Insights into the soil microbiome and prospects for its manipulation for improved pasture resilience

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Highlights

- The soil microbiome refers to the diverse collection of microorganisms present in soil, many of which can mediate essential soil functions such as nutrient cycling and plant pest and disease suppression.
- There is growing international interest in the potential to manipulate the soil microbiome to deliver improved agricultural and environmental outcomes.
- Molecular techniques are rapidly increasing understanding of the soil microbiome structure and function but manipulating soil microbiomes for pasture resilience still presents significant science challenges.

Keywords: ecosystem services, microorganisms, nutrient cycling, rhizosphere

Background

It is estimated that a gram of agricultural soil can contain between thousands and tens-of-thousands of different species of microorganisms, so just a handful of soil can contain more diversity of life than all the plants and animals present in a rainforest (de Vrieze 2015; Wakelin 2018). Microorganisms (i.e., bacteria, archaea, viruses, fungi, and other microeukaryotes) present in soil have come to be known collectively as the *soil microbiome*. The soil microbiome can be defined in many ways (Berg et al. 2020) but here we adopt the definition as the entire habitat of the soil microorganisms, which includes the microbiota themselves, their collective genetic information, and the biotic and abiotic factors within their habitat. Soil microorganisms have been described as "the eye of the needle" through which carbon (C) and nutrient transformations are mediated (Jenkinson 1977; Wakelin 2018), highlighting their essential role in cycling of organic matter and soil fertility. More recently they have also been described as "the littlest farmhands" (de Vrieze 2015), acknowledging their vital role in agriculture.

The importance of soil microorganisms has been recognized for more than a century (Fierer 2017) but the lack of methods necessary to visualise and study soil microorganisms has meant that soil has historically been regarded as a proverbial "black box". However, recent global initiatives are placing value on soil microbiology for delivery of key ecosystem services that underpin robust functioning of soil and water systems required for plant growth (e.g., Keesstra et al. 2016), and this is driving new international research efforts. There is also growing interest in the potential of soil microbiology to deliver beneficial outcomes for New Zealand's productive sectors (Wakelin 2018). Some of this new interest is driven by farmers who want to maximise soil function and may have questions about the scientific validity of the burgeoning range of microbial products and soil additives on offer today, many of which come with substantial (often unvalidated) product claims. There is also increasing interest in improving "soil health" on-farm, however being able to identify and manipulate the soil microbiome for desired outcomes remains difficult. Fortunately, with the advent of new molecular approaches, increased understanding of the soil microbiome in managed ecosystems is emerging (Chen et al. 2019).

New Zealand's pastoral sector is built upon its productive soils. Pasture production and resilience are impacted by many factors including climate, management practices and soils, including soil microbiology. Important to ensuring pasture growth throughout the year is understanding how plants respond to stress events. Here we define resilient pastures as those that incur less reduction in production and recover faster after a stress event (e.g., drought/flooding, pest/disease pressure, Walker et al. 2004). As our pastures reflect the soils in which they grow, it is axiomatic that soil microbiology will impact pasture resilience, and possibly that the resilience of soil microbial communities themselves plays an important role of supporting pasture resilience. It is important to acknowledge that in comparison with other disciplines contributing to pasture resilience (e.g., soil biogeochemistry, plant breeding), soil microbiome research is still in its infancy, especially in New Zealand. We have only relatively recently begun to consider the role of the soil microbiome in pastoral agriculture, so definitive commentary on its impact on pasture resilience *per se* will therefore be limited and to some extent speculative at this stage.

Here we briefly review the current state of knowledge on the soil microbiome, focussing where possible on New Zealand-based research but also drawing on international examples or studies from other sectors where there are gaps in knowledge. We highlight where current opportunities for progress exist and identify key knowledge gaps that must be addressed to allow realisation of the opportunities afforded by the soil microbiome to enhance pasture production and resilience.

Current knowledge
Soil microbiome – structure and function
Soil microorganisms are essential for decomposition, nutrient (e.g., nitrogen (N), phosphorus (P), and sulphur (S)) cycling and C sequestration, enhancing soil structure and improving water holding capacity by creating soil aggregates (Figure 1). While some soil microorganisms remain unidentified and many others remain poorly characterised (Fierer 2017), some have been well studied. For example, soil rhizobia bacteria can form symbioses with legumes, providing biological N fixation services for plants. Specialised fungi known as mycorrhizae can enhance plant water and nutrient (e.g., P) uptake by extending their hyphae beyond the plant roots, while many soil bacteria produce plant growth-promoting hormones and antibiotics to stimulate plant growth and protect against plant pathogens. Not all microbes are beneficial; some are pathogenic for plants, while others produce greenhouse gases (e.g., N_2O, CH_4) as by-products of their activity. Nitrifier microorganisms can convert ammonia to nitrate which, if not taken up by plants can be leached to ground water causing adverse environmental outcomes.

Just as pasture production and resilience are impacted by climate, management, and soil types, so too is the activity of soil microbial communities influenced by these factors (e.g., plant species, animal grazing, soil pH and structure, temperature and rainfall, Morales et al. 2015; Highton et al. 2020; Kaminsky et al. 2020).

