FOCUSED MEETING
2018

Microbiomes Underpinning Agriculture

Rochestown Park Hotel,
Cork, Ireland
1–2 October 2018

Agriculture and Food Development Authority

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INTRODUCTION

We bid you the warmest of welcomes (and the traditional céad míle fáilte) to our Focused Meeting on Microbiomes Underpinning Agriculture in the rebel county of Cork. Microbiomes play an undeniably fundamental role in agriculture and food production, and advances in microbiome research offer great potential to meet the many challenges agriculture faces into the future as we move towards more efficient and sustainable food-production systems. At this meeting we bring together expertise from microbiome areas across agriculture, with focused sessions on plant, soil and animal microbiomes. There is much to be gained by studying the interaction of these microbiomes, and on cross-linking expertise across disciplines. This is something which is rarely done but we hope will be achieved at this meeting. This Focused Meeting also incorporates a methods workshop for exploring microbiomes in agriculture at Teagasc (The Irish Agriculture and Food Development Authority), Moorepark, that will enable integration of skills and sharing of best practice across technical aspects of microbiome research, including sequencing and analysis/interpretation of data associated with complex microbial communities in agricultural systems. An optional tour of the genomic sequencing and microscopy facilities in Moorepark Research Centre will also be available. This meeting is the culmination of much hard work by many people and we would like to thank them sincerely for their assistance in making this meeting happen. We would particularly like to thank the Microbiology Society for their absolutely invaluable support in organising this meeting and our sponsors for their kind financial support. We hope your time at our meeting will be both productive and enjoyable, and that you will get a chance to explore some of the many delights that the most southerly county in Ireland has to offer. Beir bua agus beannacht!

Organising Committee:

Fiona Brennan
(Teagasc, Ireland)

Paul Cotter
(Teagasc, Ireland)

Kaye Burgess
(Teagasc, Ireland)

Sinéad Waters
(Teagasc, Ireland)

Ewen Mullins
(Teagasc, Ireland)

Orla O'Sullivan
(Teagasc, Ireland)

Fiona Crispie
(Teagasc, Ireland)

GENERAL INFORMATION

Certificate of attendance

A certificate of attendance can be requested by emailing profdev@microbiologysociety.org following the event.

Dietary Requirements

All disclosed dietary requirements made at the point of registration have been shared with the catering team in advance. However, please ensure you ask for advice if required and the catering team will do their best to accommodate your needs.

Photography and filming

The Society may carry out filming and photography throughout the meeting. The images and videos will be used to promote the meeting and the activities of the Society. They may be used online, in Society publications, or for other PR and marketing purposes. Please do not photograph posters or record presentations unless prior consent has been given by the presenter.

Posters

Posters will be displayed throughout the meeting and a poster abstract book will be provided online. Each poster has been assigned a number, which appears alongside the abstract in a separate poster abstract book. Presenting authors are requested to stand by their posters, when possible, to present their work. Posters must be removed by 13:30 on Tuesday 2 October. Neither the Society nor the venue are able to return any remaining posters after this time.

Programme changes

While every effort has been made to ensure the programme is accurate, changes are unavoidable. We will ensure updates are provided throughout the meeting.

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Wi-Fi

Connect to Rochestown Park Hotel network, no password required.

PROGRAMME OVERVIEW

Day 1

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Structural variability and niche differentiation in the microbiome of field-grown winter oilseed rape under different soil tillage practices	10:30	Kieran Germaine (Institute of Technology, Ireland)	12
Moss and lichen-associated microbiomes in warming Icelandic heathlands, moraines and fell fields	10:45	Oddur Vilhelmsson (University of Akureyri, Iceland)	13
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Ecological drivers and functional aspects of the plant microbiome	11:30	Angela Sessitsch (Austrian Institute of Technology, Austria)	14
A systems view of microbial establishment on growing rhizoplanes	12:00	Daire Carroll (University of Warwick, UK)	14
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Day 1

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Engineering the rhizosphere microbiome for crop parasite control	14:45	Neil Warnock (Queen's University Belfast, UK)	18
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A tale of two stories from the underground: soil microbial diversity and N-cycling	15:30	Laurent Philippot (INRA, France)	19
Harnessing the soil immune response: impacts of long-term plant residue management on soil organic matter quality, *Pseudomonas* community structure and soil disease suppressiveness	16:00	Bryony Dignam (AgResearch Ltd, New Zealand)	20
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Day 2

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SOCIAL PROGRAMME

Drinks reception and poster session

17:00–18:00, Monday 1 October

Location: Rochestown Park Hotel, Kiltegan Suite

Following the first two sessions of the conference, we would like to invite you to an informal drinks reception and poster session that will allow you to discuss the research with the authors and to catch up with old contacts and make new ones.

Drinks reception sponsored by APC Microbiome Ireland

Meeting dinner

18:00–22:00, Monday 1 October

Location: Rochestown Park Hotel, Estuary Suite

The Meeting dinner will be held in the Estuary Suite after the drinks reception.

