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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

primers at 0.3 μM each, dNTPs at 200 μM each, 1.2 U of Taq polymerase (Takara), 10 × reaction buffer, 10 ng of template DNA and water to 25 μL. Eight individual PCR amplifications were set up over a primer annealing range of 48–58 °C (Brodie et al., 2006). After hot-start enzyme activation, thermocycling consisted of 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 90 s. A final elongation step was performed for 10 min at 72 °C. PCR products from the separate 25 μL reactions were pooled, precipitated with isopropanol, washed with 80% ethanol and resuspended in 50 μL of water.

For each sample, 500 ng of PCR product was mixed with a control oligonucleotide spike and digested into 50–200 bp fragments with DNase I (Invitrogen). The 3′ ends of the fragments were labelled with biotin using terminal deoxynucleotidyl transferase (Promega), according to the GeneChip DNA labelling procedure (Affymetrix, CA). Biotinylated mixtures were denatured (99 °C for 5 min) and then hybridised on PhyloChip microarrays at 48 °C and 60 rpm for 16 h. Arrays were washed and stained (streptavidin–phycoerythrin) on an Affymetrix fluidics station according to protocols described before (Brodie et al., 2006).

The raw PhyloChip array data (Affymetrix CEL data files) were imported into PhyloTrac for primary array analysis (Schatz et al., 2010). Pixel images were resolved as perfect match (PM) and mismatch (MM) probe pairs and grouped into probe sets (OTUs). Each probe set contained an average of 24 probe-pairs per OTU, and also contained a central 17-mer not found in other OTUs. The trimmed mean fluorescence intensity (highest and lowest probe values removed before averaging) for each probe set was normalized to internal spike-in control intensities using a maximum likelihood method, then scaled to the mean overall array and log transformed (Ivanov et al., 2009). The internal spike was used to adjust for any variations in fluorescence over the probes relating to differences in staining, washing etc between the arrays. Taxa were considered present in a sample if at least 90% of the probes in its probe set passed the following criteria: PM/MM $\geq$ 1.3 and PM−MM $\geq$ 130 × background noise$^2$ (DeSantis et al., 2007).

### 2.4. Functional analysis of soil microbial communities using the GeoChip microarray

A functional gene array (FGA), GeoChip 3.0 (He et al., 2010) was used to assess the composition of functional genes associated with biological transformations affecting soil nutrient cycling processes. The array has probes covering about 57,000 gene sequences in 292 gene families involved in cycling of C, N, P, S, energy metabolism, antibiotic resistance, metal resistance, and organic contaminant remediation. GeoChip analyses were performed as described previously (Wu et al., 2006; He et al., 2010). In order to produce consistent hybridizations from all samples, whole community genome amplification (TempliPhi Kit; GE Healthcare, NJ) was used to generate approximately 3.0 μg of DNA with 50 ng purified DNA as the template. Single-strand binding protein (267 ng $\mu L^{-1}$) and spermidine (0.1 mM) were added to the reaction mix to improve the amplification efficiency. The reactions were incubated at 30 °C for 3 h and terminated by heating to 65 °C for 10 min. All products were labelled with the fluorescent dye Cy-5 using random priming method (Wu et al., 2006). After purification (Qiagen, Valencia, CA), labelled DNA was suspended in 120 μL hybridization solution containing 50% formamide, 3 × SSC, 10 μg of unlabelled herring sperm DNA (Promega, Madison, WI), and 0.1% SDS. The mix was denatured at 95 °C for 5 min and kept at 50 °C until it was aliquoted directly onto a microarray. Hybridizations were performed with a TECAN Hybridization Station HS4800 Pro (TECAN US, Durham, NC) and scanned by ScanArray Express Microarray Scanner (Perkin Elmer, Boston, MA). ImaGene version 6.0 (Biodiscovery, El Segundo, CA) was used to determine the intensity of each spot, and identify poor-quality spots. Raw data from ImaGene were submitted to the Microarray Data Manager (http://ieg.ou.edu/microarray/) and analysed with the following major parameters: (i) spots flagged as 1 or 3 by ImaGene and with a signal to noise ratio <2 were removed as poor-quality spots; (ii) the normalized intensity of each spot was calculated by dividing the signal intensity of each spot by the mean intensity of the microarray; (iii) at least two probes among all biological replicates were required for each gene.

### 2.5. Statistical analyses

For soil physicochemical data, variables were normalised to put them on a common (comparable) scale. The data sets showed little skewing and were therefore not transformed. Pair-wise correlations between variables were conducted to identify highly correlative links. Soil organic matter (OM) and organic carbon (OC) data were highly correlated ($r^2 = 0.999$) and were treated as a single variable (OM) in all subsequent analyses. Similarity in physicochemical properties among soils was calculated using Euclidean distances.

For fungal community analysis, each TRF size was treated as an OTU, and the peak height inferred as representing the relative abundance of that OTU. Peak-height data was log-transformed to down-weight the contribution of highly dominant TRFs in subsequent analyses. A resemblance matrix with similarity in fungal community TRFs between soil samples was calculated using the Chi-square distance method. This method has been shown to provide strong power to separate community types based on natural groupings (Kuczynski et al., 2010). The analysis was repeated using TRF presence/absence data (i.e. ignoring peak-height values).

