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Shifts in the phylogenetic structure and functional capacity of soil microbial communities follow alteration of native tussock grassland ecosystems

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ABSTRACT

Globally, tussock-based grasslands are being modified to increase productive capacity. The impacts of cultivation and over-sowing with exotic grass and legumes on soil microbiology were assessed at four sites in New Zealand which differed in soil type, climate and vegetation. Primary alteration of the soil physicochemical status occurred with land use change. This was driven by addition of mineral fertiliser and alteration of pH. Genes associated with several biogeochemical cycles (GeoChip data) were impacted by land-use but not sampling location. A number of functional gene families associated with biogeochemical cycling of C, N and S were present in greater relative abundance in the undisturbed soils. Similarly, soil bacterial (PhyloChip) and fungal (TRFLP) communities were strongly influenced by land-use change, but unaffected by sampling location. Alteration of land-use increased the relative abundance of Firmicutes, Actinobacteria and OD1 phyla, but many of the less-common phyla, such as Verrucomicrobia and Dictyoglomi decreased in abundance; these phyla may be important in internal soil nutrient cycling processes. This work provides evidence that tussock grassland soils are strongly dependent on microbially-mediated nutrient cycling, and these processes are highly-sensitive to exogenous nutrient inputs and/or alteration of pH. De-coupling of processes following addition of fertilisers or removal of organic matter (grazing) may make these improved grassland systems more susceptible to nutrient leakage. This has important implications for environmental quality.

1. Introduction

Temperate grasslands including savannas, woodlands, shrubland and tundra, are globally significant both in spatial extent (40% of Earth's terrestrial surface excluding Greenland and Antarctica) and in supporting biodiversity and delivering a range of ecosystem services (White et al., 2000; Henwood, 2010). Due to degradation from human activity, principally agricultural development, temperate grasslands are now considered to be the most altered of terrestrial ecosystems (White et al., 2000).

In New Zealand, grasslands dominated by tussock-forming species are a characteristic land cover, particularly in areas of low rainfall and mid-high altitude (Fig. S1). The dominant grassland species vary according to habitat (e.g. altitude and rainfall) and are described in detail elsewhere (Mark and McLennan, 1995). Briefly, tussock grasslands are dominated by the endemic genus *Chionochloa*, and the more cosmopolitan genera *Festuca* and *Poa* (Mark and McLennan, 1995). In total, some 2.65 M Ha of New Zealand's land area (~10%) is classified as tussock grasslands (www.mfe.govt.nz/issues/land/land-cover-dbase/), constituting the second largest class of indigenous plant cover. These grasslands have a high conservation value in supporting indigenous biodiversity (Mark et al., 2009; Mark and McLennan, 1995).

Following European settlement of New Zealand, native tussock grasslands have been extensively used for pastoral grazing of sheep and cattle. These ecosystems have undergone major disturbances including grazing (stock and also feral mammals), addition of

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mineral fertilisers to boost soil fertility (principally S and P), liming to increase soil pH, over-sowing with introduced grasses and legumes, invasion by weeds (e.g. *Hieracium pilosella* L.), and burning to increase establishment of over-sown seeds and provide new, more palatable, growth of tussock. Many of these management strategies have been shown to impact above-ground biodiversity (e.g. Yeates and Lee, 1997; McIntosh et al., 1999; Espie and Barratt, 2006; Barratt et al., 2009) and soil fertility (Ross et al., 1997; McIntosh et al., 1999). Plant-soil feedbacks are important to the sustainability of these ecosystems, with plants depending on soil-borne microbial communities for the supply of nutrients, and soil organisms being driven by plant energy and carbon inputs (Wardle et al., 2004). Not surprisingly, therefore, anthropomorphic modification of the plant-based component of the ecosystem impacts on below-ground soil microbial diversity and processes (e.g. Sarathchandra et al., 2005).

There are ongoing demands to increase production from tussock grasslands. These vary from intensification of intact tussock-based systems (e.g. fertilisation) to complete conversion into cultivated pasture (Mackay, 2008). Given the geographical extent of tussock grasslands across New Zealand, such land-use alterations may have broad impacts on soil biogeochemical cycling of nutrients (C storage, N cycling etc) and provision of ecosystem services such as water purification (infiltration to groundwater or catchment area runoff to waterways) (White et al., 2000). Determining how modification of tussock grasslands affects soil microbial communities is an essential step towards estimating impacts on soil function and associated ecosystem services.