Studies on the effects of land use change and individual farming practices (e.g., agricultural inputs, crop-residue management, tillage, stocking rate, crop selection) on soil microbial diversity and function have been undertaken (Wakelin 2018; Chen et al. 2019; Hermans et al. 2020a, b, c; Wakelin et al. 2021). Many of these studies report a measurable impact of farm practice on the composition and sometimes function of soil microorganisms. For example, Wakelin et al. (2012) reported shifts in the composition and functional capacity of soil microbial communities following modification of New Zealand tussock grasslands to cultivated pastures through fertiliser application and cultivation of introduced grasses and legumes. While a direct assessment of

dependency was not included, they found higher relative abundance of many functional genes involved in nutrient cycling in undisturbed tussock grasslands compared with modified grasslands, suggesting that these unmodified systems were more dependent on internal cycling of nutrients. Microbially-mediated nutrient cycling is highly sensitive to exogenous nutrient inputs and/or alteration of pH, so de-coupling of microbial processes may render improved pastures more susceptible to nutrient leakage. To some extent, management practices influence the soil microbiome through altering soil physical and chemical properties. There is increasing evidence from multiple sources that soil pH is a key factor controlling the range and richness of microbial species present in soil with other factors such as N and C availability, and temperature also playing a role (Fierer 2017).

All the factors discussed above (i.e., climate, management, and soil types) contribute to the high level of spatial and temporal variability in soil microbiome structure and function, making soil one of the most complex ecosystems on Earth. Hence understanding how the collective activity of soil microbial communities drives pasture production and resilience is challenging. Some important functions are carried out by a smaller range of species that can be present in soil at low abundance. For example, a key transformation in the nitrification pathway (ammonia to nitrate) in New Zealand pastures is predominantly carried out by a relatively small group of specialised ammonia-oxidising bacteria (Di et al. 2009), so this function is potentially at risk from environmental disturbance – this is why ammonia oxidation is widely used as an indicator of soil health by researchers (Wakelin 2018). Although many complex soil functions, such as decomposition of C-rich residues, can be undertaken by multiple different members of the microbial community and should therefore be less likely to be disrupted by disturbance/stress, this process will only be possible if sufficient N, P and other essential elements are made available through biogeochemical cycling meditated by other specialised microorganisms. As such, the complex relationships between different microbial groups and their reliance on interdependent functions will vary widely depending on the ecosystem and associated disturbances and stresses. The implications of these findings for pasture management and resilience are still far from clear.

Soil-microbe-plant interactions
In the context of agricultural ecosystems, it is difficult to consider the soil microbiome without some reference to plants and their associated microbiomes (Cordovez et al. 2019; Song et al. 2020). Plants are key drivers of the structure and function of soil microbial
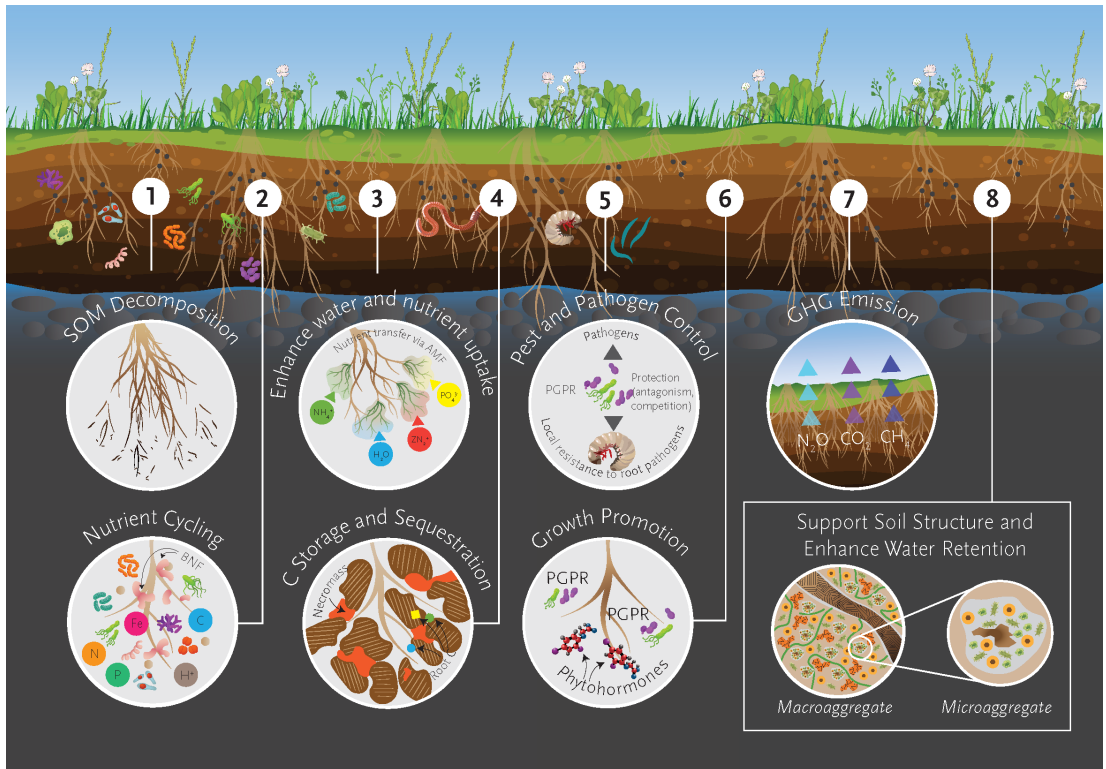
Figure 1 Overview of the functional roles that the soil microbiome may contribute directly or indirectly to pasture resilience to abiotic and biotic stresses. These include: 1) decomposition of root debris and soil organic matter (SOM); 2) nutrient cycling in soils, including N fixation and P solubilisation; 3) enhanced plant uptake of water and nutrients via extended fungi hyphae; 4) C storage and sequestration; 5) pest and pathogen control for host plants; 6) plant growth promotion by secreting phytohormones; 7) greenhouse gas (GHG) emission through microbial activities; 8) creating aggregates for supporting soil structure and enhancing water retention.