GeoChip data associated with metal resistance, antibiotic resistance, organic remediation etc were excluded from the analyses described in this study, leaving data for 16 major gene categories associated with biogeochemical cycling of major nutrients. Functional gene intensity data (log-transformed values) were aggregated to gene-category level and similarity in GeoChip profiles between samples measured based on Chi-squared distances.

For PhyloChip data (log values), a similarity matrix was created using Pearson's correlation method. This method was selected as it performs well when comparing among samples with a large number of response variants, such as 16S rRNA OTUs from the PhyloChip. Given the high number of PhyloChip variables (OTUs), interpretation of treatment effects on bacterial communities were conducted at the phylum level (described below).

PERMANOVA and CAP analyses were used to test for effects of land use type and location on soil microbial community structure, function and physicochemical properties. PERMANOVA (permutational MANOVA; Anderson, 2001a) was used to test if between-group variation explained a significant proportion of the total system variation (i.e. if natural groupings could be detected). CAP analysis (canonical analysis of principal coordinates; Anderson and Wilis, 2003), on the other hand, determined if principal coordinates axes could be found that separate *a priori* defined treatments; i.e. CAP analysis attempted to 'seek-out' pre-defined groups within the data cloud. Both tests were conducted for each data set based on the respective resemblance matrices; the significance of test effects was determined against null distributions based on 999 permutations (random allocations) of the samples (Anderson, 2001b) under a reduced model. Our sampling strategy allowed testing for the main effects of land-use across the different locations, and main effect of sampling location across the land-use types. However, testing of effects of land-use within each site was not possible as

this level of replication wasn't present. Principal coordinates analysis (PCoA; Gower, 1966) was used to visualise multivariate distances among variables (PhyloChip and fungal OTUs, or soil physicochemical properties) in a 2-dimensional space. For GeoChip data, where treatment effects were small relative to overall variation in the data cloud, CAP analysis was used to partition main treatment effects. Vector overlays, based on Pearson correlations, were used to explore relationships between variables and the ordination axes. These were supported by SIMPER analysis (Clarke, 1993), which was used to more formally determine which variables contributed to the separation between groupings resolved by PERMANOVA or CAP analysis. For PhyloChip, Phylum-level SIMPER analysis, data were first normalised to take into account differences in probe numbers between the different phyla represented on the array. For all SIMPER analyses, the over-sown and cultivated treatments were treated as a single grouping termed 'modified'; this was based on our *a priori* interest in effects of tussock management alteration and was supported by data that showed few differences between over-sown and cultivated treatments c.f. differences to the native tussock.

Associative links between soil properties and microbial community structure and function were made using the BIO-ENV test (Clarke, 1993; Clarke and Ainsworth, 1993). This method selects variables or combinations of variables that maximise the rank (Spearman; $\rho$) correlation between two data sets (the biotic set being fixed, and variables within the environmental data being tested). Permutation of variables (499 permutations) was used to generate a null-distribution to allow for probability testing.

All multivariate analyses were conducted in the PRIMER software package with the PERMANOVA+ add-on using statistical approaches described by Clarke and Warwick (2001) and Anderson et al. (2008).

## 3. Results

### 3.1. Soil properties

Summary data for the soil physicochemical properties are given in Fig. S2. Soil physicochemical properties significantly varied between land-uses, but not locations (PERMANOVA $P < 0.05$; Table 1). PCoA (ordination) showed strong two dimensional influences (nearly even variation across both axes). Native tussock samples were separated from over-sown tussock and cultivated pasture soil samples over the *Y*-axis, and this was most strongly correlated with the $SO_4$ status of the soils (Fig. 1A; Supplementary Fig. 2). The major soil properties associated with differences between the native tussock and modified land uses (SIMPER analysis) are given in the Supplementary data. The most strongly discriminating variables were soil pH (12.9% contribution), $SO_4$ status (11.6%), P (11.3%) and then other elemental factors. Soil properties associated with C and N content were of low relative importance.

**Table 1**
Summary results table for PERMAVOVA and CAP analysis of location and land-use effects on soil microbial community structure and functional gene structure.

| | PERMANOVA | | | | | CAP | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Land use | | Location | | | Land use | | Location | |
| | √cv | $P_{perm}$ | √cv | $P_{perm}$ | √cv residual | Trace | $P_{perm}$ | Trace | $P_{perm}$ |
| Soil properties | 1.35 | 0.04 | 0.08 | 0.248 | 2.67 | 1.92 | 0.003 | 1.29 | 0.256 |
| GeoChip | −0.09 | 0.676 | 0.01 | 0.452 | 0.33 | 1.99 | 0.021 | 2.18 | 0.461 |
| PhyloChip | 0.07 | 0.004 | 0.02 | 0.067 | 0.03 | 0.80 | 0.001 | 0.34 | 0.781 |
| Fungi | 0.48 | 0.075 | 0.17 | 0.429 | 1.57 | 0.17 | 0.020 | 1.49 | 0.657 |

√cv = square root of the component of variation associated for each term.
$P_{perm}$ = probability statistic derived from permutation.
Trace = sum of the squared canonical eigenvalues.

