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RESEARCH

Protecting the unseen majority: Land cover and environmental factors linked with soil bacterial communities and functions in New Zealand

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Abstract: The biodiversity in soil ecosystems is simultaneously incredibly rich and poorly described. In countries such as New Zealand, where high endemism in plant species emerged following extended geographical isolation, it is likely similar evolutionary pressures extended to soil microbial communities (our biodiversity 'dark matter'). However, we have little understanding of the extent of microbial life in New Zealand soils, let alone estimates of endemism, rates of species loss or gain, or implications for systems where plants and their microbiomes have co-evolved. In this study, we tested for the impacts of land-cover type (native forest, planted forest with exotic conifers, and pastoral agriculture) on soil bacterial communities and their functional potential, using environmental microarrays (PhyloChip and GeoChip, respectively). This evaluation was conducted across four environmentally different locations (Hokitika, Banks Peninsula, Craigieburn, and Eyrewell). The environment from which samples were collected was the largest and most significant factor associated with variation in bacterial community assemblage and function. As such, novel pockets of bacterial biodiversity, with discrete ecosystem function, may be present in New Zealand. There was some evidence to suggest that change in land cover affected soil bacterial species, but not their functions. Secondary testing found this effect was restricted to differences between native forest and agricultural land use. Bacterial communities and functions between native and planted forests were similar. Analysis of soil environmental properties among samples found that land cover effects were underpinned by changes in soil pH that typically accompanies application of lime in agricultural systems, but is uncommon in planted forests. When compared with other studies conducted in New Zealand, we conclude that: (1) different locations can harbour distinct communities of soil microbial diversity, and (2) land-use intensification, not land cover change *per se*, shifts microbial biodiversity through alteration of primary habitat conditions, particularly soil pH.

Keywords: bacterial community assemblage, bacterial community function, soil microbiology, land use type, land use intensification

Introduction

New Zealand's natural ecosystems harbour high rates of species endemism (Kier et al. 2009). For example, over 80% of New Zealand's 2500 species of native plants, spanning conifers to flowering plants and ferns, are found nowhere else. Relative to flora and fauna, we know far less about endemism and rarity of New Zealand's microbial species, particularly those living in soils. New Zealand is estimated to have 22 000 species of fungi, of which at least 1480 are likely to be endemic (Johnston et al. 2017). For the remaining microbial biodiversity, spanning bacteria, algae, protists, and so on, the catalogue of species present in New Zealand is so poorly documented that the extent of endemism is unknown (MfE 1999). This microbial biodiversity not only comprises a vast reservoir of native and endemic species, but also has a critical role in supporting native plant species; 90% of New Zealand's plants are directly reliant on these microbial associations (MfE 1999).

In addition to supporting the diversity and growth of plants, soil microbial life delivers a range of ecosystem functions including regulation of water flow and quality, carbon storage and climate regulation, through to disease occurrence and suppression (Coleman & Whitman 2005). Many of these functions directly deliver towards the United Nations Sustainable Development Goals (SDGs; Keesstra

et al. 2018). Adopted by all UN members, the SDGs provide a roadmap for sustainable use of global resources and, central to this, are robustly functioning soil and water systems.

The rich microbial life in soil also comprises a reservoir of novel chemical agents (antibiotics, pharmaceuticals, and plant growth regulators), biocontrol agents, and biological fertilisers (Bardgett & van der Putten 2014). Protection of this natural capital provides insurance against natural and human-made disruptions to sustained delivery of ecosystem function and human health and well-being (e.g. Delgado-Baquerizo et al. 2017). However, given the lack of knowledge of soil microbial biodiversity in New Zealand, we have no idea if we are gaining or losing entire clades of microbial taxa (Geisen et al. 2019), nor the consequences such losses may impart on the functioning and resilience of our ecosystems.

If the conservation and preservation of New Zealand's endemic soil microbial life is of value, it is essential to assess the extent of this diversity (i.e. which species are present and in what ecosystems). This is a necessary first step in understanding their conservation status, and ideally linking conservation values to the implicit and intrinsic value of the species itself, and its role in delivering ecosystem functions. These concepts are not new to conservation ecology in macroecological systems, but their application in soil microbiology has only recently been considered following: (1) a growing recognition of the critical role of microbial life in soil processes that underpin the SDGs and human wellbeing (Koch et al. 2013; Wall et al. 2015; Keesstra et al. 2016; Bach et al. 2020), and (2) the resolution of clear links between species and delivery of ecological functions (e.g. Trivedi et al. 2017, 2019). The contribution of data and samples from New Zealand into global soil biodiversity initiatives (Cameron et al. 2018) will be fundamental towards understanding what is unique about the New Zealand soil microbiome, and will aid in audits such as 'State of the Environment' reporting (MfE 1999).

It follows, therefore, that the second research area is "how do we appropriately conserve and restore unique microbial species in soils?" This question is fundamental given many soils, as distinct habitats, have been extensively modified globally. Indeed, the development of human society has been centred on our ability to use resources, such as soils, to support plant and animal-based agricultural systems (Meyer & Turner 1992; Parikh & James 1992). Utilisation of soils for societal agricultural needs has required chemical modification (particularly through adjustment of pH and alteration of micro- and macronutrient status), physical alteration (e.g. tillage, drainage), from inputs of agrichemicals (pest, weed, and disease control), and by fundamentally altering below- and above-ground linkages by replacing the species present (Wardle et al. 2004). Understanding how different habitat alterations (e.g. nutrient enrichment vs plant cover) have impacted on the natural capital of soils will be needed to support development of strategies directed towards soil ecosystem restoration (Heneghan et al. 2008).

The goal of this study was to determine the importance of land cover on soil bacterial species and functions in New Zealand soils. In particular, we aimed to understand the role of land use change (different cover types) in maintaining diversity to a reference normal situation of native New Zealand plant cover; significant efforts are still required (elsewhere) to define the microbial species that are endemic and/or at conservation risk. The focus on land cover in this study is (1) a reflection of the extent of land-use change (*sensu* change in plant cover from native to productive/managed systems)

that has occurred in New Zealand, and (2) recognises the growing base of scientific knowledge on the importance of land use change on soil microbial communities and functions. However, while an understanding between land use change and soil microbial communities has been observed across a broad range of biomes, most work has focussed on regions of high macroecological diversity such as the Amazon (Jesus et al. 2009; Rodrigues et al. 2013; Ranjan et al. 2015; Pedrinho et al. 2019).

