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The effect of soil type, rice cultivar and water management on ammonia-oxidizing archaea and bacteria populations

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ABSTRACT

Ammonia-oxidizing microorganisms are partly responsible for the production of N_2O, a potent greenhouse gas. Rice paddies provide a habitat where ammonia oxidizing microorganisms can be active. The influence of different agricultural practices on these microorganisms, particularly on archaeal ammonia oxidizers, is an active field of research. In this work, we conducted two greenhouse experiments where we analyzed the influence of two soil types with different organic matter contents, two rice cultivars and water management on both archaeal (AOA) and bacterial (AOB) ammonia oxidizers. We determined the AOA and AOB abundance and population structure by q-PCR and T-RFLP, respectively. The archaeal and bacterial ammonia monooxygenase subunit A gene was used as the PCR target. The AOA and AOB copy numbers were affected by sampling time in both experiments. AOA abundance was also influenced by the time of flooding. The population structure of AOA was more variable than that of AOB and was strongly determined by soil type. Changes in AOB population structure were observed mainly according to sampling time.

1. Introduction

Rice is the staple food for more than 3 billion people worldwide (FAO, 2007); it is cultivated on arable lands in tropical and subtropical areas covering approximately 20% of the global cultivated land (Peng et al., 2011).

Cultivated soil is the main anthropogenic source of N_2O, contributing 1.7–4.8 Tg of N_2O-N yr^{-1} (IPCC, 2007). Paddy rice is a significant source of CH_4, whereas N_2O emissions from paddy fields are thought of as marginal. Although most of the rice-originated N_2O is emitted by upland rice, efforts are being made to understand the dynamics of N_2O emissions in paddy rice to reduce this emission (Zou et al., 2009). N_2O has a 100-year global warming potential, 298 times that of CO_2 (IPCC, 2007), and an atmospheric lifetime of more than 100 years (Ko et al., 1991); it also degrades stratospheric ozone via NO_x.

Nitrous oxide is biologically produced in soil through the processes of denitrification and nitrification. In denitrification, a wide group of microorganisms, which include mainly prokaryotes and some eukaryotes, use NO_x as an electron acceptor in anaerobic respiration. Thus, this process takes place mainly in anoxic or microaerophilic environments (Zumft, 1997). The final product of denitrification can be either NO, N_2O or N_2, depending on the microorganisms that are involved and on the environmental conditions (Bakken et al., 2012).

Nitrification is the biological conversion of ammonia (NH_3) to nitrate (NO_3^-); it occurs in two steps by the action of two distinct groups of microorganisms. In the first step, ammonia serves as energy source to a group of chemoautotrophic microorganisms that are collectively called ammonia oxidizers and convert ammonia to nitrite (Egli et al., 2003). Ammonia-oxidizing bacteria (AOB), notably *Nitrosomonas* spp., were first described in the late nineteenth century (Winogradsky, 1891), while the first report of an archaeon that was able to grow by aerobically oxidizing ammonium was published in 2005 (Könneke et al., 2005). Thus, it is not surprising that the interest in and the knowledge about the ecological significance of ammonia oxidizing archaea (AOA) have increased exponentially in recent years. Currently, the relative importance of AOA and AOB in different environments is being questioned (Di et al., 2009; Leininger et al., 2006; Zhang et al., 2011). Although the mechanisms through which N_2O is formed during nitrification are not completely understood, it is clear that ammonia oxidation can account for up to 80% of the total emitted N_2O, depending on the environmental conditions and soil type (Gödde and Conrad, 1999).

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Ammonia-oxidizing microorganisms are slow growing (Kowalchuk and Stephen, 2001), and their cultivation is possible for a modest proportion (Smith et al., 2001). Culture independent techniques, such as terminal-restriction fragment length polymorphism (T-RFLP) (Osborn et al., 2000), have been extensively used to study both AOA and AOB (Glaser et al., 2010) using the ammonia monooxygenase subunit A (amoA) gene as a target (Francis et al., 2005; Rotthauwe et al., 1997).

Several studies have indicated the influence of plant genotype in shaping the communities of plant-associated bacteria (Germida and Siciliano, 2001). This influence is modulated by soil type and changes during the growing season (Reichardt et al., 2001). Briones et al. (2003) reported different nitrification activity and AOB populations associated with roots of different rice cultivars. In addition, different rice cultivars can have different CH_4 emission rates; therefore, the use of more-efficient cultivars is a promising strategy to mitigate greenhouse gas emissions (Setyanto et al., 2004).

Several reports have shown that alterations in water management, such as mid-season drainage, are effective options for CH_4 mitigation in irrigated rice fields (Itoh et al., 2011). However, this practice of alternate anaerobic and aerobic cycling can stimulate N_2O emission.

The main objective of the present study was to analyze the dynamics in AOA and AOB abundance and community structure in simulated paddy rice fields throughout the crop cycle. We used two rice cultivars (one *indica* and one *japonica*), two water regimes, and two contrasting soil types according to their organic matter content. We hypothesized that these factors might affect AOA and AOB in different ways. We found that only the soil type influenced the AOA community structure.

