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Life in earth – the root microbiome to the rescue?

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Manipulation of the soil microbiome holds great promise for contributing to more environmentally benign agriculture, with soil microbes such as *Pseudomonas* promoting plant growth and effectively suppressing pathogenic microorganisms. Next-generation sequencing has enabled a new generation of research into soil microbiomes, presenting the opportunity to better understand and exploit these valuable resources. Soil bacterial communities are both highly complex and variable, and contain vast interspecies and intraspecies diversity, both of which respond to environmental variation. Therefore, we propose that a combination of whole microbiome analyses with in-depth examination of key microbial taxa will likely prove the most effective approach to understanding rhizosphere microbial interactions. This review highlights recent efforts in this direction, based around the important biocontrol bacterium *Pseudomonas fluorescens*.

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Introduction

The Green Revolution boosted global agricultural production in the 20th century through innovations centred on the development of high-yielding dwarf crop varieties that respond well to chemical fertilizers and other agrochemicals. It is estimated that this process saved between 18–27 million hectares of land from being converted to agriculture [1] and that the associated yield gains prevented over one billion people from starving. However, the continued heavy use of agrochemicals is costly, ecologically damaging, and unsustainable in the medium to long term. The use of precision agriculture, involving the better use of external inputs alongside genetically modified crops with more efficient nutrient-use characteristics is likely to be hugely important in achieving future productivity gains [2]. Additionally, the soil microbiome holds great promise for contributing to more environmentally benign agriculture. Naturally occurring soil-dwelling microbes influence plant health, resource-use efficiency and biocontrol [3,4]. However, their potential has been under-exploited to date. Recent advances in nucleic acid sequencing technologies have enabled a new generation of research into soil microbial communities, and offer the opportunity to better understand, and hence exploit, this resource.

Advances in soil microbiome analysis

Soil microbiomes are intricate, highly diverse ecosystems containing thousands of interacting microorganisms—a recent analysis of the microbiome of disease-suppressive soils identified over 33 000 bacterial and archaeal OTUs in the sugar beet rhizosphere [5•]. Recently, the ability to rapidly sequence and identify DNA extracted from soil samples has enabled the development of several powerful metagenomic analysis techniques [6]. For example, interrogation of the genetics of whole microbial communities allows us to probe the physiological characteristics and potential of plant-associated microorganisms [7,8]. Amplicon sequence analysis of marker genes, typically 16S rRNA in the case of bacteria, enable us to characterize the relative abundance of different species in phyllosphere and rhizosphere communities [9], while metatranscriptomic approaches may be used to examine the metabolic activities and regulatory mechanisms that function in different environments [10–12].

While much has been learned about the relative abundance of different microbial phyla and genera, and the functional and metabolic characteristics of the plant and soil-associated microbiome [13,14], it is also imperative to understand the metabolic, natural product and genomic diversity associated with individual species in the soil system to obtain a better understanding of microbial function [15•,16–18,19•]. For example, we now know that the metabolic behaviour of the nitrogen fixing species *Rhizobium* varies profoundly between the rhizospheres of different plant species [20]. Furthermore, environmental variation profoundly influences the relative abundance of individual genes in the population of a single species group [21•,22]. In the near future, newly developed methods for microbial isolation and culturing will markedly increase our capacity to understand both the overall microbiome, and the individual species within it [23•,24]. Total microbiome approaches by definition are more superficial in their analyses, while complete assignation of functional genes to particular microbial OTUs in the soil is challenging, although the

reconstruction of a draft genome from a novel soil methanogen indicates that this may become more commonplace in the future [25]. Nonetheless, in reality the reconstruction of discrete microbial genomes will always be problematic. Bacterial genomes are composed of multiple, often plastic genetic elements, leading to problems in assembling genome complements. This is especially the case in complex communities where species complexes are commonplace. Therefore, advances in sequence analysis will most likely give rise to the creation of 'species metagenomes'. The production of broader culturable metagenomes [26], coupled with an increased ability to sequence individual microbial isolates will be useful for verifying genome reconstruction from metagenomes, and also for use in manipulative experimentation. We propose that a combination of total community studies, with more in-depth analysis of key culturable microbial taxa will further our understanding of rhizosphere microbial interactions more effectively than either approach taken in isolation.