Like many countries, New Zealand has experienced dramatic changes in plant cover. Exotic grasslands (primarily for livestock grazing) constitute 39.6% of the land cover, and exotic forests (typically single-species plantation forestry) cover a further 8% (2012 data; MfE & Stats NZ 2018). Shifts between land uses are ongoing, and often reflect movements in commodity prices (i.e. meat, milk solids, wood) and carbon trading (Anastasiadis & Kerr 2013). In the future, the introduction of pricing tools that value ecosystems services such as soil stabilisation, water regulation, and biodiversity protection (OECD 2018) will also likely drive decisions around land use and intensification in New Zealand. Current information available for New Zealand provides strong evidence for links between land cover and the assemblage of microbial communities present (e.g. Hermans et al. 2017; Wood et al. 2017).

The experimental design we used was set up such that land-cover types (native, planted forest, and grassland for livestock grazing) were sampled across an environmental gradient, enabling partitioning of the relative importance of environment compared with land cover, but also examined a range of variables against which variation in bacterial communities and functions among samples might be attributed. The methodology followed a similar approach as a previous New Zealand-based study (Wakelin et al. 2013) that analysed soil ecosystem DNA using high density phylogenetic and functional microarrays to investigate effects of land use intensification (native grassland through to high-input dairy pasture) on bacterial communities and function. As such, the results of both studies can be compared to determine the relative influence of land use cover, and land use intensification, on the biodiversity and functional ecology of New Zealand soils.

The aims of this study were to: (1) determine the importance of land cover and environment (sampling location) on soil bacterial diversity and function; (2) determine key links between changes in land-cover type, soil physicochemical conditions (e.g. shift in pH), and soil bacterial diversity and function, and (3) assess the relative magnitude of effect of change in land cover from planted forest and low-intensity grassland, and of land use intensification (from native grassland through to high-input grassland), on shifting soil ecosystem from a reference native state (native bush/forest).

Methods

Sites, soil collection, and soil characterisation

Our sampling strategy was based on a transect across the South Island of New Zealand, where contrasting environmental settings occur over a short geographical distance (Appendix S1 in Supplementary Materials). Collection of soil was made from three adjacent land cover types – pastoral agriculture (pasture), planted pine forestry (pine), and native forest/bush (native) – at four different sites (Hokitika, Orton Bradley Park on Banks Peninsula, Craigieburn, and Eyrewell). The sites

were selected to minimise distance between vegetation types within each ecological zone (all were within 1 km of each other). The environmental settings and land use descriptions are given in full in Wakelin et al. (2014), so are only briefly summarised here.

The Hokitika site is low altitude (< 100 m above sea level; a.s.l.), super humid mesothermal environment with high annual rainfall (2852 mm) and an average temperature of 11.7°C. Coldest winter temperatures occur in July with an average low of 4.4°C, and highest summer temperatures in February, with an average high of 19.6°C. The soil at the Hokitika site formed on an alluvial, infertile, flood plain. The Craigieburn site is 940 m a.s.l., receives high rainfall (1800 mm), and has an average yearly temperature of 8.1°C. However, the subalpine environment at Craigieburn results in extremes of climatic conditions among seasons. The average winter low is -2.4°C and summer high is 21.7°C. The soil at Craigieburn is comprised of greywacke loess and colluvium. The Banks Peninsula site has a temperate, maritime climate with average temperature of 12.6°C and receives 928 mm of rainfall. The soil at Banks Peninsula formed from primary loess and colluvium overlaid on weathered bedrock. The Eyrewell site is on the Canterbury plains (220 m a.s.l.) and has the lowest annual rainfall of the sites (771 mm). The annual temperature at Eyrewell is 11.2°C, and the soils were formed from moderately weathered greywacke loess over gravels. Soil description data are taken from Meurk et al. (1995); rainfall and air temperature were taken from the New Zealand National Climate Database (http://cliflo.niwa.co.nz/) and were based on 30-year average datasets. Note that, due to inadequate data at the monthly time step, average winter low and summer high temperatures for Eyrewell and Banks Peninsula could not be calculated.

At each site, soils from adjacent native forest or scrubland, plantation forest (*Pinus* spp.), and pastoral land uses were collected. All pastures were dominated by mixtures of grasses (primarily *Lolium* spp.) with a legume component (*Trifolium* spp.) and were used for sheep grazing; i.e. these were not high intensity pastoral systems (*sensu* high nutrient and water input to support high stocking / dairy). For the native sites, the vegetation varied at each location; details are given in Wakelin et al. (2014).

Soils were collected after removal of the surface organic matter layer (i.e. forest floor material, or pasture sward). A spade was used to collect soil to 5 cm depth (spade-square sampling). At each location, three random samples were collected, pooled into composite samples, and passed through a 2 mm sieve. The bulk of this soil was used for soil fertility analysis (Wakelin et al., 2014) at an accredited provider (Hill Laboratories, NZ). Briefly, pH and electrical conductivity (E.C.) were measured in 1:5 soil:water extracts, organic matter measured by combustion and CO_2 determination, total N and C using the Kjeldahl method, available P using Olsen extraction and Molybdenum blue colorimetry, extractable cations (Ca_{ext} and Mg_{ext}) were measured using atomic absorption spectroscopy, and bulk density (BD) assessed on a ratio of mass of dried and ground soil to soil volume. Sulphate sulphur (SO_4-S) was extracted in 0.02 M K_2HPO_4 and determined with ion chromatography; total S was measured after acid digest using ICP-OES, and organic S was calculated as the difference. Available N was calculated after anaerobic incubation (40°C for 7 days) KCl extraction and Berthelot colorimetry. In addition to the empirically measured variables, charcoal-C, particulate organic C (POC), quartz, kaolin, smectite, Si, Al,

Fe, Ca, and Mg were inferred from mid-infrared spectroscopy (MIR) and partial least squares analysis. Detailed methods are provided elsewhere (Haaland & Thomas 1988; Janik & Skjemstad 1995; Janik et al. 2007).

