Our Land & Water Nexus Think Piece Genomics.

Prologue

The question:
How do we achieve simultaneous improvement in productivity and reduced environmental footprint through interactions of soil-plant-animal genomics?

Hypothesis

Microbiomes associated with soil, plants, and animals contribute significantly to productivity in the pastoral sector, but are often treated as distinct communities. However, they may also be considered a meta-community (farm system biome), that interconnects though water, carbon, and other nutrient cycles. While opportunities through the manipulation of the individual microbiomes are being realised (e.g. plant endophytes, rumen CH_4), there may be greater opportunities to achieve system-wide gains by looking across two or more microbiomes. As such,

> *we hypothesize that significant additional gains in soil, plant, and animal productivity, coupled with reduced environmental footprint can be realised through appropriate matching of genotypes (plant and animal) and understanding the interactions among and across the various microbiomes.*

We believe this will also result in increased microbiome resilience in the ecosystem to internal and external disturbance events (e.g. pest and weed invasion, influences of climate and land use change, shifts in animal diet, soil cultivation, farm management) and improved environmental sustainability. Understanding multiple genotype x environment (G^xxE) interactions within New Zealand's pasture-based production systems, will identify opportunities for our key stakeholders to manage and optimize these interactions on farm and to achieve greater efficiency within production systems.

In this draft document we provide a brief background on the rapid rise of genomics technologies and provide a summary of genomic tools available for studying host and microbiomes. This is followed by an outline of the various gaps and shortcomings in our knowledge towards a system-wide approach to study the microbiome. As examples of the current state of knowledge we have included case studies describing knowledge on microbiomes in soil, plant ecology, rumen and water.
This doument is a work in progress, and the final version will be guided by the discussions and conclusions of the Nexus Think Piece Genomics workshop.

Working the Nexus Between the Soil, Plant and Rumen Microbiomes to Improve Productivity and Reduce Environmental Impacts of Pastoral Agriculture

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Abstract

The New Zealand pastoral sector relies on the biological processes occurring in soils, forage plants and ruminant animals to drive the production of dairy, meat and fibre products. Each of these biological components have their own associated microbiomes, which have been viewed previously as distinct entities. However, these microbiomes operate under similar ecological principles and are physically connected to each other via water, carbon and other nutrient cycles. Here, we summarise the microbiome work that has been done in each of these three environments and investigate what additional benefits may be possible through understanding the interactions between the various microbiomes and appropriate matching with (plant and animal) genotypes to drive greater productivity and reduced environmental footprint.

Introduction: technologies so far

Genomics introduction

The onset of the next generation sequencing (NGS) era (454, illumina) enabled a paradigm shift in tools to develop and implement genomic selection (GS) particularly in the livestock industry, and provide genome wide association studies (GWAS) to further elucidate genomic regions of importance in production traits. Genome assemblies together with re-sequencing of diverse breeds around the globe has established SNP arrays, genomic selection methods (Bayesian and GBLUP) together with imputation strategies that utilise various SNP densities in a cost effective manner for uptake by the breeding industry. Furthermore, utilisation of imputed whole genome sequence equivalent genotypes for GWAS and GS are now a reality (BayesRC). A continued decline in sequencing costs together with an improvement in longer read technology has generated more refined genome assemblies that are being annotated at the functional level via assays designed to establish chromatin architecture, accessibility,

modification and subsequent transcription and translation (methods include HiC, ATAC seq, ChIP seq, methylation). The human and mouse ENCODE projects have paved the way for the international consortium FAANG (Functional Annotation of the Animal Genome), that aims to identify all the functional elements in animal genomes. A significant challenge in the post-genomic era is connecting genotype to quantitative phenotype in basic and applied biology - the genome to phenome challenge (www.FAANG.org). Understanding the genotype to phenotype link is not only important from a genomic selection perspective but also improving the fundamental understanding of biology.

Utilisation of genomic tools in livestock has had a substantial effect on genetic gain for the industry, however, the animal genome is only one component of the "pasture to plate ecosystem". Use of NGS to characterise and understand the microbiome of the rumen, plants and soils, initially via targeted sequencing approaches, but now also global identification of species and gene expression has been established. In addition, genomic techniques can be extended to not only understand the microbiomes in the agricultural production of animals, but also in establishing and understanding sources of food or water contamination, important impacts for human health.

Connecting the genome to phenome is a significant challenge in the post-genomic era. The integration of the host genomics and their microbiomes together with other components of the ecosystem to enhance agricultural production while reducing environmental footprint is also an important challenge. A "systems genomics" approach is needed whereby the microbiomes associated with soil, plants and animals that contribute significantly to productivity in the pastoral sector, will not be treated as distinct entities.

Summary of genomic tools available for host and microbiomes:

Genotyping tools like SNP chips, Genotpying-by-Sequencing (GBS), amplicon sequencing, whole genome sequencing, functional GBS.

Single cell genomics – this opens new opportunities for biology studies whereby cultivation-independent data can be generated. These whole-genome data are a powerful tool as the majority of the microbes from most environments can not be cultivated, and we are therefore unable to study them using standard microbiology tools. It is an advancement

on using small subunit rRNA (SSU rRNA), where microorganisms with highly similar SSU rRNA genes have significant genomic and biogeographic variability.

Environmental metagenomics -the study of organisms in a microbial community based on analysing the DNA within an environmental sample.

