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Detection of nitrogen cycle genes in soils for measuring the effects of changes in land use and management

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ABSTRACT

Nutrient cycles represent key links between above- and below-ground ecosystems. But obscurity of functional diversity of nutrient-cycling organisms has constrained our understanding. We studied the microbial ecology of the nitrogen cycle using bacterial genes involved in nitrification (*amo*A), denitrification (*nap*A) and nitrogen fixation (*nif*H) from areas with highly contrasting soils, climates and land management practices within Australia. Agricultural soils had greater frequency of occurrence of nitrogen cycling genes compared with areas of adjacent remnant native vegetation. The relative abundance of the nitrate reductase gene, *nap*A, increased following the addition of urea to soils, indicating a pulse-response of nitrate-reducing bacteria. Laboratory perturbation of soils with highly saline water had no effect on the frequency of functional gene detection, but following perturbation of soils with antibiotics, the presence of *amo*A fell below detectable levels while levels of ammonia increased over time. In contrast, *nif*H and *nap*A were detected in some soils for the duration of the perturbation experiments. This observed positive relationship between gene abundance and respective process rates was indicative of an active bacterial population. As such, detection and quantification of bacterial genes involved in the nitrogen cycle represents a model system for gaining insights to soil microbial diversity and ecosystem function.

1. Introduction

Research on microbial diversity of soil ecosystems places extreme demands on taxonomic and ecological expertise. Identifying what organisms live below ground, their function and how many of them are present, has been a major constraint on developing a theoretical, functional ecology of soils. This dilemma is compounded further by the use of taxon-specific sampling and extraction techniques, making it difficult to integrate data on the distribution and abundance of the major groups of soil organisms. Microorganisms are a case in point, where traditional culture-based methods can only target about 5% of soil bacteria (Amann et al., 1995). The cultivation and identification of soil fungi presents specific taxonomical challenges relating to isolation and cultivation in laboratories followed by microscopic examination and description of reproductive structures (where present).

For many studies, techniques based on the direct extraction of nucleic acids from soils obviate the need to isolate, culture and identify individual organisms (Trevors, 1996) or extrapolate possible function based on laboratory identification. They allow the comparison of phylogenetically diverse taxa within the same soil sample. Of the molecular methods available, amplification of target genes using the polymerase chain reaction (PCR; reviewed by Sharma et al., 2007) allows for both phylogenetic and function-based approaches to applied soil microbial ecology. Phylogenetic methods involve describing microbial community structures via amplification and differentiation of ubiquitous gene sequences (e.g. 16S rRNA genes). Function-based approaches focus on distribution and abundance of organisms based on their metabolism (e.g. chemolithic autotrophs, diazotrophs) via quantifying functional genes, i.e. those that code for enzymes involved in biogeochemical nutrient cycling (Thies et al., 2001; Prosser and Embley, 2002; Nicolaisen et al., 2004). The distribution and abundance of functional genes can be related to rates of biogeochemical cycles (Neufeld et al., 2001; Bürgmann et al., 2003; Wallenstein and Vilgalys, 2005) to provide new insights into the links between microbial diversity, ecosystem functions and ecological interactions among ecologically significant groups of soil microbiota.

Biological nitrogen transformations are important in Australian production systems. Many of these, particularly dry-land grain and

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pastoral-based systems, rely on microbial nitrogen mineralisation and fixation to provide nitrogen in plant-available form (Peoples and Crasswell, 1992). However, in many agricultural systems, N budgets are controlled both as inputs (N fertilisation) and outputs (harvesting). In these systems of tightly-managed N cycling, the ecology of N geochemistry at a molecular level may be closely linked to expected inputs of C and P or other soil factors. Environmental consequences of human intervention in the N cycle (Vitousek et al., 1997; Galloway et al., 2003), have extended well beyond direct impacts on the microbial community, and include the production of nitrous oxide, a greenhouse gas with >300 times the global warming potential of carbon dioxide (Mosier et al., 1998), and the leaching of nitrate to waters (Mosier et al., 2001). These effects have further served to focus attention on those organisms involved in the biogeochemical transformation of nitrogen.

In contrast to production systems, the management of native vegetation, particularly in terms of N inputs, has been less affected by anthropomorphic N-based disturbance. In such natural ecosystems, N cycling by the native soil microbial community is likely to be closely coupled with N oxidation states in the soil. In Australia, agricultural land is often interspersed with patches of native vegetation. These patches may be of significant conservation value, but consist of plant communities with lower growth rates and nutritional requirements than the adjacent production systems. There exists a marked contrast between high production agricultural systems where N inputs and outputs are closely managed to native-vegetation systems where N cycling is relatively undisturbed and controlled by biological transformation.