A small subsample of soil (10 g) was kept at 4°C for up to five days for DNA extraction. Extractions were conducted in triplicate from 0.25 g sub-samples of each soil using the MoBio PowerSoil DNA extraction kit. Mechanical disruption of the samples was conducted using a vortex-mixer attachment (max power for 10 min). The triplicate samples of DNA were pooled providing a single representative sample for each treatment. Samples were quantified using spectroscopy (NanoDrop ND-100) and stored at -80°C until use.

Bacterial community composition

Bacterial communities were characterised using PhyloChip, a high-density oligonucleotide microarray system (Brodie et al. 2006, 2007). The PhyloChip, has probe sets covering > 8400 bacterial operational taxonomic units (OTUs) with a taxonomically-hierarchical design; i.e. samples can be analysed at individual probe level and/or aggregated up to phyla-level groupings.

Near full-length bacterial 16S rRNA genes were amplified from the soil-extracted DNA using primers 27F and 1492R (Wilson et al. 1990; Lane 1991). The methodology and PCR chemistry followed previously described methods (Wakelin et al. 2013). Eight individual PCR reactions were set up over a primer annealing range of 48–58°C, thus reducing primer bias to bacterial taxa. PCR products from the separate 25 µl reactions were pooled, precipitated with isopropanol, washed with 80% ethanol, and resuspended in water. The reaction mixtures were then processed for PhyloChip analysis as described in Brodie et al. (2006), DeSantis et al. (2007), and Schatz et al. (2010).

Soil functional genomics

A functional gene array, GeoChip V3.0 (He et al. 2010) was used to characterise the composition and abundance of nutrient cycling in the soil samples. The array design has coverage of approximately 57 000 genes spanning 292 families of functional genes associated with the cycling of nutrients (C, N, S, P, Fe, etc), metal and antibiotic resistance, pathogenicity elements, and many others (Wu et al. 2006).

DNA from each soil sample was fragmented, labelled, and hybridised to GeoChip arrays as described previously (Wu et al. 2006; He et al. 2010). For the sample collected from under native bush at Eyrewell, there was insufficient DNA to conduct GeoChip analysis. Although whole community genome amplification could be used to increase total DNA content (Wang et al. 2011), the bias associated with this method can be large and obfuscate underlying biological trends (Wakelin et al. 2016). As such, this sample was not included for processing or subsequent data analysis.

After stringent array washing, arrays were scanned by a ScanArray Express Microarray Scanner (Perkin Elmer, MA) and the intensity of each probe-spot read (ImaGene v6; Biodiscovery, CA). Probe spots with signal to noise ratio < 2 were removed and the intensity for remaining probes was then normalised by the mean intensity of the microarray.

Data analysis

Soil and environmental properties were initially normalised (scaled to a mean centred on 0 and with ± 1 standard deviation),

allowing comparison of the variables on a common scale. Similarities in soil and environmental properties among samples were then calculated using Euclidean distance, and the magnitude of main effects (sample environment and land use) partitioned using permutational multivariate analysis of variation (PERMANOVA; Anderson 2001).

Pair-wise testing among environments and land use types was conducted to determine the individual comparisons that strongly differed (PERMANOVA t-test). For comparisons in which pair-wise testing demonstrated statistically significant differences; SIMPER (Clarke 1993) was used to determine the contributions of individual variables towards overall separation among the samples. Although statistical testing was conducted on adjusted data, untransformed data (e.g. mean values based on the units the variables were collected in) are presented to allow direct interpretation of the results.

PhyloChip array data were imported into PhyloTrac (Schatz et al. 2010) and the taxa present in each sample determined. From this OTU-level data, alpha-diversity statistics to measure species (*sensu* 16S rRNA phylotype / OTU) richness (Margalef's index; d) and evenness (Pielou's index; J') were calculated. The variation of these among environments and land cover groups was determined via two-way ANOVA. Correlations (Spearman's rank) among these indices and corresponding abiotic data were used to test for underlying relationships with soil or environmental variables that were not linked to land cover or location (environment). For beta-diversity analysis, UniFrac (phylogenetic relatedness) distances were calculated among samples (Lozupone & Knight 2005), and visualised using non-metric, multi-dimensional scaling (nMDS ordination). Testing for the contribution of environment and land cover (effect size) in explaining overall bacterial community composition, and also for pair-wise differences among samples, followed the method described for the abiotic data set. In addition, the extent of dispersion (variation in community composition) among the land cover groups in each environment was calculated (PERMDISP) with deviations calculated from the median. The distribution of bacteria (Class level) among land cover and environments was visualised using a heat map generated from log-transformed abundance values (samples grouping by location). Numerically dominant taxa present in the soils were identified from the 200 most abundant OTUs (ranked based on signal intensity) and assignment of these to Phyla level phylogeny. To explore underlying relationships between the taxa present and abiotic variables, the total data set was first aggregated to Phylum and Class levels, the values log-transformed, and Spearman's rank correlations made against each of the abiotic variables.

The GeoChip data were reduced to the set of probes associated with nutrient cycling categories (C, N, S, P, etc). These data were then analysed in a highly similar manner to the PhyloChip data, with the exception that analysis of alpha-diversity was not conducted and distances among samples calculated using Bray-Curtis dissimilarity.

Biota to environmental matching (BIO-ENV matching; Clarke & Ainsworth 1993) was used to link both the bacterial (PhyloChip) and functional (GeoChip) multivariate structure to the abiotic (soil and environmental) variables. The routine used the BEST approach; Mantel-type testing that is supplemented by permutation of the variables establishing a null-distribution from which the likelihood (P_{perm} significance) of the test outcome (Spearman's ρ-correlations) can be assessed.

