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Short communication

Influence of water potential on nitrification and structure of nitrifying bacterial communities in semiarid soils

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ABSTRACT

The objective of this study was to examine the relationship between soil water potential, nitrifier community structure and nitrification activity in semiarid soils. Soils were collected after a 5-month dry period (end of summer) and subsequently rewetted to specific water potentials and incubated for 7 days prior to analysis of nitrification activity and nitrifier community structure. The approach used in this study targeted a 491bp segment of the *amoA* gene which encodes the active site of the ammonia monooxygenase enzyme, which is the key enzyme for all aerobic ammonia oxidisers. *amoA* serves as a useful target for environmental studies since it is both specific and universal for all ammonia oxidisers and reflects the phylogeny of the ammonia oxidisers. Our results suggest that in semiarid soils water potential plays a key role in determining the structure of ammonia oxidising bacteria (AOB), and that additionally AOB community structure is correlated to potential nitrification rate in these soils.

1. Introduction

Water is a critical resource and its availability regulates microbial activity within the soil matrix. This is especially true in arid and semiarid regions where the episodic nature of rainfall results in cycles of extended dry periods followed by soil rewetting (Schjonning et al., 1999; Fierer and Schimel, 2002). Water availability is known to affect the osmotic status of microbial cells and indirectly affects substrate availability, diffusion of gases, soil pH and temperature (Schimel et al., 2007). Periods of moisture limitation may affect microbial communities through starvation, induced osmotic stress, and resource competition eliciting strong selective pressure on the structure and functioning of soil microbial communities (Drenovsky et al., 2004; Griffiths et al., 2003). Rapid rewetting of a dry soil causes microorganisms to undergo osmotic shock, possibly inducing cell lysis and a release of intracellular solutes (Kieft et al., 1987; Fierer et al., 2003). Furthermore, rewetting of dried soils is known to cause increased mineralisation of both carbon and nitrogen (Lundquist et al., 1999; Murphy et al., 1998). The exact role of microorganisms in mediating these processes is still largely unresolved and very little is known about how complex microbial communities respond to changing water status.

Autotrophic ammonia oxidising bacteria (AOB) are a microbial functional group that mediate nitrification, and

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are influenced by a variety of environmental factors, including water content, that dictate community parameters, i.e. numbers, diversity and activity *in situ* (Stark and Firestone, 1995; Hastings et al., 2000; Griffiths et al., 2003). Studying the ecology of ammonia oxidisers has been challenging as most cannot yet be cultured and those that can are very slow growing and do not always remain active after cultivation (Koops and Pommerening-Roser, 2001). Although a number of studies have revealed the effect of moisture on nitrification (Stark and Firestone, 1995; Avrahami and Bohannan, 2007), no inference has been made to changes in diversity of bacterial populations that underlie these processes. The use of molecular methodologies currently offers the most potential for assessing the diversity of soil nitrifier communities, yet few studies were found applying such approaches to an investigation of moisture effects on nitrifier populations (Avrahami and Bohannan, 2007; Singh and Kashyap, 2006; Horz et al., 2004; Hastings et al., 2000). The overall aim of this study was to examine differences in the molecular diversity and potential nitrification of AOB in soils incubated either dry or subjected to a rewetting stress. We propose that some members of the AOB community in these soils have increased moisture tolerance as a result of exposure to regular episodic rewetting events, and therefore we hypothesised that changes in AOB community structure would take place as a result of the intensity of the rewetting stress applied.

