ILSI 2021 ANNUAL SYMPOSIUM
April 13-15 & 20-22
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ILSI 2021 Annual Symposium Session 2: The Microbiome beyond the Gut

Transcript of the presentation, Soil Microbiomes and Crop Health: Are soil microbiomes the key to sustainable cropping? **Linda Kinkel**, PhD, University of Minnesota, United States

Thank you. And thank you to the organizers. I'm pleased to be here today and talk a little bit about microbes and crop health with you today. I think we're all aware of the profound challenges we face in feeding a world that will have a population well above nine billion by 2050. And even at current population levels, we have a significant challenge to reduce global malnutrition. Estimates are that we'll need to increase food production 20 to 70% to meet the need by 2050. So, this raises a question. Beyond the challenge of food waste, which we're becoming much more aware of, there's multiple significant factors that limit crop yields at the field level. And these include inadequate moisture, lack of nutrients. Oops, I'm seeing everybody's faces now instead of my screen. There. Inadequate moisture, lack of nutrients, sub optimal soil conditions. Soil degradation is a significant challenge globally. Pests and pathogens reduce yields. And climate change is beginning to represent a significant challenge. Suboptimal temperatures either too high or too low have significant negative impacts on crop yield.

Traditional responses or solutions to these are inputs. That is irrigate, add inorganic fertilizers, add pesticides. This is our traditional approach. And in fact, we bought ourselves many decades of increased productivity through inorganic inputs. Yet these inputs all have consequences, mostly negative for our environment. We recognize that the challenges of water shortage is globally, and increasing soil salinization due to over irrigation, impending mobile phosphorus shortages are real. Inorganic soil amendments tend to be quite costly and not available in developing countries. And they tend to also have limited effectiveness. And finally, we know that the classes of pesticides on the market have negative impacts for non-target organisms, including humans with pesticide residues in our food.

So, these negative consequences have led many of us to begin to say it's time for a fundamentally different approach. That as we had the green revolution of the 60s and 70s is the time now coming from a microbial revolution in agriculture? That is, is it time for us to begin to explore how we can optimize plant and soil microbiomes to support global food production. And to what extent is this a practical strategy for us to close the yield gap, to reduce reliance on external inputs, to restore degraded lands, and to expand productivity and capacity of marginal lands globally.

Now we all know over the past years, it's clear microbes are everywhere. And this year's pandemic is especially a great reminder that microbes are everywhere, and they will find you. Plants evolve and exist in intimate symbiosis with microbes. Microbes predate plants evolutionarily. They've always been together. And plants and the ecosystem services they provide globally, rely profoundly on the services that the microbiomes provide to them. Crop yields are influenced by microbial partners, but the reality is our history of agriculture is focused predominantly on pathogens, on one very tiny slice of the microbial world.

But briefly to let you know the densities and diversities of microbes that are in close association with plants is enormous. So, what is the potential here? In soil, in the vicinity of a crop plant root, there can be up to 10 to the 10th cells of bacteria in a single gram of soil, the tip of your pinky finger. 10 to the 10th cells. Inside plants, bacteria can proliferate dramatically. On the leaf surface, we see bacteria in association with a stalemate. Here's a leaf surface. We see fungal hyphae, yeast cells and bacteria. Plants are bathed in microbes in the soil and on the plant surface. Looking at the fungi, which could be president densities of 10 to the fifth to 10 to the seventh cells per gram. And this beautiful closeup of soil. Look at the fungal hyphae ramifying through this soil. The fungi are critical to maintaining soil structure.

Soil aggregation. And you can see here in this red pine seedling whose root system is encompassed by these thick roots but is colonized by a fungus that expands the effective root system up to 80%, so fungi. And finally, viruses. Viruses of course are not free-living, but they're densely colonizing if you will, soil. Both due to their presence in plant tissue, but also inside fungi and bacteria. There can be 10 to the 10th, excuse me, 10 to the nine virions per gram of soil. So, plants are living in an incredibly rich array of microbes in that soil community.

So, what can these microbes do? Beyond the pathogens, which we've spent most of our intellectual capital on for the past century. Beneficial microbes that are common in soil can help plants acquire nutrients. In addition to expanding root capacity, microbes can fix nitrogen and make it available to plants. They can solubilize phosphorous also making it available to plants. Microbes could protect plants against pests and pathogens by directly antagonizing and killing pests and pathogens. Microbes can also turn on plant resistance to help plants keep from becoming infected by other microbes. Microbes can help plants tolerate environmental stresses, including salinity stress, drought stress, temperature stress, and environmental pollutants. And finally, microbes can help plants grow better. Many soil microbes produce plant hormones. When those microbes are present, plants grow bigger, stronger, and faster.