The impact of land-use intensification compared with land cover change in affecting bacterial community composition (PhyloChip data) was investigated by comparing data collected in this study (land cover groups) with a study of similar design but in which intensification of grazed grasslands was assessed across different environmental locations in New Zealand (Wakelin et al. 2013). While these studies used the same methods for sample collection, DNA extraction, and analysis (PhyloChip), the calculation of ecological distances in community composition differed; distances in the previous study were calculated using Pearson's method. Therefore, to enable valid comparisons across studies, PhyloChip data for both studies were recalculated using log-transformed, Bray-Curtis generated ecological distances. Thus, comparable ecological distances between unaltered, semi-modified, and modified grasslands samples were determined (intensification gradient) and could be validly compared alongside the data for the pasture, pine, and native cover soil bacterial communities. Fisher's LSD test was used to compare treatment means among samples.

Results

Abiotic properties

While soil and environmental properties varied strongly across sampling environments (locations) and between land cover groups, environment was the strongest factor (Table 1). The main and pair-wise treatment effects are summarised in Table 1, and differences in variables among treatments given in Appendix S2 and S3 in Supplementary Materials.

For the environmental properties, Hokitika significantly differed from all other sites (Table 1). Hokitika and Craigieburn sites mostly varied with regards to soil structure, with Hokitika having higher % sand and lower % clay (MIR predicted as smectite) than at Craigieburn. Soil from Craigieburn contained greater levels of charcoal than at Hokitika, reflecting a natural history of fire at this site (Kelly 1995). Hokitika and Eyrewell were separated based on rainfall (considerably higher at Hokitika; Appendix S2) and this was also reflected in lower exchangeable Ca (due to leaching) in the Hokitika soil (Appendix S2). The abundances of smectitie and kaolin also varied between these two sites (Appendix S2). Soil fertility was a major factor defining differences in soils from Banks Peninsula and Hokitika (Appendix S2), with higher total N, organic S, available N and organic C in the Banks Peninsula soils.

Pair-wise comparisons between land-cover types found strong separation in soils planted to pasture and native forest ($P = 0.031$), and when pasture was compared with exotic forest ($P = 0.055$; Table 1). However, there were no differences in soils sampled from under native or plantation forest ($P = 0.61$) (Table 1). When compared with native forest, pasture soils had lower levels of SO_4^{2-}, POC and a lower C:N ratio, but higher pH, Olsen P, and total Mg. Similarly, when compared with exotic forest, pastures had a lower C:N ratio, POC, but higher Mg, and Olsen P. Appendices S2 and S3 summarise how environmental variables varied between locations and between land-use types (SIMPER analysis).

Bacterial α-diversity

Neither the richness (d) nor evenness (J') of the soil bacterial communities were directly linked to location ($P_d = 0.689$; $P_{J'} = 0.273$) or land-cover type ($P_d = 0.914$; $P_{J'} = 0.553$). Similarly, when community richness values were correlated to

Table 1. PERMANOVA main effects summary table. P derived from 999 unrestricted permutations of the raw data; √CV = square root of the components of variation; [1]PhyloChip bacterial community microarray; [2]GeoChip functional gene microarray; n.d. = not determined as the main test for land cover was not approaching meaningful significance. BP = Banks Peninsula.

	Environmental variables		Bacterial community composition[1]		Ecosystem functional genes[2]	
Main tests	P	√CV	P	√CV	P	√CV
Environment	0.002	3.35	0.015	0.10	0.007	30.05
Land cover	0.036	2.01	0.078	0.07	0.636	-8.78
residual		4.23		0.16		38.07
Pair-wise tests	P		P		P	
Hokitika v Craigieburn	0.045		0.172		0.093	
Hokitika v Eyrewell	0.034		0.046		0.161	
Hokitika v BP	0.013		0.204		1.000	
Craigieburn v Eyrewell	0.159		0.214		0.030	
Craigieburn v BP	0.033		0.031		0.020	
Eyrewell v BP	0.775		0.055		0.138	
Native v pasture	0.031		0.022		n.d.	
Native v exotic	0.610		0.474		n.d.	
Exotic v pasture	0.055		0.119		n.d.	

combinations of soil and environmental variables (BIO-ENV testing), no associations were evident ($P = 0.75$). However, the evenness (Pielou's index) of the bacterial community was strongly correlated with soil pH and exchangeable Ca ($\rho = 0.677$; $P = 0.017$). Linear regression was then used to explore these relationships. In both cases, these were significant ($P < 0.002$) and positive; i.e. as acidic soils became more neutral (increase in pH), or concentrations of exchangeable Ca increased, bacterial communities became increasingly even (Appendix S4 in Supplementary Materials).

Bacterial β-diversity

Location was the most important factor linked with variation in the structure of the bacterial community ($P = 0.015$; Table 1). Pair-wise comparisons revealed significant differences between bacterial communities from Hokitika and Eyrewell, Craigieburn and Banks Peninsula and there was some evidence to support differences between Eyrewell and Banks Peninsula ($P = 0.055$; Table 1). These effects are evident in the nMDS ordination, where environmental effects (groupings of samples by location) are stronger than those of land use (Fig. 1a). Also evident are differences in multivariate dispersion within each site, i.e. how far land-use management effects could affect or drive separation in community composition (Fig. 1a). At the Banks Peninsula site, the extent of variation caused by land cover was small (multivariate dispersion = 0.11) when compared with sites such as Craigieburn where dispersion was 1.63 (i.e. > 90% more variation).

