SOIL BACTERIAL COMMUNTIES ARE AFFECTED BY SOIL PH AND PHOSPHOROUS CONCENTRATIONS

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Many of the processes that sustain life on earth are at least partly catalysed by bacteria and similar organisms which carry out critical ecological functions. Specifically, the soil environment is home to many organisms, both eukaryotic and prokaryotic which are important contributors to the overall functioning of the ecosystem. Soil dwelling prokaryotes (i.e., bacteria and archaea) in particular play important roles within soil ecosystems. They are involved in key soil biogeochemical cycles (Ranjard *et al.*, 2000), can interact with plants both directly and indirectly to affect their growth (Compant *et al.*, 2005; Wu *et al.*, 2008) and contribute to the formation and structure of soils (Tisdall *et al.*, 1978; Wu *et al.*, 2008). Altogether, the interaction between bacteria and many components of the soil ecosystem highlights their vital roles, and therefore importance for the maintenance of soil ecological health and production potential.

Since soil bacteria are a crucial component of the soil ecosystem, it is imperative that we better understand their presence in our soils, and how land use, in particular the intensification of agricultural practises, impacts these communities. However, to date, our knowledge regarding the composition of soil bacterial communities across New Zealand is incomplete, and more importantly, we are limited in our understanding of how they respond to either natural or anthropogenic factors at large spatial scales. As agricultural and forestry products generate almost 70% of New Zealand's export earnings, we are heavily reliant on the health and productivity of our soils (Ministry of Agriculture and Forestry, 2011). Increasing our understanding of what bacteria are present in our soil and why they are there is therefore important.

Further benefits from studying the biogeography, or distribution, of soil bacterial communities could come in the form of using them as indicator organisms. There are many examples of macroorganisms, both invertebrates and vertebrates, being used as indicators of environmental health or biodiversity (Cairns Jr and Pratt, 1993; Neher, 2001; Whitfield and Elliott, 2002; Marchant, 2007). However, despite their presence in most environments, bacteria are not commonly utilised in this manner. This is likely due to the historic difficulties in determining which bacteria are present where, and the biases associated with previously having to rely on culturing them. However, the development of molecular methods for describing bacterial diversity allow us to study in detail the bacterial communities present in our environment. These tools enable us to gain a better understanding of which environmental or anthropogenic variables may influence, or correlate with, changes in bacterial communities. This will allow us to determine whether the diversity and composition of bacterial communities can be used to measure the health of an environment and the impacts of anthropogenic activity. Developing a bacterial tool to monitor the health of our soils could greatly benefit New Zealand's agricultural industry. However, before this can be

achieved, we first need to expand our knowledge around the bacteria in New Zealand's soils and what influences their distribution.

Our aims were to determine:

1. If bacterial communities in soils from different land uses are significantly different to each other in composition
2. If there are any patterns in the extent to which bacterial communities are different to those that are found in natural forests
3. Which environmental variables correlate with the extent to which bacterial communities in human impacted environments are different to those found in natural forests

To achieve these aims, a large number of soil samples were collected throughout the Auckland and Waikato regions of New Zealand (Figure 1). Within these regions, five different land uses were sampled: horticulture, dairy, dry stock, exotic forests and indigenous forests (Table 1).
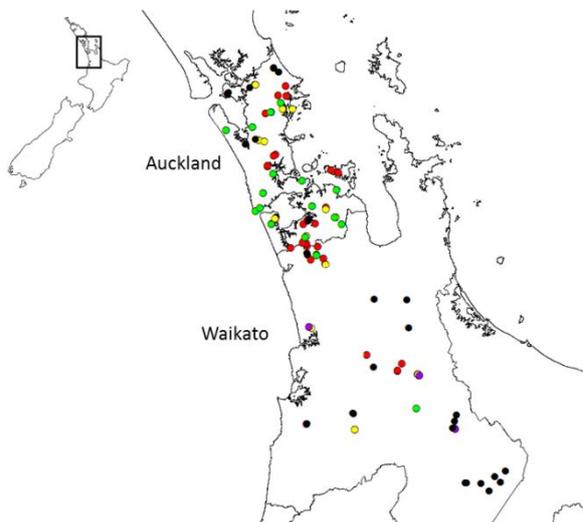
Figure 1. Location of the sites sampled within the Auckland and Waikato regions. Five different land uses were sampled: indigenous forest (●), exotic forest (●), horticulture (●), dairy (●) and dry stock (●).

Table 1. Description of the land uses sampled for this study, adapted from Hill and Sparling (2009).

Land Use Type	Description
Horticulture	Mostly market gardening, orchards or vineyards. Typically in rows, with or without grass cover between rows.
Exotic forest	Typically exotic pine or Eucalyptus to produce timber for construction, pulp and paper
Indigenous forest	Typically beech or broadleaf Podocarp forest with understorey species (ferns etc.); but could also include wetland and coastal habitat and indigenous dominated scrubland.
Dairy farming	Permanent grass-legume pastures, typically used for dairy farming.
Dry stock farming	Permanent pastures or grasslands (including tussock grasslands). Typically used for beef cattle.