communities with the root-soil interface - known as the rhizosphere- considered a hotspot of soil microbial activity (Kuzyakov & Blagodatskaya 2015). Evidence that the soil-plant microbiome plays a critical role in protecting plants from abiotic and biotic stresses is now indisputable (de Vrieze 2015). Despite this, soil-plant microbiome research focussed on New Zealand-relevant pasture species is limited.

Plant microbiomes evolved with their host plants and significantly contribute to their environmental adaptation. Plants play an active role in the recruitment of specific microbes from the soil environment (Walters et al. 2018) and structure their soil microbiota to their advantage; this includes recruitment of selected soil microorganisms to alleviate/ameliorate impacts of abiotic and biotic stressors (Compant et al. 2019). The presence of the right microbes can improve pasture resilience, for example the bacterium *Bacillus amyloliquefaciens* GB03 increased ryegrass growth under drought conditions by improving survival rate and maintaining chlorophyll content in pot trials (Su et al. 2017). Further, wheat plants under drought stress had five-times greater survival and 78% higher biomass when they were inoculated with selected drought-tolerant rhizobacteria (Timmusk et al. 2014). Some potential mechanisms for enhanced drought tolerance of plants by rhizosphere bacteria include: production of hormones (e.g., abscisic acid, auxin) to enhance plant growth and enzymes to reduce the level of ethylene in plants; induction of plant systemic resistance; and formation of bacterial biofilms on plant roots that enhanced water retention (Timmusk et al. 2015; Jansson & Hofmockel 2020). Beneficial microbes may also be responsible for disease and pest resistance at the plant variety level, and this microbe-mediated resistance can be transferred to susceptible varieties (Bell et al. 2016; Kwak et al. 2018). Evidence is now mounting that certain keystone microbial species are the major determinants of the microbiome network; Agler et al. (2016) suggested that the plant host genotype acts on these keystone species which then modulate the whole microbial community to modify host plant fitness, highlighting the complexity of plant-soil-microbe interactions.

Soil microbial resilience

Soil microbes can enhance plant adaptation and tolerance to stresses thereby increasing plant resilience. Therefore, the ability of the soil microbiome to respond and recover from stress or disturbance will likely underpin plant resilience. Stability of soil ecosystems depends on both the resistance of the soil microbiome (quantified as the amount of change induced by disturbance) and its resilience (extent of recovery of function back to the undisturbed status). Many ecosystem functions performed by soil microorganisms are spread across diverse microbial species. As such, deleterious effects caused by any disturbance (e.g., climatic stress) can be reduced by so called "functional redundancy", and it is assumed that soils with high biodiversity are much more resilient to stressors (abiotic or biotic) and recover much faster after the event.

While some work has been undertaken on resistance and resilience of New Zealand soils (e.g., Orwin et al. 2006), these studies have not typically been undertaken in pasture ecosystems. Using a C utilisation profile methodology, Wakelin et al. (2014) examined functional resistance and resilience of New Zealand soil microbes collected from under different land uses (pasture, forest) at four locations, and imposed a freeze/thaw disturbance. The study found that functional resilience of soil microbes was not linked to land use or location but was negatively correlated with soil Olsen-P levels (less resilience in soils with higher P availability). The functional stability of the soil microbiome (post-disturbance) was greater in soils from high rainfall sites. While in this study alteration of the P status of soils impacted on the capacity of soil microbial communities to rapidly respond to disturbance, a multitude of other factors play important roles in maintaining soil microbiome resistance and resilience, including soil organic matter (SOM, Gregory et al. 2009) and the composition of the soil microbial community itself. Research indicates that soil microorganisms with varying life history strategies (i.e., fast vs. slow growth rates) play key roles in recovery and resilience, with different components of the microbial community delivering required functions over time (Orwin et al. 2006). If further studies can confirm the link between the stability of soil microbiomes with pasture resilience to stress, then management practices could be developed to support soil microbiome stability to encourage improved pasture resilience.