### 3.2. Soil fungal communities – TRFLP

Variation in the soil fungal community structures was strongly linked to land use (PERMANOVA $P = 0.075$; CAP $P = 0.02$), but not location (Table 1). Ordination by PCoA revealed a trend in the data set whereby the effects of land use were separated evenly by interactions over both axes (Fig. 1B). SIMPER analysis showed that differences in fungal communities between native tussock and modified land uses were explained by variation across a large number of fungal TRFs, each contributing to a small percentage to the overall dissimilarity (Supplementary data); as such, vector overlays on the ordination plot are not given (Fig. 1B). However, several fungal TRFs present in native tussock samples were not found under modified land use types and vice versa. Transformation of the fungal data set to presence/absence and re-testing by PERMANOVA supported the finding that land-use ($P = 0.013$), but not location ($P = 0.262$), strongly affected OTUs of fungi present in the soils.

The relationship between fungal community structure and soil physicochemical properties was explored using BIO-ENV matching. Strong links were made to soil pH ($\rho = 0.481$; $P = 0.004$); other single variables had much lower correlation to fungal community structure. For example, the next strongest were $SO_4$, Ca, and K with rank correlations of 0.252, 0.242, and 0.031 respectively. The best overall solution included a combination of pH and $SO_4$ ($\rho = 0.619$; $P = 0.11$).

### 3.3. Soil bacterial communities – PhyloChip

A total of 1908 bacterial OTUs, distributed over 279 families and 41 phyla, were detected across the study sites examined in this study. Detected taxa included all major soil-associated phyla, such as Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria (alpha, beta, gamma, and epsilon sub-divisions), Verrucomicrobia and many others. A full list of taxa detected is available in the Supplementary data (microarray data file).

The structure of the soil bacterial community, as determined by PhyloChip analysis, was strongly affected by land use (PERMANOVA $P = 0.004$), which explained most of the variation in the data set (√CV values; Table 1). The effect of land use is clear in the PCoA ordination plot, with primary separation of samples over the *X* axis, which describes 85.5% of the total variation (Fig. 1C). Several phyla were strongly correlated with distances in overall bacterial community structure between samples (Fig. 1C), especially KSA1, Chlamydiae, Planctomycetes, OD1 and Actinobacteria.

BIO-ENV testing found highly significant rank-correlation between bacterial community (all OTU data; Pearson correlation) and soil properties. The strongest single variable correlation was with soil Ca content ($\rho = 0.585$; $P = 0.001$), and the strongest overall correlation was to Ca, pH, P, $SO_4$ and Mg ($\rho = 0.643$; $P = 0.003$). Independently, however, the other soil variables were not important at Ca (pH, $\rho = 0.222$; P, $\rho = 0.286$; $SO_4$, $\rho = 0.340$; Mg, $\rho = -0.029$) and investigations of the output files repeatedly demonstrated the primary importance of Ca.

SIMPER analysis on normalised phylum data (taking into account variation in total probe numbers among taxa on the PhyloChip) showed that overall differences in the bacterial communities between samples were due to a range of taxa each contributing a relatively small percentage of the differences (Supplementary data). The responses of the phyla ranked to be most discriminatory are shown in Fig. 2A. This plot (over all land uses) shows how the abundance of phyla such as KSA1, marine group A,

