The abundance of nitrogen cycle genes *amoA* and *nifH* depends on land-uses and soil types in South-Eastern Australia

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ABSTRACT

Nitrogen is a critical nutrient in plant-based primary production systems, therefore measurements of N cycling by microorganisms may add value to agricultural soil monitoring programs. Bacterial-mediated nitrogen cycling was investigated in soils from two broad land-uses (managed and remnant vegetation) across different Soil Orders from three geomorphic zones in Victoria, Australia, by examining the abundance of the genes *amoA* and *nifH* using quantitative polymerase chain reaction (qPCR). The aim of the study was to identify parameters influencing bacterial populations possessing the genes *nifH* and *amoA*, and examine their distribution at a regional scale across different management treatments. The gene *amoA* was most abundant in the neutral to slightly alkaline surface soils from Calcarosols in North-West Victoria. There was a highly significant ($P < 0.001$) interaction between land-use and geomorphic zones in terms of the abundance of *amoA*. Detection of the gene *nifH* was site specific with low copy number (less than 100 copies per nanogram of DNA) observed for some strongly acidic surface soil sites in North-East Victoria (Dermosols) and South-West Victoria (Sodosols/Chromosols), while *nifH* was more abundant in selected Calcarosols of North-West Victoria. The gene *amo*A was detected across more sites than *nifH* and was strongly influenced by land-use, with almost consistently greater abundance in managed compared to remnant sites, particularly for North-West and South-West Victoria. The abundance of *nifH* was not related to land-use, with similar copy numbers observed for both managed and remnant sites at some locations. For the gene *nifH*, there was no significant interaction between land-use and geomorphic zones, between managed and remnant sites or between the three geomorphic zones. Regression tree analysis revealed a number of likely soil chemical and microbial variables which may act as drivers of gene abundance of *amoA* and *nifH*. Variables identified as drivers for *amoA* included pH, Olsen P, microbial biomass carbon, nitrate and total nitrogen while for *nifH* the variables were microbial biomass carbon, electrical conductivity, microbial biomass nitrogen, total nitrogen and total potassium. Measures of N cycling genes could be used as an additional indicator of soil health to assess potential ecosystem functions. The spatial scale of the current study demonstrates that a landscape approach may assist soil health monitoring programs by evaluating N cycle gene abundance in the context of the different microbial and chemical conditions related to Soil Order and land-use management.

1. Introduction

Australian soils are under increasing pressure to provide a range of agroecological goods and services to meet the growing global demand for food, fibre and bioenergy. Current land-use practices are likely to influence soil ecosystem health and the underlying microbially mediated functions related to plant nutrient supply (Kibblewhite et al., 2008). Nitrogen (N) is a critical nutrient in all plant-based systems, and in agriculture is applied to crop and pasture based systems to promote plant biomass, grain protein levels, animal feed intake quality and ultimately farm gate profit. Nitrogen is also associated with greenhouse gas production, groundwater contamination and soil acidification, hence management of this critical nutrient is paramount for economic and environmental sustainability (Ridley et al., 2004).

Microbial communities are structurally and functionally variable, depending on a range of factors including soil type, water content, temperature, pH, oxygen status, land-use (intensity) and

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plant cover (Bossio et al., 1998; Saleh-Lakha et al., 2005; Jangid et al., 2008). The soil microbial consortia responsible for the various N transformations of N₂-fixation, N-mineralisation, nitrification, ammonia oxidation, and denitrification are also likely to be influenced by these factors. Importantly, microbial N cycling in below-ground ecosystems directly impacts above-ground plant-based ecosystems. Biological N₂-fixation, the conversion of atmospheric N₂ gas to biologically available ammonium by free-living, associated and symbiotic diazotrophs from a wide range of bacterial phyla, provides an important source of nitrogen to natural ecosystems (Zhang et al., 2006). Autotrophic ammonia oxidation is a two-step process consisting of the conversion of ammonia to nitrite and its subsequent conversion to nitrate. The first step is carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Leininger et al., 2006; Prosser and Nicol, 2008) and the second by nitrite-oxidizing bacteria (NOB) (De Boer and Kowalchuk, 2001). Ammonia-oxidizing bacteria and archaea perform a rate-limiting step of nitrification and play a key role in the regulation of soil nitrogen dynamics (De Boer and Kowalchuk, 2001; Prosser and Nicol, 2008).

Genes associated with the N cycle have been quantified using quantitative or real time polymerase chain reaction (PCR) to make inferences about the function of soil microbial communities associated with soil geomorphology and land-use (Colloff et al., 2008), pH (Nicol et al., 2008), pasture management (Wakelin et al., 2009), N-fertilisers (Okano et al., 2004; Cavagnaro et al., 2008) and tillage (Cavagnaro et al., 2008). Two of the most widely quantified bacterial genes using quantitative PCR (qPCR) are *amoA*, which regulates the production of the enzyme ammonia monooxygenase in ammonia oxidation, and *nifH* which encodes the dinitrogenase reductase component of nitrogenase in N₂-fixation.