2. Materials and methods

2.1. Greenhouse experiments

Soils were collected from paddy fields in two consecutive years from the top layer (15 cm in depth) two months before rice sowing. Two sites were selected, representing the main geographical zones where rice is cultivated in Uruguay. Soil 1 was collected at the Instituto Nacional de Investigación Agropecuaria (INIA) (33°16′10.00″ S; 54°10′04.00″ W), and soil 2 was collected from a private paddy field (31°22′10″ S; 57°27′45″ W). Soil 1, referred to hereafter as "lower OM" (lower organic matter), is a Typic Argiudoll, with pH 5.7, 3.4% organic matter, 13 μg/g P Bray I, and 10 μg/g NO_3-N. Soil 2, referred to hereafter as "higher OM" (higher organic matter), is a Typic Hapludert, with pH 5.6, 5.8% organic matter, 10 μg/g P Bray I and 15 μg/g NO_3-N.

In two consecutive years, two greenhouse experiments were carried out. The experimental unit consisted of 12 plastic boxes (40 × 60 × 35 cm), each containing approximately 60 kg of sieved soil. The boxes were set in a randomized block design, comprising three replicates of each treatment. The rice cultivars that were used were El Paso 144 (*Oryza sativa* ssp. *indica*) and Tacuari (*O. sativa* spp. *japonica*). Seeds were sowed in two parallel lines by box at a density equal to field conditions. At sowing, ammonium nitrate was applied at the equivalent of 100 kg ha^{-1}. Subsequent fertilization was applied as urea at the equivalent of 20 kg ha^{-1}, at tillering and at the flower primordium stage.

The first experiment consisted of a 2 × 2 factorial design. The factors were rice cultivar and water management. The soil that was used in this experiment was from the INIA site (lower OM). Flooding was established at tillering in all treatments. In the drained treatment, boxes were drained at the beginning of the flowering stage and flooded again 16 days after draining. In the not-drained treatment, flooding was maintained until one week before harvest. The treatments were as follows: Tacuari, drained (TD); Tacuari, not drained (TnD); El Paso 144, drained (EPD); and El Paso 144, not drained (EpnD). Each treatment had three replicates. The definitive draining for all boxes was performed one week before harvest.

Soil samples were taken at tillering, at the flower primordium stage, five days after draining (draining), and at harvest. From each plastic box, six randomly collected soil cores (2 × 5 cm) were taken and mixed together.

The second experiment consisted of a 2 × 2 factorial design. The factors were soil type and time of flooding. The soils that were used were "lower OM" (LOM) and "higher OM" (HOM). The water regime consisted of an early flooding (at the tillering stage) and late flooding (after flower primordium). The treatments were as follows: lower OM, early flooding (LOMEF); lower OM, late flooding (LOMLF); higher OM, early flooding (HOMEF); and higher OM, late flooding (HOMLF). Each treatment had three replicates.

The final draining was performed one week before harvest. Soil samples were taken one week after sowing, one week after tillering, two days after the flower primordium stage, and at harvest.

2.2. Nucleic acid extraction

Soil samples were air-dried at 26 °C and 40% relative humidity for 48 h prior to DNA extraction. DNA was extracted from 0.25 g (dry weight) of soil using the MoBio Power Soil® DNA Isolation Kit (San Diego, CA, USA). The extracted DNA was checked on a 0.8% agarose gel. The DNA concentration and purity were determined with NanoDrop® 2000c UV–vis spectrophotometry (USA).

2.3. q-PCR analysis

The abundance of AOA and AOB was determined by real-time PCR using a LineGene K Thermocycler (Bioer Technology) using the fluorescent dye SYBR-Green I. All of the samples and standards were quantified in triplicate.

The reaction mixture for AOA (12.5 μl) contained 6.25 μl of 2× Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific Inc.), 10 μg of bovine serum albumin, 2.5% (v/v) dimethyl sulfoxide (DMSO) and 0.5 μM each primer: Arch-amoAF (5′-STA ATG GTC TGG CTT AGA CG-3′) and Arch-amoAR (5′-GCG GCC ATC CAT CTG TAT GT-3′) (Francis et al., 2005). Thermal cycling was as follows: 95 °C for 10 min and 40 × (95 °C, 45 s; 53 °C, 45 s; 72 °C, 45 s; and 79 °C, 15 s for data collection), and the program ended with a melt curve from 65 °C to 90 °C. Standard curves were generated by amplifying 10-fold dilutions of a mix of linearized pJET1.2/blunt plasmids containing five different archaeal amoA genes. The PCR efficiency ranged from 85.39% to 101.35%, averaging 94.36%, and the correlation coefficient ranged from 0.977 to 0.998, averaging 0.990.

The reaction mixture for AOB (12.5 μl) contained 6.25 μl of 2× SYBR® Premix Ex TaqTM (TaKaRa Bio, Inc.), 10 μg of bovine serum albumin, 2.5% (v/v) dimethyl sulfoxide (DMSO), 1× ROX reference dye and 0.4 μM each primer: amoA-1F (5′-GGG GTT TCT ACT GGT GGT-3′) (Rotthauwe et al., 1997) and amoA-2Rs (5′-CCT CKG SAA AGC CTT CTT C-3′) modified from those originally published by Rotthauwe et al. (1997) to minimize dimmer formation. Thermal cycling was as follows: 95 °C for 10 min and 40 × (95 °C, 30 s; 58 °C, 30 s; 72 °C, 45 s; and 80 °C, 15 s for data collection), and the program ended with a melt curve from 72 °C to 90 °C. Standard curves were generated by amplifying 10-fold dilutions of linearized pJET1.2/blunt plasmid containing *amoA* gene from *Nitrosomonas europaea*. The PCR efficiency ranged from 90.60% to 105.82%, averaging 98.55%, and the correlation coefficient ranged from 0.986 to 1, averaging 0.994.