Three-course dinner exclusively for the conference delegates will provide a great opportunity to continue the discussions from the day in a relaxed atmosphere.

DAY 1

Monday 1 October

DAY 1 SPEAKER ABSTRACTS

Please note: abstracts are published as received from the authors and are not subject to editing

09:30

Invited speaker: Davide Bulgarelli (University of Dundee, UK)

Defining the host genetic control of the rhizosphere bacterial microbiota

My group uses barley (*Hordeum vulgare*) as a model to gain novel insights into the genetic basis of plant-microbiota interactions in the rhizosphere. We previously demonstrated that Elite varieties and wild barley ancestors host distinct microbiotas, possibly representing a footprint of plant domestication on the microbial communities inhabiting the rhizosphere. We recently extended this line of investigation by characterising the microbiota of a bi-parental population between an elite variety and a wild barley ancestor. By combining 16S rRNA gene sequencing profiles, as 'quantitative traits', with thousands of SNPs in the barley genome we compiled a map of the plant loci shaping the rhizosphere microbiota. Strikingly, we did not observe a linear relationship between number of loci and bacteria putatively controlled by them. Rather, our data suggest that microbial community assembly in the barley rhizosphere is controlled by a few major alleles with a major effect. In particular, we identified a single locus on barley chromosome 3H significantly associated with the recruitment of nine, phylogenetically unrelated, bacteria. Here I will discuss the experiments that led us to these discoveries and their implications for basic and applied science.

10:00

Offered paper: Sarah Worsley (University of East Anglia, UK)

The chemical ecology of protective plant root microbiomes

The development of effective biocontrol agents is a major challenge facing modern agriculture. Bioactive microbial species found within the plant root microbiome could be better exploited in arable systems. We present several endophytic strains of *Streptomyces* bacteria, isolated from the roots of *Arabidopsis thaliana*, that demonstrate broad-spectrum inhibitory activity against pathogenic fungi and bacteria *in vitro*. The severity of wheat take-all (*Gaeumannomyces graminis*) fungal infections is also reduced by adding *Streptomyces* spores as seed coatings in vivo. Furthermore, whole genome sequencing followed by biosynthetic analysis, indicated that isolates encode between 24 and 35 secondary metabolite gene clusters. The protective role of predicted antifungal products is being demonstrated via CRISPR/Cas9 deletion. Several of the *Streptomyces* isolates also enhance plant biomass and encode gene pathways associated with plant growth promotion. It is hypothesized that root exudates may be involved in attracting and maintaining bioactive bacteria within plant roots. Illumina sequencing coupled with stable isotope probing showed that although *Streptomyces* were prominent members of the *A. thaliana* root microbiome, they were outcompeted by more abundant Proteobacterial genera for the use of exudates. However, *Streptomyces* isolates could grow on purified root exudates in the absence of competition; their root exudate preferences are now being evaluated using a comparitive metabolomics approach. Defining factors that influence the competitiveness of protective bacteria within the plant root microbiome will have implications for the development of more consistent biocontrol strategies. This work also demonstrates that endophytic *Streptomyces* species may represent a diverse source of novel anti-infective agents.

10:15

Offered paper: Ville-Petri Friman (University of York, UK)

Phage selection can constrain plant disease dynamics via resistance-virulence trade-offs

Parasitic bacteria-specific viruses, phages, could constrain bacterial disease dynamics via density regulation or evolutionary trade-offs where phage resistance mutations lead to a reduction in bacterial virulence. While such eco-evolutionary dynamics are often observed in the laboratory, it is less clear if this takes place in more natural environments. We studied this in the context of bacterial wilt diseases of tomato, which is caused by notorious bacterial plant pathogen *Ralstonia solanacearum*. We isolated both pathogen and its phages from the rhizosphere microbiome of tomato in four geographically distant fields in China. We found clear patterns of local phage-bacteria adaptation. First, phages were more infective to pathogens isolated from their own fields, which indicates local host-parasite adaptation within every field. Second, we found that phage resistance correlated negatively with the pathogen growth and virulence measured *in vivo*. To verify this causally, we conducted additional selection experiments *in vivo* to show that evolution of broad phage resistance range leads to a clear reduction in pathogen virulence and growth, and crucially, very low levels of bacterial wilt disease. Together these results suggest that phages could be important at controlling bacterial disease dynamics in agricultural environments via evolutionary trade-offs between phage resistance and virulence.

10:30

Offered paper: Kieran Germaine (Institute of Technology, Ireland)

Structural variability and niche differentiation in the microbiome of field-grown winter oilseed rape under different soil tillage practices

Microbes play vital roles in many soil ecosystem functions and services, many of which are crucial for agricultural productivity. Among different agricultural management practices, soil tillage methods can result in significant changes in a soil's physical, chemical and biological properties, including the soil microbiome. In addition, crop type and the plant developmental stage are important drivers of rhizosphere bacterial microbiota structure and composition. Oilseed rape (Brassica napus L.) is the world's third largest source of vegetable oil used in human nutrition and as a source of oil for biodiesel production. It is also an important break crop in cereal crop rotation and can significantly reduce the rate of fungal disease incidence in subsequent crops in the rotation, leading to improved crop yields. The promotion of agricultural practices that maintain the natural diversity of the oilseed rape microbiome is an important element for a sustainable production system that ensures crop productivity and quality while reducing chemical inputs. We used high-throughput 16S amplicon sequencing to explore the Oilseed rape microbiome structure, diversity and composition in two contrasting tillage practices; conventional-plough based tillage and conservation strip tillage over three different plant growth stages (vegetative, flowering and harvesting stage). We uncovered the core and accessory microbiome associated with field grown Oilseed rape and examined the effect that tillage practices and plant developmental stage had on this microbiome.

