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Deep influence of soil microbes

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Soil microorganisms have long been known to aid plants through nitrogen fixation and water and nutrient exchange. Now researchers are unearthing new ways in which this subterranean biome affects plant performance.

A single gram of soil is said to contain more than 8 billion bacterial cells, representing up to 50,000 different species[1]. These microorganisms play important roles, from tweaking how plants interact with their surroundings to influencing the chemical composition of the environment[2]. Now, the increased affordability of DNA sequencing technologies has enabled scientists to gain a deeper understanding of the diversity and function of the soil microbiome.

So how important are these tiny organisms? Their role in plant nutrition is well established. As noted by John Howieson, director of the Research Institute of Crops and Plant Sciences at Murdoch University in Perth, Western Australia, "soil bacteria are…the engine room for delivering nutrients to plants". Legumes, for example, rely on rhizobial bacteria that dwell in their roots and convert nitrogen from the environment into chemical forms that the plants can use. Ken Giller, professor of plant production systems at Wageningen University, the Netherlands, is leading a project that explores the potential of these nitrogen-fixing microbes to boost food production in sub-Saharan Africa. N2Africa (www.N2Africa.org) has developed rhizobial inoculates that improve the productivity of legume crops such as soybean (*Glycine max*), chickpea (*Cicer arietinum*), the common bean (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*). "N2Africa's large scale dissemination campaigns have demonstrated that rhizobial inoculants together with a small amount of P fertilizer can almost double soybean yields from 0.95 t ha^{-1} to 1.75 t ha^{-1} on average in northern Nigeria," says Giller. "In some cases inoculation alone can give more than 1 t ha^{-1} of extra yield and the costs are only US$5 per hectare. Farmers in Ghana and Nigeria talk of rhizobial inoculants as 'the magic black powder'," he adds. Giller estimates that the project could potentially help over 600,000 farmers in poor regions of 11 countries in sub-Saharan Africa.

But soil microbes modify plants in many other ways beyond their role in nutrition. Now, researchers are looking at how soil microbes can help plants fight drought and influence other, more subtle, properties.

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Soils in the Gaborone area of Botswana are nitrogen-poor and prone to drought.

Fighting drought with microbes

For the past five years Flora Pule-Meulenberg, at the Botswana College of Agriculture, has been digging through Botswana's dry, nutrient-poor soils to find 'elite' nitrogen-fixing rhizobial strains that are adapted to low-moisture conditions and high soil temperatures. After isolating bacteria from hundreds of root nodules, she discovered that the majority were not typical rhizobia, but other types of growth-promoting bacteria. Now, she is working with Ann Hirsch and Maskit Maymon at the University of California, Los Angeles (UCLA) to see whether these bacteria can help plants to grow under severe water and salt stress. In so doing, the team hopes to find clues to help both African and Californian crops deal with extreme drought.

So far they have identified about 150 species of plant-growth-promoting bacteria in Botswanan soils that may provide valuable drought-resistant traits to plants. They are now inoculating agriculturally important plants (such as sorghum, millet, cowpea and Bambara groundnut) with cocktails of these bacterial strains under different moisture conditions to assess their effects in the field. "We are growing plants on California soil inoculated with different combinations of these bacteria, and hope to use some to boost production in local legume species, particularly alfalfa, that are struggling with the ongoing drought" says Hirsch. Furthermore, the UCLA team has been working with environmental DNA — short fragments of DNA, each representing a unique microbal species — to identify members of the underground soil community that cannot be cultured.

Meanwhile in Western Australia, John Howieson at Murdoch University is more worried about when rain falls, rather than how much is falling. Some Australian soils can be very dry and inhospitable, with soil temperatures in the summer reaching well over 50 °C, and rain absent for over 150 days of the year. In fact, annual rainfall has decreased by 20% in the past 50 years in the main cropping areas of Western Australia[3]. But in the past decades, rains have not only become scarce, they have also shifted in season. "We are seeing a trend towards unreliable spring rains, but more frequent early summer rain in November and December" says Howieson. And this is a big problem for some plants, such as those used for forage. These plants need water by mid-October for their spring flush; any rain after that is too late, says Howieson. As a result, livestock in Western Australia are failing to gain weight.

But Howieson, along with Julie Ardley and colleagues at Murdoch University, plan to change this. They have spent more than a decade searching for legume crops, and associated rhizobial bacteria, that can withstand the harsh soil and climate of Western Australia. "What we wanted was a perennial plant that could live eight months without rain, would grow deep roots, be tolerant of acidic and low nutrient soils, and produce many harvestable seeds, all while being delicious and nutritious for animals," says Howieson. "If such a plant could be identified and incorporated into Australian farming systems it would be a great deal," adds Ardley. "A drought-tolerant perennial pasture legume would also help to prevent soil erosion and salinity, and provide green feed and shelter for sheep and other animals in the hottest months."