Climate change is expected to cause an increasing frequency of stress events, and recent research has shown climate change inevitably impacts on the functions of the soil microbiome. Increasing atmospheric CO_2 can increase photosynthesis and C allocation to soils through root growth and exudation, stimulating microbial growth and decomposition of soil C. Analysis of soil microbiomes from five long-term elevated CO_2 field FACE sites found that key microbial functional genes involved in C decomposition, C and N fixation and greenhouse gas (GHG) emissions were enhanced under elevated CO_2 levels (He et al. 2020). Warming stimulates microbial growth and activities in grasslands (Sheik et al. 2011; Zhang et al. 2014); in particular, warming can result in decreased fungal to bacterial ratios which has potential implications for nutrient cycling and plant disease expression. Drought is predicted to result in declines in microbial function as soil becomes drier. Studies have shown that drought can have long-lasting impacts on the soil microbiome in grassland ecosystems, as the vegetation shifts to more drought-tolerant plant species and they subsequently select for different root-associated soil microbes (de Vries & Shade 2013; de Vries et al. 2018). Network analysis of a multi-year field study showed that bacteria are more sensitive to drought than fungi in grasslands (de Vries et al. 2018) and, potentially, fungi may play key roles in nutrient cycling with hyphae bridging spatially discrete resources for plants. High rainfall and flooding can result in soil pores becoming anaerobic, which can provide ideal conditions for production (CH_4 and N_2O) by methanogens and denitrifiers.

Opportunities to optimise soil microbiology for improved pasture

The diversity in the soil microbiome is so staggering that finding out which organisms benefit plants most, how they do it, or what combinations work best is a gargantuan task (Thiele-Bruhn et al. 2012; de Vrieze 2015). Targeted manipulation of established soil microbiomes to modify function remains a significant science challenge, let alone actually applying this knowledge on farm. However, some opportunities to harness the potential of the soil microbiome for agriculture in New Zealand have been highlighted previously (e.g., Andrews et al. 2011; Wakelin et al. 2013; Wakelin 2018). These tend to be focused on single organisms with specific functions which are easier to manipulate than a more complex microbiome. Below we focus on those which may have potential to deliver gains in pasture resilience.

Soil rhizobia for improved N fixation

Prior to intensification and heavy use of N fertilisers, biological N fixation (BNF) by legumes, particularly white clover, has played a key role in the sector's competitiveness by increasing pasture production. While there has been long-term and extensive investment in improvement of clover cultivars, this has largely excluded consideration of clover's symbiotic microbial partner, rhizobia.

Rhizobium leguminosarum bv. *trifolii* bacteria,

which form symbioses with white clover, are not native to New Zealand soils and inoculation of clover seeds with exotic rhizobium strains has been widely used since the 1970s. Over time, exotic rhizobium strains have become naturalised in New Zealand pasture soils and are sometimes present in high numbers. A recent field survey of 26 pasture sites across New Zealand found that abundance of white clover rhizobium varied from <100 to >10^8 rhizobia per g soil. Significantly, the abundance of rhizobia did not correlate with their symbiotic effectiveness (i.e., how effectively rhizobia fix N and support clover growth), indicating that in some situations, the presence of rhizobia in soil does not always guarantee optimal BNF (Wakelin et al. 2018). In addition, the size and effectiveness of rhizobia populations were highly variable across farm and paddock scales, further suggesting reliance on naturalised rhizobia populations may not be the best strategy. Recently, New Zealand rhizobia strains with improved N-fixation efficiency and competitive ability over the current commercial strain TA1 have been identified (Shi et al. 2019).

The effectiveness of soil rhizobia populations can be impacted by soil properties. Wakelin et al. (2018) found symbiotic effectiveness of soil rhizobia was positively correlated with soil pH, total base saturation, and Ca and Mg concentrations. More research is needed to elucidate these relationships, but these results suggest at least the potential to select for and maintain effective rhizobia populations through farm practices.

Reliance on BNF is likely to increase because of increasing public concern about environmental impacts and corresponding regulatory changes around N application to pasture (e.g., N-caps). BNF occurs in response to plant demand and plant N uptake can reach 100% so is important for pasture resilience. Soon after a climatic/environmental stress, rhizobia can provide N for legume plants and help pastures to recover. However, more research on developing stress tolerant rhizobium strains is important, together with improving delivery systems (Baena-Aristizábal et al. 2019) to ensure effective rhizobia can persist in soils. The potential to select for desiccation-tolerant New Zealand rhizobia has already been demonstrated (van Ham et al. 2016), and these findings now need to be validated under field conditions.

Phosphate solubilising microorganisms

Pastoral agriculture is also dependent on regular inputs of inorganic P in the form of soluble mineral fertilisers but only 5-30% of applied fertiliser P is recovered by plants with the remainder immobilized in soil (Trolove et al. 2003). Diverse soil and plant-associated microorganisms can mobilise P into plant-available forms through many mechanisms (Richardson 2001). A wide range of soil bacteria capable of solubilising P (at least *in vitro*) have been identified from New Zealand's three long-term pasture fertiliser trials (Whatawhata, Winchmore and Ballantrae). The abundance and diversity of soil phosphate-solubilising bacteria (PSB) differed between sites and the frequency of P-solubilisation in the bacterial population was greater in low P-status soils (Mander et al. 2012). Further work at Winchmore has validated that some soil bacterial communities (*Actinobacteria*) and mycorrhizal fungi, proposed to be involved in supplying P to pasture plants, are closely linked to soil P status (Wakelin et al. 2012).