10:45

Offered paper: Oddur Vilhelmsson (University of Akureyri, Iceland)

Moss and lichen-associated microbiomes in warming Icelandic heathlands, moraines and fell fields

Lichens and mosses form essential parts of arctic tundra vegetation and play important roles in soil formation in the Arctic. Lichens, generally described as a mutualistic association of a fungus and an alga, are known to harbor species-specific bacterial communities, typically dominated by Proteobacteria. Similarly, mosses possess their own microbiome, although research into bryophyte-microbe interactions has been very limited thus far. Among the potential functional roles of these bacteria are inorganic phosphate mobilization, nitrogen fixation, and nutrient scavenging. Microbial communities on the woolly moss Racomitrium lanuginosum and the "Iceland moss" lichen Cetraria islandica were assessed from community profiles obtained by MiSeq sequencing of V3/V4 16S rDNA amplicon libraries prepared from both cDNA and genomic DNA extracted from samples taken from warmed and cold heathlands, and from glacial moraine chronosequence transects. For both moss and lichen, Proteobacteria dominate the associated communities, but Acidobacteria, Armatimonadetes, Verrucomicrobia, and Planctomycetes are also richly represented in most samples. Of the Proteobacteria, dominant orders include the Rhodospirillales, Rhizobiales, Burkholderiales, Pseudomonadales, Caulobacterales, and Sphingomonadales, although the abundance of these orders vary considerable between moss and lichen and between 'active' (cDNA) and total (genomic) populations. While the microbial communities were on the whole found to be somewhat resilient to a warming climate, co-occurrence analysis revealed complex interactions among the individual taxa, suggesting that climate warming decreases community stability in both the moss and lichen-associated communities, and indicating several key drivers of community composition in the microbiomes of these important members of Arctic tundra and fell field vegetation.

11:30

Invited speaker: **Angela Sessitsch (Austrian Institute of Technology, Austria)**

Ecological drivers and functional aspects of the plant microbiome

Plant microbiome analyses have shown that the plant genotype, tissue, the vegetation stage as well as stress factors influence the structure and possibly functioning of endophyte communities. Metagenome analysis as well as genome, transcriptome and functional analysis of individual strains have provided important functional insight leading to as well as of a rice root community revealed characteristics like various plant growth promoting characteristics, cellulolytic enzymes, quorum sensing, degradation of aromatic compounds, methane oxidation, nitrogen fixation as well as nitrification and denitrification. In addition, isolated endophytes serve as a source of microbes to be applied as bio-fertilizers or biocontrol agents. Some strains tested in detail proved to efficiently colonize many plants such as maize, strongly promote plant growth, reduce drought stress and to induce systemic resistance responses. In addition, the analysis of individual strains and their interaction with plants provide valuable information on the mechanisms responsible for beneficial effects.

12:00

Offered paper: **Daire Carroll (University of Warwick, UK)**

A systems view of microbial establishment on growing rhizoplanes

The rhizosphere is a region of soil holding incredible biological complexity and diversity with microbial activity contributing to processes such as plant pathogenesis and nutrient cycling. Previous studies of soil microbial dynamics have viewed the rhizoplane as static, failing to explain large shifts in microbial composition taking place when roots colonise new regions of soil. Using Lettuce and Pseudomonas fluorescens (Psf), this project aims to identify and quantify factors contributing to early stage root colonisation when dynamic processes, such as root elongation, exudation and bacterial motility, are important. Assays have been designed measuring bacterial motility, chemotaxis and adherence to roots, along with root growth rate. The adherence of Psf to lettuce roots during the early stages of root growth has been quantified. Through confocal imaging of fluorescently transformed Psf on roots, the localisation of bacteria to the different root regions during colonisation has been described. The motility of Psf and the chemotaxis of Psf towards lettuce root exudate has also been measured. This data has been fed into a basic model of bacterial adherence to roots during the early stages of interaction. Going forward; the challenge will be to expand this model and to include other important aspects of microbial establishment on root tips, showing how dynamic plant and microbial traits can be used to explain observed shifts in the rhizosphere community. This model will be valuable for enabling predictions about root colonisation to be made.

