



## Short Communication

## Response of ammonia oxidizing archaea and bacteria to changing water filled pore space

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## ABSTRACT

This study examined the effect of water filled pore space (WFPS) on gross N fluxes and community structure and abundance of ammonia oxidizing archaea and bacteria in a semi-arid soil. Different WFPS altered the community structure of both AOA and AOB. Ammonia oxidizer communities (for both archaea and bacteria) from 'wet' soils (95, 85 and 75% WFPS) and 'dry' soils (25, 45 and 55% WFPS) were distinctly different from one another. Additionally there was a significant relationship between community structure and gross rates of nitrification. There was also a significant relationship between WFPS and bacterial *amoA* abundance but not archaeal *amoA* abundance suggesting that bacterial ammonia oxidizers are more responsive to changes in soil water availability. These results are in agreement with other studies suggesting that both groups of ammonia oxidizers have distinct physiological characteristics and ecological niches with consequences for nitrification in response to WFPS. Overall findings from this study indicate that nitrification, both in terms of process rates and populations responsible for nitrification activity, is highly responsive to soil water availability.

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In soils, ammonia oxidizers are particularly sensitive to water stress which affects their activity through both dehydration and substrate limitation (Stark and Firestone, 1995). Although a number of studies have revealed the effect of soil water on nitrification (Stark and Firestone, 1995; Avrahami and Bohannan, 2007; Gleeson et al., 2008) few inferences have been made to changes in AOB structure and none to how AOA populations respond to changing water status. It had been believed that microbial ammonia oxidation was performed solely by bacteria, which uniquely possessed the *amoA* gene for ammonia monooxygenase. However, metagenomic studies (Schleper et al., 2005; Treusch et al., 2005) have demonstrated co-occurrence of archaeal 16S rRNA genes and genes homologous to those encoding bacterial ammonia monooxygenase (AMO). Archaeal *amoA* genes appear to be ubiquitously distributed in soils (Nicol et al., 2008) and detection and quantification of bacterial and archaeal *amoA* genes indicate a greater abundance of archaeal over

bacterial ammonia oxidizers (Leininger et al., 2006; He et al., 2007). Recent work has demonstrated that in marine ecosystems, archaeal nitrifiers have much higher affinities for ammonia than bacterial nitrifiers and thus, likely occupy a different niche (Martens-Habbena et al., 2009). However, discrimination of ammonia oxidizing activity by bacteria and archaea is currently not possible at the process level. It remains unclear whether ammonia oxidation is predominately linked to Archaea, as implied by their current generally reported higher abundance over bacteria. We tested the hypothesis that AOA and AOB respond differently in terms of their community structure and abundance to changes in water-filled pore space (WFPS).

Soil (0–5 cm) was collected from a semi-arid field site on the Cunderdin Agricultural College, Western Australia (311360S, 1171130E) and sieved <2 mm prior to use in incubations. Climate and soil characteristics are reported in Barton et al. (2008). Target WFPS values of 25, 45, 55, 65, 75, 85 or 95% were achieved by adding appropriate volumes of water to 20 g dry soil, mixing well and packing into 30 mL tubes to a bulk density of 1.4 g cm<sup>-3</sup>. Tubes were incubated at 25 °C for 7 days prior to addition of <sup>15</sup>N (1 mL of 100 µg ml<sup>-1</sup> solution to give final concentration of 5 µg g<sup>-1</sup> soil dry weight). Rates of gross N

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transformations were estimated by  $^{15}\text{N}$  isotopic pool dilution (Kirkham and Bartholomew, 1954; Murphy et al., 2003). Ammonium-N and nitrate-N (nitrate and nitrite) concentrations in  $\text{K}_2\text{SO}_4$  extracts were determined colorimetrically by automated segmented flow analysis (San Plus System, Skalar Analytical, Breda, The Netherlands). Gross N mineralization and nitrification (Kirkham and Bartholomew, 1954) were calculated using the relationship:

$$m \text{ or } n = \frac{M_0 - M}{t} \frac{M(\log H_0)/M_0(H)}{\log M_0/M} \quad (1)$$