The use of P-solubilising microorganisms as potential bio-fertilisers has been the focus of research over several decades (Khan et al. 2007) and some products have been commercialised overseas, although to date these have not been developed for pastoral agriculture. Field efficacy of these products has not often been validated through robust experimentation but the drive towards reduced dependence on synthetic P fertilisers is leading to a rapid expansion in such "biologically-based" products. However, there are many inherent challenges in augmentation of pastoral soils with beneficial microorganisms and rather than inoculation of specific P-solubilising microorganisms into soil, there is growing interest in the potential to enhance development of a diverse, active soil microbial community (that includes P-solubilising microorganisms) through management as an alternative strategy (Raymond et al. 2021). Previous work (Mander et al. 2012; Wakelin et al. 2012) suggests that both abundance and composition of PSB are under strong selection pressure affected by farm management strategies (fertiliser application). More detailed understanding of the ecology of PSBs should provide the opportunity to increase the availability of soil P for plant-uptake. Progress towards this goal is being realised through the application of emerging metagenomic tools that can track changes in the abundance and activity of microbial P transformation genes. For example, recent research has demonstrated that in diverse agroecosystems, long-term N inputs decreased microbial P-solubilising and mineralising capacity while P inputs favoured microbial immobilisation by altering the microbial functional profiles (Dai et al. 2020).

Microbial suppression of soil-borne pests

Insect pests are well recognised as key disruptors of pasture persistence and resilience, with pest feeding damage resulting in economically significant losses of productive species and ingress of weeds (Ferguson et al. 2019). Insect-infested pastures are also more susceptible to drought and root damage allowing ingress of plant pathogens. Some of New Zealand's

most damaging pests (e.g., grass grub and porina) are soil-borne or spend part of their life cycles in soil. Soil microbes have a role in supressing these insect pests, with a wide range of naturally occurring diseases reported from New Zealand pasture pests (Glare et al. 1993), with some of these entomopathogens implicated in population collapses of their hosts. Two species of the soil-dwelling bacteria *Serratia* spp. have evolved in close association with grass grub and can cause disease outbreaks in high density grub populations, suppressing grub populations to beneath economically-damaging thresholds (Bourner et al. 1996). Another bacterial insect pathogen *Yersina entomophaga* is highly effective against many pasture pests, including grass grub, porina, black beetle, manuka beetle and plantain moth (Hurst et al. 2011). Soil-borne fungal diseases of grass grub and porina are also common in pasture soils and occasionally cause insect disease outbreaks that can reduce pest populations (e.g., Townsend et al. 1995) but their activity is often limited by soil temperatures.

These entomopathogens represent an important resource for management of endemic (and some exotic) pasture pests. However, pathogen populations in soil can be disrupted, particularly by abnormally dry weather in late spring and early summer. Similarly, cultivation during pasture renewal and large-scale land conversion can disrupt established pathogen populations. It can take several years for these microbial communities to recover, and a few years after pasture renewal, pasture pest outbreaks occur because of a decrease/removal of their associated pathogens that would normally have kept them under control. Careful maintenance of mature pastures to preserve soil-borne pathogens and maintain their pest suppressive capacity may be a useful option in some circumstances.

Deliberate augmentation of the soil microbial community with high numbers of artificially cultivated and formulated pathogens targeting specific pests may also be successful. The potential of this biopesticidal approach has been demonstrated; application of the bacterium *Serratia entomophila* resulted in longer term suppression of grass grub than diazinon, where pest populations rebounded in subsequent seasons (Zydenbos et al. 2016). Use of New Zealand's insecticidal microorganisms as biopesticides is feasible where they can be easily and cost effectively cultured and many pot and field trials with various pest/pathogen combinations have provided proof-of-concept for this approach. De-registration of several synthetic pesticides and on-going review of others used in pastures may yet drive adoption of pathogen-enhanced integrated pest management systems.

Harnessing the full benefit of the insecticidal component of the soil-insect microbiome requires knowledge of the presence and distribution of insect pathogens in soil. To date, there has been limited work on development of molecular probes for selected pathogens (e.g., Monk et al. 2010). Work is also needed to determine the combination of effective insect pathogens required to maintain pest populations at low levels and predict where it may be necessary to augment the soil microbial community to greatest effect.

