application, as evidenced by differences in net N mineralization and N_2O emissions (Byrne, 2006; Scharenbroch et al., 2005; Flessa et al., 2002). In oxic soils, nitrification is considered to influence N_2O fluxes to a greater extent than denitrification (Wrage et al., 2001), and N_2O flux has been found to be correlated with the abundance of ammonia oxidizing bacteria (AOB) (Di et al., 2009a). Thus, the effects of mulching on the diversity of nitrifying organisms, specifically the ammonia oxidizers, warrants particular attention. Although previous studies have documented changes in bacterial diversity following mulch application (Tiquia et al., 2002), ammonia-oxidizer

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http://dx.doi.org/10.1016/j.soilbio.2012.10.016

communities in mulched soils have not been investigated to date. Since ammonia oxidation is the first and rate-limiting step of nitrification, changes in ammonia oxidizer communities have significant potential to affect the concentration of soil nitrite and nitrate, two forms of nitrogen influencing the production of air-polluting aerosols and greenhouse gases (Kulmala and Petäjä, 2011 and Su et al., 2011). Due to the absence of plant roots for nitrate uptake, nitrate is more susceptible to leaching and denitrification losses in mulched soils than in vegetated soils (Byrne et al., 2008).

Ammonia oxidizers are found within bacterial and archaeal domains (Treusch et al., 2004; Leininger et al., 2006). The abundance and activity of both groups of ammonia oxidizers have been shown to be affected by N availability (He et al., 2007; Di et al., 2009b, 2010) and the amount of soil organic matter (Chen et al., 2008; Leininger et al., 2006). Although the effects of soil management on ammonia oxidizers have been relatively well studied in agricultural systems, much less is known about how this keystone microbial group responds to other soil management practices, especially those associated with urbanization. Further, most of what is known about the effects of land use change on nitrification has focused on AOB. Recent studies have shown that ammonia-oxidizing archaea (AOA) are also widespread and active in soils and can be affected by soil management (Nicol and Schleper, 2005; Francis et al., 2007; Herrmann et al., 2008; Urich et al., 2008; Schauss et al., 2009; Zhang et al., 2010).

The objectives of this study were to investigate AOA and AOB abundance and AOA diversity in experimental plots established in an old field for a land-cover study begun in 2003 (Byrne, 2006). In that study, soils in mulched plots were warmer and wetter than in vegetated plots, and gravel-mulched plots had consistently lower $NH_4^+ - N$ and higher $NO_3^- - N$ concentrations than bark-mulched or vegetated soils (Byrne et al., 2008). Based on N cycle process observations, we hypothesized that AOA and AOB abundance would be higher in gravel-mulched soils than in other soils and that AOA diversity would differ between mulched and vegetated soils. We tested these hypotheses on soil collected three years after the experimental plots were established. We used quantitative PCR for AOA and AOB genes for ammonia monooxygenase subunit A (*amoA*) and analyzed AOA diversity by constructing *amoA* clone libraries.

2. Methods

2.1. Study site and soil sampling

Soils in this study were obtained from 10 × 10 m plots established in an old field in April 2003 for an urban land cover experiment (Byrne, 2006; Byrne et al., 2008). The old field site is located at the Russell E. Larson Research Farm at Rock Springs, Centre County, Pennsylvania (40° 43′ N, 77° 55′ W, 350 m elevation, 975-mm mean annual precipitation). Soils at this site (0.84-ha or 200 × 42 m) are well-drained, shallow (<1 m to limestone residuum), and have a silty clay loam texture (Opequon series, clayey, mixed, active, mesic Lithic Hapludalfs) (Natural Resources Conservation Service, 2012).

Prior to the experiment, orchard grass (*Dactylis glomerata*) and Kentucky blue grass (*Poa pratensis*) were the dominant vegetation in the old field. For at least the previous 25 years, the field received no N fertilizers and was managed by mowing once a year. The experimental plots were established using a complete randomized block design with four replications and included the following land-cover treatments: unaltered vegetation, regularly mowed lawn (newly planted *P. pratensis*), and bark- and gravel mulches. For plots that were mulched, the old field vegetation was mowed down and killed with one application of glyphosate, followed by gentle raking to remove dead aboveground vegetation but to retain root biomass within the soil. Commercially available mulches of bark (dark brown, finely shredded, mixed Pennsylvania hardwoods) and gravel (grade 2B light gray limestone, 2–4 mm diameter) were placed into respective plots and spread to a depth of 5–8 cm by hand raking. During the next three growing seasons, weeds were removed from mulched plots by hand or with minimal, targeted glyphosate treatments on dandelions. One additional bark mulch application was made in April 2005.

For the present study, triplicate soil cores (4-cm deep × 2.7 cm wide) were collected aseptically from three plots in two experimental blocks in September 2006. Each plot had one of the following treatments: unmanaged vegetation, bark mulch, or gravel mulch. The three cores were obtained within one 100-cm^2 area per plot but processed separately to assess within- and between-plot variation. Distances between sample collection sites among all plots ranged from 18 m to 100 m. Cores were stored in a cooler until transported to the laboratory, where they were kept at 4 °C. Each core was subdivided into aliquots and stored at −80 °C until further processing. Additional composite samples of soils collected per plot were air-dried, mixed and used to measure total C and N and pH. Total C and N were measured by combustion at the Agricultural Analytical Services Laboratory at Penn State University. Mineral N was not measured in this study. Soil pH in water was measured twice for each sample using a pH electrode and a 1:1 ratio of soil to water. Gravimetric soil moisture was determined after drying soil for 24 h at 105 °C.

2.2. Soil DNA extraction

Genomic DNA for standard PCR amplification and archaeal *amoA* clone library construction was extracted from 0.5 g of moist soil using the MoBio Ultra Clean Soil DNA extraction kit (MoBio Laboratories, Inc., Carlsbad, CA). Manufacturer's instructions were followed except for differences in physical lysis methods, since preliminary trials indicated that DNA in gravel-mulched soils was more readily sheared by bead-beating than by vortexing. Thus, soil samples from the unmanaged vegetation and bark-mulched plots were extracted by bead-beating the sample for 30s, while those from the gravel plots were extracted by vortexing the soil for 10 min.

Further testing in our laboratory indicated that higher soil genomic DNA yields were obtained using the MoBio Power™ Soil DNA Isolation Kit. Thus, DNA used for quantitative PCR (qPCR) was extracted from 0.3 g of moist soil from a second set of subsamples using the latter kit. We again compared DNA yields using vortexing and bead-beating, since lysis methods have been reported to affect *amoA* quantification (Leininger et al., 2006). Because bead-beating resulted in inconsistent DNA yields, only qPCR results DNA extracts obtained by vortexing were statistically analyzed. Nevertheless, similar trends in gene copy abundances among treatments were observed for DNA obtained by the two methods. Due to sample limitations, DNA for qPCR was obtained from only two of three cores initially collected from the second block of each treatment. DNA concentration of all samples was determined using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc.).

2.3. Quantitative PCR

Copy numbers of putative archaeal *amoA* genes were quantified using an ABI 7300 Sequence Detection System for each of the five soil cores analyzed per treatment. Optimization of a SYBR Green assay for the detection of archaeal *amoA* using primers ArchamoF and ArchamoR (Francis et al., 2005) proved to be challenging after