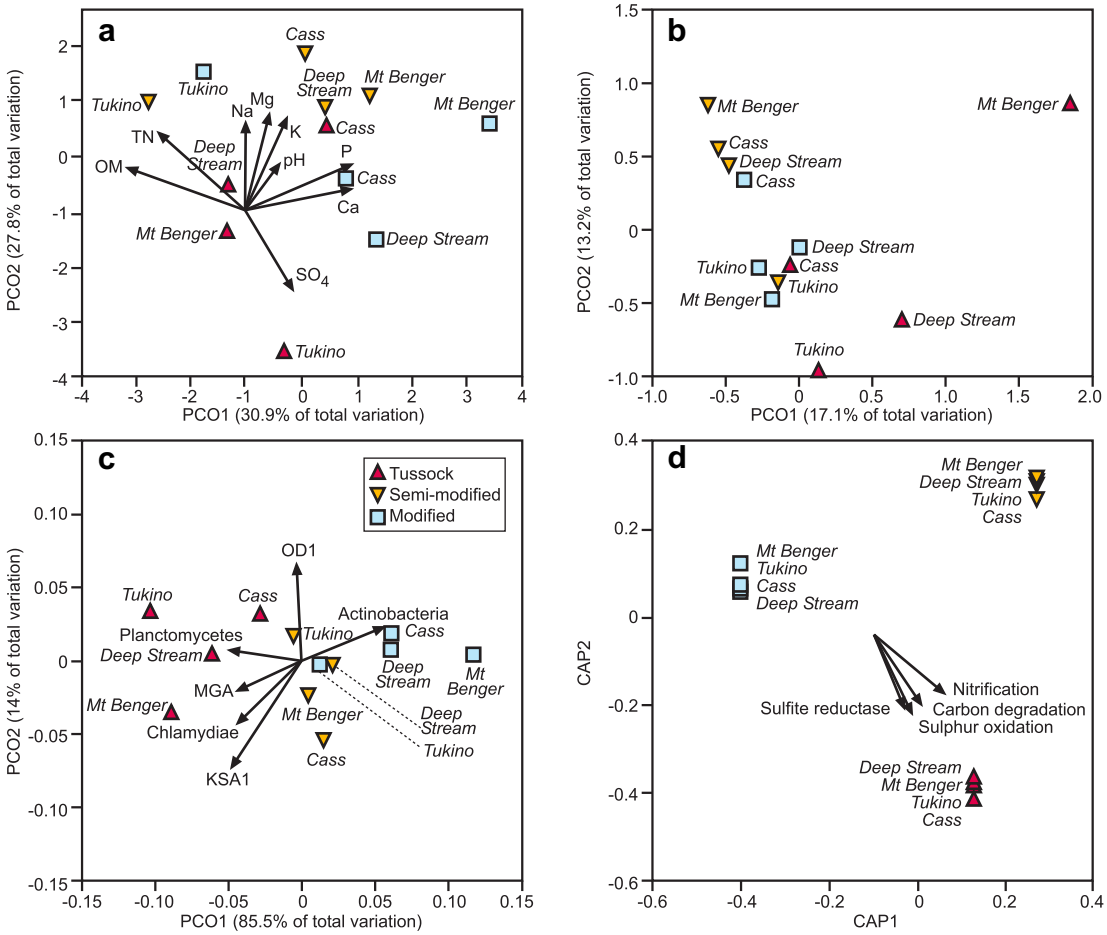


**Fig. 1.** PCoA ordination plots of (A) soil physicochemical properties, (B) fungal community structure (TRFLP), and (C) bacterial community structure (PhyloChip). (D) Canonical analysis of principal coordinates (CAP) plot of GeoChip functional analysis of soils analysed for differences according to land use ($P = 0.021$). Vector overlays represent variables strongly associated (Pearson correlation) with differences between land uses (defined as a significant treatment effect). Vectors not given for fungal community PCoA plot, as differences between samples were partitioned among many species (TRFs).

Verrucomicrobia, and Planctomycetes are reduced with modification of tussock grassland, and other phyla such Firmicutes, Actinobacteria and OD1 increase in relative abundance.

### 3.4. Soil functional gene distribution – GeoChip

There was a large variation in the distribution of functional gene properties across the soil samples included in the study, and therefore no significant treatment effects were detected using PERMANOVA (variation between treatments explained a small component of overall variation; Table 1). However, when analysed by CAP, a clear effect of land-use on soil function genes was evident ($P = 0.021$; Fig. 1D). The major gene categories that were correlated with land-use partitioning were carbon degradation, sulphur oxidation, sulphite reduction and nitrification (Fig. 1D). This result was directly supported by SIMPER analysis of soil function between the native tussock and modified land-uses (Supplementary data) – in this analysis, C degradation and S-cycling gene categories contributed to >60% of the total variation, but the contribution of nitrification was minimal. For all functional gene categories that contributed >5% to separation across the land use types (Supplementary data), the normalised abundance of these gene categories declined with change in land use from native tussock to over-sown tussock and cultivated pasture (Fig. 2B). The functional gene categories that were non-responsive were acetogenesis, methane cycling, ammonification, assimilatory and dissimilatory N reduction, and ANNAMOX.

Variation in GeoChip data (gene category level; $Chi^2$ distances) was correlated with soil pH ($\rho = 0.301$; $P = 0.05$), but not with any other soil properties. For example, the next strongest association was with total N, but this was very weak $\rho = 0.044$. The best fit of the GeoChip data to soil properties included pH, $SO_4$, Mg, and organic matter ($\rho = 0.367$), however this was not significant ($P = 0.65$).

## 4. Discussion

Modification of tussock grassland significantly altered the structure and relative abundance of genes involved in biogeochemical processes within the soil ecosystem. Land-use impacts were greater than variation in soils taken from different sampling locations spanning over 800 km in distance. Furthermore, key ecosystem properties such as tussock species composition, rainfall, elevation, soil type, slope and aspect varied among these sites (Barratt et al., in press). Thus, despite the large variation in study sites across the examined transect, clear general patterns of land use change impacts could be observed. Given the generality of the trends observed, it is reasonable to predict that such impacts are not unique to New Zealand, but might also hold for similar grassland ecosystems in other regions of the world, such as Argentina,