Although partitioning of variation due to land-cover was not as strong as environment (Table 1), bacterial communities under native forest differed significantly from those under pastoral agriculture ($P = 0.022$). This separation is not well represented in the nMDS ordination (Fig. 1a) but is evident on 3D plots, i.e. collapsing the multivariate distances to two dimensions does not sufficiently represent the structure and effects present within the underlying dataset. SIMPER analysis (Class level), showed that most of this difference (57.6%) could be explained by variation in Gammaproteobacteria, Betaproteobacteria, and Bacilli (more abundant in pasture), along with Alphaproteobacteria and Actinobacteria (more
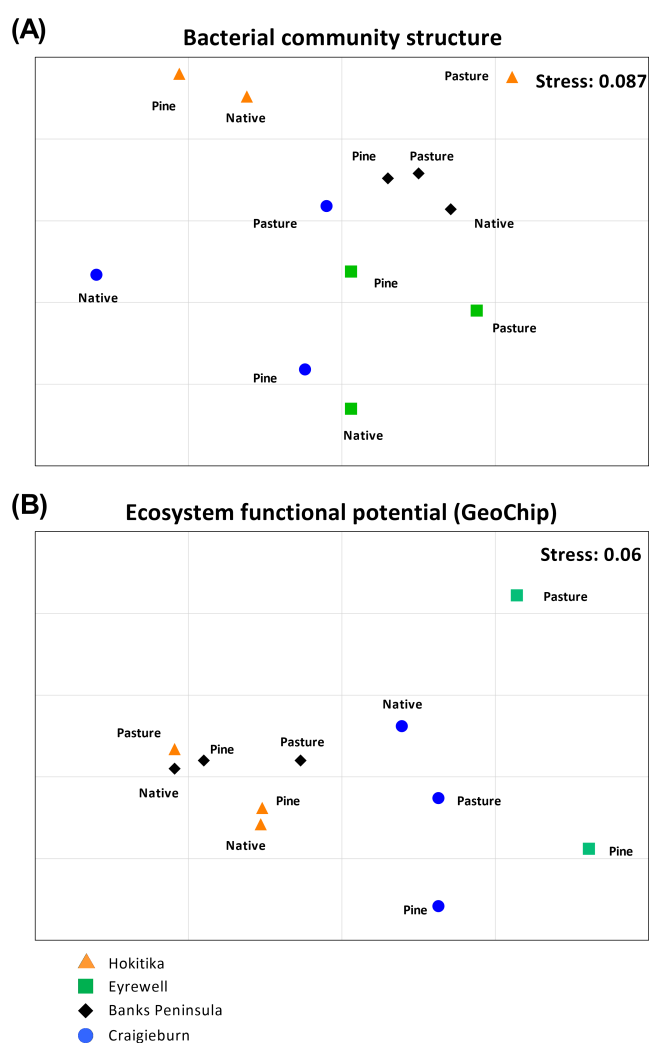

Figure 1: nMDS ordination plot showing similarity among samples based on (a) phylogenetic distances (UniFrac) and (b) functional gene similarity. Samples closer in the ordination plot have similar phylogenetic or functional structure c.f. those further apart.

abundant in soils planted to natives).

The numerically dominant bacterial community, defined as those represented in the top 200 OTU probe intensity data set, comprised 18 assignable Phyla (other unknown taxa were present). This comprises approximately half the total Phyla detected in the entire data set (n = 43). Over all the samples, the dominant Phyla were Proteobacteria (59.7%), Acidobacteria (10.6%), Actinobacteria (8.1%), Firmicutes (7%), and Bacteroidetes (3.3%); others were all below 3%. The distribution of Phyla across locations and land-use types is given in Appendix S5 in Supplementary Materials.

The abundances of all dominant Phyla (defined as present at > 3% abundance), except the Firmicutes and Bacteroidetes, were significantly correlated with abiotic ecosystem properties (Fig. 2). When the Phyla were split into respective Classes, the contributions of different groups are evident (Fig. 2). Overall, the soil organic S concentration was the variable most frequently associated with abundances of bacterial taxa; this was expressed across a wide range of Phyla. Clay and bulk density (soil texture) were the next most important factors followed by C:N ratio and exchangeable Ca (Fig. 2). Environmental factors (rainfall and temperature) were only correlated to the abundances of two taxa; a group of unclassified Proteobacteria (rainfall) and Actinobacteria (temperature).

The relative effect size of land use intensification compared with land cover type on bacterial community composition was tested by comparing data from a previous study (Wakelin et al. 2013) with this study (Fig. 4). Generally, the mean values for all land use intensification comparisons, i.e. transition from unaltered grassland to semi-modified and fully modified states, had higher impact on bacterial communities than change in cover (Fig. 4). Importantly, the shift of tussock to semi-modified grassland, essentially representing a shift in soil fertility from a non-improved state to one where minimal fertiliser and lime have been added (Wakelin et al. 2013), had a stronger magnitude effect than movement of land use from native cover to either pasture or pine forestry.

Phyla	Class	pH	EC	Char	C/N	Olsen P	Organic S	Clay	BD	Exch-Ca	Rainfall	Average temp
Proteobacteria							-0.6054	-0.6084				
	Alphaproteobacteria						-0.6514	-0.6713				
	Betaproteobacteria		0.6294				-0.5841					
	Gammaproteobacteria						-0.5735					
	Deltaproteobacteria					-0.5926						
	Epsilonproteobacteria						-0.5961	-0.6089	-0.6503			
	Unclassified	-0.6316		0.6154	-0.575		-0.5983			-0.7483	0.6909	
Acidobacteria						0.5944	-0.6032	-0.5874				
	Acidobacteria					0.5944	-0.6032	-0.5874				
	Acidobacteria-5	-0.5895				0.6084				-0.5944		
	Acidobacteria-6											
	Acidobacteria-10						-0.5912	-0.6643				
	Sloibacteres	-0.6456				0.6224	-0.7196	-0.6014		-0.6294		
	Unclassified						-0.6491			-0.5874		
Actinobacteria				0.6224					-0.6294			-0.5614
Firmicutes												
	Bacilli											
	Clostridia											
	Desulfotomaculum											
	Gut clone group											
Bacteroidetes												
	Bacteroidetes											
	Sphingobacteria						-0.5735					
Chloroflexi							-0.6408					
	Chloroflexi-3											
	Anaerolineae						-0.5877					
	Dehalococcoidetes						-0.7399					
	Unclassified					-0.5785	-0.6124					
Verrucomicrobia						0.6923		-0.5947	-0.6853			
	Verrucomicrobiae				0.7063	-0.6067	-0.577	-0.6434				
	Unclassified						-0.6266	-0.6923				
OP10							-0.6054	-0.5874				
	CH21 Cluster						-0.5735					
	Unclassified						-0.57					
Planctomycetes										-0.5874		
Cyanobacteria								-0.6154				
	Cyanobacteria							-0.5874				
	Unclassified						-0.5877					
Gemmatimonadetes						-0.582	-0.6054					
SPAM												
Synergistes												
Natronoanaerobium							-0.5877					
TM7							-0.6585					
OP9/JS1							-0.5806					
Nitrospira												
OP3												
Unclassified							-0.616					

Figure 2: Significant (Spearman's ρ > 0.05) correlations between the abundance of bacterial taxa (Phyla and Class level) with environmental and soil variables. Blue/teal are positive correlations, and red to yellow negative. Colour intensity is a guide to the degree of correlation.

