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Effects of inorganic fertilizer and manure on soil archaeal abundance at two experimental farms during three consecutive rotation-cropping seasons

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ABSTRACT

Soil archaeal population dynamics at two experimental sites of the same clay-loam type in Ottawa and Woodslee, Ontario, were investigated to determine fertilizer and manure effects following their different long-term crop rotation and fertilization schemes. Phylogenetic analysis of cloned soil archaeal 16S rRNA gene libraries of both sites identified them with group 1.1b of Thaumarchaeota. The gene population dynamics subtly varied in the order of 10^7 copies g^{-1} soil when monitored by quantitative real-time PCR during three growing seasons (2007–2009). In Ottawa, where plots were amended with dairy-farm manure, soil thaumarchaeal gene abundance was double of the unamended plots. At the Woodslee N-P-K-fertilized plots, it remained at least 30% fewer than that of the unfertilized ones. These cultivated plots showed soil carbon limitation while the fertilized ones were low in soil pH (ca. 5.5). Surface soils from an unfertilized sod plot and an adjacent deciduous forest had higher total carbon content (C:N ratio of 9 and 11, respectively). Their thaumarchaeal gene abundance varied up to 4.8×10^7 and 7.0×10^7 copies g^{-1} soil, respectively. The former value was also attained at the manure-amended plots in Ottawa, where the C:N ratio was just below 10. Where soil pH was above 6.0, there was a weak and positive correlation between soil total C and the estimated gene abundance. Such gene population dynamics consistently demonstrated the stimulating and suppressive effects of dairy-farm manure (Ottawa site) and inorganic fertilizers (Woodslee site), respectively, on soil thaumarchaea. At both sites archaeal *amoA* and 16S rRNA gene abundance were similarly affected. Archaeal *amoA* gene abundance also outnumbered bacterial *amoA* abundance, suggesting that ammonia-oxidizing archaea might be dominant in these soils. Only minor crop effects on gene population dynamics were detected.

1. Introduction

Thaumarchaeota is the current phylum designation of the Archaea domain (Brochier-Armanet et al., 2008; Spang et al., 2010), which was initially known as mesophilic Crenarchaeota and regarded as uncultivated archaeal group 1 (Delong, 1998). They are ubiquitous and commonly found in bulk soil (Sliwinski and Goodman, 2004). Although they globally constitute a relatively small fraction (ca. 2%) of soil prokaryotes compared to the bacterial population (Bates et al., 2011; Ochsenreiter et al., 2003), thaumarchaea are abundant in the rhizosphere of various agroecosystems (Simon et al., 2000), including rice (Großkopt et al., 1998), maize (Chelius and Triplett, 2001) and pine seedlings (Bomberg et al., 2003). From cultivation-independent molecular evidence (Leininger et al., 2006; Wuchter et al., 2006), as well as physiological characterization of the first purified marine isolate *Nitrosopumilus maritimus* (Könnecke et al., 2005), many thaumarchaea are understood to be active and numerically dominant ammonia-oxidizers relative to their well-characterized autotrophic bacterial counterpart. Hence, they play an important role in affecting soil fertility and nitrogen cycling.

Assessments of archaeal and bacterial contributions to soil ammonia oxidation reaffirmed their functional redundancy and significance (Prosser and Nicol, 2008; Schauss et al., 2009), which could be niche-dependent (Wessén et al., 2010). However, they are reported to be differentially affected by pig manure, antibiotics (Kleineidam et al., 2011; Schauss et al., 2009; Taylor et al., 2010), the type and amount of organic amendment (Wessén et al., 2010) in agricultural soils. Jia and Conrad (2009) suggested the possibility of heterotrophic growth of soil archaea supplemented with energy derived from autotrophic ammonia oxidation, implying that they are capable of mixotrophy. Soil archaeal CO_2 fixation via the hydroxyproprionate-hydroxybutyrate cycle was demonstrated (Offre et al., 2011; Zhang et al., 2011), and coupled with ammonia oxidation (Pratscher et al., 2011; Xia et al., 2011). Autotrophic

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ammonia oxidation has been confirmed in the first purified soil archaeon *Nitrososphaera viennensis* str. EN76, which can also grow on urea and at an enhanced rate with extraneous pyruvate (Tourna et al., 2011). Recently, an obligate autotrophic, acidophilic and ammonia-oxidizing archaeon (*Nitrosotalea devanaterra*), was enriched from an acidic agricultural soil (Lehtovirta-Morley et al., 2011). Hence, thaumarchaea are versatile, diverse, dynamic and important nitrifiers that significantly affect soil fertility, and contribute to global carbon and nitrogen cycling.

Some effects of fertilizer type, soil properties and temporal changes on archaeal population dynamics in cultivated field soils or microcosms have been investigated. Generally, soil ammonia-oxidizing archaea (AOA) were suggested to be more stable and numerous than ammonia-oxidizing bacteria (AOB) in their abundance and composition in long-term fertilizer treatments (He et al., 2007; Shen et al., 2008; Wessén et al., 2010). However, their relative distribution and potential contribution under changing environmental conditions as affected by fertilizer type, soil properties and weather remain lacking and unclear without repeated sampling. It was hypothesized that soil amendments with manure or inorganic fertilizer would influence the dynamic change in soil AOA and AOB abundance, and employing regular and systematic monitoring would help better understand the underlying changes of these microbes in soil nutrient cycling.

Two long-term crop rotation experiments with soil amendments of farm manure or inorganic fertilizer were conducted on the same type of soils in Ontario, Canada. Our main objective was to clearly discern potential effects of different fertilizers and crop rotation systems on soil Archaea distribution and population dynamics.