Biocontrol pseudomonads in the soil microbiome

As the harmful environmental impacts of chemical pesticides become more apparent, manipulation of the soil and plant-associated microbiota is gaining increasing recognition as a potential alternative treatment for a range of crop diseases and pests. This may occur on a whole-microbiome level, for example through the development of suppressive soils or the control of potato scab by irrigation, or alternatively through the stimulation/introduction of key biocontrol microorganisms, such as *Bacillus* or *Pseudomonas* spp. Many important fungal and bacterial diseases including fire blight (*Erwinia amylovora*, [27]), potato scab (*Streptomyces scabies*, [28]) and take-all (*Gaeumannomyces graminis* var. *tritici*, [29]) are effectively suppressed by members of the *Pseudomonas fluorescens* species group. These important, widespread soil-dwelling microbes have an established role in the development of take-all suppressive soils [29–33], where the fungal pathogen is maintained at a low level in the soil but is unable to cause disease. Take-all is a destructive fungal crop disease that causes substantial losses in cereal crops [34,35], and is therefore an attractive target for the development of *Pseudomonas* biocontrol agents. However, to date efforts in this direction have been plagued by inconsistency [36], in large part due to the huge complexity of the plant/pathogen/soil ecosystem.

Pseudomonas fluorescens

P. fluorescens are a diverse clade of Gram negative, γ-proteobacteria that non-specifically colonise a number of different plant species. They represent a major constituent of the rhizosphere microbiome, and exploit root exudates as source of nutrients and energy. *P. fluorescens* spp. are flexible, generalist bacteria that are able to colonise many different environmental niches and carbon sources. Their genomes are correspondingly complex, encoding around 6000 genes, and with a high degree of intraspecies diversity—the *Pseudomonas* core genome represents as little as 20% of an individual bacterial genome [19•], with much of the accessory genome given over to signal transduction, phenotypic output loci and secondary metabolism [15•,19•]. The high degree of genomic and metabolic plasticity among the soil pseudomonads allows both individual bacteria, and the microbial population as a whole, to effectively adapt to different plant–soil–microbiome environments.

Pseudomonas plant colonisation is a complex, tightly controlled process that begins with chemotaxis into the rhizosphere along a gradient of root exudates, followed by surface association and migration on the rhizoplane [37], and ultimately the formation of a bacterial biofilm [38•]. The early stages of colonisation are facilitated by flagella and type IV pili, and the production of biosurfactants, which together enable coordinated swarming motility [37,39]. The later stages are characterised by the formation of micro-colonies on the plant surface, then establishment of a mature biofilm. In addition to bacterial cells this protective matrix is composed of proteinaceous adhesins [40], lipopolysaccharide [41] and various exopolysaccharide molecules [38•,42]. To successfully colonise the plant rhizosphere, many *Pseudomonas* spp. produce enzymes that enable them to manipulate plants, encouraging growth and disrupting stress responses. For example, enzymes that synthesise and catabolise auxins [15•] and plant growth-promoting volatiles such as 2-3-butanediol and acetoin [43] have been identified in several *Pseudomonas* genomes [15•,19•]. In addition, many *Pseudomonas* spp. produce ACC deaminase, which protects plants from environmental stresses by short-circuiting ethylene production [44].

P. fluorescens in the rhizosphere is under continuous attack from other members of the soil microbiome. This takes the form of competition and antagonism from other microorganisms, as well as predation by nematodes and insects. To counter this second threat, and to prevent insect predation of their host plants, many *Pseudomonas* spp. produce insecticidal molecules such as the Mcf, IPD072Aa and Fit toxins [15•,45,46]. Meanwhile, to fight against hostile bacteria, oomycetes and fungi, soil *Pseudomonas* spp. secrete bacteriocins [47,48], alongside toxins and other natural products using specialised protein secretion pathways. Type III and Type VI complexes inject toxins and effector proteins into eukaryotic and bacterial cells, and contribute to various cytotoxicity and virulence-associated phenotypes [49]. Type II secretion systems are diverse protein exporters, and facilitate the secretion of bacteriocins, surface adhesins and extracellular enzymes [40]. *Pseudomonas* secrete a number of these exoenzymes including plant tissue-degrading lyases, proteinases and chitinases that contribute to

biocontrol by hydrolysing fungal cell walls [50,51]. As well as affecting plant behaviour, some *Pseudomonas* spp. also disrupt signal transduction by other rhizosphere microorganisms, for example by producing AHL lactonase to suppress quorum sensing [52].