Gene category	Sub category	EC	Organic S	SO4	Clay	Sand	Smectite	Exch-Mg
Carbon cycling			-0.6791					
	Carbon degradation	0.6182	-0.7391					
	Carbon fixation		-0.6375					
	Acetogenesis		-0.6375				-0.6364	
	Methane		-0.6976					
Energy process			-0.6929					
Nitrogen cycling			-0.6329					
	Denitrification		-0.5959					
	Dissimilatory N reduction		-0.6929					-0.6364
	Assimilatory N reduction	0.6545	-0.6837		-0.6455	0.6182		-0.6636
	Nitrogen fixation		-0.7114					
	Ammonification							
	Nitrification							
	Anammox	0.6455	-0.656					
Phosphorus cycling			-0.7022					
Sulphur cycling			-0.6976					
	Sulfite reductase		-0.6976					
	Sulphur oxidation		-0.7391	-0.6179				
	Other sulphur		-0.7761					

Figure 3: Significant (Spearman's $\rho > 0.05$) correlations between the ecosystem functional potential (GeoChip gene families and sub-families) with environmental and soil variables. Blue/teal are positive correlations, and red to yellow negative. Colour intensity is a guide to the degree of correlation.
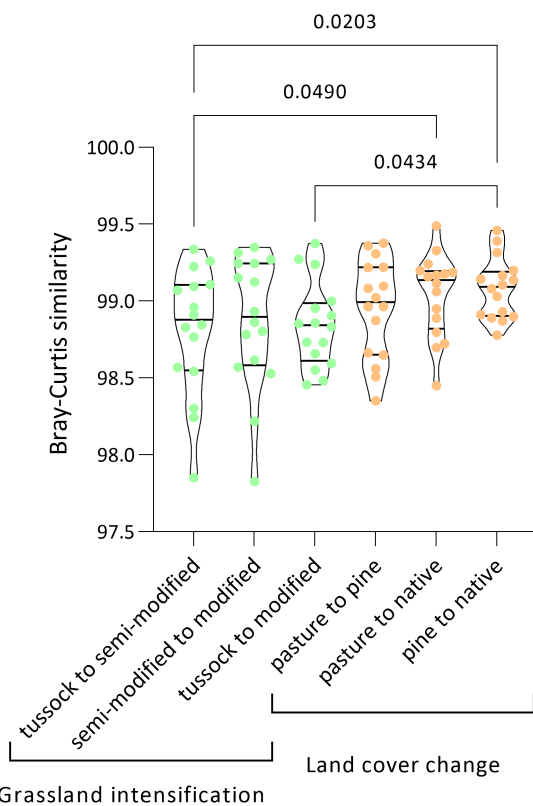
Figure 4: Relative impact of land use intensification compared with different land cover types on changes in soil bacterial community composition among different environments. Bray-Curtis distances were compared among pairs of treatments. With increasing similarity between treatments, i.e. conserved community composition, samples are higher on the Y-axis (100 = completely similar communities). Treatments connected pairwise (overhead line) have significantly different mean values (test *p*-values are provided).

Ecosystem functional potential

Analysis of similarity in functional gene abundances (GeoChip) between samples showed that sampling location ($P = 0.007$) was strongly associated with potential ecosystem function (Table 1). Significant differences were present when comparing Craigieburn and the other sites, particularly Banks Peninsula and Eyrewell ($P < 0.05$; Table 1). In contrast, land-cover did not affect soil functional gene profiles ($P = 0.636$; Table 1). The relative influence of environment c.f. land-cover on functional genes is evident in the nMDS ordination plot (Fig. 1b).

The abundances of nearly all functional genes were significantly related to the organic S status of soils, the exceptions being nitrification (*amo*A gene family) and ammonification (*ureC* and *gdh* gene families) (Fig. 3). Soil E.C. correlated with abundances of C degradation (29 genes), anammox (*hzo* genes), and assimilatory N reduction (*nir* and *nsa* gene families). Assimilatory N reduction was also correlated with soil structure (clay and sand) and exchangeable Mg. In addition to organic S, the abundance of sulphur oxidation genes (*sox*) was significantly correlated with the sulphate content of the soils.

Discussion

The Anthropocene epoch has been coined in recognition of human impact on Earth's biomes and systems (Lewis & Maslin 2015). In particular, it recognises that human activity now directly influences more than three-quarters of the global land area (Ellis & Ramankutty 2008). Alteration of these systems, particularly through land-use/cover change and intensification, is impacting many ecosystem services including biodiversity. Given the high level of endemism in New Zealand's flora and fauna, and the role of soil microbial communities in supporting these directly (e.g. as symbionts) or via delivery of ecosystem

functions, it is critically important to understand the impacts of land cover change and land use intensification on soil microbial life. As New Zealand's flora and fauna have an intrinsic value, so too should microbial species. As yet, however, we have a poor understanding of the magnitude of diversity present in New Zealand, the extent of endemism within this, nor the conservation status assigned to rare, threatened, or keystone species (Banerjee et al. 2018).