12:15

Offered paper: **Jake Newitt (University of East Anglia, UK)**

Investigating the role of nitric oxide in plant root colonisation by *Streptomyces coelicolor*

Streptomyces is a genus of soil dwelling Actinomycetes, that play an important role in plant health, through association with plant roots. They provide an array of benefits to the plant host such as infectious disease prevention and plant growth promotion. We investigate the role of nitric oxide (NO), an ubiquitous signalling molecule used by plants and bacteria alike, in root colonisation by *Streptomyces coelicolor*. Plating studies were conducted for Arabidopsis thaliana and *Triticum aestivum*. Relative colonisation was determined by comparing recovery of marked mutant strains, with a marked wild-type control. The effect of Increased endogenous NO was studied with a knockout mutant of nsrR/hmpA - genes responsible for NO detoxification in *Streptomyces*. We also explored the impact of exogenous NO, through addition of a donor (deta-NONOate) to the systems. We present results to show that *S.coelicolor* ΔnsrR/hmpA is significantly more competent at colonising *T.aestivum* rhizosphere, compared the control (0.031). Despite natural variation in the endosphere, there is a trend to support improved colonisation by the mutant in wheat. Greater sampling is required for analysis. Preliminary data indicates that addition of deta-NONOate to the A.thaliana system leads to a visible increase in growth on the plant root, as well as viable colonies recovered in a plating study. We are excited to present promising new evidence to support an as yet undescribed link between NO and plant root colonisation by *Streptomcyes coelicolor.* Understanding the mechanisms that underpin this process is the first step in eploiting these interactions for agricultural technology.

13:30

Invited speaker: **Richard Bardgett (University of Manchester, UK)**

Harnessing ecological knowledge for sustainable management of grassland soils

Soils are at the heart of many of the major challenges that face our planet, including the provision of food, climate mitigation, water security, and conservation of biodiversity. However, they are also under threat, especially intensive land use and climate change, which in many places are causing extensive soil degradation and limiting soils capacity to deliver ecosystem services. This degradation has led to increasing calls to halt soil degradation, but also to devise sustainable ways of managing soils in order to use resources, such as nutrients and water, more efficiently, and increase the resilience of key soil functions to future environmental change. In this talk I will focus on how recent advances in plant and soil ecological research might contribute to the challenge of sustainable management of grassland soils at local and regional scales. In particular, I will consider recent developments in our understating of how: (a) the functioning of soils is influenced by interactions between plants, their roots, and highly complex soil microbial communities, and (b) how these biotic interactions can promote properties key to soil health, such as carbon sequestration, nutrient retention, stable aggregate formation, and resilience of soil functions to climate extremes. I will also consider some of the hurdles and constraints that need to be overcome to reap the benefits of such ecological knowledge.

14:00

Invited speaker: Spencer Diamond (University of California, USA)

Depth and rainfall shape soil microbiomes that include genomically novel Acidobacteria and Thermoplasmata

Acidobacteria are often the dominant bacterial group in soil and Verrucomicrobia, Gemmatimonadetes, Chloroflexi, Armatimonadetes and Rokubacteria occur at lower abundances. The metabolic functions of the vast majority of these bacteria remain unknown. Here, we genomically and proteomically resolved sixty metagenomic datasets from a grassland soil ecosystem and recovered 790 near-complete microbial genomes from 21 phyla, representing around one third of all organisms detected. This enabled extensive metabolic predictions, including for representatives of at least 10 classes for which there are no previous sequences or isolates. Our results indicated that complex carbon degradation, CO oxidation, methanol oxidation and nitrogen compound transformations were phylogenetically widespread, highly represented in the soil proteome, and were detected within many groups of soil organisms with no previously sequenced representatives. Notably, a novel Euryarchaeote within the Thermoplasmata that is highly abundant in our system is predicted to oxidize ammonia to nitrite. Metabolic analysis of genomes statistically stratified with depth indicated increased soil denitrification capacity and carbohydrate degradation near the soil surface and breakdown of C1 and nitrogen compounds at depth. Additionally, analysis of organisms enriched in soil subjected to extended spring rainfall suggested a further increase in denitrification capacity. Overall, soil depth, spatial gradients and experimentally extended spring rainfall are statistically significant determinants of community composition and metabolic functional distribution.

14:30

Offered paper: Adriano Sofo (Università della Basilicata, Italy)

Long-term sustainable soil management applied in Mediterranean orchards can affect the genetic and functional diversity of soil microbiota

The aim of this work was to evaluate the long-term effect (20 years) of two soil management systems, so called 'sustainable treatment' (ST) and 'conventional treatment' (CT), on the bacterial genetic and functional diversity in soils of different orchards located in Southern Italy. ST system included minimum tillage, and endogenous organic matter inputs from spontaneous cover crops and pruning material. CT soil was tilled, without cover crops, and pruning residues were removed. Microbial analyses were carried out on soil composite samples (0-30 cm depth) by culture-based (plating, Biolog®) and molecular-based approaches (DGGE, 16S DNA and metagenomic analysis, pPCR). In ST soils, significant increases were observed for the following parameters: a) number of some groups of culturable bacteria (actinomycetes, ammonifying, proteolytic and free N-fixing bacteria); b) activities of microbial enzymes (β-glucosidase and protease); c) diversity indexes (Shannon's diversity index, evenness and richness) obtained from Biolog® assay; d) number of bacterial groups involved in C and N dynamics, particularly symbiont N-cycling bacteria and the abundance of their related genes (nifH, amoA). On the basis of the molecular analysis, the composition of the microbiota of ST and CT soils resulted to be significantly different, particularly for the bacteria involved in soil N cycle, lignin degradation and humification. A sustainable orchard management is a key factor for increasing the functionality and diversity of soil microbiota, that in turn enhance soil microbiological fertility. This amelioration leads to a higher soil quality, stability and multifunctionality, positively affecting plant physiological status and crop productivity.