Their search, spanning nearly 15 years, has taken them to many places around the world, from North America to the Middle East, and finally to South Africa, where they found soils remarkably similar to those in Western Australia: dry, infertile and highly acidic. In South Africa, the team found legumes that thrived under these conditions and, unlike the endemic legumes in Western Australia, were palatable to animals. "A lot of them weren't suitable — for example, the rhizobia didn't survive in Western Australian soils and therefore the plants died too," says Howieson. After testing several candidate plants under both greenhouse and field conditions, Howieson and his team focused their attention on one of these legumes, *Lebeckia ambigua*, a pasture plant found in the fynbos biome of the Western Cape of South Africa.

Thanks to the successful establishment of rhizobial bacteria, *Lebeckia* has now been successfully grown across various localities in Western Australia where farmers have allowed researchers to use their land as experimental plots. The plant grows deep roots that can reach underground water reservoirs, and is palatable for farm animals, so it holds great potential to serve as grazing pasture. "Even in this year's extreme winter and spring, the second driest since the 1900s, *Lebeckia* plants have survived where all other crops and pastures died," says Ardley. The next step is to see whether a *Lebeckia*-exclusive diet can nourish sheep all year round.

Wines with a microbial spirit

As well as growth and drought resistance, soil microorganisms may influence other aspects of their plant hosts. One example can be found in the wine industry, where at least some researchers believe that the mix of microorganisms associated with a grapevine can influence the basic characteristics of the resulting wine — and they are trying to prove it.

Microbes are found all over vines: in the soil as well as in every part of the plant, including the grapes. Recent studies have revealed, with unprecedented detail, the diversity of these microorganisms. Clear differences in the microbial makeup of grapes coming from different vineyards and wine-producing regions are emerging. In a Californian-based study, for example, microbiologist David A. Mills at the University of California, Davis (UC Davis) and his team found that Chardonnay musts from the Napa Valley have a different bacterial and fungal profile compared with musts from the Central Valley[4]. "Since we know many of these microbes can influence wine flavour we speculate that this is an element of regional character in wines" notes Mills; "an element of *terroir*."

Grapes growing in Oakville, California, showing a bloom containing spores of wild yeasts and other microorganisms.

Terroir is an important but ill-defined concept in viticulture, linking the characteristic taste and flavour of a wine to the environment in which it is produced. Kerri Steenwerth, a soil scientist in the US Department of Agriculture, also sees a reflection of *terroir* in soil microbiota. She found that the composition of soil microbial communities in the Napa Valley varies as a function of the features of a specific grape-growing region. These features include soil pH, the carbon and nitrogen content of fractionated soil organic matter pools, and climate[5]. "This is really exciting," Steenwerth notes. "The soil may act as a reservoir for bacteria that play a role in enabling distinctions in grapevine health and growth that would impart, indirectly, regional characteristics in wine quality."

In New Zealand, a team led by Matthew Goddard at the University of Auckland focused their attention on the yeast *Saccharomyces cerevisiae*[6]. They assessed the effect of different strains of *S. cerevisiae* from vineyards across New Zealand on a batch of sterilized Sauvignon blanc grape juice from the Marlborough wine region of New Zealand. The different geographical strains of *S. cerevisiae* yielded different wines. The researchers believe their findings to be the first experimental evidence linking a microorganism present in grape juice to the resulting wine.

Finding differences in soil microbial communities between vineyards is an exciting discovery, but it doesn't prove that these microbes influence wine taste, says Jack Gilbert, a microbial ecologist at the Argonne National Laboratory in Illinois, USA, and one of the leaders at the Earth Microbiome Project, which seeks to catalogue the diversity of microbial life. Driven by their desire to understand how bacteria affect plant growth and crop properties, Gilbert and his post-doctoral researcher Iratxe Zarraonaindia, now based at the University of the Basque Country in Bilbao Spain, analysed the microbiota of grapevines and their associated soil from different locations: two sites in the US (California and Suffolk, New York) and one site in the Bordeaux region of France. As well as significant differences in below- and above-ground bacterial communities, they identified about 400 bacterial lineages that were present across leaves, flowers, grapes and roots, suggesting that the soil may serve as a reservoir for some of the microorganisms found in the plant[7].

That the yeasts and other microorganisms present on the outside of grapes have an influence during fermentation, especially on the mixture of volatile compounds produced, is relatively uncontroversial. But whether microbes below ground have any effect is another matter. More than 600 volatile compounds are known to exist in any typical wine, points out Anita Oberholster, an oenologist at UC Davis, and Gilbert's is only a single study. "Are the 400 microbiota shared compared to those not shared enough to show a link between soil and plant and specifically grape and wine composition?" she asks.