Disease suppressive soils

Soil-borne plant diseases in New Zealand pastures are a large, unrealised and probably underestimated problem; for example, losses in the Waikato region due to the soil nematodes and pathogens are estimated at least $900 - $1506/ha/year (Wakelin et al. 2016a). Specific soil-borne diseases can sometimes be managed through identification and application of biological control agents (Goldson et al. 2015; Niu et al. 2020) and new research suggests the potential to enhance "soil immune response" where in disease suppressive soils, susceptible plant hosts are protected from virulent soil-borne pathogens by either the competitive or antagonistic activities of the wider soil microbial community (Raaijmakers & Mazzola 2016). Although further research is needed, the potential of this concept is large. In the future, we may be able to harness the natural defensive activities of the soil microbiome for sustainable disease management, thus increasing the resilience of multi-plant, multi-pathogen agricultural systems (Bell et al. 2016; Dignam et al. 2016; Wakelin 2018; Figure 2). Using high throughput DNA sequencing with culture-independent community characterisation approaches, we are now able to explore how the presence of a consortium of microbial taxa contribute to disease suppressiveness. From soil with differing wheat cropping histories, Chng et al. (2015) were able to identify specific assemblages of microorganisms associated with soils characterised as having either specific, general, or non-suppressive activities against take-all disease. Pastoral management decisions that affect SOM quality (chemical composition and decomposability) have been identified as key opportunities to manage soil microbial communities to favour development of a disease suppressive state. Dignam et al. (2019) found that frequent addition of organic residues to grassland soils enhanced some members of the *Pseudomonas* bacterial community which were associated with soil disease suppression and with changes in SOM quality. This creates the opportunity to direct the natural processes that lead to enhanced disease suppression via manipulation of soil pH, fertility or addition of amendments to soil that change organic matter quality towards a state that encourages the development of soil disease suppression (Dignam et al. 2018, 2019). Associations between farm management, SOM quality and soil disease suppressive

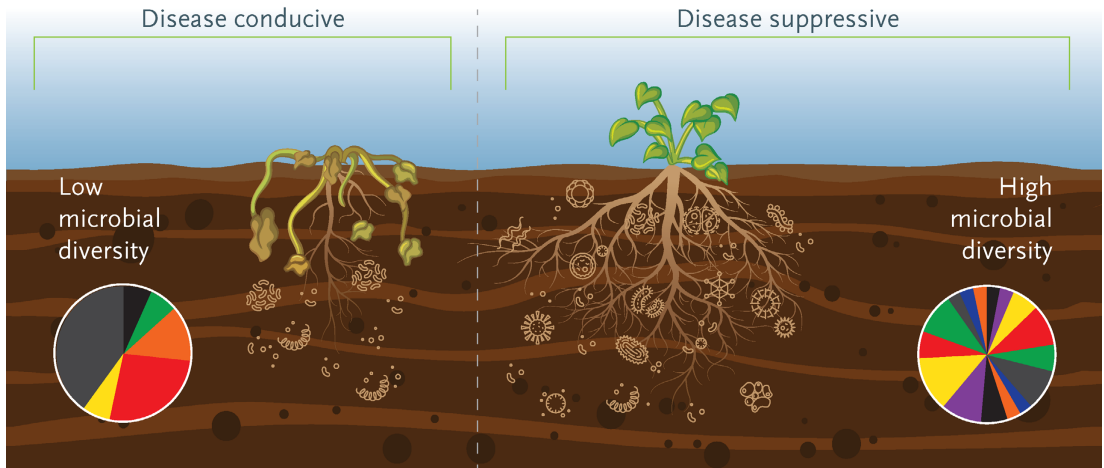Change in structure and diversity of the soil microbial community

Figure 2   Low microbial diversity and low SOM quality soils associated with plant disease-prone (conducive) soil state (left) while more diverse and higher quality organic matter soils associated with disease suppression (right).

communities must now be challenged and validated to allow prediction of manipulations to the soil microbial community that optimise productivity and resilience.

Matching pasture genetics with the soil microbiome

Given the intimate associations between the soil and plant microbiomes, greater consideration should be given to the soil microbiome during selection and development of pasture species for enhanced persistence and resilience. Some of New Zealand's strains of soil rhizobia appear to perform better than the current commercial strains (Shi et al. 2019), and new research is showing that better matching rhizobia strains with different clover genotypes can lead to better clover performance (Pers. comm. A. Griffiths, AgResearch). Whether these effects will lead to improved pasture resilience is yet to be determined, but it seems reasonable that pasture performance and resilience could be optimised if legume plant breeding programmes included traits for microbial symbiosis. In addition, there is potential to pair emerging clover varieties with desiccation-tolerant rhizobia strains (van Ham et al. 2016).

In order to capture the benefits of plant-soil microbiome interactions means moving beyond consideration of the plant genotype alone, or even plant genotype × environment focus to much broader plant genotype × microbiome genotype × environment interactions (including management practices) (Attwood et al. 2019). This will make understanding the impact of pasture management and resilience more complicated but ultimately more fruitful as many of the

"missing" variables that determine outcomes between the interactions of soil, plant, and livestock will become clearer.

Challenges and research needs

As discussed above, soil microbiome research is still in its infancy, in comparison with other disciplines that already underpin pasture production and resilience. Opportunities based on single organisms for specific soil functions may be able to be utilised to enhance pasture production and resilience in the short term. However, moving towards manipulating multiple microorganisms within the complex microbiome will require significant knowledge gaps to be addressed.