14:45

Offered paper: Neil Warnock (Queen's University Belfast, UK)

Engineering the rhizosphere microbiome for crop parasite control

Plant parasitic nematodes (PPNs) impose a significant burden on global agriculture, equating to an estimated $100 billion in losses each year. The soil-borne infective PPN juvenile locates a host plant root system by following concentration gradients of root secreted chemicals; termed plant root exudates. Here we aimed to develop a novel approach to preventing crop parasite invasion by genetically engineering the soil microbe *Bacillus subtilis*, to secrete a peptide that disrupts nematode perception of root exudates, preventing normal host-finding and invasion. The peptide is assimilated by the PPN sensory neurons, and dysregulates normal sensory signalling process. We have demonstrated that modified *B. subtilis* cultures secrete the peptide, and that inoculation of these cultures onto seedling roots can prevent PPN host-finding and reduces invasion by up to 90% relative to controls, in the laboratory. We have also shown that the activity of this peptide is specific to PPN species, having no negative impact on the behaviour of non-target soil-borne nematodes. This proof of principle study demonstrates that the soil microbiome can be manipulated to improve crop productivity in a targeted and robust manner.

15:30

Invited speaker: Laurent Philippot (INRA, France)

A tale of two stories from the underground: soil microbial diversity and N-cycling

Soil microorganisms form one of the largest biodiversity reservoirs on earth. They also play essential roles in ecosystem functions such as biogeochemical cycling. However, the importance of microbial diversity for ecosystem functioning is still debated. In this talk, I will give examples of how we addressed this knowledge gap using denitrifying microorganisms as model functional guilds. Denitrification is a microbial respiratory process during which soluble nitrogen oxides are used as alternative electron acceptor when oxygen is limiting. Denitrification can results in considerable losses of nitrogen, which is the most limiting nutrient for crop production in agriculture. It is also responsible for emissions of nitrous oxide (N_2O), the dominant ozone depleting substance and an important greenhouse gas with a global warming potential over 100 years of about 298 times that of CO_2. I will show that microbial diversity loss can alter nitrogen cycling and discuss studies showing the importance of microbial community composition for emissions and consumption of the greenhouse gas N_2O.

16:00

Offered paper: Bryony Dignam (AgResearch Ltd, New Zealand)

Harnessing the soil immune response: impacts of long-term plant residue management on soil organic matter quality, *Pseudomonas* community structure and soil disease suppressiveness

Diverse microbial communities harboured by agricultural grassland soils provide ecosystem services essential to soil health and plant productivity, including suppression of soil-borne plant diseases. Understanding soil properties impacting upon these microbial communities will provide opportunities by which soil microbes may be managed to enhance soil suppressiveness and, subsequently, plant yield. A New Zealand long-term grassland field trial was used to investigate the impact of 20 years of plant residue management, as a factor influencing soil chemical and microbiological properties, on the suppression of disease caused by a broad host range pathogen.

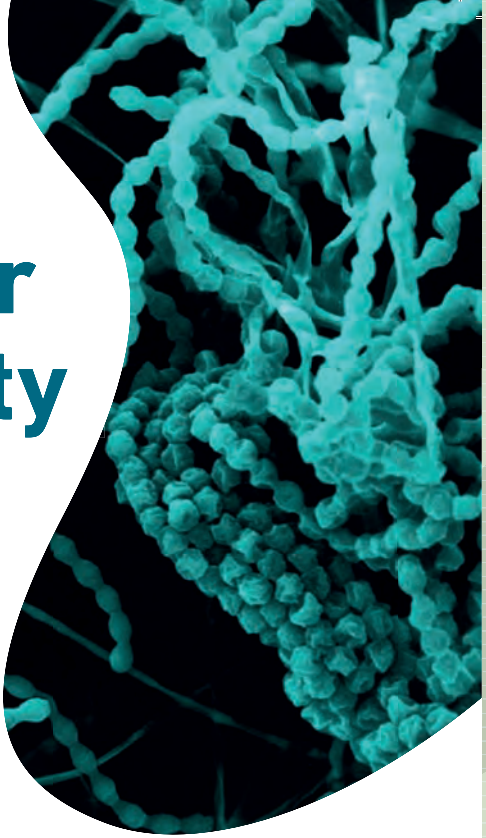
Phylogenetic community analysis using the Illumina MiSeq platform targeted *Pseudomonas* spp., and a plant-pathogen bioassay (*Rhizoctonia solani*-induced damping-off of kale) was utilised to provide a comparative measure of disease suppression. Plant residue management (mowing and plant biomass removal) led to significant variation in *Pseudomonas* bacterial community structure ($P<0.001$) and soil organic matter quality was responsible for 80% of the observed variation ($P=0.01$). *Pseudomonas* species diversity (Shannon's index) and richness were the primary parameters explaining the greatest proportion (>30%) of variation in the suppressive capacity of soils across treatments. Further, increased *Pseudomonas* species diversity, microbial activity, soil organic matter content, and carbon availability distinguished suppressive soils from high disease soils. Our results suggest that management-induced shifts in *Pseudomonas* community composition provide a better indicator of soil susceptibility to disease than soil chemical parameters. Furthermore, we demonstrate that management practices that result in the frequent addition of organic residues to grassland soils enhance the diversity and activity of plant-beneficial bacterial taxa.