- extremely limited prior to the advent of NGS.
- NGS -capability to profile entire microbial communities from complex samples, discover new organisms, and explore the dynamic nature of microbial populations under changing conditions.
    - Shotgun metagenomics
    - 16S rRNA sequencing- this is a common amplicon sequencing method used to identify and compare bacteria present within a given sample. NGS 16SrRNA is a culture-free method enabling analysis of the entire microbial community within a sample. With the ability to combine many samples in a sequencing run, NGS-based 16S rRNA sequencing as a cost-effective technique to identify strains. Depending on the design of the experiment this is usually limited to bacteria.
- microbial whole-genome sequencing and *de novo* assembly.
    - Unlike capillary sequencing or PCR-based approaches, NGS allows sequencing hundreds of organisms via multiplexing. Compared to traditional methods, NGS-based microbial genome sequencing doesn't rely on labour-intensive cloning steps and will also identify low frequency variants and genome rearrangements that may be missed or are too expensive to identify using other methods.
- Microbial metatranscriptomics- RNA-Seq enables unbiased strand-specific identification of common and novel transcripts

System genomics methods - big data, big databases

- systems-level analyses of genetic adaptations and interactions between organisms in their natural ecosystem
- QC of the ecosystem e.g. changing the forage, changes the animal microbiome therefore production traits and environment (methane, zoonosis)
- Nutrigenomics (animal genotype to plant) and interactions with microbiomes.

Where to next to achieve additional gains through understanding the microbiomes' interactions?

Look at 'current' knowledge with new eyes: Principal advantage of using genomic tools is that these provide more precision in identification and quantification of the structure of the microbial communities (number of different species, number of specimen within the species, detection of unculturables).

- What new questions can now be asked about the interactions along the ecological continuum which could not be accomplished with previous technologies?

Numerous microbiome studies are performed using 16S rRNA PCR–based approaches. While this is working well for many examples (though often limited to bacteria only), with the drop in cost of genome sequencing and the increasing automation in data analysis. the time has come to consider moving onto newer technologies.

- How long will 16S rRNA remain competitive versus shotgun sequencing?

Rapidly increasing volumes of sequence data of microbiome samples mean that large databases of sequence information are building up in the public domain. To get to where the community is now has been an expensive and long process.

- Could mining these empirical data could be the key to better understanding our current (and future) microbiome samples?

Deciding on some quick gains: For soil/plant/animal and based on current knowledge:

What would be the 'top pick' of what can be done now to make a difference?
What are the top routes for each for application of the knowledge?
How to implement it?
How does that impact the other areas? (E.g. how does making a different to the plant microbiome affect the soil or animal microbiomes?)
What would you/could you do long-term?

Holistic view: Understanding of the various microbiomes (soil, plant, animal) 'in isolation' can be fairly detailed, but connecting up the knowledge to get a more holistic view of ecosystems is rare/non-existent. Genomic tools can provide new knowledge to allow this ecosystem investigation. Questions that arise:

- How do the different microbiomes interact with each other?
- How do we connect up the knowledge on different microbiomes to make a difference in agriculture?

- How do we move forward to an ecosystem-wide approach to understand the microbiomes across the ecosystem?

Microbiome phenotype: In order to understand the effect of changes present in or made to the microbiome we need to be able to determine the phenotype of a microbiome. Genomics have allowed for a vast amount of data to be generated, but the knowledge on how to translate the genome knowledge into a phenotype is not always present.

- What is the experimental methodology to determine the phenotype?

The challenges are

- how to define a microbiome phenotype and how to determine it;
- how to determine the phenotype of a (deliberate) change in the microbiome;
- how to predict the phenotype.

Microbiome composition, quality and quantity: With the growing amount of genomic data available for every single microbiome sample, increasingly microorganisms present in minute quantities can be detected with accuracy. We do not understand whether there is a relationship between the quantity of microorganisms in a sample and the contribution ('quality') of that organism.

- Do microorganisms which are present in high frequency (quantity) contribute more to the overall phenotype than microorganisms which are rare (quality?)
- How to determine whether it is quantity over quality?

Manipulate the microbiome to our advantage: Being able to manipulate the microbiome has several possible advantages. Examples are the potential of keeping nutrients in the soil through e.g. using diverse plant genotypes/ plant species to manipulate the microbiome. The soil microbiome is influenced by the plant genotypes/ plant species that are grown (e.g. different potato cultivars grown have greater impact on soil microbiome than same potato cultivar with/without antimicrobial transgene)

- Can we increase the resilience and resistance of pastoral systems (and pasture persistence) by manipulating the microbiome?
- How do we achieve this with less or the same environmental input while increasing productivity, i.e. make efficiency gains?

Case study 1: soil microbiome

Goal:

What simultaneous improvement in productivity and reduced environmental footprint can be gained from the interaction soil-plant-animal genomics

- Encompass recent developments in genomics that provide new tools to understand the microbiome along the soil-plant-animal continuum.
- These tools provide more precision in the identification and quantification of the structure of the microbial communities (numbers of species, strains etc).
- How emerging tools in genomics can be applied

Foreword

The biology of soils has long been recognised as central to the productivity capacity of natural and managed ecosystems (Coleman and Whitman, 2005; Ogunseitan, 2005). The diversity of life present in soils provides a reservoir of species that may support or inhibit the growth of plant and animal directly, e.g. as beneficially symbionts or pathogen, or indirectly through the actions of soil biology on affecting the biological availability of nutrients and toxins in soil (Roper and Gupta, 1995). Furthermore, functions supported by soil biology provides a range of enabling and provisioning ecosystem services that support the natural environment, including interaction between above and below ground terrestrial biomes, aquatic ecosystems (rivers, lakes, groundwater's), and the global atmosphere (e.g. Orwin et al., 2015). It is paradoxical that while we can not directly observe the biology in soil, that its function shapes the world around us.