New research tools and multi-disciplinary research is needed

Critical to furthering our understanding of the soil microbiome and its role in supporting pasture resilience is the need to be able to identify and interpret the huge diversity present within our soils. While the abundance and function of certain microorganisms (e.g., N-cycling microorganisms) can now be tracked very precisely through advances in molecular technologies, other groups of microorganisms are not as well studied. The characterisation of both the structure and activity of soil microbial communities as well as functional gene markers (i.e., genes linked to biogeochemical processes) can provide understanding of the mechanisms by which specific groups of soil microorganisms mediate ecosystem services. The next step is greater application of ecological genomics and other '-omics' methodologies; we have already seen this

approach supporting advances in human, animal and plant microbiome analysis. These techniques remain very expensive in complex environments like soil but as technologies develop, the costs will decline, opening future opportunities for understanding the complex soil microbiome. Such knowledge is key for successful manipulation and optimisation of the microbiome for soil ecosystem resilience and developing evidence-based on-farm practices.

While there has been some effort to standardise techniques and build this knowledge base (Wakelin et al. 2016b, 2021; Lear et al. 2018), effort is still very fragmented; a long term and carefully coordinated national effort is needed before we can manipulate soil microbiology as effectively as soil physicochemical properties to drive pasture production. Soil type, fertility and management all have important influences on both pasture resilience and the soil microbiome. Hence opportunities to sample and analyse the soil microbiota during sample collection from transects across different soils on defined land management units used to measure soil fertility, should be identified and acted upon. The potential gains may be just as great, especially if the required metadata that defines the ecosystem is collected concomitantly with sequencing efforts (Wakelin 2018). Once better able to identify the diversity of microorganisms together with a greater understanding of what comprises a desirable microbial community for pastoral soils, then we can rationally manipulate the microbiome through inoculation or alter management to optimise the soil microbiome.

As illustrated by *Epichloë* and the recent rhizobia research reviewed here, matching microbial genetics with appropriate plant genotypes can lead to improved plant performance (Smith & Goodman 1999; Saikkonen et al. 2010; Gagic et al. 2018). This highlights the importance of collaboration between different disciplines. If desired plant traits (e.g., drought tolerance) can be provided or enhanced by using a microbial partner, early collaboration would likely lead to greater success and save limited research resources. Broader multidisciplinary research is needed to fully understand the potential effect of new pasture cultivars/microbiome associations within the wider ecosystem (e.g., Duckworth et al. 2017; Ochoa-Hueso 2017). Equally, as with any technological changes, questions around the unintended impacts will also need to be answered; for example, "what are the potential effects on other beneficial microbes in soil and plant?", "what are the environmental cost-benefits (e.g., GHG emissions, C loss, pollution)?", and "are there any indirect effects on beneficial insects (e.g., bees) or livestock grazing these cultivars?". Answering these types of questions will require cross-disciplinary collaboration as has been successfully applied in other areas of research, e.g., human health (Shah et al. 2020).

Identification and understanding of novel soil and plant microbiota involved in plant stress response and resilience

The identification of novel soil and plant microbiota involved in plant stress response and resilience, is the essential first step to be able to manipulate the soil microbiome for resilience of pasture systems. Successful manipulation also requires a mechanistic understanding of observed benefits. A great example in New Zealand is the *Epichloë* endophytes for enhancing grass productivity and longevity. Decades of research have provided considerable understanding of the different alkaloids produced by different *Epichloë* strains and strain-ryegrass cultivar interactions, which has supported commercially successful use of these endophytes in improved pasture varieties (Johnson & Caradus 2019; Caradus & Johnson 2020). Similar effort applied to other potentially beneficial microorganisms would likely lead to equally successful advances. Our experience with identification of several new-to-science New Zealand-unique insect pathogens (Glare & O'Callaghan 2019) suggests potential for targeted selection for other novel soil and plant microorganisms that may have a role to play in pasture resilience. This is an area of intense research effort internationally, but New Zealand research in this space is limited (apart from *Epichloë*) and fragmented. For example, New Zealand isolates of the fungus *Trichoderma* have recently been shown to protect perennial ryegrass from take-all disease (Umar et al. 2019) and desiccation-tolerant rhizobia isolates (van Ham et al. 2016) have been identified; however, these and other initiatives require research effort (similar to that applied to *Epichloë* studies e.g., mechanisms, interaction with host and environment) before they can contribute to enhanced pasture growth and resilience.

Manipulating the soil microbiome through inoculation

There is currently a diversity of microbial-based bio-stimulants, bio-fertilisers and bio-amendments available on the market and this global market is rapidly expanding with large international fertiliser companies joining forces with biological products companies to offer these options to farmers (e.g., New AG International 2020). However, in many cases, there is a lack of consensus in mechanistic understanding of their effectiveness and the context in which they might be effective, and few products have been used in pasture systems to date. Key challenges include failure to establish the inoculant in soils at a sufficiently high level to have consistent effects on

plant growth, and strong competition for inoculants with the established indigenous microbial community for forming interactions with plants or to colonise appropriate soil niches. Further fundamental research will be required to understand the microbial genes that influence their fitness and competitiveness in soil and understand soil microbiome community assembly and development over time to identify the best time for inoculant treatment (e.g., during seedling germination is useful for symbiotic soil microbes). In addition, the below-ground nature of many pests and diseases makes visual detection of a potential pest/disease outbreak difficult, as well as hampering application of targeted treatments. An ability to forecast outbreaks through the development of models is required to allow early application of microbial biopesticides.

