

Genome Insider S5 Episode 2: Forest Fungi, Seagrass, and a New View of Symbiosis

Menaka: At a broad level, a lot of researchers that the JGI supports are following nutrients. This is a little like following the money in investigative journalism – if you really want to know what's going on, trace what's valuable.

And for plants, microbes, fungi, and viruses, nutrients like carbon, nitrogen, and phosphorus are crucial. So JGI users are often trying to figure out how organisms process these nutrients, and what shifts as they use or store them.

This work often focuses on one piece of what's happening with these nutrients, but all these pieces add up. They create crops and products, and of course, bigger processes like our global carbon cycle.

So in this episode, we've got a few different stories from these many, many projects that the JGI supports.

We've got fungi from the forest floor, a flowering plant that lives in the sea, and a new view of cooperation between a plant's roots and fungal mycelium. All of them with interesting things to teach us about the nutrient cycles that are constantly at work on our planet.

This is Genome Insider from the US Department of Energy Joint Genome Institute. Where researchers discover the expertise encoded in our environment — in the genomes of plants, fungi, bacteria, archaea, and environmental viruses — to power a more sustainable future. I'm Menaka Wilhelm.

And this episode is a variety show, rather than a single story. We'll hear from a few different researchers about work that's come out recently, with the JGI's support. Each of these teams is working with different species, in different parts of the world, but all with the same big goal of understanding how organisms on our planet manage nutrients like carbon and nitrogen.

So – our first story is on fungus. And this work comes from France. Specifically, mycologist Francis Martin's research group at the National Research Institute for Agriculture, Food and Environment.

Menaka: To study fungi at the forest floor, Francis Martin has sampled a lot of soil.

Francis Martin: It's very nice places usually, very nice forest sites, we have to collect using soil corers, and we have to snap freeze very quickly the samples. So you need to carry dry ice, or liquid nitrogen on the site. And for some of the sites, it was very, very challenging because, this was forest up in the mountains. So you have to — not me, I'm getting too old — my students had to carry the dry ice on their shoulders and to hike up.

Menaka: His goal is to understand how fungi function in a forest ecosystem. Because underground, fungi help trees store carbon and cycle nutrients — and if we know how those processes work, it could open up avenues to help forests thrive in the future. So for decades, he's combed fungal DNA and RNA for clues about what's going on at the forest floor.

In the early 2000s, lots of researchers were surveying fungal diversity in forest soils — they could use ribosomal DNA metabarcoding to look at fungi.

Francis Martin: But back to this time, we were not able to assess the function expressed by these microbial communities.

Menaka: So in 2012, he started work on a Community Science Program proposal with the JGI, where he would take a look at the functions fungi were taking on -- he'd ask questions like, which fungi were recycling forest debris, and which were shuttling nutrients around? And lately, he's finishing some results from that project.

Menaka Wilhelm: So it sounds like you could ask who's here, who's around before projects like this, and now, you're able to ask what are they doing?

Francis Martin: Yeah, that's the point. We know who are there, and now with these new tools, metatranscriptomics, RNA sequencing of soil RNA, we can even access — what are they doing, how do they interact.

Menaka: These are protocols that he's excited to share with the community. Check our show notes for links to recent papers, and a write-up on the JGI website about what they're starting to find. Here, in the next few minutes, we'll hear from Francis and a collaborator about how they got these results together. Because as with many research projects, he originally thought this work would come together a bit more quickly than it did.

Francis Martin: I was very confident that in five years, Yeah, everything would be done. You know, I'm a very optimistic guy. I say, okay, thanks to the JGI-powered machines and so on. We will do it in five years. It took almost 12 years because we faced three main bottlenecks. The first one was to extract RNA from soil, because soil is very dirty. There's a lot of bugs and contaminants, so it was very easy to degrade the RNA. And the quantity of eukaryotic RNA, poly-A mRNA is very, very low in soil. So this was really challenging. It took us almost two or three years to design the procedure.

Menaka: Next -- managing the RNA from fungi in that soil was its own challenge. Here's Lucas Auer, a research engineer in bioinformatics who's worked with Francis to analyze this fungal data.

Lucas Auer: We have a very, very huge diversity of microorganisms in this forest soils and, the huge diversity has consequences. It's difficult to assemble transcripts with it because there are

too many different transcripts. And the consequence of that is that we often have incomplete genes. And it's quite difficult to annotate these incomplete genes.

Menaka: One way of handling that is using reference genomes -- so, leaning on existing genomes to figure out what's going on in a new transcript. But early on in this project, there weren't many references for them to use. Only about 10% of the genes this team found mapped to existing fungal genomes at the JGI. It was almost like they knew the destination they wanted to drive to, but the road wasn't paved yet.

Francis Martin: So we had to increase the number of sequenced genomes, and thanks to the project I'm also coordinating — the 1000 Fungal Genome Project — we were able to move from about 200 genomes of fungi to now almost 2,600.

Menaka: Okay, got it. So the 1000 Fungal Genomes Project really opened up a new highway for this project.