New undertakings are aimed at realising opportunities based on understanding and management of soil biology for productive an environmental gains (Vogel et al., 2009; Bissett et al., 2016). These increasingly utilise various environmental genomics approaches (*sensu* van Straalen and Roelofs, 2006) where soil is treated as an ecosystem hosting a rich diverse 'microbiome' of species and across these harbouring diverse 'functional' genetic elements (e.g. genes conferring antibiotic resistance or nitrogen fixation). Assessing these,

at an ecosystem level, is technologically challenging, requires development and application of new advances in (bio)computational and statistical tools for ecological analysis (e.g. Xu et al, 2014; Deng et al., 2012), and most importantly necessitates a shift in conceptual thinking from organism or gene x ecosystem property/function interactions, to embracing the complexity of interactions among organisms, their genetic elements, and the abiotic and biotic factors that collectively express to deliver ecosystem processes (Torsvik et al., 2002; Myrold et al., 2013). Clearly, understating the complexity of the soil ecosystem, across spatial and temporal scales, must be viewed as a key opportunity rather than hindrance for future advances in soil ecology and function (Konopka, 2009; Mendes et al., 2015; Dignam et al., 2016).

Characterising and understanding soil ecosystems

Soils are hyper-diverse ecosystems, comprising complex assemblages of bacterial, archaea, and eukaryotic taxa. Indeed, soils are currently considered the most diverse ecosystems on Earth (Curtis et al., 2002). Estimates of the total of life in soil vary widely, and depend on the method used, the definition of what constitutes a 'species', and so forth. However, for bacteria alone, estimated richness is in the order of thousands to tens-of-thousands of species per gram of agricultural soil (Curtis et al., 2002; Torsvik et al., 2002; Schloss and Handelsman, 2006).

Assessment of species rank-abundance curves (RAC's) show that the soil microbiome contains many rare species (Caporaso et al., 2011; Huse et al., 2010). This has particularly been brought to light with NGS-based community sequencing analysis; with increasing depth of sequencing, more species (generally SSU rRNA phylogeny) are discovered. That is, the tail of the RAC's generated for soil microbial ecosystems are very long. However, do these rare species matter in relation to soil-provided ecosystem function?

In many cases, the rare biosphere is the reservoir of many novel lineages, colloquially referred to as 'microbial dark matter' (Rinke et al, 2013). Our understanding of these taxa is slight, particularly as many of these novel, and sometimes candidate, phyla, remain to be isolated in pure culture (Stewart, 2012). As such, the ecological importance of the rare biosphere is unequivocal. Genomic analysis has shown that these taxa harbour unexpected metabolic features (Rinke et al, 2013), and are therefore a potential source of novel

enzymes and ‘stored ecosystem potential’ (Lynch and Nufield, 2015). Furthermore, the recruitment of taxa, with unique ecophysiological adaptations, has been shown to be essential in recovery of soil ecosystem function, such as ammonia oxidation, after disturbance events (Mertens et al., 2009). Thus the rare biosphere has wider impacts on ecosystem function than the size of the community suggests, and represent an important seed bank of organisms with which we may begin to have a functional role as opportunities arise, for example recruitment by a host plant or animal, or edaphic or environmental changes.

The application of molecular-based tools has been essential towards efforts to characterise and understand soil ecosystems. However, the hyper-diversity of life in soil means that the ecosystem is also genetically complex. A single gram of soil is estimated to contain up to 1,000 Gbp of metagenome DNA (Vogel et al., 2009; Frisli et al., 2012). As such, even current next generation sequencing (NGS) platforms only provide small, partial coverage (depth of sequencing) of soil metagenomic DNA. Therefore, most soil metagenome research to date has have relied on the characterisation of specific elements within the metagenome. For example, the use of meta-barcoded primers to assess community composition (e.g. Fierer et al., 2012), application of NGS or high-density environmental microarrays to determine functional status/composition of the community (e.g. He et al, 2007; Nelson et al., 2016), or functional screening of libraries of cloned DNA fragments for novel enzymes and bioactive compounds (Lee and Lee, 2013). With rapid development of sequencing and biocomputational ability, the shotgun (random) sequencing of significant components of entire soil communities may be achievable and at a reasonable cost (particularly computational and bioinformatic time). Whilst acknowledging there remain other significant hurdles to overcome (Thomas et al., 2012; Prosser, 2015), achieving this point would represent a remarkable advance in our ability to characterise and understand complex ecosystems. Given so little is known about the vast majority of life in soils, how these interact to support ecosystem functions such as nutrient cycling, and opportunities for discovery of novel metabolites or pathways, the application of metagenomics to soil ecosystems provides an exciting opportunity to make gains in ecosystem production and environmental outcomes.

Example metagenomic application: Disease suppressive soils

Disease suppressive soils are defined as those in which the activity of the resident soil microbiota reduce the occurrence or severity or occurrence of plant disease caused by soil-borne pathogens (Weller et al., 2002). Given the high cost of soil disease on agricultural production (e.g. estimated costs of 28-50% of pasture production in New Zealand; Skipp and Watson, 1996; Wakelin et al., 2016b), and the lack of practicable and economic control options, the development of disease suppression in soil microbial communities represents an important soil service that serves to maintain agricultural production and the food and fibre it produces (Dignam et al., 2016).