For pristine or relatively unmodified soils, which have evolved from different parent material and/or formed under different pedogenic processes, classification schemes (e.g. Hewitt 1998) are linked to soil physicochemical parameters, and thus provide useful classification habitats for microbial communities (Lauber et al. 2008, 2009; Wakelin et al. 2008). As such, previous studies in New Zealand have shown that the distribution of microbial species is linked to underlying soil type (e.g. pallic, recent, ultic, gley and other NZ soil orders) (Kaminsky et al. 2017). The modification of the parameters that underlie soil groupings, either directly through agricultural inputs or indirectly through the effects of plant growth on soil conditions, can therefore increasingly mask the background soil type effect. Likewise, Kaminsky et al. (2017) reported that prokaryotic communities (beta-diversity) vary primarily with pH and land-use. Furthermore, to observe a soil type effect on soil bacterial communities, variation due to pH must first be accounted for (Kaminsky et al. 2017). For example, in high-intensity agricultural systems, where soil conditions are highly modified to optimise the productive potential of a pasture or crop, the primacy of soil type being an effect is obviated as the physiochemical attributes that comprise a soil type are overly modified (Wakelin et al. 2013). Anthropogenic modification of soils, therefore, profoundly impacts the diversity and ecology of soil-dwelling species. As there has been no systematic monitoring of these ecosystems over time, it is impossible to know if mass extinctions or invasions have occurred, nor if key components of the ecosystem (e.g. keystone endemic taxa) are inadvertently being maintained. Although the technology is still developing, the analysis of archived soil samples using ancient DNA techniques (e.g. Martin-Laurent et al. 2001; Ivanova et al. 2017), may eventually be able to help reconstruct New Zealand's soil ecosystems over extended time periods and address these critical biodiversity and conservation questions. Perhaps New Zealand can create another catalogue of lost species?

A key goal of this work was to determine the importance of land cover and environment as drivers of bacterial species and functions in soils. We have been able to build on a body of knowledge demonstrating the importance of land-use on soil bacterial communities (compositional-based diversity) in New Zealand's natural and managed ecosystems. For example, Wood et al. (2017) found all microbial (and invertebrate) taxa significantly differed across a range of land use types including natural and planted forests, unimproved and improved grasslands, and vineyards. Natural forests had high abundances of Solibacterales and Chromatiales (purple sulphur bacteria) c.f. other land uses, immediately providing examples of taxa potentially susceptible to land use change. Underlying these changes were key associative links between bacterial communities and soil pH, base saturation, and C:N ratio. Hermans et al. (2017) also assessed geographic distance and land cover factors associated with soil bacteria in New Zealand. For most land cover types (native and planted forest, horticulture, dairy, and dry stock), the effect of geographic distance on bacterial communities were minimal.

Rather, compositional change in bacterial communities was associated with variation in soil pH, C:N and Olsen P (Hermans et al 2017), all of which typically differ between land use/cover types. Finally, Kaminsky et al. (2017) also demonstrated the importance of land-use intensification on bacterial communities in New Zealand soils, by comparing high country, sheep and beef, and dairy-based grazing systems (stocking type and rates). As described previously, these studies also further highlight the importance of soil pH as a primary factor associated with bacterial community change in New Zealand soils, and were achieved using meta-barcoded next generation sequencing (NGS) which allowed for analysis of large sample numbers. Overall, these findings are entirely consistent with those presented in an earlier study (Wakelin et al. 2013) in which a smaller set of samples (3 land use types × 4 locations) were analysed using environmental microarrays. Thus, even with 12 samples analysed, the underlying influence of soil pH, cations, and sulphate-S on bacterial community composition was evident, supporting the case for a close and constant relationship between these factors and microbial (bacterial) diversity in soils.

An important outcome was confirmation that microbial species and functions present in soils can vary strongly across locations (Wakelin et al. 2008, 2012, Kaminsky et al. 2017). For most species, this is likely to be linked to differences in soil and environmental conditions across sites, and not geographic distance per se (Hermans et al. 2017; Dignam et al. 2018). Regardless, there are likely to be environments in New Zealand that are unique from a soil biology perspective; these may warrant consideration for protection from anthropogenic alteration.

The influence of land cover on soil bacterial community composition varied within each environmental setting. At sites such as Craigieburn and Eyrewell, relatively large shifts in taxa and functions were observed among land cover types, but these shifts were relatively minor at, for example, the Banks Peninsula site. Across all the data, however, the only significant land-cover effects were between soils under native cover compared with grassland pastures. Importantly, the bacterial taxa in soil under pine could not be significantly distinguished from those under native cover.

An important difference in soil properties separating pasture from native forest soils was soil C:N, P and S content, and particulate organic matter. These variables are an expression of the role of fertiliser inputs typically used to support agriculture (soil P and S status), and the land cover itself (POC and C:N). Despite these influences, shifts in the soil bacterial composition between native cover and pastures remained most closely linked with soil pH (Spearman's correlation ρ = 0.642). This pH effect on soil bacterial communities has been often been found in studies globally (Lauber et al. 2008, 2009; Kaminsky et al. 2017; Dignam et al. 2018). In places such as New Zealand that have naturally low soil pH, the shift of land use from native cover (average pH 4.93) to pasture (5.63) typically involves application of agricultural lime. Thus, the extent of agriculture and pastoral land cover across New Zealand has likely had a profound influence on the underlying biology and functioning of soil ecosystems. The change in land cover from native cover to pasture was associated with a shift in dominance of Alphaproteobacteria and Actinobacteria taxa towards more Gammaproteobacteria, Betaproteobacteria, and Bacilli. Makiola et al. (2019) showed that the alpha-diversity of plant pathogens (fungal, oomycete and bacterial) was much higher in modified land-uses than natural forests. Furthermore,

changes in microbial communities that occur within a land-use type can impact adjoining ecosystems. For example, the evolution of plant pathogens originating from remnant wild vegetation bordering cultivated land has been demonstrated. The presence of herbaceous perennials at this agro-ecological interface provides opportunities for pathogens to survive crop cycles, even when fallowed (Papaix et al. 2015).

Some relationships between soil bacterial abundance (relative proportions of bacterial groups in soil) and carbon cycling / mineralisation have been established (Fierer et al. 2007). The abundance of Actinobacteria has been negatively correlated with C mineralisation (oligotrophy), while the abundance of Betaproteobacteria are positively correlated (copiotrophy). These results provide evidence that shift in soils from under native cover to agriculture, supported by fertiliser and pH alteration (lime), may affect overall ecosystem attributes between K towards r type ecology/selection (sensu Fierer et al. 2007). However, this remains to be formally tested.