So, we recognize the significant potential that microbes have to help plant productivity. So, this isn't a new phenomenon, right? How do we capture the benefits of microbes to enhance sustainable production? Well, over the past decades, we've tried many different things. Inoculants, inoculate microbes. Here's a microbe that can do a good thing. Let's inoculate it. We've been doing that with rhizobium for decades.

More recently, there are many, many different microbial inoculants on the market. Alternatively, we can manage indigenous population. Every soil has microbes with tremendous potential to support plants. How do we enrich and enhance the capacity of the already existing communities to provide benefits? And finally, there's active efforts among plant breeders to manipulate plant genotypes to support colonization of beneficial microbes.

Today, I want to go through an example, talking about a strategy for developing a mindset for managing indigenous populations to support beneficial functions. Now, this is important. I have to note that we've been managing soil microbiomes as long as we've done agriculture. Every time we plow, or irrigate, or add NPK, we're managing, we're changing soil microbiomes. But we have virtually no insight into what those impacts are. But what we need to do is we move towards a soil microbiome management, we have to fundamentally change the way we think about these management strategies from controlling a population, that is let's minimize a pathogen to understand the entire network of organisms that are interacting in that soil. And there's fundamental questions we don't know anything about. That is to date, there still is limited information on what the characteristics of a good microbiome look like. What should a microbiome look like to optimize productivity? And how can management achieve that outcome?

In our research, we have taken a full head-on strategy to try to address this question, looking at a naturally occurring disease suppressive soil. This is a soil that after 34 years of potato monoculture, so for 34 years potatoes and only potatoes were grown in a small plot of land. And this was because it was a plot that had very, very high levels of two important potato diseases, potato scab, and verticillium wilt. So, this plot was useful for breeders to screen breeding lines. If they planted potatoes in that field and they came up with disease, they threw them out of the breeding program.

But after 30 odd years of continuous potato monoculture disease disappeared from that field. Even the most highly susceptible potatoes would come out of that field with no disease. When I started my career at the University of Minnesota, the first thing that I did was to say, what happened? So, I went to that field to try to understand why that soil suppressed pathogens. And the first thing we found in that soil was lots and lots of bacteria in the genus streptomyces. And in the laboratory, we found that those streptomyces were outstanding at inhibiting lots of different plant pathogens, including bacterial pathogens, aphanomyces pathogens, fungi and even nematodes.

And so, we did the simple thing, which was first let's inoculate. We took some of the best healers, the best antibiotic producing isolates. We inoculated them into a potato field in April at planting. At harvest in September, we had inoculated potatoes that came out quite lovely. And the non-inoculated with the characteristic symptoms of the disease potato scab. We've inoculated in many, many different crops. Here's an example of an inoculation on alfalfa. Here we had soil that's naturally infested with a pathogen by Tathra. These are non-inoculated pots.

And these are pots that at the time of planting of the alfalfa seeds, each pot received one milliliter of 10 of the eight spores of one of our really good antagonistic streptomyces. At 28 days later, you see the benefits of that inoculant. But realizing that I could spend an entire career finding isolates from that soil and trying to inoculate in other locations, the more important question, and the question that's the frameshift, let's move away from an inoculated vision. Understanding what happened in that community. That is why is that soil suppressive or more to the point, how did it get that way? How did that soil microbiome move from highly disease, rich intense disease to no disease?

Now, unfortunately, that field was initiated back in the 1940s, and we don't have soils that go back that far. But on the research station adjacent to the suppressive soil, we have a field that remains fully conducive to both potato scab and verticillium wilt. Well, so these two soils are about 10 meters apart. These two fields, they different cropping history. There are indistinguishable in NPK, but one is suppressive, and one is conducive.

So, we compared the streptomyces communities. The question was, is this something comprehensive about the streptomyces communities that is in fact associated with disease suppression in a comprehensive way? And we asked three simple questions. We said, maybe suppressive soils are suppressive because they have higher densities of streptomyces. And that was true. We had significantly higher streptomyces densities in the suppressive than in the conducive soil. So, more streptomyces. Maybe they're better at killing pathogens. We randomly collected 300 streptomyces from the suppressant and 300 from the conducive soil. And we looked at the ability of every one of those streptomyces to kill 21 different plant pathogens. And we found for every pathogen, a greater proportion of streptomyces could inhibit that pathogen when the streptomyces were from the suppressive soil. And the main inhibition zones, the zone sizes of killing were significantly greater against every plant pathogen for populations from the suppressive and the conducive soil. So, there's more streptomyces, they're better antibiotic producers. And in fact, they produce a higher diversity of antibiotic inhibitory phenotypes. So, you might say that's interesting, but does it tell you how?