Pseudomonas spp. also produce a diverse array of secreted natural products. These have varied functions, although many serve to kill or suppress plant predators and competing microorganisms [15•,53]. Even those molecules with a well-defined alternative function often function as antimicrobials. These include the metal ion-chelating siderophores, which also inhibit pathogenic fungi by inducing metal ion starvation in model rhizospheres [54]. Phenazines; flavin coenzyme analogues that function as electron shuttles in microoxic environments [55] also inhibit electron transport in plant pathogens [56,57], and are linked to ecological fitness in take-all infected wheat rhizospheres [58]. Likewise, viscosin and other cyclic lipopeptides act both as surfactants to enable swarming motility [37], and antibiotics that solubilise cell membranes [59]. Soil *Pseudomonas* spp. also produce a host of dedicated antimicrobials, such as the antifungal compounds pyoluteorin and pyrrolnitrin [60,61], phloroglucinols like 2-4-DAPG [62], and hydrogen cyanide [63]. A recently conducted metabolic profiling analysis based on soil isolates from Rothamsted Research (Harpenden, U.K.) demonstrated a remarkable level of natural product diversity within the rhizosphere *Pseudomonas* population, with isolates from a single wheat field producing a comparable natural product complement to an extensive library of global isolates from diverse environmental sources [64•].

Depending on the exact conditions in their environment, *P. fluorescens* populations select from the huge potential within the accessory genome to produce an optimal genetic and metabolic response. Clearly, if we can define the genetic loci and phenotypic characteristics that contribute to rhizosphere colonisation and biocontrol, and determine how these change with different plant/soil environments, we will be much better placed to exploit the soil *Pseudomonas* population to develop better crop management strategies and novel biocontrol agents.

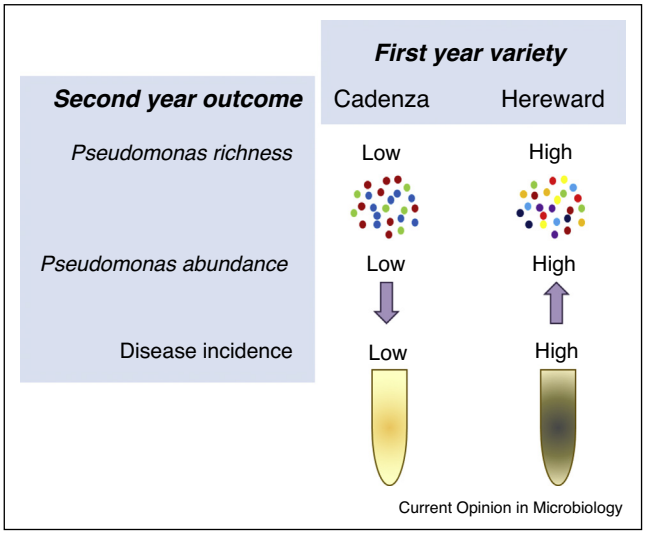
Analyzing genomic diversity in plant associated *Pseudomonas* populations

A recent two-year experiment at Rothamsted [65] presented us with an opportunity to examine the relationship between the *Pseudomonas* genome and the environment, in the context of infection with take-all. This experiment compared high (Hereward) and low (Cadenza) take-all inoculum building (TAB) wheat varieties, and the impact on crop yield in the second wheat [65]. We isolated hundreds of *Pseudomonas* CFUs from the rhizospheres of second year wheat plants, and subjected them to extensive phenotypic, genotypic and genomic analysis, including whole genome sequencing of 19 isolates.

A phylogenetic tree of all *Pseudomonas* isolates based on ERIC PCR profiles and housekeeping gene sequences showed that the wheat variety grown in year one exerted considerable selective pressure on both the extent and nature of *Pseudomonas* genomic diversity. Hereward plots showed increased take-all build-up and *Pseudomonas* genomic richness, alongside yield losses of ~3 t/ha (Figure 1) [66•]. However, while distinct clusters of genotypes were observed when year one wheat variety was considered, no pattern was observed with cultivars from year two. These findings agree with a 16S rRNA gene amplicon sequence analysis of the rhizosphere soil in each plot, which showed that year one Hereward plots contained significantly larger *Pseudomonas* populations, alongside several different genera of saprophytes [21•].