16:15

Offered paper: **Aaron Fox (Agroscope, Switzerland)**

Agricultural management and agro-climatic region are important determinants of bacterial community composition in European permanent grasslands

The BIOINVENT project aims to further our understanding of how the composition of the grassland microbiome is influenced along gradients of both management and agro-climatic regions in Europe. For a pan-European survey, a North-South transect was established with 4 participating countries: comprising Sweden, Germany, Switzerland, and Portugal (Azores). Each country was divided into a ‘favorable’ agricultural region, where productivity is optimal and a ‘less favorable’ region where productivity may be constrained by certain environmental/climatic conditions. In the summer of 2017, soil samples from 3 permanent grasslands management types; intensive, low intensive and extensive, were taken from all regions (288 sites in total). The bacterial 16S rRNA gene was PCR amplified from these samples and an amplicon-based Illumina Miseq sequence analysis was conducted. Agro-climatic region (country plus favourability region) had a highly significant influence on bacterial community composition ($P < 0.001$). Furthermore, the bacterial community significantly differentiated according to favourability region in each country with the exception of Portugal (Azores). Management type also displayed a significant effect, with the intensive management harbouring a significantly distinct bacterial community when compared to both the low intensive and extensive managements ($P < 0.05$). Agro-climatic region was, however, the stronger driver of bacterial community composition compared to management along the transect. Further work will examine fungal community composition in the same samples. The knowledge gained on how both agro-climatic region and management influence the grassland microbiome will be used to develop strategies for monitoring soil microbial biodiversity trends in these systems.



DAY 2

Tuesday 2 October

DAY 2 SPEAKER ABSTRACTS

08:30

Invited speaker: Sharon Huws (Queen's University Belfast, UK)

Advancing our understanding of the rumen microbiome to create the utopian ruminant

The rumen microbiome is complex, containing bacteria, protozoa, fungi, methanogens and viruses, which interact both symbiotically and competitively. These complex, multi-kingdom interactions, largely control the ruminant phenotype. Ruminants are adept at converting high fibre forages to human-edible protein, due to the digestive capacity of the rumen microbiome. Nonetheless, feed nitrogen use efficiency is only approx. 30%, with the remaining approx. 70% of ingested nitrogen being excreted to the environment. The function of the rumen methanogens also results in livestock producing approx. 37% of the anthropogenic methane released into the environment. Red meat and milk are also often defined as 'unhealthy' due to their high levels of saturated fatty acids (SFA), which is also a consequence of the rumen microbiome. Ruminant diets are typically high in human health beneficial polyunsaturated fatty acids (PUFA), but as these are toxic to many rumen microbes, they biochemically remove the double bonds, a process known as biohydrogenation, resulting in ruminant products which are low in PUFA and high in SFA. The complex ecology of the rumen microbiome does, however, pose major challenges for improving their function in order to produce the utopian animal. This paper discusses how the recent explosion in meta-omics have advanced our understanding of the rumen microbiome in order to improve animal phenotype.

09:00

Offered paper: Stephen Moore (University of Missouri, USA)

Long-term effects of the endometrial microbiota on the endometrial transcriptome in postpartum dairy cows

The endometrial microbiota plays a major role the development of metritis, an inflammatory uterine disease that develops in 20-25% of cows and reduces pregnancy rates by 50%.We hypothesized that the microbiota can affect endometrial function via the transcriptome. Endometrial biopsies were collected from 15 Holstein dairy cows at 1, 5, and 9 weeks postpartum and processed for 16S rRNA gene sequencing and RNA sequencing. Data were analysed by multivariate (microbiota; PAST) and univariate (transcriptome; limma, R) modelling.

Endometrial differentially expressed genes (DEG) were identified by fitting ovarian cyclicity status (cycling, not cycling), week (1, 5, 9), microbiota principal components (PC1, PC2), and their interactions as fixed effects with cow as a random effect. Endometrium DEG (BH $P \leq 0.05$) were submitted to Ingenuity Pathway Analysis. Permanova analysis revealed the microbiota at week 1 was significantly different from week 5 ($P=0.0001$) and week 9 ($P=0.0001$) and that the week 5 and 9 microbiota were similar ($P=0.45$). Fusobacteriales bone C3G7, *Porphyromonas*, and *Actinobacillus seminis* had the strongest loadings on week 1 but were not detected thereafter.