where gross mineralization ( $m$ ) or gross nitrification ( $n$ ) rate per unit mass of dry soil per unit time ( $\text{mg N kg}^{-1} \text{d}^{-1}$ );  $M$  is  $\text{NH}_4^+\text{-N}_{\text{total}}$  (mineralization) or  $\text{NO}_3^-\text{-N}_{\text{total}}$  (nitrification);  $H$  is  $\text{NH}_4^+\text{-N}_{\text{labeled}}$  (mineralization) or  $\text{NO}_3^-\text{-N}_{\text{labeled}}$  (nitrification);  $t$ , time (days) between the first ( $M_0$ ,  $H_0$ ) and subsequent ( $M$ ,  $H$ ) soil analysis. Net mineralization was calculated from the change in the size of the  $\text{NH}_4^+$  plus  $\text{NO}_3^-$  pool, and net nitrification as the change in  $\text{NO}_3^-$  pool, through time. The difference between gross and net rates reflects  $\text{NO}_3^-$  consumptive processes.

Soil DNA was extracted following the method of Griffiths et al. (2003) with the modification that the samples were incubated overnight in polyethylene glycol (PEG). For T-RFLP PCR amplification of the archaeal *amoA* gene was based on the method of Francis et al. (2005) using the primer set Arch-amoAF and Arch-amoAR. Amplification of the bacterial *amoA* gene was based on the method of Horz et al. (2004), using the primer set amo-1F and amo-2R (Rotthauwe, 1997). In each case the forward primer was labeled with 5-carboxyfluorescein (FAM). All PCRs were performed in duplicate and pooled for subsequent restriction and fragment analysis. Approximately 100 ng of PCR product was used in a restriction digest with the restriction endonuclease *MspI* (archaeal *amoA*) or *HaeIII* (bacterial *amoA*) (New England Biolabs Inc.). Terminal restriction fragment lengths were determined by electrophoresis using a capillary electrophoresis system (Applied Biosystems) and analysis of fragment profiles using Genemapper. Abundance of archaeal and bacterial *amoA* genes present in soil DNA extracts was determined by performing quantitative real-time PCR (qPCR) using the QuantiTect™ SYBR® Green PCR Master Mix real-time PCR kit and an ABI 7500 real-time PCR machine (Applied Biosystems). The primer set Arch-amoAF/Arch-amoAR was used for the archaeal *amoA* assay (Park et al., 2006) and amoA-1F/amoA-2R used for the bacterial *amoA* assay (Mintie et al., 2003) with copy numbers determined based on the size of the fragments (Fogel et al., 1999). Univariate statistical analyses were performed using GenStat (9th edition; Lawes Trust, Harpenden, UK) and multivariate statistical analyses of T-RFLP profiles using Primer 6 (Primer-E Ltd, UK) and the Bray-Curtis similarity index. Tests of the null hypothesis among *a priori* defined groups were examined using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001). Data were transformed as necessary.

There was no significant effect of WFPS on either net or gross N mineralization rates ( $P=0.13$ ), however WFPS had a significant effect on both net ( $P=0.001$ ) and gross ( $p=0.001$ ) nitrification rates. Between 25 and 65% WFPS nitrification rates (both net and gross) rates did not change as a function of WFPS, while at 75–95% WFPS nitrification rates decreased substantially (Fig. 1a). At WFPS >75% gross nitrification rates were less than  $1.5 \mu\text{g N}^{-1} \text{g soil}^{-1} \text{day}^{-1}$  and net nitrification was negative indicating a higher demand for  $\text{NO}_3^-$  consumption than was able to be supplied. The decline in gross nitrification rates at 75–95% WFPS was not attributable to a lack of  $\text{NH}_4^+$  availability as (i) the gross nitrification rates were assessed in the presence of added  $\text{NH}_4\text{Cl}$  thus ensuring that gross nitrification rates were not substrate limited and (ii) gross N mineralization rates were greater than gross nitrification rates indicating that ammonium production from soil organic matter mineralization was not