2. Materials and methods

2.1. Soil description and treatments

The study site was located near Dangin, Western Australia (GPS Coordinates: WGS84, UTM50H, 0528737, 6449071). Daingin has a Mediterranean-type climate, with an annual rainfall of 376 mm (http://www.bom.gov.au) mainly during the winter months (with the exception of a small number of summer rainfall events), a mean daily maximum temperature of 25.1 °C and a mean daily minimum temperature of 11.4 °C (Commonwealth Bureau of Meteorology, http://www.bom.gov.au/climate). Soil samples were taken from three different soil types across a 500 m transect (different soil types were located close together thus excluding climate – rainfall, temperature, etc., effects): sand; loamy sand and clay loam. All soils were assessed for particle size distribution (measured using a modification of the pipette method), pH (water (1:1)), total C and N (measured by Leco total combustion) and microbial biomass carbon (MBC); soil data are given in Table 1. Soils had received 165 mm rainfall (occurring mostly in five significant summer rainfall events) in the 5 months preceding collection and the average daily maximum temperature was 30.1 °C with high evaporation. At each site (i.e. soil type), four replicate composite soil samples (total 12 cores per single bulked sample, 4 replicates total per soil type) were taken to a depth of 0–10 cm at the end of summer (March 2006) and thus were dry on collection (gravimetric moisture of all soils was 0.5%). Soils were sieved at 4 mm and thoroughly mixed prior to incubation.

2.2. Experimental design

The experiment was established in 1 l Kilner jars: 100 g soil (dry weight equivalent) was weighed into each jar and soil water amended to achieve the target water potentials of −100 (dry soil control), −5 and 0 kPa. Initially, soil moisture curves were determined using the ceramic pressure plate method (Cresswell and Hamilton, 2002); saturated cores were equilibrated at a series of water potentials (−0.1, −10, −50, −100, and −1500 kPa). Soils were subsequently incubated at −100, −5 and 0 kPa. Incubation at different water potentials rather than water contents allowed us to compare the effect of water availability on nitrifier community structure in soils of differing textures, in response to rewetting after a significant dry period. All soil treatments were incubated at +15 °C for 1 week. Net ammonification and mineralisation rates were determined over a 7-day incubation period. Potential nitrification was assessed after the incubation period and samples were also collected and stored at −20 °C for molecular analysis.

2.3. Analytical methods

2.3.1. Microbial biomass carbon

Microbial biomass C was determined by fumigation extraction (Vance et al., 1987) using 20 g soil shaken in 80 ml of 0.5 M K_2SO_4, for 1 h followed by filtration using pre-washed Whatman 42 filter paper. Non-fumigated soils were also extracted. Oxidisable-C in the K_2SO_4 extracts was determined by TOC analyser (Shimadzu, Japan). C rendered extractable by fumigation was converted to microbial-C using a k_{ec} factor of 0.45 (Wu et al., 1990).

2.3.2. Mineral nitrogen

Mineral N in soil samples was extracted by shaking with 0.5 M K_2SO_4 (soil:extractant ratio of 1:4) for one hour. NH_4-N and NO_3-N concentrations in the extracts were determined colorimetrically by automated flow injection analysis (Skalar Analytical B.V., The Netherlands).

2.3.3. Potential nitrification

Nitrification potential was measured using the shaken-slurry method (Hart, 1994). From each treatment, 15 g soil (dry weight

Table 1 – Selected soil characteristics (mean values of four replicates; numbers in parenthesis are standard error) (microbial biomass carbon: MBC)

	Sand (%)	Silt (%)	Clay (%)	Total C (%)	Total N (%)	C-to-N ratio	MBC ($\mu g\ g^{-1}$ dry soil)	pH
Sand	97.3 (0.3)	1.3 (0.5)	1.5 (0.3)	0.55 (0.01)	0.04 (0.001)	13.2 (0.4)	57.2 (5.8)	5.1 (0.03)
Loamy sand	88.8 (1.1)	4.3 (0.5)	6.8 (0.9)	0.89 (0.01)	0.06 (0.01)	14.8 (0.2)	84.8 (18.5)	5.5 (0.3)
Clay loam	41.5 (2.6)	25.0 (4.8)	33.3 (3.5)	1.75 (0.01)	0.15 (0.01)	11.9 (0.2)	307.7 (44.7)	7.1 (0.1)