Disease suppressiveness has been observed in a number of soils, with different disease-host interactions, and can develop naturally over time (Mazzola, 2002; Weller et al., 2002). In instances where disease suppression develops, it is underpinned by alteration in the soil microbial community structure towards a greater number of disease suppressive taxa, or expression of potential (latent) disease suppressive activity (reviewed in Mazzola, 2004; Dignam et al., 2016). Not surprisingly, the development of disease suppression in soils is highly desirable, and there have been considerable efforts to understand how this can be facilitated through changes in system management (e.g. fertiliser use and plant residue management; Weller et al., 2002). However, the characterisation of the community and functions associated with general disease suppression has been very difficult, particularly as they potentially represent a small fraction of the total microbial diversity in soils (Raaijmakers et al., 2009). Furthermore, in the case of 'general' disease suppression (Hornby, 1983), underpinned by phylogenetically diverse consortia of microbiota, functions spanning lytic enzyme production, antibiotic secretion, through elicitation of plant defence mechanisms, may be collectively responsible (Weller et al., 2002).

Advances in understanding changes within the soil community during development of disease suppression are being supported through application of ecological genomic tools (see Dignam et al., 2016 review). These include the application of high density oligonucleotide microarrays (Mendes et al., 2011), tag-based NGS (Kyselkova et al., 2009), and shotgun metagenomics (Penton et al., 2014; Chapelle et al., 2015) to characterise the disease suppressive microbiome in various soils. In each case, phylogenetically diverse microbial consortia were associated with disease suppression. It would be difficult to have deciphered these communities against the rich background of soil microbial diversity without an ecological genomics approach.

More recently, the focus on assessment of soil-borne disease suppression has been extended from approaches largely focused on identifying the taxa responsible, towards assessing soil ecosystems based on functional-genes. This has followed recognition that, in many instances, a phylogenetic description of a microorganism can be a poor reflection of the metabolic (functional) ability outside of its base metabolism. This is particularly important where functions, such as antibiotic resistance, antibiotic production, host compatibility, virulence etc are borne on mobile/transferrable genetic elements such as plasmids (Rankin et al., 2011). Indeed, the acquisition or loss of a plasmid can change the biology and wider ecology of individuals of the same species in the soil; in these cases, the identification of species only indicates the presence of a 'potential host' that may or may not harbour the functional genes of interest (e.g. Young et al., 2006). As such, the detection of multiple functional genes associated with disease suppression is likely to provide a richer understanding of the ecosystem potential for this important ecosystem function (Raaijmakers and Mazzola, 2012). To achieve this, technology platforms such as functional environmental microarrays (He et al., 2007) are being constantly updated to include information on genes either directly or putatively associated with disease suppression. These include many antibiotic production genes, such as *phz*F and *phz*A (phenazine production), *bac*A (bacilysin), *pab*A (chloramphenicol), *phl*D (DAPG), *lgr*D (gramicidin), *lmb*A, (lincomycin), *prn*D (pyrolnitrin), *str*R (streptomycin), *spa*R (subtilin), and *pcb*C (β-lactam) genes, alongside sub-sets of existing gene probes for detection of lytic enzyme production, *hcn*B (cyanide) formation, etc (Dignam, 2016). By assessing the abundance and distribution of these genes in soil eDNA samples, the functional ecology of disease suppressive communities may be determined. Impacts such as farm management practices, on disease suppression, can then be interpreted through the lens of functional changes in the soil biology. Over time, this knowledge is expected to provide novel opportunities for on-farm management of soil biological resources towards enhanced disease suppression. Furthermore, molecular-based tools may enable the rapid identification of soils suppressive to specific diseases. These soils will represent important natural resources enabling the transmission of suppression from one soil to another by deliberate soil inoculation (Weller et al., 2002).

The transfer of biology from one soil to another can confer new ecosystem phenotypes. This has been extensively shown for movement of various microbial species as mycorrhizal fungi, plant pathogens, and rhizobia; the movement of these taxa among soils has direct impact on the productive capacity and success of various plant species in the receiving environment (Schnitzer et al., 2011; Lau and Suwa, 2016). These examples, and others, demonstrate the

potential to manage soil biology for specific production-based and/or environmental outcomes.

Soil genotype x (genotype or environment) interactions?

As described previously, associations between soil biology, plants, above and below-ground animals, and the environment are massively complex. Thus, while there are many examples of the importance of management of individual and simple consortia, most ecosystem outcomes are supported by the activities of a wide consortium of microorganisms. These outcomes are the result of multitudes of species, with a collective of functional traits that emerge together to result in an altered ecosystem phenotype. The opportunities to harness these interactions are immense, and are potential opportunities if these can be directed.

Critically, the soil microbiome has a number of direct influences on plants. With the exception of seed-borne (vertically transmitted) endophytes, the soil biology provides the primary reservoir of microorganisms that colonise the root rhizoplane, rhizosphere, and ultimately the wider endophytic microbiome within the plant (Gaiero et al., 2013; Nallanchakravarthula et al., 2014; Schreiter et al., 2014; Rascovan et al., 2016). The discovery of endophytes remain in its infancy, and estimates of 1 million endophytic species of higher plants maybe reasonable (Ganley et al., 2004). The consequences of this are profound, as the plant microbiome has wide range impacts on expression of the phenotype. Microbiomes have been shown to confer drought tolerance in plants (Zolla et al., 2013), alter flowering phenology and timing (Wagner et al., 2014; Panke-Buisse et al., 2014), plant shoot dry matter production (Schreiter et al., 2014), and induce system resistance to diseases (Babu et al., 2015). The interaction between the microbiome and the plant genetics also affects aspects of plant quality via altering changes in the production of plant metabolites, or providing additional metabolic capacity by the microbiome (ancillary metabolic pathways) (Brader et al., 2014). The quantity and profile of strawberry flavour, for example, is influenced by microbiome regulation of fuaranol synthesis (Zabetakis, 1997; Verginer et al., 2010), while the soil microbiome influences the community on the grape and wine properties (Zarraonaindla, et al., 2015). It is likely that microbiome affect the quality of resins, fruit, honey, and essential oils (Brader et al., 2014). While these are presently vastly understudied, the manipulation through the microbiome presents opportunities for novel products, or additional value of current products, that may lay outside the opportunities that can be expressed by the plant genome alone. Indeed, the microbiome background in which