Some winemakers are on board with the idea that soil microbes influence wine quality, although they acknowledge that more research is still needed. The Noon Winery in South Australia, a family business that has been producing wine since 1976, has always believed in the importance of the soil. "Soil microbes matter and will influence the way plants grow in ways we don't fully understand yet", says Drew Noon. "We use compost, mulches and so-called 'biodynamic preparation' (for example, BD500, a cow-manure-based preparation) in our vineyards in an effort to improve soil life which will in turn affect the grapes and so the wine." Unlike most winemakers, the Noon winery uses naturally occurring yeasts found on their grapes, which they hope will add an extra dimension to the resulting wine. "We acknowledge that is a little more risky, but we know and trust the vineyards that we work with and we are chasing that extra small percent of excitement that the naturally occurring yeast from the vineyard may bring."

Crowd-sourcing soil

Whether the goal is to grow crops in harsher environments or to produce a medal-winning vintage, it is clear that surveying what lies beneath the surface of the soil and how it changes between locations could prove to be a worthwhile endeavour. And that is exactly what a citizen science project called MicroBlitz, based in Perth, Western Australia, is doing (www.microblitz.com.au).

Headed by Andy Whiteley, Winthrop Research Professor at the School of Earth and Environment in the University of Western Australia (UWA), the project is the first of its kind in Australia. The basic goal is to generate a baseline soil microbial map of Western Australia, and to gain an understanding of the environmental factors that control the geographic distribution of microbes using data collected by volunteers across the region. "Once we begin to take this knowledge and layer other criteria such as land use, history and productivity, we can begin to work out what an optimum soil microbial community should look like for any particular application, be it agriculture, land reclamation or conservation," says Whiteley. "This baseline understanding is critical if we are to increase the efficiency of soil microbes and the processes they generate for us, such as a healthy soil and a productive rehabilitated landscape."

One central question Whiteley hopes to tackle with the data generated is "how to rehabilitate our environment after environmental change and help to feed the next billion people through smart soil-microbe based interventions, based upon key scientific observations of why microbes function in the places that they do". The data generated by MicroBlitz will help farmers assess soil health, and will help conservation efforts in ecological restoration. More broadly, the massive dataset will allow researchers to discover which factors are driving the biogeography of microbial populations.

Crucial to the success of the project are its volunteers, over a thousand, dispersed throughout Western Australia. They receive an envelope containing bags, strings and a ruler with which to collect small samples of soil. "We never would have been able to get this far without the amazing support we have received from our volunteers," says Deborah Bowie, project manager at MicroBlitz. These volunteers help overcome one major difficulty of the MicroBlitz project: the expense of sampling.

So far the team has analysed the microbial composition of about 500 samples using next-generation sequencing technologies. They have identified a large number of bacterial and archaeal lineages, most of which are dominated by sequences belonging to three different bacterial phyla, says Deepak Kumaresan, an assistant professor at UWA who oversees the data analysis aspect of MicroBlitz. However, a complex level of clustering suggests genetic differences between the samples.

DEBORAH BOWIE/MICROBLITZ

Trays of soil collected by the MicroBlitz project.

Whiteley headed up a similar project in the UK a couple of years ago. At that time researchers thought that the millions of soil microbial species would be randomly distributed through the landscape, due to their tremendous diversity, small size and ease of dispersal. But Whiteley notes that "when we completed the analyses, it was clear that different regions harboured different microbial taxa, in essence a biogeography for microbes as we see for plants and animals". They found that pH was a major driver of the distribution of different groups of soil microbes, along with plant cover and land use. "This all led to formulating the ideas that we may be able to predict where 'healthy' microbial communities are for operations such as agriculture," he says. With MicroBlitz, Whiteley is targeting an area ten times the size of the UK. "It is one of the few biodiversity hotspots on the globe but has significant pressures on the landscape, from agricultural needs through to high volume mineral extraction and the need for substantial reclamation/restoration, all of which require an optimum soil–microbe composition," he says.

Microbiome engineering

"In the future, tweaking with plant health and other traits may be as easy as pouring some beneficial bacteria on the plants," says Matthew Wallenstein, a microbiologist at Colorado State University, who is also heading a new company called Growcentia that is producing soil probiotics to enhance plant growth through phosphorus solubilization. Their probiotic consists of a consortia of four bacteria found naturally in soil and further improved through breeding. "When inoculated into soils, the bacteria continuously produce enzymes, chelators and organic acids that all aid in phosphorus solubilization," explains Wallenstein. The cocktail, called Mammoth-P, increases the yield of plants such as turf grass, tomatoes, peppers, wheat and hemp by an average of 16%.

From drought protection and fertilization to the cultivation of character in wines, it is clear that soil microorganisms influence plants in a multitude of ways. Indeed, the answers to many of the challenges facing modern agriculture may truly be said to lie in the soil. ❐

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