Optimising soil microbiome through management practices

On-farm practices can impact soil microbiomes and their function (Chen et al. 2019). There is increasing understanding of factors that influence the soil microbiome, and hence this offers the opportunity to alter management to optimise the microbial community. We understand that fertiliser, pesticides and insecticides (Wang et al. 2006; Thiele-Bruhn et al. 2012; Meena et al. 2020) and altering C-rich residues (Raymond et al. 2021) impact the soil as well as the soil microbiology; however, how to practically achieve desirable microbial community assemblages on-farm requires further research. For example, the link between soil pH and the symbiotic effectiveness of New Zealand's soil rhizobia populations capable of nodulating white clover also suggests the potential to select for and increase benefits to be gained from soil rhizobia by regulating soil pH through liming. However further research is needed to determine the underlying mechanisms and timescales needed to achieve significant responses in pasture N nutrition.

There is growing interest in more diverse and complex agroecosystems. Diverse plant communities are expected to lead to more diverse, interconnected and stable soil microbiomes (Tosi et al. 2020) which could be more resilient to changes. However, more research is needed to provide clarity around what is a minimum or optimal degree of plant diversity required to promote a stable soil microbiome, i.e., what keystone plant species need to be present in the community?

As discussed earlier, it is also important to understand the broader plant genotype × microbiome and genotype × environment (including management) interactions. While not easy, understanding such interactions is important when applying microbiome science to improve plant health, yield and resilience (Busby et al. 2017). In addition to lab-based experimental investigation, well-designed field experiments incorporating advanced statistical analyses (e.g., machine-learning techniques) and comprehensive metadata will be needed to disentangle these complex interactions which can further inform management decisions. Another challenge to successfully apply microbiomes for agriculture is to define microbial consortia that can persist and function in a variety of agricultural ecosystems within a range of climates, soil types and farm management. To achieve this goal, fundamental knowledge of core microbiomes associated with plants across different ecosystems is needed. Although challenging, rapid development of tools and multi-disciplinary research will accelerate research progress in this crucial area.

Scientifically validated tools for farmers

In contrast to the wide range of tools used by farmers to evaluate chemical properties of their soils, there are very few tests currently available commercially in New Zealand that directly evaluate soil microbial activity and ecosystem function. Most of the few tests that are available are either not calibrated for our soils or are not standardised across labs. The lack of biological indicators in most soil health assessments reflects the fact that they often require context-specific ecological knowledge or are difficult to assess and interpret because they are not benchmarked (van Leeuwen et al. 2017). Further research to develop soil biological indicators of healthy soil with functionally stable microbiomes is urgently needed. This is not easy but can be achieved through improving our fundamental understanding of keystone species present in stable pastoral microbial communities, identifying soil microbial genes involved in functions important to the resilience of our pasture systems (e.g., N, P cycling, pathogenic genes), and establishing linkages between abundance and the ability to function under New Zealand conditions. These keystone species, if identified, can be used as indicators for healthy and stable microbial functions in soils, and can be used to measure changes resulting from soil inputs and pasture management.

Quantitative measurement of key functional genes can be used for estimating biogeochemical cycling processes or early detection of soil-borne pathogens or pests. For example, the SARDI PREDICTA® B tests used in Australia to quantify some soil-borne crop pathogens and determining the disease risks for soils (Ophel-Keller et al. 2008). Farmers can use this information to select low-risk crops for their land, and as a guide for selecting management practices to reduce disease risk. Developing such tests for New Zealand pasture systems would benefit farmers as losses from below-ground pathogens and pests are normally noticed too late for effective management. These tests cannot be simply "imported" from overseas

but need to be developed based on New Zealand soil microbiomes, pasture species, and climate conditions. Ultimately, making these tests commercially available (and affordable) for farmers would assist in working towards soil microbial communities for enhanced pasture resilience.

Conclusion

Increasing evidence supports the vital role of soil microbes in agriculture, particularly supporting plant response to abiotic and biotic stresses. Currently soil microbiology is underutilised across all agricultural systems in New Zealand. While it remains a difficult and complex task to manipulate or optimise the soil microbiome, the potential to enhance plant growth and pasture resilience is huge. Additionally, including microbial-based approaches (together with other 'green' strategies) into farm management practices are generally considered environmentally sustainable options. Examples such as rhizobia for fixing N and use of microbial biocontrol agents for pasture pests demonstrate how soil microbiology can support the pasture sector. Although many of the current examples mentioned are linked to individual microbes, investigations targeting microbial consortia are becoming more common (Kong et al. 2018; Woo & Pepe 2018; Aguilar-Paredes et al. 2020). While there are still many challenges, soil microbiome research has made great progress in the past decade with continuous development of improved 'omics' technologies and data analysis techniques. Now is the time to explore the potential of this vast diversity of normally 'unseen' microbes in the soil. Scientists from different disciplines, industries, and land managers need to work closely together to develop applicable strategies and long-term plans to discover and utilise microbial resources for the New Zealand pastoral sector.

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