Our power to explore relationships between land use changes and soil microbial ecology has greatly increased with development of tools which assess microbial communities based on cultivation-independent methods targeting both phylogenetic (typically rRNA gene sequence variation) as well as functional gene markers (i.e. genes linked to biogeochemical processes). In particular, the detection and characterisation of sets of functional genes covering multiple transformations of a single nutrient (such as N cycling), or one or more transformations of multiple nutrients (C, N, P cycling/soil fertility), has provided new insights into nutrient flow and impacts of various forms of disturbance on soil biogeochemical cycling (Colloff et al., 2008; Hallin et al., 2009; Lindsay et al., 2010; Reeve et al., 2010). By coupling such approaches with phylogenetic analysis, links between structural shifts in microbial communities and ecosystem processes can be made (Yergeau et al., 2009; He et al., 2010).

In this study, we investigated how modification of native tussock grasslands through fertilisation and over-sowing of introduced (predominantly European) grasses and legumes, or full conversion to cultivated pasture, impacts on soil microbial communities and functional processes associated with soil biogeochemical cycling (principally nutrient cycling). Shifts in bacterial and fungal communities were assessed using the PhyloChip microarray (Brodie et al., 2006) and fungal-specific TRFLP, respectively. For functional gene analyses covering a range of biogeochemical processes, GeoChip microarrays were employed (He et al., 2010). By integrative assessment of changes in microbial community properties against soil physicochemical changes, the wider impacts of land-use change on system biogeochemistry could be explored.

2. Materials and methods

2.1. Sites and sampling

Soil was sampled from four tussock-grassland sites as described in Barratt et al. (2005, in press). In brief, these sites included two locations in Otago (Deep Stream and Mt Benger), one site in Canterbury (Cass), and one site at Tukino in the central North Island (Suppl. Fig. 1). The sites varied in elevation, rainfall, composition of dominant vegetation, soil type, slope and aspect; full details, of the sites and the treatments are provided elsewhere (Barratt et al., 2005, in press).

At each sampling location, three contrasting land-uses were sampled: Native tussock, tussock over-sown with exotic grasses and clover, and cultivated pasture (previously tussock). Fertilisers have been applied to both the over-sown and cultivated-pasture treatments to increase the primary level of soil fertility. Sampling was conducted in January 2004 (full details in Barratt et al., in press). From each treatment, 15 soil cores of 25 × 100 mm were randomly collected and the samples combined to provide a homogenous, representative sample for each treatment level at each site. DNA was extracted from duplicate 0.5 g soil samples using the FastDNA SPIN Kit for Soil according to the manufacturer's instructions (Qbiogene, Inc.). The method included physical disruption using a mini Bead-beater (Biospec). Duplicate DNA extracts were pooled, purified by a GENECLEAN genomic DNA kit (Qbiogene Inc.), and stored at −80 °C until use.

Similarly, the physicochemcial properties of the soils were measured on the bulked, representative sample. Total N was measured using the Kjeldahl method; P was measured using Olsen (bicarbonate) extraction and Molybdenum blue colorimetry; SO_4-sulphur was extracted in 0.02 M K_2HPO_4 and determined with ion chromatography; cations were measured using atomic absorption after 2 min extraction in ammonium acetate solution; pH was measured in 1:5 soil soil:water extracts; organic matter was measured by combustion and CO_2 analysis.

2.2. Fungal community TRFLP analysis

The soil fungal community structure was characterised via TRFLP of PCR-amplified nuclear rRNA ITS fragments. Fungal ITS regions were amplified in 25 μL reaction volumes using modified ITS1F(FAM) and ITS4 primers (Gardes and Bruns, 1993; White et al., 1990), using conditions described previously (Wakelin et al., 2007). Resulting amplicons were purified from the PCR mixture (Promega Wizard columns) and 100 ng digested with 20 U HaeIII, TaqI and MspI as per manufacturer's instructions (Promega). Digested amplicons were purified from pooled digests (SigmaSpin Post-Reaction Clean-Up plate; Sigma-Aldrich). The samples were analysed by the Australian Genome Research Facility (Adelaide, Australia) on an ABI 3730 Genetic Analyser. For each digest, 5 μL aliquots were mixed with 4 μL of formamide and 1 μL of a size standard (GeneScan-500 LIZ, ABI). The samples were denatured at 94 °C for 5 min, and then chilled on ice prior to capillary electrophoresis. Length (bp) and peak heights of TRFs were determined using the GeneMarker AFLP/Genotyping software program (SoftGenetics LLC Version1.8) using a detection limit of 200 fluorescence units (FU). TRFs that deviated by less than 1 bp in length were considered to be within the same bin set; each bin set was defined as an operational taxonomic unit (OTU).

2.3. PhyloChip microarray analysis of bacterial community structure

A high-density oligonucleotide microarray system (G2 PhyloChip; Brodie et al., 2006, 2007) was used to characterise the taxonomic composition of soil-borne bacterial communities. The array system has probes targeting the 16S rRNA genes of 8434 different bacterial OTUs (Brodie et al., 2006). Bacterial 16S rRNA genes were first PCR amplified from soil DNA using the primers 27F and 1492R (Wilson et al., 1990; Lane, 1991). PCR mixtures included