The abundance of N cycle genes will most likely be directly and indirectly influenced by soil and land-use. In South-East Australia, soils are highly variable ranging from Australian Soil Classification Soil Orders (Isbell, 2002) such as the alkaline Calcarosols of the arid zone of North-West Victoria (average annual precipitation <300 mm) to the more texture-contrast Sodosols and Chromosols and the acidic Dermosols in the higher (>600 mm) rainfall regions of Victoria. Additionally, agroecosystems in Australia are generally characterised by extensively disturbed areas interspersed with patches of native vegetation (i.e. remnant vegetation) with lower growth rates and nutritional requirements than the adjacent production systems (Colloff et al., 2008). Colloff et al. (2008) measured gene abundance for three N cycle genes at three locations in Australia for both high production agricultural systems where N inputs and outputs were closely managed, and native vegetation (remnant) systems where N cycling was relatively undisturbed and controlled by biological transformation. While only relative gene abundance was measured, not gene copy number, some differences in the detection of genes was reported with *amoA* more commonly found in production systems while *nifH* was detected at both agricultural production and remnant sites for some locations (Colloff et al., 2008). While previous studies have examined strongly contrasting soil types we focussed on sampling a large number of sites to enable a regional scale comparison of gene copy number of *amoA* and *nifH* within each Soil Order and from different land-uses.

Here we report on the measurement of two key bacterial-mediated N cycling genes *nifH* and *amoA* in Victoria, Australia, using soil DNA extracted from 360 soil samples collected from two broad land-use sites (managed and remnant vegetation) across different Soil Orders from three geomorphic zones. Agricultural soil monitoring programs rely heavily upon soil chemistry and physical measures with the biological component of soils measured by microbial biomass and enzyme activity (Mele and Crowley, 2008).

The use of qPCR for assessing the potential or expression of N cycling activities in soil or other environments is becoming increasingly common, therefore we tested these assays on soils of known characteristics to examine the utility of the genetic assays as part of a soil health monitoring system that includes traditional microbiology and soil chemical measurements. In this study, we attempt to identify parameters influencing bacterial populations possessing the functional genes *nifH* and *amoA* and examine their distribution across different management treatments and ecosystems across South-East Australia. We specifically address three major questions for two N-cycle genes: 1) is the detection and abundance of *amoA* and *nifH* associated with Soil Order?; 2) how does soil chemistry influence the abundance of *amoA* and *nifH*?; 3) does land-use have an effect on the abundance of these genes?

2. Materials and methods

2.1. Study sites and sampling

Soils samples were collected from three major geomorphic zones of Victoria represented by four major Soil Orders (Calcarosols, Dermosols, Sodosols and Chromosols) and two broad land-uses (i.e. 'managed' and 'remnant' vegetation) within each Soil Order. The 'managed' sites were predominantly agricultural sites that were cropped or under pasture, with varying levels of agricultural inputs. The 'remnant' sites comprised small parcels of land that were either State Parks or shelter belts where native remnant ecosystems remained. These sites were minimally disturbed with no agricultural inputs.

Ten locations were selected on the basis of Soil Order within the three geomorphic zones: 'Eastern Uplands' in North-East Victoria (Dermosols), 'Western Plains' in South-West Victoria (Sodosols/Chromosols) and 'North-Western Dunefields and Plains' in North-West Victoria (Calcarosols) totalling 30 locations (Table 1, Fig. 1). The FAO World Reference Base classification (IUSS Working Group WRB, 2007) for these soils is given in Table 1. Soil characterisation was carried out by a soil pedologist, using existing soil maps of Victoria and four hand auger checks at each location. Mean total annual rainfall data for the year of sample collection was obtained from the Bureau of Meteorology (http://www.bom.gov.au).

Samples were collected at the 30 locations around Victoria, with paired study sites at each location representing the two major land-uses (managed or remnant) giving a total of 60 sites (see Table 1 for site codes). The paucity of suitable remnant sites in the South-West region required the inclusion of an additional Soil Order so that a mix of Sodosol/Chromosol sites was sampled. Both of these soil orders are characterised by strong texture contrast between surface horizons and the more clayey subsoil horizons. Sodosols, however, are distinguished by sodic (i.e. exchangeable sodium percentage of 6 or greater) subsoils as opposed to the non-sodic subsoils for Chromosols. Dermosols and Calcarosols, are characterised by a lack of strong texture contrast between surface and subsoil horizons (soil texture often gradually increasing with depth). The Dermosols in North-East Victoria are usually well structured and have strongly acidic surface and subsoil horizons. The Calcarosols, however, are characterised by neutral to slightly alkaline surface horizons that become strongly alkaline with depth and abundant calcium carbonate in subsoil horizons (for further information see Victorian Resources Online http://www.dpi.vic.gov.au/vro). Soil samples were collected from South-West Victoria in November 2005, North-West Victoria in 2004 and 2006, and North-East Victoria in 2004 and 2005.

Paired managed and remnant land-use sites were selected on the basis of similar Soil Order and close proximity, with adjacent plots sampled where possible at each of the 30 locations. The