

7.2 Soil Bacteria composition

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Preamble: The most numerous organisms in soil are bacteria, which play a crucial role in nutrient cycling and other soil biogeochemical processes. Bacteria respond to major soil disturbance, such as droughts, floods, and land management activities e.g., tillage, lime addition. changes and thus there can be a change in composition of bacteria. Bacterial composition can be measured in different ways which provides different levels of detail about composition. For example, composition described by phospholipid fatty-acid analyses can be expressed as amount of gram positive and gram-negative bacteria with only selected bacterial taxa identified, while more recent analyses have focussed on using molecular approaches, in particular eDNA metabarcoding approaches, which provide a finer level of detail about the composition of bacteria in a given soil sample.

State of knowledge of the ‘Bacteria composition in soil’ attribute: **Poor / inconclusive** – based on a suggestion or speculation; no or limited evidence

There is some knowledge on bacterial compositional change in relation to land use types or activities. However, while we can answer the question of ‘Who is there?’, we are still struggling to understand the ‘What are they doing?’, and therefore the significance of compositional changes. ‘What are they doing?’ can be done on a smaller scale, but our current limitation is cost effective analysis of soil samples at a national scale.

Part A—Attribute and method

A1. How does the attribute relate to ecological integrity or human health?

Soil bacteria are key players in soil nutrient cycling and their composition is known to influence the ecological integrity by either enabling or stalling some of those nutrient cycling functions. When examining bacterial composition in soil, information on which bacterial taxa are present can be provided and some information on abundance and function they perform in soil can be provided although these relationships are variably known. In relation to human health, soil bacterial composition in itself is not a risk, however if there are pathogenic bacteria present and growing in

soils, that can become an issue from a human health perspective if these bacteria enter waterways or food sources. However, bacteria are not the only soil organisms that contribute to biogeochemical processes. Fungi, protists, and invertebrates are just as important, and they all contribute to soil nutrient cycling. They are also just as sensitive to external changes in soil as bacteria and it is important to encompass total soil microfauna when talking about soil biodiversity and soil health [1].

Bacterial composition in soils can be measured in different ways which provides different levels of detail about composition. For example, composition described by phospholipid fatty-acid analyses can be expressed as amount of gram positive and gram-negative bacteria with only selected bacterial taxa identified – alongside information on fungal community. More recent analyses have focussed on molecular analysis using eDNA metabarcoding approaches, which provides a finer level of detail about the described taxa of bacterial population present in a given soil sample. When talking about bacterial composition, we often compare alpha and beta-diversity measures to look for patterns occurring between different soil samples. Bacteria are often very sensitive to soil disturbances and with their (generally) fast growth rate, their composition gives us a good insight into which bacterial communities respond to an external factor of interest (e.g., fertilisation, tillage, drought, heavy metals). Since bacteria are major drivers of soil biogeochemical processes, we can extrapolate what soil functioning changes in soil based on the taxa present.

A2. What is the evidence of impact on (a) ecological integrity or (b) human health? What is the spatial extent and magnitude of degradation?

Several studies looked at the relationship between soil bacterial composition to soil health, but they are mainly limited in the scope or defined by the specific case study. When studies look into bacterial composition as an indicator of soil health, they often correlate microbial alpha and beta diversity measures to soil disturbance. Internationally, [2] found that bacterial and fungal diversity is lower in undisturbed systems (woodlands) and high in grasslands and disturbed environments (croplands). [3] searched for bacterial biomarkers that would be directly correlated to soil health after major disturbance and found that genome size, interpolated from bacterial community data is the best predictor of soil disturbance (tillage) that they measured. Nationally, [4] also looked at correlations between bacterial composition in soil and soil chemical properties. However, the evidence of direct impact of bacterial composition on ecological integrity is very weak, with studies only looking at correlation of bacterial beta diversity with soil type. While there are very clear trends emerging from individual studies when examining correlations between bacterial diversity and soil type, these analyses are very site specific. When looking at different soil types and land uses in different environments, the correlation will change and that makes current microbial measurements not suitable for predicting soil health metrics to be used across the globe [5, 6]. In the recent years, there has been increasing international evidence that microbial functioning (spanning wider than just bacterial) is a better soil health indicator than diversity measures [7].

A3. What has been the pace and trajectory of change in this attribute, and what do we expect in the future 10 - 30 years under the status quo? Are impacts reversible or irreversible (within a generation)?

This is unknown as there have been no studies that provide consistent information over time in New Zealand. How soil disturbance affects bacterial composition depends on the readiness of the soil bacterial community to change. Some soils can be considered more resilient and more ready for change when multiple members of soil bacterial community can support the same soil function (e.g., nitrification). These soils will perform better over time and will adapt to new conditions faster [8].

Currently, studies are only studying one or two disturbances in the study system. This gives us important oversight into potential resilience of soil community, but we are still unable to predict the response of microbial community when they are exposed to multiple disturbances, such as successive weather events (e.g., prolonged drought, followed by prolonged flooding). These are already happening as our climate keeps changing and it will be an even more common occurrence in the future [9].