plants are grown can be seen to contribute to the wider *terrior* of the plant product, and maybe used to value add to the provenance of product grown in different soils.

Soil biology is also the 'engine room' that recycles plant material, either from direct inputs (leaf fall, root senescence), or secondary deposition (animal manure, urine) (Coleman et al., 2004). The nutrients in these material are either recycled within the biosphere, or mineralised into the geochemical matrix of the soil (Coleman et al., 2004). In terrestrial systems, the soil microbiology provides interface between the biological and abiotic worlds, affecting movement of essential major and trace elements between the geologic reserves and the biosphere. As such, there are a broad range of opportunities to harness the potential of soil ecosystems to optimise nutrient cycling. These include increasing the supply of many major and minor essential elements for plant use, stimulating the long-term storage of carbon in soils, and promoting 'closed' nutrient cycling such that NO_3 and N_2O, for example, stay on-farm. Given our current lack of understanding of soil biology, we still have a rudimentary knowledge of the extent of species interactions that may potentially affect critical gate-ways in the mineralisation, immobilisation, and cycling of nutrients and the coupling of nutrient cycles. Indeed, it is highly likely that cryptic species and/or functional processes will have hitherto unrecognised importance in many aspects of soil nutrient cycling.

Indeed, there are a multitudes of direct and indirect interactions between below and above ground ecosystems, and these converge and are expressed into terrestrial ecosystem function (Hooper et al., 2000; Wardle et al., 2004). These collectively express as an ecosystem 'ecotype', or 'functional status' to the soil. Across a multitude of functions, a 'normal operating range' of soil ecosystems can be defined. By assessment of these across a range of different samples, a generalised understanding of the performance of a soil with others (measured v expected system function) can be gained (van Straalen and Roelofs, 2006). This framework can be expanded to investigation of factors such as expression of plant-genotype effects, impacts on soil ecosystems due to disturbance (human, climate, etc), assessing ecosystem recovery, and so on. Future decisions about plant or cultivar selection for different farming systems (pastoral, arable, horticulture, and forestry) are likely to include an understanding of the soil biology present. Furthermore, this is likely to extend to precision use of fertilisers, agri-chemicals, seed dressings (incl. biological), that consider the wider ecosystem parameters. These opportunities will converge to enable a balance

between optimal productivity and environmental outcomes which are not obtainable with the current laissez-faire approach.

Opportunities for multi genotype x environment interactions ($G^x \times E$).

Emerging opportunities centre on understanding the interactions between the soil, plant, and animal microbiomes within different environmental situations. Such holistic, community-level approaches to asses complex, multi-trophic linkages and communication (signals) among microorganisms, plants, and animals, against influences of edaphic and environmental spatiotemporal heterogeneity, will require application of a range of emerging tools and approaches such as those based on ecological genomics (Wakelin et al., 2016a). These will need to deliberately embrace the inherent complexity of microbiomes as 'meta-ecosystem' containing an assortment of biological elements (species, mobile genetic elements), with different functional potential, that express an overall ecosystem phenotype. An integrative ecological genomics approach, that explores interactions among and across these meta-ecosystems and their collective ecological control, will be required to translate the biology to outcomes in a control-analysis approach (van Straalen and Roelfs, 2006).

Case study 2: Pasture Ecology

Pasture ecology case study

Grassland composition and productivity is finely balanced under the influence of interactions among many factors: the physical environment (soil water and nutrient availability, temperature, and extreme climatic events), management (particularly of grazing process), plant genetics, and the soil and plant microbiome. For the most part, New Zealand's pasture-based livestock industries use relatively simple mixtures of temperate grass and legume species as the main feed source for ruminant animals. Yet even these 'simple' vegetation communities vary greatly in space and time, often for reasons that are not obvious using traditional scientific monitoring or analysis methods (let alone through farmer observation).

The propositions behind this article are 1) that the microbiomes associated with soils, plants and animals contribute significantly to productivity in the pastoral sector, but that 2) they are often treated as distinct entities whereas they operate under similar ecological principles,

and 3) are interconnected through water, carbon and nutrient (especially nitrogen) cycles. Where, then, is the evidence for these propositions?

A case study: dairy pasture dynamics in the upper North Island

New knowledge of the ecology of pasture communities in this region has revealed a clear instance where it is the microbiome that drives change in community structure, and the consequent feedback loops that engage other microbial communities. Increasing prevalence of predation of perennial ryegrass root systems by insect larvae, combined with other stress factors particularly increasing soil moisture deficit, has led to widespread but spatially disaggregated instances of near-complete perennial ryegrass pasture failure. For example, when the insect pest is black beetle, and the ryegrass population contains an endophyte strain that offers minimal protection against this species, pasture collapse is observed within 2 years after sowing whereas infection with an effective endophyte strain maintains ryegrass populations. The signature of ryegrass failure in this instance is the content of white clover in the pasture, which increases rapidly above that seen in pastures with effective endophytes (Figure 1). It is notable that the plant genome does not explain this different survival pattern – though there can be subtle host genotype x endophyte strain interactions that mediate the speed and scale of change.