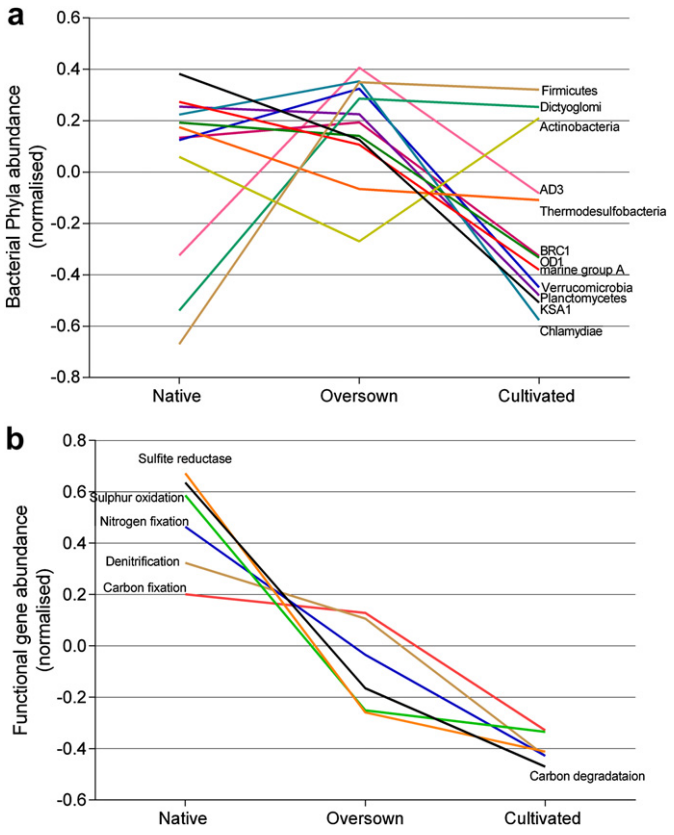


**Fig. 2.** Change in abundance of bacterial phyla (A) and functional genes (B) across three land uses. Variables plotted are those identified by SIMPER analysis to contribute towards differences between tussock and altered land uses. Bacterial phyla or functional gene families that did not contribute strongly to differences between land uses (<2.4% for bacterial phyla; <5% for functional gene families) are not shown. All data have been normalised (*Y*-axis).

North America, Brazil and northern China (Canant et al., 2001; Barratt et al., 2005).

The results associated with shifts in soil physicochemical properties and microbial community (phylogeny and function) provide strong evidence that alteration of grasslands is likely to strongly impact soil biogeochemical cycling. The primary driver of this is alteration of the base-level physicochemcial status of the soils following addition of mineral fertilisers (S and P), adjustment of pH ($CaCO_3$), and increased inputs of C from plants (possibly through livestock). The abundance of many functional genes (GeoChip data) was greater in soil under native tussock than under cultivated pasture (Fig. 2). We propose that the higher relative abundance of these genes is related to the greater dependence on internal cycling of nutrients in the unaltered tussock soils, compared with that in the modified systems. These soil ecosystems are strongly dependant on microbially-mediated nutrient cycling, and these microbial functional processes are sensitive to exogenous nutrient inputs (e.g. Lindsay et al., 2010). In particular, the decreased dependence of closed nutrient loops, and 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, as nutrients such as N can act as pollutants when leached to ground waters ($NO_3$) or lost to the atmosphere as greenhouse gases ($N_2O$).

On average, conversion of tussock grassland to cultivated pasture resulted in only small changes in the size of the soil organic matter pool (Suppl. Fig. 2); however, the effects varied greatly between sites. Net OM losses occurred at Deep Stream and Mt Benger (30–40% OM loss), but gains of 20% occurred at Cass and 36% at Tukino.

Stocks of soil C and organic matter are highly sensitive to land use change (Guo and Gifford, 2002). Worldwide, improvement of grasslands (fertilisers, legumes etc) often leads to significant increases in soil C (Canant et al., 2001). However, in systems with high initial soil carbon content, land use change often results in net loss of C (Post and Kwon, 2000). In New Zealand, some pastures have lost organic matter (Schipper et al., 2007), possibly as a result of increased microbial respiration, which exceeds photosynthate inputs. Across our sites, net change in organic matter was not correlated to rainfall or elevation, nor linked to soil type, but given the latitudinal gradient covered in the study, we propose mean annual temperature as a possible link. This would support the findings of Schipper et al. (2007), as sites with the greatest net primary productivity (temperature dependent) would be expected at the Tukino site (most northerly) and soil organic matter pools increase in time. The loss of soil organic matter at the most southern sites indicates that land-use alterations in colder zones could result in soil carbon depletion. Loss of carbon from grassland ecosystems, which typically hold more C in the soil than in the vegetative cover, has been identified as a major source of greenhouse gas emissions (White et al., 2000; Bellamy et al., 2005).

Analysis of the GeoChip data using PERMANOVA and CAP appeared to provide different outcomes in relation to the presence or absence of treatment effects. Analysis by PERMANOVA found neither a land-use or location effect. The primary reason was the high residual level of variation; the √CV for 'residual' was much greater than for treatments. Within this high overall variation the effects of treatments could not be partitioned. However, based on *a priori* definition of groupings, CAP analysis was able to find axes that separated the multivariate data cloud by the treatment-groups. This allowed for more accurate linking of variables to groups than could be achieved by using unconstrained ordination (where they would be affected by the total variation).