A4-(i) What monitoring is currently done and how is it reported? (e.g., is there a standard, and how consistently is it used, who is monitoring for what purpose)? Is there a consensus on the most appropriate measurement method?

The attribute is not currently monitored or reported as such either nationally or internationally and there is no clarity of use [6].

Improvements in technology accelerated the scale of which the soil bacterial composition can be measured. Initially, phospholipid-fatty acid profiles were the main way to determine bacterial composition by determining Gram-positive:negative ratios which is correlated to C, N availability and drought resistance, however this method is limited by the number of samples that can be processed at the same time. With development of next generation sequencing, metabarcoding became a primary way to sequence and determine bacterial composition in soils, with the ability to sample a wide range of samples in one sequencing run. However, this still only gives us information on microbial taxonomy which can only be linked to soil health and functioning via correlation. While there has been an evolution in methodology, it is still unclear how to best measure microbial response to soil disturbance and how to interpret/standardise [9].

There has been an EU funded study (Benchmark programme - <https://soilhealthbenchmarks.eu/scientific-articles/>) that looked at soil indicators in Europe and while they have acknowledged soil bacteria composition as an option, it is not consistent enough to include it on the list on indicators.

A4-(ii) Are there any implementation issues such as accessing privately owned land to collect repeat samples for regulatory informing purposes?

Private land may be required to be accessed for soil sampling for regulatory informing purposes, and thus there can be issues with access.

A4-(iii) What are the costs associated with monitoring the attribute? This includes up-front costs to set up for monitoring (e.g., purchase of equipment) and on-going operational costs (e.g., analysis of samples).

To monitor bacterial composition via eDNA, soil must go through DNA extraction and then barcoding and library preparation before being sequenced. While the cost has reduced significantly in the past decade, the attribute needs to be analysed in a molecular laboratory. Sequencing is the main cost of the analysis and it is costed per run, which can include ~200 samples in one MiSeq (Illumina) run.

One MiSeq run with 200 samples cost can be estimated between \$6000 and \$8000 (from DNA extraction to sequence output), with additional time needed for a professional bioinformatics analysis. The analysis time can be estimated to 20h for a standard community analysis, with more time required if there is any more detailed analysis needed. This costing is under the assumption that

soil samples do not need any further optimisation and DNA can be extracted using standard methodology. Some soils (such as allophanic soils) bind DNA to soil particles and can be particularly difficult in DNA extraction [10]. Those soil samples would increase the cost of any molecular analysis by at least 3-fold or more.

A5. Are there examples of this being monitored by Iwi/Māori? If so, by who and how?

We are not aware of any soil bacterial profile monitoring being undertaken by iwi/hapū/rūnanga. However, we are aware of many soil health monitoring programmes being led by Māori (see, e.g., [18]).

A6. Are there known correlations or relationships between this attribute and other attribute(s), and what are the nature of these relationships?

Bacterial composition is directly linked to Soil nitrogen and phosphorus and Soil carbon, especially since bacteria are major drivers of cycling associated with those attributes. Soil compaction and Soil water storage, capacity and fluxes will have an impact on bacterial composition in relation to water and oxygen availability that would drive the bacterial composition. Soil bacterial composition is also heavily dependent on aboveground plant diversity, so Indigenous plant dominance attribute would be related to the Bacterial composition attribute.

Part B—Current state and allocation options

B1. What is the current state of the attribute?

Currently there is no routine monitoring of the soil bacterial composition and there is also no consensus on how to interpret the diversity data to link it to soil quality indicators, since these relationships are primarily based on correlation, rather than establishing causality. There is a call internationally for validation and standardisation of microbial bioindicators (i.e., specific bacterial taxa or genome size) as soil health attributes [11].

Overall, current state of bacterial diversity attribute is only known for local samples (i.e., individual studies), but it remains unknown on a national or even regional scale.

B2. Are there known natural reference states described for New Zealand that could inform management or allocation options?

The study by Hermans *et al.* [4] is the most extensive study to date on the use of bacterial composition as a soil health metric in New Zealand. They were able to demonstrate that soil bacterial community composition correlates to land use and that when modelling the site land use, the best way forward was when combining chemical/physical data with microbial diversity data. However, that still only gives us a high-level granularity – land use and is not easily converted into the quantitative measure for soil health metrics. Generally, native forest is considered a reference state however as a study by Hermans *et al.* [4] demonstrate, native forest has a lower bacterial diversity than managed land (often considered “unhealthy”), which makes it difficult to set a reference point for this attribute.

B3. Are there any existing numeric or narrative bands described for this attribute? Are there any levels used in other jurisdictions that could inform bands? (e.g., US EPA, Biodiversity Convention, ANZECC, Regional Council set limit)

To our knowledge, there are no existing numeric or narrative bands.