The outcome of clover dominance (resulting from reduced competitive pressure from the grass) leads to rhizobium symbiosis becoming a dominant process in the community, and illustrates the connectivity between the plant and soil microbiomes – mediated through ecological processes (competition). Furthermore, clover dominance changes the nutritional composition of the feed eaten by livestock – reducing total fibre content and increasing soluble protein, which in turn creates a feedback loop in the rumen as the rumen microbial composition changes to utilise the altered substrate. Hence a further point of connection among microbiome levels, this time to the animal, is established. A direct result outcome of this connection is increased ammonia release in the rumen, flowing through to increased excretion of surplus N via urine, i.e. higher urinary N concentrations and increased risk of nitrate leaching. This way, via three feedback loops, precipitated by a mis-match between the plant microbiome and the environment, a grass-dominant and relatively N-efficient pasture is transformed to a legume dominant pasture with a leaky N cycle.

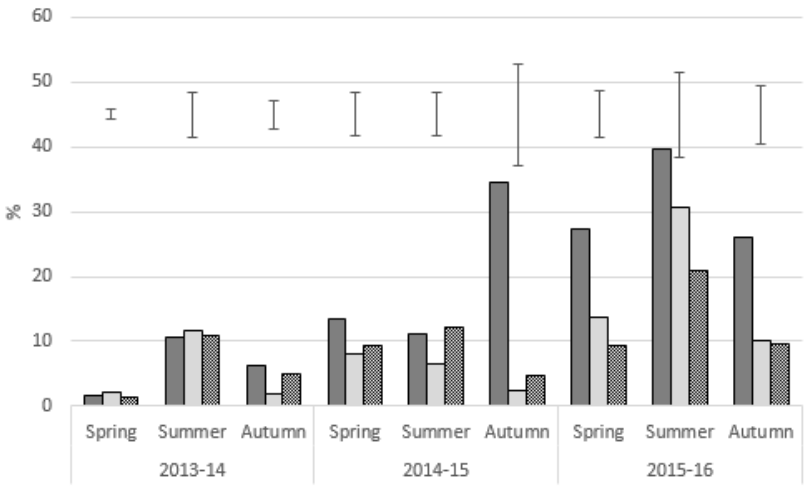


Figure 1. The content of white clover (% total dry matter yield) in perennial ryegrass pastures sown with AR1 ■, AR37□, and standard ▩ endophyte at Newstead, Waikato. Bars are ± SED.

Obviously the endophyte strain can be manipulated, to close down the negative feedback loops to pasture persistence and the environment. What do we lose by so doing?

- We lose clover content, and therefore total DM production
- So, some loosening of the competitive noose around the clover neck is beneficial
- Can we control the equilibrium via the microbiome – in this case, either the endophyte or the rhizobium, or both? Or neither, but through other microbial populations?

Thus, evidence does exist for the influence of, and in some case control by, the microbiome on the productivity of pastoral ecosystems. Questions that arise include: Where and how should we intervene to manipulate the microbiome in an ecological system such as this? With what purpose and consequences? And what benefit can we expect, relative to the manipulation of the plant or animal genome itself, from going down this pathway?

Case study 3: rumen microbiome

The rumen microbiome

By virtue of converting human-indigestible plant polymers (cellulose and hemicellulose) into edible animal protein, ruminants enable high value food production from pasture plants resources. Ruminant animals are therefore an important part of the NZ pastoral sector and produce a wide range of food and fibre products of considerable value to the NZ economy. The digestion of plant material is achieved via the ruminant's specialised digestive systems, consisting of a multi-chambered stomach which supports the growth and fermentation activities of a diverse array of anaerobic microbes. The main digestive processes are carried out in the first two stomach compartments, called the reticulo-rumen, where microbes colonise and breakdown forage plant material. The microbes ferment the released sugars into volatile fatty acids, which are absorbed from the reticulo-rumen and used by the animal to fuel animal growth and productivity. The process is regulated so that only a partial fermentation occurs, allowing the ruminant host to absorb and utilise the intermediate fermentation products for its own metabolism and growth. The ruminant also benefits from the provision of vitamins and from the microbial proteins flowing further down the digestive tract.

There has been a continual drive by livestock breeders and farmers to improve the efficiency of digestion in the rumen. Studies of the rumen microbiome have focused on understanding the contribution that the microbes make to the digestion and metabolism of particular feeds, or that are involved in productivity traits that are selected during animal breeding. However, microbiome analyses are increasingly being used to identify new ways to manipulate microbial metabolism itself, to enhance its digestive capacity and drive greater productivity in the host animal, while reducing waste or detrimental end products of the fermentation that have negative impacts on digestive efficiency, rumen function or the environment. There are many previous examples of microbial manipulations in ruminants to influence digestion, including additives such as buffers, antibiotics (ionophores and non-ionophores), protease and deaminase inhibitors, fats, methane inhibitors, vitamins, minerals, iso-acids, enzymes, and exogenous bacteria and/or yeast. These additives target different processes in the rumen and have varying degrees of effectiveness, depending on the ruminant species targeted and the diet fed to the animals. Many of these additives are non-selective or have unknown modes of action, and there is a need to have a better understanding of rumen microbiome responses so that these manipulations can be more precisely tailored to deliver the desired improvements while removing, or minimising any unintended consequences.