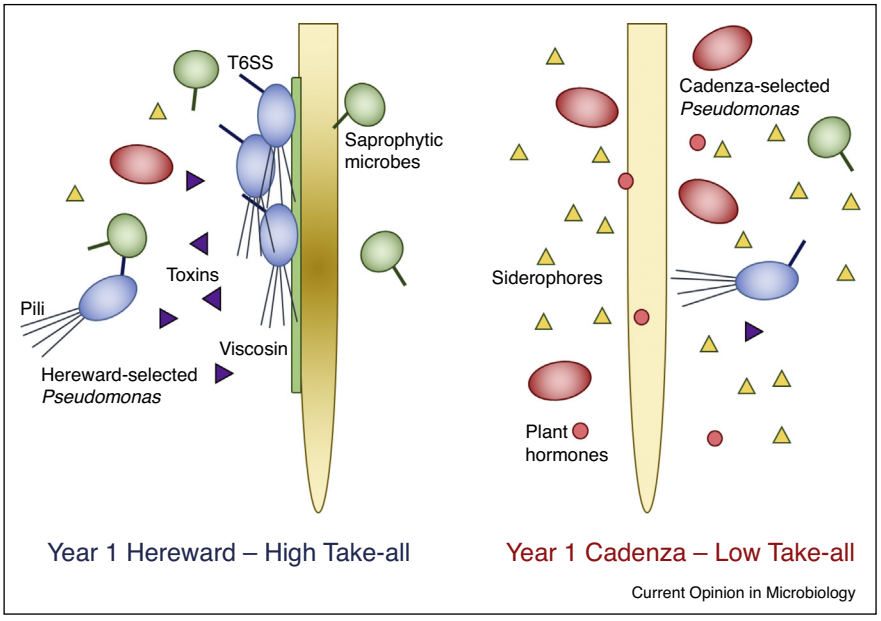
We then took a statistical approach to combine our various datasets, conducting correlation coefficient analyses to identify the phenotypes and genes that were selected by different cultivar combinations over the course of the field trial. This analysis identified several interesting correlations between phenotypes, genotypes, and the wheat varieties from which strains were isolated [21•]. At least two distinct, mutually exclusive phenotypic/genotypic groups emerged from our analysis. The first of these showed increased levels of antimicrobial activity towards *Streptomyces* spp., and contained operons for cyclic-lipopeptide and LPS biosynthesis, type VI secretion and toxin production. The second group produced

Figure 1



Soil *Pseudomonas* genotypic richness is associated with more severe disease incidence in wheat roots.
Differences in *Pseudomonas fluorescens* genotypic richness and take-all disease incidence after year two, in response to cultivar planted in year one (figure adapted from Mehrabi *et al*. [66•]).

Figure 2



A model for year 1 wheat cultivar selection of soil *Pseudomonas* genotypes.
High take-all levels in the soil of first year Hereward plots lead to increased plant disease and root senescence. This in turn leads to increased populations of saprophytic microorganisms (green), and an associated shift in the *Pseudomonas* population towards a more aggressive, 'territorial' morphotype (blue). Conversely, where take all levels are low, the *Pseudomonas* population shifts towards phenotypes including metal ion scavenging and plant hormone production (red).

high levels of fluorescent siderophores, a phenotype that strongly correlated with acetoin catabolism loci [21•].

Excitingly, we also saw correlations between individual *Pseudomonas* genes and the wheat varieties grown in the first year. The operons associated with year one Hereward cultivation (high TAB) also positively correlated with *Streptomyces* suppression, while loci that positively correlated with year one Cadenza (low TAB) strongly associated with increased pyoverdin production [21•]. In addition, *Pseudomonas* isolates from this field experiment were used to construct synthetic community fungal antagonism assays [1]. Increased *Pseudomonas* spp. richness positively correlated with *in vitro* pathogen growth. This supported the field observation that first year Hereward plots, with a higher rhizosphere genotypic richness than first year Cadenza, developed more severe take-all disease, demonstrating a negative biodiversity effect (Figure 1). We propose that the increased levels of senescent root tissue and saprophytic microorganisms that accompany Hereward growth in year one may lead to an increased abundance of pseudomonads that are adapted to niche competition with other microbes, whereas the comparatively benign environment associated with Cadenza rhizospheres favours *Pseudomonas* genotypes that are better adapted to plant–host communication and increased production of metal scavenging siderophores (Figure 2).

Clearly, it remains to be established whether the model we propose for the interplay between wheat, take-all and *Pseudomonas* is correct, or whether there is a different reason for the population shifts we see. Nonetheless the impact of the first year wheat cultivar was still detectable two years after the beginning of the experiment, consistent with substantial selective pressure on the first-year rhizosphere population. A second long-term wheat experiment that will capture the full disease epidemic is underway, as are several laboratory experiments including root exudate metabolomic analysis, to strengthen and refine our initial conclusions. Our experiments indicate that first year wheat genotype affects both the overall *Pseudomonas* population, and also the distribution of individual genotypes in the second year rhizosphere [21•,65,66•]. In turn, these experiments support our contention that a better understanding of the soil microbiota, combined with smart manipulation of plant cropping systems may present a reliable future route to sustainable yield improvement and biocontrol.

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