Alteration in the abundance of soil functional genes occurred alongside changes in microbial community structure. Alteration of native tussock system resulted in a relative decline of many phyla considered to be at low abundance in soils, i.e. excluding Acidobacteria, Actinobacteria, Proteobacteria, Bacteroidetes, and Firmicutes, which typically dominate soil 16S rRNA gene libraries (Janssen, 2006; Lauber et al., 2009). The parallel decline in the numbers of low abundance phyla (Fig. 2) and functional genes suggests a link between these bacteria and high energy maintenance systems (i.e. where nutrient and energy flow is dominated by within-system cycling and processes). However, the link between increases in the relative abundance of Firmicutes and Actinobacteria and soil organic matter cycling is not clear, as the abundance of these taxa do not vary in a predictable manner with respect to changes in soil C availability (Fierer et al., 2007). Jesus et al. (2009) found that Firmicutes, Actinobacteria and Bacteroidetes were the most responsive phyla to land use changes, and these authors attributed these effects to changes in soil pH. However, Rousk et al. (2010) demonstrated that the pH effect is mostly restricted to variation in Acidobacteria abundance and Lauber et al. (2008) found that Firmicutes and Actinobacteria were not strongly influenced by soil pH (Lauber et al., 2008), particularly over the narrow range found in these soils (Lauber et al., 2009). Fertilisation is unlikely to affect abundance of soil Firmicutes (Wessén et al., 2010), but may be linked to reduction in the Verrucomicrobia (Nemergut et al., 2008). Nevertheless, previous work supports the finding that pasture improvement and cultivation appears to select for these organisms over other land uses (Lauber et al., 2008; Kuramae et al., 2010). Given that Firmicutes

and Actinobacteria are able to form recalcitrant spores, we propose that their abundance in cultivated systems is indicative of higher periods of stress in these environments, such as soil moisture deficit, rather than the underlying nutrient status. Firmicutes and Actinobacteria, but not Proteobacteria, Acidobacteria or other dominant taxa, both increased in abundance in soils treated with Cu (Wakelin et al., 2010), indicating a more general capacity to tolerate ecosystem stress.

It has long been recognised that fungal biomass and the fungal contribution to soil nutrient cycling (relative to bacteria) are generally greater in undisturbed soil systems (Garrett, 1963). This was reflected by a previous study (Sarathchandra et al., 2005), which demonstrated that soil fungi are a diverse and significant component of the soil ecosystem in native tussock grasslands. Our results demonstrate that the composition of the fungal community was highly affected by alteration of the tussock-grassland system. Importantly, many compositional changes were due to presence/absence changes in TRFs between land-use types, indicating potential species loss or replacement. These findings are supported by culture-based identification of fungi at these sites (Sarathchandra et al., 2005). A notable change is the increase in *Fusarium* spp. in cultivated soils. This genus was 'conspicuous by its rarity' within the native tussock soils. *Fusarium* spp. have been found to have a wide range of roles in soil nutrient cycling and are highly responsive in both the quantity and species composition imposed by land-use changes (Wakelin et al., 2008). The structural composition of general soil fungal communities has also been shown to be highly responsive to land-use change, including intensification in agricultural production systems through application of fertilisers and increased stocking rates (Wakelin et al., 2009), directly supporting the findings of this study.

Samples for array-based work and the fungal TRFLP PCRs were all conducted with a standard amount of template DNA. As such, all results and findings are related to 'per unit' community size, and variation in abundances of functional and phylogenetic genes represent changes in community composition. However, the total size of the microbial communities may also be affected by sampling location or land-use alteration. As soil microbial biomass is often tightly linked to soil organic matter content (e.g. Schnürer et al., 1985), %OM values could potentially be used to adjust the data with respect to total community size.

## 5. Conclusions

This work has demonstrated strong and significant effects of modification of tussock grassland on soil microbiology and biogeochemistry. We propose that under unmodified tussock, there is greater internal re-cycling of nutrients than in cultivated pasture. This is reflected in the greater abundance of microbial functional genes associated with transformation of many nutrients under unmodified tussock grassland. Following fertilisation, pH correction and over-sowing with introduced species or full conversion to cultivated pasture, nutrient cycles are affected as less C is needed to drive internal cycling of S, P, N etc. Changes in soil microbial community structure were linked to changes in land use, but not sampling location. Shifts in relative abundance of key bacterial phyla were concomitant with changes in soil function, and indicate a role of many rare Phyla in internal nutrient cycling processes. Given the consistency of effects of land-use alteration across sites, we conclude that these impacts may be interpreted across a wide geographical range. As soil microbiota have a central role in nutrient cycles and other processes, the alteration of these communities may have far-reaching impacts to wider ecosystem services such as water quality, soil carbon storage and greenhouse gas emissions.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.soilbio.2012.07.003.