At each of the 110 sites, up to 25 samples (i.e. soil cores to 10 cm depth) were collected along a 50 m transect line and the DNA extracted from each sample separately, using PowerSoil® - htp 96 well DNA Isolation Kits (Mo Bio Laboratories Inc., CA, USA). From each of these DNA extracts, a region of the bacterial 16S rRNA gene (V3-V4) was then amplified using PCR and sequenced on the Illumina MiSeq Platform. Bioinformatic analyses was conducted using a combination of USEARCH (Edgar, 2010) and QIIME (Quantitative Insights into Microbial Ecology) to ultimately generate a table that shows both the presence and abundance of thousands of OTUs (operational taxonomic units) within each site. An OTU is a unit commonly used to describe microbial diversity based on 16S rRNA sequence data. Organisms belonging to the same OTU can broadly be thought of as belonging to the same bacterial species, an approach which is highly debated, but remains in common use in microbiology (Rosselló-Mora and Amann, 2001).

To complement the bacterial data that was generated, soil chemistry and physical characteristics were assessed as described in Hill and Sparling (2009). The parameters measured included nutrients such as carbon, nitrogen and phosphorus, as well as the soil pH, bulk density, macroporosity and the concentration of a wide range of metals. Additionally, ArcGIS 10.3 (Environmental Systems Research Institute (ESRI), Redlands, CA) was used to extract climatic data for each site, using the site locations and NIWA (National Institute of Water and Atmospheric Research) databases.

Overall, statistical analyses revealed significant differences among soil bacterial communities from each land use. Pairwise PERMANOVAs showed that each comparison of bacterial community composition from different land uses varied significantly (p-value < 0.001, after corrections for multiple comparisons).

When assessing the extent to which the bacterial communities in our human impacted sites differed to those found in our indigenous forest, it was found that there was a gradient. Our two most intensive land uses, horticulture and dairy, harboured communities which were the most different to those found in the native, indigenous forests. The exotic forests and dry stock farms had communities which were still significantly different to those communities found in the indigenous forests, but to a lesser extent than our more intensive land uses.

Out of all the soil and climatic variables collected, three were highlighted as being significantly correlated with the extent to which bacterial communities in human impacted environments differed to those in our indigenous forests. The most important variable was Olsen Phosphorus (Olsen P) followed by the pH of the soils. Lastly, the slope of the sites seemed to correlate with a small subset of the bacterial communities. These results highlight the correlation between bacterial communities and anthropogenic activity since both Olsen P and pH are linked to land use. Olsen P, which measures the biologically available phosphorus in soils, is strongly linked to anthropogenic activity, as phosphorous is an important fertiliser applied to New Zealand soils (During, 1984; Olsen, 1954). Similarly, the pH of soils used for agriculture is often intentionally altered through practises such as liming (During, 1984).

Overall, the results from this large scale bacterial study have shown that bacterial communities are indeed affected by what is happening above the ground. That is to say that our anthropogenic activities alter the soil environment which in turn is reflected in changes in the bacterial communities. This provides more insight into the variability of bacterial communities and which environmental variables correlate with the changes in composition. Additionally, by highlighting the link between land use and soil bacteria, this study has also confirmed that bacterial communities could be used as a biologically relevant indication of the effect of anthropogenic activity on New Zealand's soil.

Following this investigation at a regional scale, we will now be assessing the changes in composition of bacterial communities using samples collected on a national scale, both from the North and South Islands. This data will be used to map the biogeography of soil bacterial communities across New Zealand to identify the correlations between bacterial community attributes and soil physicochemical factors. This will ultimately aid the development of novel bacterial indicators of soil health, which will help us manage nutrient use to ensure the long term sustainability of our agricultural industry.

References

Cairns Jr J, Pratt JR. (1993). A history of biological monitoring using benthic macroinvertebrates. In: Freshwater biomonitoring and benthic macroinvertebrates, Rosenberg, DM & Resh, VH (eds), Chapman and Hall Inc.: New York, USA, pp 10–27.

Compant S, Duffy B, Nowak J, Clément C, Barka EA. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71: 4951–4959.

During C. (1984). Fertilisers and soils in New Zealand farming. Sigma Print Limited: Petone, NZ.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460–2461.

Hill RB, Sparling GP. (2009). Soil quality monitoring. Land and soil monitoring: A guide for SoE and regional council reporting. Land Monitoring Forum: NZ.

Marchant R. (2007). The use of taxonomic distinctness to assess environmental disturbance of insect communities from running water. Freshw Biol 52: 1634–1645.

Ministry of Agriculture and Forestry. (2011). Situation and outlook for New Zealand agriculture and forestry (SONZAF) 2011. Ministry of Agriculture and Forestry: Wellington, NZ.

Neher DA. (2001). Role of nematodes in soil health and their use as indicators. J Nematol 33: 161–168.

Olsen SR. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department Of Agriculture: Washington, D.C, USA http://krishikosh.egranth.ac.in/handle/1/2041713 (Accessed November 11, 2015).

Ranjard L, Poly F, Nazaret S. (2000). Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. Res Microbiol 151: 167–177.

Rosselló-Mora R, Amann R. (2001). The species concept for prokaryotes. FEMS Microbiol Rev 25: 39–67.

Tisdall J, Cockroft B, Uren N. (1978). The stability of soil aggregates as affected by organic materials, microbial activity and physical disruption. Soil Res 16: 9–17.

Whitfield AK, Elliott M. (2002). Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. J Fish Biol 61: 229–250.

Wu T, Chellemi DO, Graham JH, Martin KJ, Rosskopf EN. (2008). Comparison of soil bacterial communities under diverse agricultural land management and crop production practices. Microb Ecol 55: 293–310.