And we think actually these data suggest an important way of thinking about what happened in that soil. So, potatoes were grown for 34 years. Where do potatoes put their carbon? It pumped their carbon below ground in big juicy tubers. And they have root systems that actually turn over fairly rapidly. This is a great way to feed your saprophytic community and streptomyces are saprophytic. So, we hypothesize that long-term potato monoculture enrich the streptomyces density. There are more of them. Now you have lots of streptomyces competing in the soil at much higher densities under these high density, highly competitive settings, antibiotic production should confer a greater fitness benefit than in a lower density setting. And this should generate a density dependent selection for antagonistic or antibiotic producing streptomyces, which should yield this increased frequencies and intensities of inhibitory phenotypes.

So now you have a rich streptomycin community with lots of antagonistic activity. Yet think if all those populations are producing the same antibiotics, they're wasting their time. Yet that clever streptomyces that is able to produce a novel, or a rare antibiotic will obtain a greater fitness benefit from that rare antibiotic then from a common antibiotic. And this is a model for frequency dependent selection that is to diversify within that community, the distinct antibiotic inhibitory phenotypes. And so, we hypothesize that this long-term monoculture induced straightforward density and frequency dependent selection that generated a disease suppressive soil.

So, we can rephrase, we can reconceptualize this model as a basic story about coevolutionary selection. We think of soil communities with an evolutionary potential that's determined by the density, and the diversity, and compensation. But we've highlight in our predictive model, microbial species interactions. We argue strongly that microbial competition is a critical driver for this functional outcome we care about, this disease suppression. And in particular, we predict that competition can generate an antagonistic arms race. There was a plate of cookies. I kill you, that imposes tremendous, I get all the cookies, but this imposes tremendous selection pressure on these co-existing populations to become resistance and generate their own weapons. This arms race is what we hypothesized generates that highly diverse, highly antagonistic community yet in a lot of data that I'm not going to have time to show today, we also show that communities might undergo a distinct coevolutionary trajectory. That is well, antibiotic competitive interactions are extremely valuable for disease, suppression, and pathogen control.

Competing microbes might have a different co-evolutionary outcome. That is there's a plate of cookies. Certainly, if I produce antibiotics, I get all the cookies. Yet antibiotics are metabolically costly. And it may be that if there's chocolate cookies and peanut butter cookies, my fitness may be optimized if I eat the chocolate cookies, you eat the peanut butter cookies, somebody else eats the fig Newtons, and collectively this niche differentiation in a soil microbiome can also lead to stable coexistence. But the community in that case has a very different functional outcome that is rather than antagonistic, you have populations that are highly growth efficient on a small range of substrates yielding a community that's very efficient and effective in nutrient cycling.

So, these functional outcomes reflect really different species interaction trajectories, which raises the next critical question. What determines which trajectory a soil microbiome might follow? And I'm skipping a little back on information. In fact, naturally occurring disease suppressive soils had been documented for nearly a dozen agricultural systems globally and are virtually always associated with long-term monoculture. We think that monoculture, if you think about monoculture growing the same crop year after year, it's just like you only have one kind of cookie.

We argue that monoculture might be a really good way to force an arms race co-evolutionary trajectory. Well, in contrast, polyculture, this idea of lots of different resource availability establishes a habitat in which niche differentiation is possible. And so, we have three testable hypotheses embedded in this model. One is that monocultures will generate an arms race, and antagonistic co-evolution in pathogen suppressive communities. Polyculture will generate niche differentiated microbiomes. And that it's a difference in the nutrient diversity or nutrient richness between these that determine that outcome. We actually had, we're studying some long-term field plots at a national science foundation long-term ecological research site, which allowed us to test these three hypotheses. So, the first question was, are streptomyces populations in long-term plant monocultures, more inhibitory in populations in polycultures? We look at four different plant species, two C4 grasses and two legumes that have been growing for 14 years in a monoculture or in a community of plants four, eight, or 16 species rich.

We've looked at those microbiomes from many different perspectives. And today I'm just going to give you a tiny snapshot focusing on, first of all, are populations more pathogen suppressive in monoculture? And what we do is we do a soil dilution. We wash the soil, we plate it. We let it grow for four days and we overlay with a pathogen. So, we test it against five different important fungal and bacterial plant pathogens. And we count for every community, what are the number of inhibitory isolates? And what's the meaning of inhibition zone and size. And we compared this across diversity levels, that is with four different plants species growing in monoculture versus species of communities up to 16 species. The main proportion of streptomyces that are inhibitory against pathogens is significantly higher at high diversity. And the mean inhibition zone is also significantly greater in monoculture than in high diversity communities. That is monocultures do support significantly more inhibitory populations.