## References

Anderson, M.J., 2001a. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32–46.
Anderson, M.J., 2001b. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58, 626–639.
Anderson, M.J., Wilis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511–525.
Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth, U.K.
Barratt, B.I.P., Ferguson, C.M., Barton, D.M., Johnstone, P.D., 2009. Impact of fire on tussock grassland invertebrate populations. Science for Conservation 291, 1–75.
Barratt, B.I.P., Ferguson, C.M., Logan, R.A.S., Barton, D., Bell, N.L., Sarathchandra, S.U., Townsend, R.J., 2005. Biodiversity of indigenous tussock grassland sites in Otago, Canterbury and the central North Island of New Zealand I. The macroinvertebrate fauna. Journal of the Royal Society of New Zealand 35, 287–301.
Barratt, B.I.P., Worner, S.P., Affeld, K., Ferguson, C.M., Barton, D.M., Bell, N.L., Townsend, R.J. Biodiversity of indigenous tussock grassland sites in Otago, Canterbury and the central North Island of New Zealand VI. Coleoptera biodiversity, community structure, exotic species invasion, and the effects of disturbance by agricultural management. Journal of the Royal Society of New Zealand, in press.
Bellamy, P.H., Loveland, P.J., Bradley, R.I., Lark, R.M., Kirk, G.J.D., 2005. Carbon losses from all soils across England and Wales 1978–2003. Nature 437, 245–248.
Brodie, E.L., DeSantis, T.Z., Joyner, D.C., Baek, S.M., Larsen, J.T., Andersen, G.L., et al., 2006. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. Applied and Environmental Microbiology 72, 6288–6298.
Brodie, E.L., DeSantis, T.Z., Parker, J.P.M., Zubietta, I.X., Piceno, Y.M., Andersen, G.L., 2007. Urban aerosols harbor diverse and dynamic bacterial populations. Proceedings of the National Academy of Sciences USA 104, 229–304.
Clarke, K.R., 1993. Non-parametric multivariate analysis of changes in community structure. Australian Journal of Ecology 18, 117–143.
Clarke, K.R., Ainsworth, M., 1993. A method of linking multivariate community struc- ture to environmental variables. Marine Ecology Progress Series 92, 205–219.
Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: an Approach to Statistical Analysis and Interpretation, second ed. PRIMER-E Ltd, Plymouth, U.K.
Colloff, M.J., Wakelin, S.A., Gomez, D., Rogers, S.L., 2008. Detection of nitrogen cycle genes in soils for measuring the effects of changes in land use and management. Soil Biology and Biochemistry 40, 1637–1645.
Canant, R.T., Paustian, K., Elliott, E.T., 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecological Applications 11, 343–355.
DeSantis, T.Z., Brodie, E.L., Moberg, J.P., Zubieta, I.X., Piceno, Y.M., Andersen, G.L., 2007. High density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microbial Ecology 53, 371–383.
Espie, P., Barratt, B.I.P., 2006. Biodiversity of indigenous tussock grassland sites in Otago, Canterbury and the Central North Island of New Zealand IV. Vegetation