Rapid advances in DNA and RNA sequencing, and new high throughput screening technologies for proteins and metabolites, are now making a complete description of the rumen microbiome an achievable goal. Combined with the ability to interpret the "omics" information using new bioinformatics approaches, this is transforming our understanding of the rumen microbial ecosystem, and will inevitably lead to new ways of manipulating ruminal fermentation processes. Although these technologies are new, they are being used to address recurring questions about the contribution the rumen microbiome makes to the nutritional functions of the ruminant, i.e. what types of microbes are present, how many organisms are there and what are they doing? Furthermore, as a better appreciation is gained of the importance of gastrointestinal microbes to their host, new questions around their protective, immunological, and developmental benefits to the host are being posed (Hooper, 2004)(Xu and Gordon, 2003, Egert *et al.*, 2006, Gill *et al.*, 2006).

Case study: methane yield differences in sheep is related to expression of genes encoding the hydrogenotrophic methane formation pathway.

Methane is produced in the rumen by the methanogenic archaea and while the main rumen methanogens are known, the process of methane formation is not clearly linked to either the number (Yanez-Ruiz *et al.*, 2008, Mosoni *et al.*, 2011, Popova *et al.*, 2011) or a particular community structure of methanogens (Zhou *et al.*, 2010, Morgavi *et al.*, 201X). However, it is known that the concentration of methanogenic substrates (mainly hydrogen and methyl compounds such as methanol and methylamines) and interactions between methanogens and microbes producing and consuming hydrogen in the rumen (Janssen, 2010, Morgavi *et al.*, 2010) are important factors contributing to methane emissions. To better understand methane formation, there has been a concerted effort to measure methane emissions from ruminant animals, to examine the variation in methane yield (g methane/Kg dry matter intake/day) between animals, and to assess the effects of different diets or dietary additives on methane output. Measurements made in sheep have shown methane yields vary considerably between individual animals within flocks (Pinares-Patiño et al., 2011a; 2011b; 2013), by as much as 34% between the low and high methane emission phenotypes. These variations in methane yield have been linked to differences in particle retention time in the rumen (Benchaar et al, 2001; Smuts et al., 1994; Pinares-Patiño et al., 2011a) and to rumen volume (Goopy et al 2013), and were found to persist under different grazing conditions and to be a heritable trait in sheep (Pinares-Patiño et al., 2013). Because methane is produced solely by the action of methanogenic archaea, rumen methanogens must make some contribution to the methane phenotype in sheep, either directly or via changes to the microbial community in the rumen.

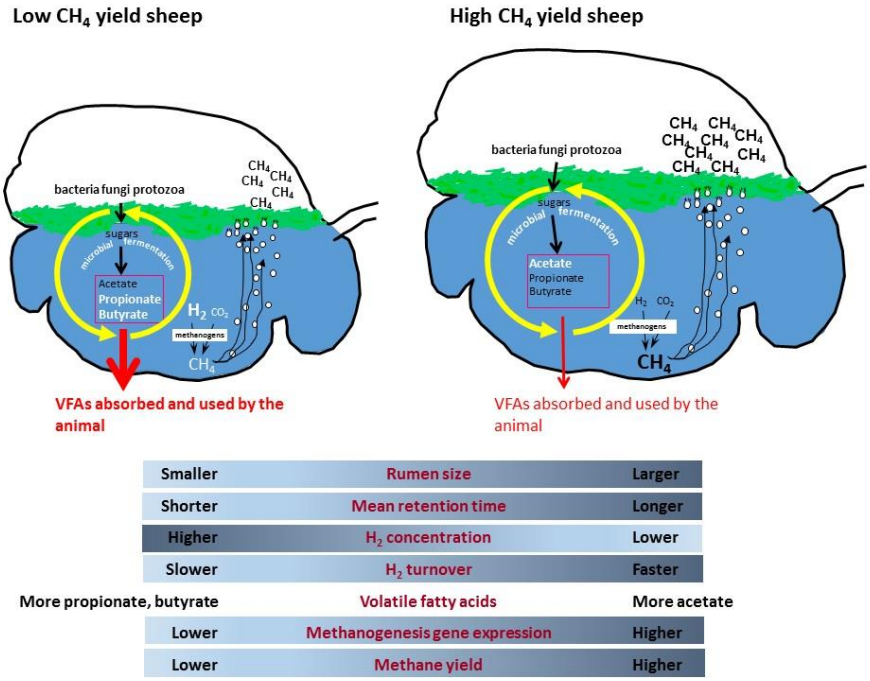
To examine the contribution that the microbiome makes to methane yield, sheep with high or low emission status were rumen sampled and DNA and RNA were extracted to enable both metagenome and metatranscriptome analysis of their rumen microbiomes (Shi et al 2014). The metagenome DNA sequencing generated a total of 1,020 Gb unamplified whole genome shotgun (WGS) data, while 132 Gb of metatranscriptome sequence from mRNA-enriched samples was also generated to explore the possibility that gene expression might explain some of the differences in methane yields. Additionally, bacterial, archaeal and protozoal small subunit rRNA gene amplicons were generated and sequenced to assess differences in rumen microbial community profiles.