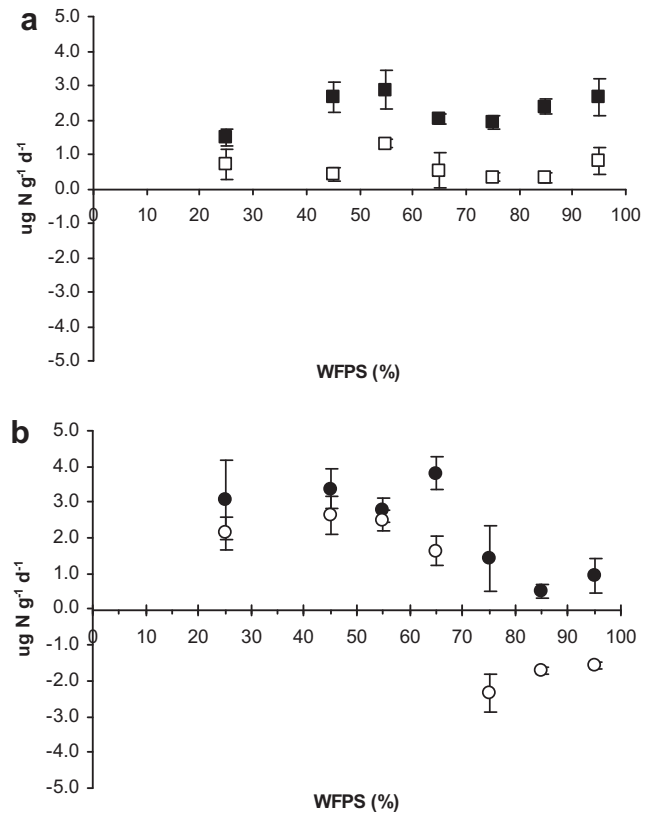


Fig. 1. Influence of change in water filled pore space (WFPS) on (a) gross (■) and net (□) mineralization and (b) gross (●) and net nitrification (○) rates ( $\mu\text{g N g}^{-1} \text{dry soil d}^{-1}$ ) on a semi-arid soil incubated at specific WFPS for 7 days (SE bars are shown,  $n=3$ ).

a limiting the substrate availability for nitrification at these WFPS. Nitrification activity is generally more sensitive to changing environmental parameters such as temperature (Hoyle et al., 2006), pH (De Boer and Kowalchuk, 2001) and water (this study) than N mineralization activity. This is likely because only two groups of organisms, archaeal and bacterial nitrifiers, are involved in nitrification whereas N mineralization is driven by a large variety of bacteria and hence has greater functional redundancy.

PERMANOVA indicated that WFPS altered both archaeal ( $F=4.46$ ;  $P<0.0001$ ) and bacterial ( $F=5.86$ ;  $P<0.0001$ ) ammonia oxidizer community structure (with the exception of 55% and 25% WFPS bacterial *amoA* gene fragments). There appeared to be two distinct populations of bacterial ammonia oxidizers, i.e. those adapted to growing under oxic conditions and those adapted to growing under sub-oxic conditions (Fig. 2). The community structure of AOB responded to shifts in WFPS with a distinct clustering of AOB from 'wet' soil (95, 85 and 75% WFPS) and 'dry' soil (25, 45 and 55% WFPS). There was also a significant relationship between both archaeal ( $\delta^2=0.92$ ;  $P<0.001$ ) and bacterial ( $\delta^2=0.920$ ;  $P<0.0001$ ) *amoA* communities and WFPS. Additionally, there was a correlation between archaeal ( $\delta^2=0.71$ ;  $P<0.002$ ) and bacterial ( $\delta^2=0.70$ ;  $P<0.0003$ ) *amoA* communities and gross nitrification rate. There was no significant relationship between either archaeal ( $P<0.17$ ) or bacterial ( $P<0.51$ ) *amoA* communities and gross N mineralization rate. We have previously shown that AOB community structure was correlated to potential nitrification rate in a similar semi-arid soil (Gleeson et al., 2008). This is in agreement with other studies that also report a link between microbial community structure and soil processes (Schimel and Gulledege, 1998). The relationship with community structure indicates that a soil with a more diverse population is likely to be able to maintain nitrification under a wider range of soil conditions.

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