Francis Martin: Yeah, really, it was really amazing how the quality of the data improved thanks to that massive amount of new genomes. And then the first steps was how to deal with all these genomes, how to map all this RNA and to make sense of them.

Menaka: So they set up bioinformatics pipelines and protocols, using data from reference genomes in the JGI data portal MycoCosm.

Lucas Auer: And the availability of thousands of genomes in MycoCosm allowed us to use another approach, which is a BLAST-like approach. So it's not done with BLAST because it would be too long. But it's with another BLAST-like tool, which is called Diamond.

Menaka: That way, they're able to check each transcript against many reference genomes, to understand what kind of organism expressed a gene, and what it's for. After working their way through these challenges, they've got a couple of papers out now — to start.

Francis Martin: But we have at least half a dozen of papers in the pipeline. So there will be also another paper comparing the conifer trees with the broadleaf tree. And then we have one which I'm really excited about. It's one dealing with truffle. One of the sites is a truffle orchard.

Menaka: Oh, wow.

Francis Martin: And so the fungal community should be overwhelmed by truffle mycelium. But we don't know, in fact. So thanks to that project, we are going to check whether truffle is competing with other fungi and so can we correlate some function with the truffle productions.

Menaka: And Francis sees this approach going even farther as time goes on.

Francis Martin: In the next few years, I think we will have a kind of atlas, a kind of global map of

the fungal diversity, but we are still missing the functions. So thanks to that kind of program that we have developed with the JGI, we have the tools to really get information on the functions of this fungal community from the poles to the tropics.

Menaka: So again that was Francis Martin and Lucas Auer, from the National Research Institute for Agriculture, Food and Environment, in France. We'll link to the work they've talked about in our show notes.

Francis Martin will also be a keynote speaker at JGI's annual meeting this year, hopefully you can join us.

Next, Allison Joy has a story about a plant that lives at the bottom of the ocean. Seagrass.

Allison: Yes, so seagrass lives in oceans all over the world and does a ton to keep our carbon cycle in balance. I talked to two different researchers who've studied it — they don't work together, but they've both used JGI resources in their work. Here's Adam Healey. He's a computational biologist at the HudsonAlpha Institute for Biotechnology.

Adam Healey: Seagrass meadows pull down a huge amount of carbon compared to the space that they occupy. They really punch above their weight, so to speak. Per square meter, they sequester three times more carbon than a rainforest, seven times more than just a temperate forest that you see in the US.

Allison: And so our researchers today want to know how these grasses got so good at pulling in carbon, and part of that involves looking at the plant's evolutionary history. As they've evolved, seagrasses have forged unique paths.

Plants, in general, evolved from algae — over millions of years, they developed the features they'd need to live on land. Seagrass also followed that path — but then after evolving to live on land, it returned to the sea. Which means it has a lot in common with land plants -- except that it lives underwater. Xiao Ma is our second seagrass researcher. She's a postdoc now in bioinformatics at Ghent University with the VIB-UGent Center of Plant Systems Biology.

Xiao Ma: It can still have the flower, but the flower is in the seawater. And the pollination also occurs in the ocean.

Allison: How do they pollinate?

Xiao Ma: Seagrass can pollinate by the current, by the ocean current.

Allison: Even though it's called a grass, this plant is much more than the lawn of the sea. Unlike the greenspace on golf courses, seagrass is a boon for biodiversity.

Xiao Ma: Seagrass meadows can provide the shelter and the nursery for many invertebrates and fishes living in the seawater.

Adam Healey: Yeah, like one acre can support 40,000 fishes and 50 million crustaceans and everything that goes along with it. So it's a pretty complex food web just within a small area of these meadows.

Allison: And there are many different kinds of seagrass around the world. So the hope is that by looking back at how this plant has evolved, we can understand the adaptations that work well. These adaptations could keep these grasses going strong, securing shorelines, protecting an array of species, and keeping carbon out of the atmosphere.

The JGI has supported multiple teams who are working to understand seagrass. Adam and Xiao are good examples — they both had papers on seagrasses come out over the past year, with their approaches focused on opposite ends of the question. Xiao's team looked more broadly at genome sequences of multiple species across three independently evolved lineages.

Adam tracked evolutionary patterns over time of one specific species. It's a type of eelgrass — so a subset of seagrass — called *Zostera marina*. *Zostera* is a big deal among seagrasses because it has managed to spread all over the world, thriving in a wild variety of environments.

Adam Healey: So the ancestral home of eelgrass, like where we think it originated from, is in the coastline surrounding Japan. But it grows all over the coastlines around the world. Like, how on earth did something that originates in Japan, how can it grow in Bodega Bay in California, in Alaska, off the coast of Portugal, off the coast of Norway?

Allison: This species tolerates a giant temperature range, along with a bunch of different conditions. So, Adam's work looked at exactly how eelgrass made its way around the world.

Adam Healey: So, prior to the study, because *Zostera marina* is so widespread, researchers assumed that this took millions of years to happen. It just needs a long timescale because it had flourished in so many different places at once, and that takes a lot of evolution. But by sampling different populations from around the world and calibrating what we call a molecular clock, we were able to find out that these colonizations are actually very young.