The second prediction was, of course that polyculture, populations in polyculture should be more niche differentiated. And we determined nutrient use for every isolate on 95 different carbon substrates. And then we measured, and I apologize. We actually measured niche overlap, which is of course the inverse of niche differentiation. Niche overlap is less in polyculture than in monoculture, which tells us that the polyculture populations are more niche differentiated. They tend to use different nutrients. So, the core finding that we think is critical in this work was that microbial species interactions are clearly driving the functionally different outcomes in these monoculture, in these polyculture communities. Now this doesn't mean the plants are not important. Remember the third prediction was that plant diversity would influence soil carbon, which is really, these interactions are mediated by the richness, the number of different kinds of carbon. Is there one kind of cookie or are there many?

And so, the final thing we did is we looked at carbon compound richness using pyrolysis GC mass spec for one of the grass species growing in monoculture and in polyculture. And one of the legumes growing in monoculture and polyculture. And the simple outcome is it soil carbon richness is significantly smaller in monocultures than in polycultures. So collectively these data show that the microbiomes and their functional capacities are exquisitely adapted to that local environment, but critically it's interactions across multiple scales. That is microbes interacting with one another in the rhizosphere of an individual plant host embedded within a community with its own characteristics, monoculture, polyculture. All of these factors together are determined that functional capacity of the soil microbiome.

And so, thinking of microbiomes, I think one of the critical things that we needed to think of with the managing soil microbiomes is moving away from our strategy to manage a population, to actually consider managing the biotic interaction. That is managing populations has been our historical legacy. Let's kill the pathogen, let's inoculate one rhizobium. But instead by untangling these communities, can we actually manage the ecological and selection dynamics that determined functionality. And in doing this, I have no illusions this is simple. We have to balance a diversity of functions. Do you want honey nutrient cycling efficient? Do you want antagonistic? Do you want p-solubilizing? Understanding these different interactions that generate these functions and recognizing you might need something very different from a high input dependent potato system versus a grassland grazing system. But microbiomes offer us the potential to optimize for sites. This not a one size fits all approach.

And getting close to the finish. I want to note that this session is a lot about the microbiome, but we have to think bigger than just the microbiome. The phytobiome collectively, the plant and the microbes in the soil with the animals and the climate and the nutrients. It's an integrated unit of production. And microbiomes in particular are not only interacting with the plant. They're actually critical to mediating nutrient availability, soil structure, and even climate through ice nucleation activity.

And so, beginning to integrate microbiome thinking, bigger thinking into this broader unit of production is critical for agriculture moving forward. I was asked to touch on what we need to build a science. And I just want to make a couple of key points. We need much more big thinking, collaborative science and public private partnerships. And in fact, there's a tremendous amount of activity in this area. I'm part of an International Agricultural Microbiomes Research Coordination Network. That's seeking to integrate research projects globally. International Phytobiomes Alliance is also playing a leadership role in this area. The EU Microbiome Support Project, and many, many international projects, especially focusing on soil microbial databases, or soil microbiomes, including the African soil microbiome project.

So, there's a lot of activity here. We need to do a little bit better job than we're doing, bringing it altogether, but the efforts are moving forward. Microbiome focus databases are a critical need globally. US investment through the Department of Energy and a national microbiome data collaborative, I think is a really bright spot. And I'm excited to see where that goes. And the National Academy of Sciences of the US is just going to be putting out a white paper on the need for a publicly accessible soil database that has the rich content that we need to manage microbiomes. And finally, data and metadata standards, as in all fields are critical for progress.

And moving forward. It's a great time to be a microbiome scientist. The next decade is going to be rich with discovery. We still don't know who, and where, and when, and what, what are these microbes doing with, what other microbes, where and when? And what are the higher order structures or dynamics that we need to be able to capture for benefits? We also have to be committed to invention. We need better conceptual frameworks and predictive models for thinking about microbiomes and soil. We need more creative and complex analytics and AI and machine learning are becoming incredibly critical to this. And we need inventive ways for translating to management. I should note that translation is advancing rapidly probably a little bit faster than the science itself. There're huge investments by major agricultural companies, the big players. There are over 200 agricultural startups, presently active with microbial inoculants. And in fact, efforts in the developing world, I think are going to be especially significant to moving the needle over the next decade.

And collectively, I do see a future in which microbes and soil microbiome management will play a central and really significant role in securing an adequate, safe, and sustainable global food supply. Finally, I want to thank the wonderful collection of colleagues I've had the opportunity to work with over the decades and funding, especially from the USDA and the National Science Foundation for supporting our work. Thank you.