Nationally and globally, there is a recognition that soil microbiota (combined bacteria, archaea, fungi, viruses, nematodes) is a major factor in soil health and it can be influenced by land use and management practices, but no numeric or narrative bands have been defined for bacterial composition. Even if the bands would be well defined, this would be very difficult to do on a national scale, but it would have to be on a more local scale and land-use dependant.

B4. Are there any known thresholds or tipping points that relate to specific effects on ecological integrity or human health?

To our knowledge, there are no known thresholds or tipping points for bacterial composition.

B5. Are there lag times and legacy effects? What are the nature of these and how do they impact state and trend assessment? Furthermore, are there any naturally occurring processes, including long-term cycles, that may influence the state and trend assessments?

On a broader scale, it is well understood that land use change leads to a lasting effect on soil microbial community composition. That has been demonstrated both in New Zealand and abroad [12, 13]. However, these broad community patterns do not take into consideration differences between active part of the bacterial community and inactive, dead, or even just free-soil DNA. Bacterial communities in soil react extremely quickly to any changes in their environment, often within minutes, however the growth of the microorganism or persistence of DNA in soil, lags behind those changes and is not responsive enough to make conclusion with high confidence. There are different techniques available, that can overcome these issues (such as stable isotope probing), but they are not cost-effective for routine monitoring. At the moment we are unable to predict how fast bacterial composition adapts to changes in ecological integrity, since this is directly connected to bacterial resilience at individual soils and is site specific.

B6. What tikanga Māori and mātauranga Māori could inform bands or allocation options? How? For example, by contributing to defining minimally disturbed conditions, or unacceptable degradation.

Māori have high interest in soil health, and this encompasses a holistic approach to assessing the condition of all parts of the soil ecosystem (and beyond). In addition to discussing this attribute directly with iwi/hapū/rūnanga, there is likely to be tikanga and mātauranga Māori relevant to informing bands, allocation options, minimally disturbed conditions and/or unacceptable degradation in treaty settlements, cultural impact assessments, environment court submissions, iwi environmental management and climate change plans, etc.

Part C—Management levers and context

C1. What is the relationship between the state of the environment and stresses on that state? Can this relationship be quantified?

No, since the use of microbial composition measurement itself is not very clear. There are patterns in soil bacterial composition that directly correlate to various land management practices, but the measurements of beta diversity are too variable to base management interventions on [4, 14].

C2. Are there interventions/mechanisms being used to affect this attribute? What evidence is there to show that they are/are not being implemented and being effective?

C2-(i). Local government driven

Local government, e.g. the regional councils via the land monitoring forum have an interest in using biological indicators to assess soil health in SOE soil health monitoring and have supported research into the use of bacterial composition using eDNA metabarcoding approaches, although use as a soil health indicator is still in a scoping phase.

C2-(ii). Central government driven

None existing, other than the current attribute scoping.

C2-(iii). Iwi/hapū driven

Iwi planning documents such as Environmental Management Plans and Climate Change Strategies/Plans may contain policies/objectives/methods seeking to influence soil bacterial quality outcomes for the benefit of current and future generations. We are not aware of any other interventions/mechanisms being used by iwi/hapū/rūnanga to directly affect this attribute.

C2-(iv). NGO, community driven

C2-(v). Internationally driven

Internationally, soil microorganisms are well recognised as key drivers of soil functioning, but there is no consensus on how to measure that in a validated methodology. Bacterial composition measurements (alpha and beta-diversity) were the primary way to assess the state of soil bacteria, but research focus has now shifted into the area of measuring functions using metagenomics and looking at genes involved in soil nutrient cycling [11] as better ways to provide meaning in the context of environmental state and ecological integrity.

Part D—Impact analysis

D1. What would be the environmental/human health impacts of not managing this attribute?

This is unknown since connections between bacterial composition and environmental or human health effect are largely inconclusive outside of specific cases where there was an issue with release of contaminated water onto land or pathogenic bacteria entering drinking water reservoirs from soils due to agricultural or industrial practices.

D2. Where and on who would the economic impacts likely be felt? (e.g., Horticulture in Hawke's Bay, Electricity generation, Housing availability and supply in Auckland)

Soil bacteria are often a source of new antibiotic resistance genes [15], which would impact public health sector. Soil can also harbour pathogenic bacteria if there is a spill of wastewater or effluent on crop, which would have an economic impact both on agriculture (via loss of crop) and public health.

Biosecurity is another economic threat, with pathogens in soil can not only target humans, but also agricultural products (plants and animals).

D3. How will this attribute be affected by climate change? What will that require in terms of management response to mitigate this?

Bacterial populations in soil will and already are reacting to climate change via changes in the community compositions and functioning they perform. There is a call from national and international scientific community to improve the way we measure bacterial response to environmental stressors using new methodologies and more scales that would represent future climate change conditions [6, 9, 16, 17]. There is also a greater need to properly standardise the measures of soil bacterial response and resilience if we want to use this information to form management strategies.

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