In this paper, we present a systems-based approach for the detection of functional genes in soil bacteria involved in the N cycle. We use detection of genes for enzymes involved in nitrification (ammonia mono-oxygenase, *amo*A), denitrification (periplasmic nitrate reductase, *nap*A) and N fixation (di-nitrogen reductase, *nif*H). Our overall aim was to develop a molecular approach for determining the performance and effectiveness of bacterial N cycling in soils in Australian production systems and under native vegetation. To test the robustness and applicability of this approach we chose field sites that provided a high degree of contrast in terms of climates, soils, production systems and native vegetation types. We attempted to determine the relationship between gene frequency and abundance and N cycle process rates following (i) the management of N by addition of urea to soil samples under controlled conditions, and (ii) the addition of saline water and antibiotics as perturbations of the soil microbial community.

2. Materials and methods

2.1. Study sites and soil sampling

Three sites were selected for this study (Fig. 1). They have been foci of long-term agricultural field trials and are extensively documented. Soil physical, chemical and biological properties, measured at the time of sampling, are detailed in Table 1. All soils were sampled to a depth of 10 cm during August and September 2002, from replicated plots and treatments. In the field, samples were spread onto a thick PVC sheet and any stones, coarse debris and plant material removed. Samples were then sieved (3 mm mesh), placed on ice and transported to the laboratory where they were mixed thoroughly and frozen (−20 °C). It should be noted that soils from all sites were of poor structure (i.e. relatively ped-free) and of very low moisture content at the time of sampling. Sufficient soil was collected from each replicate treatment for DNA extraction and pot-based laboratory trials of substrate addition and perturbation.

2.1.1. Tully, North Queensland

This site at the Bureau of Sugar Experiment Stations, Tully (17°59′S, 145°55& prime;E) is a focus site for the Sugar Yield Decline Joint Venture (Pankhurst et al., 2005). It has a tropical, high rainfall climate (mean monthly temperature, maximum 27.5 °C, minimum 19.6 °C; mean total annual rainfall 3813 mm, mostly in late summer). Soils were sampled from three replicate plots each of a continuously-cultivated cane treatment, and a treatment that had been continuous cane but was planted to a grass/clover pasture in 1995. Samples were also collected from three random points within an adjacent area of remnant rainforest vegetation. Soils under rainforest were basaltic red loams (red ferrosols) while those of pastures were leached yellow tenosols (Isbell, 2002).

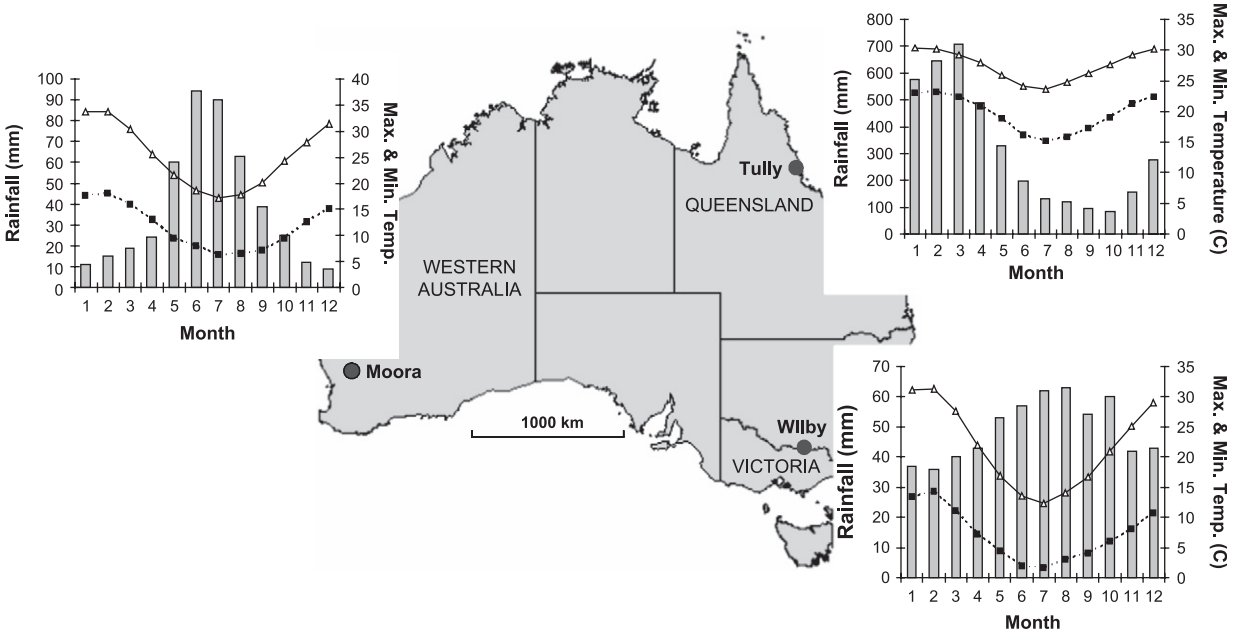

Fig. 1. The location of sampling sites and their associated climate data: mean annual rainfall (bars) and mean monthly maximum and minimum temperatures.