There were 516 and 151 DEG at week 5 and 80 and 64 DEG at week 9 in response to the week 1 microbiota PC1 and PC2. Inflammation within the week 9 endometrium was associated with PC2 of the week 1 microbiota and potentially mediated by lipopolysaccharide, interleukins, and toll-like receptor. Bacteria affecting gene expression are gradually cleared from the uterus after calving but are associated with longer-term transcription of genes involved in an inflammatory response.

09:15

Offered paper: Gillian E. Gardiner (Waterford Institute of Technology, Ireland)

Faecal microbiota transplantation in gestating sows and neonatal offspring alters lifetime intestinal microbiota and growth in offspring

Previous studies suggest a link between intestinal microbiota and porcine feed efficiency (FE). Therefore, we investigated whether faecal microbiota transplantation (FMT) in sows and/or neonatal offspring, using inocula derived from highly feed efficient pigs, could improve offspring FE. Pregnant sows were assigned to control or FMT treatments and the subsequent offspring to control treatment, FMT once (at birth), or FMT four times (between birth and weaning). FMT altered sow faecal and colostrum microbiota compositions and resulted in lighter offspring body weight at 70 and 155 days of age when administered to sows and/or offspring. This was accompanied by FMT-associated changes within the offspring intestinal microbiota, mostly in the ileum. These included transiently higher faecal bacterial diversity and load and numerous compositional differences at the phylum and genus levels (e.g., Spirochaetes and Bacteroidetes at high relative abundances and mostly members of Clostridia, respectively), as well as differences in the abundances of predicted bacterial pathways. In addition, intestinal morphology was negatively impacted, duodenal gene expression altered, and serum protein and cholesterol concentrations reduced due to FMT in sows and/or offspring. Taken together, the results suggest poorer absorptive capacity and intestinal health, most likely explaining the reduced body weight. An additive effect of FMT in sows and offspring also occurred for some parameters. Although these findings have negative implications for the practical use of the FMT regime used here for improving FE in pigs, they nonetheless demonstrate the enormous impact of early-life intestinal microbiota on the host phenotype.

10:45

Invited speaker: **Jana Seifert (University of Hohenheim, Germany)**

Influence of phosphorus availability on the composition and function of the microbiome in fowl and pigs

The microbiome of the animal's gastrointestinal tract plays an essential role for the growth and health of the animal. It is well accepted that the structure and function of the microbiome is influenced by external parameters like composition of the feed, addition of pro-, pre- and antibiotics, environmental factors as well as the by animal itself. Gathering knowledge about the activity and function of the gut microbiome under different feeding conditions can be important to develop highly adapted animal feed and stimulate the microorganisms in order to increase the efficiency in livestock production. This is of particular interest in respect of phosphorous which is a limited resource but essential for each living organism. The sustainable use and saving of phosphorous in animal feeding can only be achieved with efficient utilization of the organic phosphorous resources. This is achievable by the use of supplemented phytases and the intrinsic microbial enzymatic activity. In our studies, the influence of phosphorous on the microbiome in fowls and pig were analyzed to gather more information about this topic of interest.

11:15

Offered paper: Paul Smith (University College Dublin, Ireland)

Effect of grazing perennial rye grass swards with and without the inclusion of white clover on the rumen microbiome of dairy cattle

Methane (CH4) production arising from enteric fermentation negatively impacts the environment and the efficiency of livestock production. Reducing the amount of CH4 derived from ruminant livestock is a challenge for agriculture globally. White clover (WC) inclusion in the grazing sward has been shown to reduce methane yield (MY). To understand the mechanisms involved, the rumen microbial populations of dairy cattle grazing perennial rye grass swards with (WCPRG) and without (PRG) the inclusion of WC was investigated using an amplicon sequencing approach.

The diets were shown to have no significant impact on milk yield or milk solids however WCPRG cattle had a higher milk fat% ($P<0.05$). Numeric, but non-significant, differences were found between groups in daily grams of CH4 however WCPRG animals were shown to have a significant reduction in MY compared to those grazing PRG ($P<0.02$).

16S rRNA amplicon sequencing of the V4 region on the illumina Miseq targeting bacteria and archaeal populations, found no significant difference in the diversity of the rumen microbial populations between the two groups. However, Deseq2 analysis identified differences in the bacterial populations between the diets including an increased abundance of *Pseudobutyrivibrio* in the PRG group and higher abundance of *Saccharofermentans* and *Lachnospira* in the WCPRG group. Also, numerically the WCPRG group had a lower abundance of the methanogen *Methanobrevibacter*.

While no significant difference in microbial diversity was found between both diets, the observed changes in specific bacteria and a numerically lower abundance of methanogens could help explain the divergence in MY between both groups.