The pH in soils under native (pH 4.95) and planted forest (pH 4.93) were similar and, accordingly, hosted similar bacterial communities and functions. The finding that soil pH is similar under native and planted forest is important as there is a perception that forests based on exotic conifers, particularly *Pinus radiata*, result in soil acidification and a reduction in soil biological health. These results show the average level of pH reduction was only 0.02 units when compared with soils collected under native land cover. Furthermore, given the close association between soil pH, fertility, and C-cycling with the biological communities and functions in soil, the soil ecosystem attributes were similar across exotic and native forest systems.

Changes in overall ecosystem function – determined by GeoChip – were conserved over land cover types despite changes in taxa, demonstrating redundancy in function across wide ranges of soil bacteria. Changes in functions were linked to environmental conditions and this was strongly associated with the organic S status of soils. This single property (organic S) was linked with a broad range of gene categories, spanning aspects of the carbon, nitrogen, phosphorus and sulphur cycles. Similarly, the organic S content of soils was also correlated with the abundance of many groups of bacterial taxa, but this single property was not as dominant as for associations determined on ecosystem functional genes (i.e. many other variables also correlated with taxa abundance). The extent of association between organic S and the soil ecosystem function was unexpected, as this has rarely been reported, although the organic S status of soils is rarely measured as part of soil microbial ecology studies (Wakelin et al. 2008). In studies where various sulphur forms are measured, such as a survey of 50 New Zealand pasture soils, associations between bacterial abundance (Dignam et al. 2018) and function (Wakelin et al. 2016) have been evident. These findings indicate that sulphur may have a regulatory influence on the biogeochemical processes underpinning a range soil functions (including C, N and P cycling) and should be routinely measured in soil microbial ecology.

Studies have previously investigated the effects of plant species, soil type, management practices, environmental change (e.g. precipitation, elevated CO_2, temperature), and many other factors on soil ecosystems. However, unless these are conducted using similar (preferably identical) experimental and analytical frameworks, the determination of the effect size of the various treatments is difficult to establish. Given similarity in experimental system used in this study and one conducted previously in New Zealand (land use intensification; Wakelin et al. 2013), we were able to validly compare treatment size effects. Across both studies, environmental drivers (soil conditions) constituted the primary factors associated with variation in bacterial species and functions present in soils (as discussed above). However, while change in management of soils under native grasslands or forest into productive ecosystems was concomitant with change in soil microbiology, the magnitude of belowground effects was not associated with those observed above-ground. For example, the soil ecosystem difference between native tussock and low-input, extensively grazed grassland was of greater magnitude than the difference between native forest and pine plantation. The large relative differences in soils between tussock and low-impact grasslands is due to the impact of soil modification from fertiliser and lime use; changes in the above ground species composition comprise much weaker secondary effect.

Given the impact of soil pH and fertility on bacterial communities, alongside the potential conservation importance of New Zealand's below-ground biodiversity, appropriate questions are (1) how much biodiversity has been lost due to the expansion of agriculture; and (2) can we restore biodiversity and soil ecosystem functions? This may prove challenging as the impacts of agricultural practices can persist for many decades even after shifts in plant cover. For example, the legacy effect of pastoral agriculture is strongly evident in soils even 60 years after afforestation into *Pinus radiata* (Addison et al. 2019). Ongoing impacts of agricultural land use on aboveground biodiversity have also been well established (Isbell et al. 2019; Li et al. 2019). In addition to affecting the biodiversity present, these historic land management practices have ongoing effects on the ability of ecosystems to respond to alterations in management (Addison et al. 2019; Li et al. 2019), and may impact the ability of the ecosystems to respond to disturbance such as climate disruption (Hulme 2017). The restoration potential of soil ecosystems will likely require alteration of soil physicochemical conditions, such as fertility and pH. Even if it is possible (or practicable) to engineer soils as 'a receptive habitat for desired taxa', ecological restoration may still require novel approaches to reconstruct the ecosystem, including management of soil physical and chemical conditions, vegetation (Heneghan et al. 2008), and potentially the biology (e.g. via soil transfer; Bulot et al. 2017).

A significant impact of the Anthropocene is change in land use/cover and intensification to meet the food, fibre, energy and other needs of society. The changes can alter properties of soils that are fundamental in shaping the biological diversity within these ecosystems. In places such as New Zealand, where endemism is relatively high, the resulting effect on gain/loss of species and functions is unknown. However, as these taxa coevolved with native flora they are likely to have a direct role in supporting New Zealand's plant diversity. They also support delivery of a range of other ecosystem functions. We demonstrate that environmental locations across New Zealand harbour distinct communities of bacteria, and the alteration of soil pH, typically through use of agricultural lime, dramatically changes community composition. The impact of soil pH disturbance has been shown to be persistent (many decades) with enduring impacts on ecosystem outcomes. Given demand for food, fibre, and fuel from land use, anthropomorphic alteration of systems is not only needed, but likely to grow, and changes in land cover and intensification are likely to continue. Within an eco-pragmatic approach, trade-offs between loss or change to native/endemic soil biodiversity and maintaining the potential to restore ecosystems to a historic state, need to be considered alongside demands for other ecosystem services.

Acknowledgments

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Supplementary material

Additional supporting information may be found in the supplementary material file for this article:

Appendix S1. Sampling locations across the South Island, New Zealand. (1) Hokitika, (2) Craigieburn, (3) Eyrewell, and (4) Banks Peninsula. The total distance between sampling locations (1–4) is 172 km.

Appendix S2. SIMPER analysis determining contributions of soil and environmental variables to defining differences between sampling environments (over all land cover types).

Appendix S3: SIMPER analysis determining contributions of soil and environmental variables to defining differences between land-use types (over all sampling environments).

Appendix S4: Relationships between bacterial community evenness and soil pH and soil exchangeable Ca. Lines of best fit are based on simple regression; goodness of fit (R^2) and significance (p-values) are given on the charts. Correlation coefficients were based on non-parametric (Spearman) rank method; these were 0.87 and 0.80 for pH and Ca, respectively. Both correlations are significant ($P < 0.002$).

Appendix S5: Dominant Phyla present in the New Zealand soil samples (by environment and land cover type). The results are generated from the probe intensity data for the top 200 OTU's. Eighteen Phyla are present as well as unclassified.

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