and the effect of disturbance by agricultural development and fire. Journal of the Royal Society of New Zealand 36, 69–82.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology Notes 2, 113–118.
Garrett, S.D., 1963. Soil Fungi and Soil Fertility. Pergamon Press Ltd, London.
Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53, 325–338.
Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360.
Hallin, S., Jones, C.M., Schloter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME Journal 3, 597–605.
He, Z., Deng, Y., Van Nostrod, J.D., Tu, Q., Xu, M., Hemme, C.L., et al., 2010. GeoChiop 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. ISME Journal 4, 1167–1179.
Henwood, W.D., 2010. Toward a strategy for the conservation and protection of the world's temperate grasslands. Great Plains Research 20, 121–134.
Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., et al., 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139, 485–498.
Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology 72, 1719–1728.
Jesus, E.D., Marsh, T.L., Tiedje, J.M., Moriera, F.M.D., 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. ISME Journal 3, 1004–1011.
Kuczynski, J., Liu, Z., Lozupone, C., McDonald, D., Fierer, N., Knight, R., 2010. Microbial community resemblance methods differ in their ability to detect biologically relevant patterns. Nature Methods 7, 813–819.
Kuramae, E.E., Gamper, H.A., Yergeau, E., Piceno, Y.M., Brodie, E.L., DeSantis, T.Z., Andersen, G.L., van Veen, J.A., Kowalchuk, G.A., 2010. Microbial secondary succession in a chronosequence of chalk grasslands. ISME Journal 4, 711–715.
Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. JohnWiley & Sons Chichester, England, pp. 115–175.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75, 5111–5120.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influences of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40, 2407–2415.
Lindsay, E.A., Colloff, M.J., Gibb, N.L., Wakelin, S.A., 2010. The abundance of microbial functional genes in grassy woodlands is influenced more by soil nutrient enrichment than by recent weed invasion or livestock exclusion. Applied and Environmental Microbiology 76, 5547–5555.
Mackay, A.D., 2008. Impacts of intensification of pastoral agriculture on soils: current and emerging challenges and implications for future land uses. New Zealand Veterinary Journal 56, 281–288.
Mark, A.F., Michel, P., Dickinson, K.J.M., McLennan, B., 2009. The conservation (protected area) status of New Zealand's indigenous grasslands: an update. New Zealand Journal of Botany 47, 53–60.
Mark, A.F., McLennan, B., 1995. The conservation status of New Zealand's indigenous grasslands. New Zealand Journal of Botany 43, 245–270.
McIntosh, P.D., Gibson, R.S., Saggar, S., Yeates, G.W., McGimpsey, P., 1999. Effect of contrasting farm management on vegetation and biochemical, chemical and biological condition of moist steepland soils of the South Island high country, New Zealand. Australian Journal of Soil Research 37, 847–865.
Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., et al., 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. Environmental Microbiology 10, 3093–3105.
Post, W.M., Kwon, K.C., 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6, 317–327.
Reeve, J.R., Schadt, C.W., Carpenter-Baggs, L., Kang, S., Zhou, J., Reganold, J.P., 2010. Effects of soil type and farm management on soil ecological functional genes and microbial activities. ISME Journal 4, 1099–1107.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., et al., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal 4, 1340–1351.
Ross, D.J., Speir, T.W., Tate, K.R., Feltham, C.W., 1997. Burning in a New Zealand snow-tussock grassland: effects on soil microbial biomass and nitrogen and phosphorus availability. New Zealand Journal of Ecology 21, 63–71.
Sarathchandra, S.U., Burch, G., Sayer, S.T., Waipara, N., Cox, N.R., Ghani, A., et al., 2005. Biodiversity of indigenous tussock grassland sites in Otago, Canterbury and the central North Island of New Zealand III. Soil microorganisms. Journal of the Royal Society of New Zealand 35, 321–337.
Schatz, M.C., Phillippy, A.M., Gajer, P., DeSantis, T.Z., Andersen, G.L., Ravel, J., 2010. Integrated microbial survey analysis of prokaryotic communities for the PhyloChip microarray. Applied and Environmental Microbiology 76, 5636–5638.
Schipper, L.A., Baisden, W.T., Parfitt, R.L., Ross, C., Claydon, J.J., Arnold, G., 2007. Large losses of soil C and N from soil profiles under pasture in New Zealand during the past 20 years. Global Change Biology 13, 1138–1144.
Schnürer, J., Clarholm, M., Rosswall, T., 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biology and Biochemistry 17, 611–618.
Wakelin, S.A., Chu, G.X., Lardner, R., Liang, Y.C., McLaughlin, M., 2010. A single application of Cu to field soil has long-term effects on bacterial community structure, diversity, and soil processes. Pedobiologia 53, 149–158.
Wakelin, S.A., Colloff, M.J., Harvey, P.R., Marschner, P., Gregg, A.L., Rogers, S.L., 2007. The effects of stubble retention and nitrogen application on soil microbial community structure and functional gene abundance under irrigated maize. FEMS Microbiology Ecology 59, 661–670.
Wakelin, S.A., Gregg, A.L., Simpson, R.J., Li, G.D., Riley, I.T., McKay, A.C., 2009. Pasture management clearly affects soils microbial community structure and N-cycling bacteria. Pedobiologia 52, 237–251.
Wakelin, S.A., Warren, R.A., Kong, L., Harvey, P.R., 2008. Management factors affecting size and structure of soil *Fusarium* communities under irrigated maize in Australia. Applied Soil Ecology 39, 201–209.
Wardle, D.A., Bardget, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between above ground and belowground biota. Science 304, 1629–1633.
Wessén, E., Hallin, S., Philippot, L., 2010. Differential responses of bacterial and archaeal groups at higher taxonomical ranks to soil management. Soil Biology and Biochemistry 42, 1759–1765.
White, T.J., Bruns, T.D., Lee, S., Taylor, J., 1990. Analysis of phylogenetic relationship by amplification and direct sequencing of ribosomal RNA genes. In: Innis, M.A., Gelfond, D.H., Sainsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp. 315–322.
White, R., Murray, S., Rohweder, M., 2000. Pilot Analysis of Global Ecosystems: Grassland Ecosystems. World Resources Institute, Washington, DC.
Wilson, K.H., Blitchington, R.B., Greene, R.C., 1990. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. Journal of Clinical Microbiology 28, 1942–1946.
Wu, L., Liu, X., Schadt, C.W., Zhou, J., 2006. Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Applied and Environmental Microbiology 72, 4931–4941.
Yeates, G.W., Lee, W.G., 1997. Burning in a New Zealand snow-tussock grassland: effects on vegetation and soil fauna. New Zealand Journal of Ecology 21, 73–79.
Yergeau, E., Schoondermark-Stolk, S.A., Brodie, E.L., Déjean, S., DeSantis, T.Z., Gonçalves, O., Piceno, Y.M., Andersen, G.L., Kowalchuk, G.A., 2009. Environmental microarray analyses of Antarctic soil microbial communities. ISME Journal 3, 340–351.