11:30

Offered paper: Catherine Burgess (Teagasc, Ireland)

Studying the relationship between the composition of the microbiota at the bovine rectoanal junction and the shedding dynamics of Shiga toxin-producing *E. coli* in faeces

It is known that some STEC (*E. coli* O157) can potentially colonise the bovine rectoanal junction. The number of *E. coli* O157 shed in cattle faeces varies widely, with some cattle termed 'super-shedders'. A previous study undertaken in Ireland showed bovine shedding rates for STEC E. coli O157 (4.18 %, 55/1317; 2.13% (28/55) super-shedding) and STEC O26 (0.76%, 10/1317; 0.23% (3/10) super-shedding), indicating that almost half of the positive animals had super shedding status (4 -7.7 log10 CFU/swab). The aim of this study was to analyse the relationship between the microbial community composition at the rectoanal STEC colonisation site and shedding dynamics of STEC by bovine animals. Based on the STEC results and metadata, swabs were selected from super shedders (SS, n=30), low shedders (LS, n=32) and STEC negative animals (neg, n=54) and subjected to a 16SrRNA gene-based compositional metagenomics approach using the Illumina MiSeq. The principle phyla across the three animals groups were *Bacteroides* (~40%) and *Firmicutes* (~50%) and the principle genera were *Ruminococcoae* (30%), *Prevotella* (10%) and 60% a variety of genera. Overall, the results indicated a high level of variability in OTUs between the animal groups. Principle co-ordinate analysis (PcoA) showed no significant difference in microbiota composition at the RAJ, based on shedding status, with intra-group variability potentially masking inter-group variability. Additionally, PCoA indicated no significant differences in microbiota composition between animals of different ages, suggesting a stable microbial community in the adult beef cattle studied. Further research including younger animals would be of interest.

13:30

Invited speaker: Orla O'Sullivan (Teagasc, Ireland)

Exploiting sequencing technologies for agriculture

Microbes occupy every ecological niche on earth and until recently we only had knowledge of those microbes we could culture in the lab. The advent of next generation sequencing technologies, which circumvents the need for culturing, has allowed us to further catalogue and understand these microbes and begin to exploit this knowledge for use in agriculture. Here we present what sequencing technologies are available and how they can be used to benefit and complement agricultural research.

14:00

Invited speaker: **Chris Quince (University of Warwick, UK)**

Resolving de novo strain variation from metagenomes for high resolution analysis of microbiomes

The importance of strain level variation in microbial communities is well established. Short read metagenomics has the resolution to resolve strains but most current approaches require reference genomes. Methods for de novo resolving strains are still an area of active development. Strains cannot be simply assembled from metagenomes due to the complexity of the assembly graph when multiple closely related genomes are present. This results in fragmented assemblies of potentially millions of contigs. It is possible to cluster or bin contigs into metagenome assembled genomes (MAGs) using composition and co-occurrence across multiple samples. I will introduce our binning algorithm CONCOCT and illustrate its application through a of study of antimicrobial resistance in the Thames catchment. However, the MAGs that result from metagenome binning are still composites of multiple strains. I will also demonstrate how co-occurrence across samples can also be used to resolve sub populations within MAGs, using our DESMAN (De novo extraction of strains from metagenomes) pipeline. This leads to a higher resolution picture of changes in microbial communities.

14:30

Invited speaker: Leighton Pritchard (James Hutton Institute, UK)

Pathogen communities in agriculture: devil's in the details

Plants exist in intimate association with microbial communities, the structure and composition of which determines their health and productivity. High-level determination of microbial community structure is central to understanding the complex networks of interactions that support a healthy environment, and plant development. In addition to this, precise identification of potentially pathogenic community members is of great importance to trade, disease control and epidemiology, and plant and human health. This presentation will discuss lessons learned relevant to plant microbiome studies from three project areas: (i) identification, characterisation and diagnostics of soft-rot enterobacteria in Scotland; (ii) identification and diagnostics of *Phytophthora spp*. in UK plant nurseries; (iii) comparative genomics of environmentally-persistent *Escherichia coli* populations in Ireland.

15:00

Invited speaker: Chris Creevey (Queen's University Belfast, UK)

Identifying genomic signatures of niche specialisation in the rumen microbiome

Many microbes in complex competitive environments share genes for acquiring and utilising nutrients, questioning whether niche specialisation exists and if so, how it is maintained.

This is especially relevant to the rumen microbiome, a highly competitive, anaerobic environment, with limited nutrient availability determined by the biomass consumed by the host.

We have addressed this question over a series of recent studies that combine 16S, metatranscriptomics, Nanopore long-read sequencing and novel tool development.

This has allowed us to 1) Ascertain the niches of microbes involved in temporal successional change on freshly consumed grass in the rumen of cattle. 2) Identify the population-level genomic signatures that underpin their competitiveness in these niches. 3) Develop novel tools that allow reconstruction of the gene haplotypes involved and 4) Provide ¬in vitro verification of the results.

The methods and results provide a novel insights into the critical early activity of the rumen microbiome as biomass is degraded and represents a novel approach for untangling complex interactions between microorganisms in natural environments.

EXHIBITORS

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NOTES

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