2. Materials and methods

2.1. Experimental sites, soil properties and sample collection

The two long-term crop rotation experiments are located in eastern and south-western Ontario, Canada. They are situated on the Central Experimental Farm (CEF), Ottawa (45°22′N, 75°43′W), and the Eugene Whelan Experimental Farm, Woodslee (42°13′N, 82°44′W), respectively.

The soil at the Ottawa site has been designated as a Brandon loam (Orthic Humic Gleysol), consisting of fine loamy, mixed, mesic Typic Endoaquoll with an average pH of 6.5 and particle size of 34% clay, 27% silt and 39% sand (Ma et al., 2003). Since 1992, triplicate plots selected for soil sampling have been subjected to an alfalfa-maize (*Medicago sativa–Zea mays*) annual rotation in a 2-year cycle (Ma et al., 2003). During the 2007–2009 cropping period hybrid maize, alfalfa and hybrid maize were successively planted according to the following annual rotation schedule:

Alfalfa →	Maize →	Alfalfa →	Maize
2006 ↑	2007	2008 ↑	2009

Post-harvest manure application

After alfalfa harvest manure was applied in November for maize planting in the following year. The plots were treated with or without partially composted (PCM) or uncomposted (UCM) dairy-farm manure at 50 Mg (fresh weight) ha^{-1}. PCM and UCM contained 19–24 and 18–21 g kg^{-1} total N, respectively. The bulk of the manure consisted of straw mixed with dairy cow feces and urine.

Orthic Humic Gleysol was also found at the Woodslee site but was designated as a Brookston clay loam (Drury and Tan, 1995). The soil contains an average of 37% clay, 35% silt and 28% sand. Since 1959 the experimental field has been following a 4-year annual rotation scheme of maize-oat (under-seeded with alfalfa)-alfalfa-alfalfa. The selected plots used for this study (2007–2009) started with one pair (A) of maize plots and one pair (B) of alfalfa plots in an offset rotation scheme, with or without inorganic fertilizer according to the following scheme shown as a 4-year cycle:

Plots A (±fertilizer) :	Maize	→	Alfalfa + Oat	→	Alfalfa (1st Yr.) 2009	→	Alfalfa (2nd Yr.) 2010

	2007		2008			
Plots B (±fertilizer) :	Alfalfa 1st Yr.	→	Alfalfa 2nd Yr.	→	Maize	→ Alfalfa + Oat

Each crop consists of fertilized or unfertilized treatments without plot replication. At planting the fertilized plot received 16.8, 29.3 and 27.4 kg ha^{-1} y^{-1} of inorganic N, P and K, respectively. Soils were sampled annually as described below. In addition, soils from an unfertilized adjacent continuous sod (bluegrass, *Poa pratensis*) plot (2007–2009) and from a natural mixed deciduous forest (2008–2009) were also sampled for comparison. The former was mowed several times a year to 13–15 cm, leaving the clippings on the plot without tilling.

Soils at the Ottawa site were collected from the A_p horizon with a 7-cm-diameter core sampler twice annually in the summer (June or July) and after fall harvest in October. Three soil cores were taken from each plot, sieved (4.75-mm mesh) to remove stones, plant debris and litter, and kept at 4 °C if not immediately used for soil DNA extraction. The Woodslee soil samples were similarly collected four times (August 2007, April and November of 2008, and October 2009).

2.2. Soil DNA preparation, gene amplification, cloning and qPCR

Soil DNA was isolated and purified by using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) within 2 days of soil collection. DNA concentration was determined by using a NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific Inc., Wilmington, DE). From experience the prepared soil DNA when used at 10-X or higher dilution in all PCR experiments was adequate for quantification without inhibition of the PCR. As a standard procedure, all templates and plasmids were heated to ensure their relaxation and linearization, and verified by gel electrophoretic analysis before initiating qPCR.

As an initial survey of archaeal diversity at the two experimental sites, PCR-based cloning was performed with the archaea-biased primers 23FPL (Bintrim et al., 1997) and 958R (Delong, 1998) for the 16S rRNA gene. For each site a composite soil sample (1 g) pooled from the triplicated cores of a representative plot was used. PCR amplification was performed on a Biometra® TProfessional Basic thermocycler (Biometra GmbH, Göttingen, Germany) with an initial denaturation at 95 °C for 3 min followed by 40 cycles of 45 s at 95 °C, 45 s at 60 °C, and 1.5 min at 72 °C, and terminated by 8 min at 72 °C for extension. The amplicons were isolated from agarose gel and purified by using PureLink™ Quick Gel Extraction Kit (Invitrogen, Carlsbad, CA), ligated into the pGEM-T Easy vector (Promega, Madison, WI) overnight at 4 °C, and transformed into *Escherichia coli* DH10B competent cells (Invitrogen). Transformed colonies were grown in Luria-Bertani broth at 37 °C overnight. Plasmid DNA was prepared by using GenElute™ Plasmid Miniprep Kit (Sigma, St. Louis, MO).

The 16S rRNA inserts were sequenced by using ABI BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and run on an ABI 3100-Avant automated sequencer (Applied Biosystems/Hitachi). Sequences were edited in SeqMan (ver. 8, DNAStar, Madison, WI). Together with relevant environmental archaea 16S rRNA gene clones published in GenBank, cloned sequences from a maize plot and an alfalfa plot, respectively, in Ottawa and Woodslee, were assembled, edited and aligned by ClustalW. The neighbor-joining phylogeny based on maximum composite likelihood was performed with the Molecular