The simplest explanation of variations in methane emission yields from sheep is that they harbor different methanogen communities, therefore the rRNA gene sequences were analysed to compare microbial community profiles between the high and low methane yield animals. Surprisingly, these analyses showed no differences in the relative abundance of bacteria, archaea or eukaryotes between the low and high methane yield sheep (Shi et al. 2014). Even detailed genus-level analysis of methanogens showed only slightly elevated levels of *Methanosphaera* spp. in the low methane yield sheep and slightly higher *Methanobrevibacter gottschalkii* in the high methane yield sheep, however these were not sufficient to explain the differences in animal methane yield. An analysis of abundance of genes encoding the methanogenesis pathway also showed no significant differences, which confirmed our rRNA gene analyses. However, when the metatranscriptomic data were examined, there were clear increases in transcripts of genes encoding the methane metabolism pathway (KEGG: ko00680) in high methane yield sheep. In particular, the genes encoding the hydrogenotrophic methanogenesis pathway (in which methane is formed from hydrogen and carbon dioxide) were significantly up-regulated compared to the methylotrophic methanogenesis pathway (where methane is formed from methyl compounds). Specifically, high methane yield sheep had high transcript levels of the methyl coenzyme M reductase enzyme (*mcr*, EC: 2.8.4.1) which catalyses the final step in the methane formation pathway. A detailed comparison of these *mcr* genes found that they clustered into three distinct groups, called Sheep Rumen MCR groups 1, 2 and 3. The SRMR1 group of *mcr* genes were derived from a new group of rumen methanogens which belong to the order Methanomassiliicoccales. The SRMR2 group was identified as encoding an isozyme of methyl coenzyme M reductase (MCRII encoded by the *mrt* gene) and was found in both *Methanobrevibacter* spp. and *Methanosphaera* spp., while the SRMR3 was derived from *Methanobrevibacter* spp. only. The vast majority of methyl coenzyme M reductase transcripts were from the SRMR1 and SRMR3 groups, and were 2.84- and 2.85-fold more abundant in high methane yield sheep, respectively, while SRMR2 transcripts

were very low. These results showed that transcriptional up-regulation of the hydrogenotrophic methanogenesis pathway was an important microbial mechanism contributing to higher methane yield in sheep.

It makes biological sense that an up-regulation of methanogenesis genes in rumen methanogens results in more methane emissions from animals, but why does this happen in some sheep and not in others? A possible mechanism has been proposed which incorporates differences in rumen size and feed particle retention time, leading to altered microbial growth kinetics and fermentation thermodynamics which affects ruminal hydrogen levels (Janssen, 2010). It is proposed that low methane yield sheep have a smaller sized rumen, which causes increased particle passage rate that leads to higher rumen hydrogen concentrations (Fig. 2). The higher hydrogen concentration causes a negative feedback that results in less hydrogen formation by fermentative microbes, leading to less methane formation. Conversely, high methane yield animals are predicted to have a larger rumen with slower particle passage, which results in lower hydrogen concentrations, enhanced hydrogen formation during fermentation, and more methane. Under ruminal conditions of slower particle passage rate and lower hydrogen concentrations, it is predicted that there is a higher turnover rate of a smaller hydrogen pool through the methanogenesis pathway to account for the elevated methane formed. The lower ruminal hydrogen concentration means that methanogens have to increase expression of methanogenesis genes to produce more enzymes to scavenge the hydrogen and maintain its turnover rate. This is because enzyme concentrations as well as substrate concentrations can limit the flux through a pathway, and increasing enzyme expression partially overcomes the limitation of lower substrate concentrations. Conversely, a high particle passage rate and high hydrogen conditions would require a lower level of expression of methanogenesis pathway genes to permit the same flux.

This strong relationship between expression levels of the hydrogenotrophic methanogenesis pathways in rumen methanogens and methane yield in sheep, is the first example of rumen microbial gene expression being directly linked to an animal phenotype of relevance to environmental sustainability and production. These studies have confirmed current targets within existing methane mitigation programmes and also provide new microbial and metabolic pathway as future targets for manipulation in ruminants.



Smaller	Rumen size	Larger
Shorter	Mean retention time	Longer
Higher	H_2 concentration	Lower
Slower	H_2 turnover	Faster
More propionate, butyrate	Volatile fatty acids	More acetate
Lower	Methanogenesis gene expression	Higher
Lower	Methane yield	Higher

Figure 2. Proposed rumen model for methane yield phenotypes in sheep.

Case study 4: Water Quality Effects

While focusing on increasing the benefits of enhancing agricultural production through the genomics of the soil, plant and animal there is a need to consider the potential effects of the zoonosis carried by these animals. We know that changes in animal diets and/or farm systems can affect the zoonosis carried by farm animals (Callaway et al., 2003; Rapp et al., 2014; Vanselow et al., 2005). These zoonosis in farm animals can impact on human health via multiple pathways. The first is direct animal contact which impacts predominantly on the people who work in the industry as well as non-occupational contact (Klous et al., 2016). The second pathway is via contamination of the food products consumed (Hussein and Sakuma, 2005; Strachan at al., 2006). The third major pathway is via water contamination (Dufour et al., 2012) which in itself can exhibit three separate pathways such as drinking water (Hurdey et al., 2003), contact recreation (Soller et al., 2010) and irrigation of food crops (Pachpesky et al., 2011). These outbreaks of zoonotic disease events can have

considerable economic cost to the agricultural industries (Bennet et al., 1999; Christou, 2011; Torgerson and Macpherson, 2011). Genomic techniques have shown considerable potential in linking and understanding sources of food or water contamination (Christen, 2008; Cornelius et al., 2014; Lou et al., 2011; Stale and Sadowsky, 2016). Therefore, when studying the microbiome of animals in order to enhance agricultural production of animals it would be of considerable benefit to include screening of zoonotic organisms in the tools being developed.

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