Allison: So this wasn't an evolution that took millions of years. The correct timeline involves removing at least one, or maybe three, zeros. Eelgrass started to spread just 250,000 years ago — but a lot of its movement actually happened only within the last 40,000 years.

Adam Healey: So once conditions became right, eelgrass sort of spread out very quickly and established itself, which is something that we didn't know previously.

So from Japan, it came across the ocean current and colonized, sort of the California coastline

down near Mexico, like that area. And then a hundred thousand years later, it came across again from Japan, instead of going south this time, it sort of started to move north and it went up towards Washington state and then off the coast of British Columbia, Alaska, and there it sort of sat for a little bit. When the Bering Strait opened up, it was no longer covered by sea ice, there was a new ocean current that opened up that allowed eelgrass to catch a new ocean current and actually go across, over top of the Arctic and get established into the Atlantic.

Allison: I asked Xiao what she thought about this discovery.

Xiao Ma: It's a very nice paper. But after reading this paper, I have one question. I'm very curious why only the *Zostera marina* is the most widely distributed.

Allison: Fair enough. *Zostera* is just one of the handful of species Xiao studied. There are lots of different seagrasses, and they're all able to travel in ocean currents. So how did *Zostera marina* end up all over the place, while other species Xiao studied — ones that have been around even longer than *Zostera* — had smaller, less varied, footprints? She had one idea.

Xiao Ma: Based on genomic results, we found that *Zostera marina* has a very strange genome structure. Compared to other seagrasses like *Posidonia*, *Cymodocea* and *Thalassia*, we investigated, you know, this paper, and we found that *Zostera marina* have a lot of rearrangement in their genome structure. However, the genome structure of *Posidonia*, is quite conserved. So I would like to know whether the rearrangement is related to the wide distribution.

Allison: That's an interesting idea, because rearrangement basically widens the combinations of genes that plants could inherit. So you could imagine that rearrangement might have helped *Zostera marina* adapt — but... they could also be totally unrelated. So there's a lot more to analyze there.

Allison: *Zostera marina* is the most widely studied seagrass — and Xiao's team is working to widen that lens. In their new paper, they've produced a lot of new data.

Xiao Ma: Most of the research about seagrass is about *Zostera marina*, because *Zostera marina* is the first published seagrass genome.

Allison: That was done at the JGI. It's a resource to help researchers all over the world understand these carbon sinks better.

Xiao Ma: So they always study the seagrass *marina*, because if they want to study other seagrasses, they don't have, they don't have the genomic data. So now our paper provides the reliable genomic data for other seagrasses.

Allison: In Xiao's study, she examined additional sea grasses that prefer specific climates, like tropical and freshwater seagrasses. For which the JGI also provided sequencing. Xiao and her team studied species of three independently evolved lineages: *Posidonia oceanica* or Mediterranean tapeweed, *Cymodocea nodosa* or Neptune grass and *Thalassia testudinum* or turtle grass — as well as our all-star *Zostera marina*. Xiao's team also included a draft genome for *Potamogeton acutifolius*, a sort of freshwater sister lineage.

Xiao Ma: So we would like to know whether the sequences from the different lineage have the convergent evolution. And whether they have the same strategy to adapting to the marine environment.

Allison: And Adam was also stoked about this data's potential to enable future work.

Adam Healey: Previously when we studied this, it was sort of a one-off comparison. We only had the genome of *Zostera* to compare to. So that was our reference point.

Allison: But the genomes from Xiao's paper change that.

Adam Healey: So you can compare saltwater species to freshwater and look at what differences in their genetics exist between those species.

Allison: With all these data, it could be possible to run more studies across many species, to find out what features could help seagrasses thrive as our environments shift in the future. Because ultimately, this kind of data is a more-is-more situation — the more info we have, the more we can learn. ... And to get all of this information together, these collaborative projects require many hands on deck.

Adam Healey: It really brought together people who have their own little expertise and everyone's sort of working together to answer a really big scientific question.

Allison: They involve researchers in ecology, microbiology, and bioinformatics — so, researchers who've been in the field, and scientists who have done this work from afar. Adam and Xiao are both on this data side, they told us — so sampling is not part of their everyday work. In fact, Xiao said she'd never even seen a whole seagrass itself in person — but with warmer weather, she might try to finally catch these unique plants in the wild.

Xiao Ma: So maybe in the summer I will go to Italy and I will find some seagrass and to see how they look like.

Allison: Hey, we hope that happens! Maybe she'll even catch a couple of seagrass blooms — but either way, any seagrass she spots is a top notch example of carbon storage that we're learning more about everyday.

Menaka: So again, that was Adam Healey from the HudsonAlpha Institute for Biotechnology,

and Xiao Ma from Ghent University. You can find both of their papers linked in the show notes. Next up, we've got researchers looking into how fungi and plants cooperate.

And this story takes us below ground, to the networks between fungi and plants – mycorrhizae.

Fungi grow these tiny tubes through soil to connect with plant's roots. But these networks are for sharing in both directions. Fungi send plants nutrients and water would otherwise be out of reach, and in return, fungi get carbon from their plant partners. It's a symbiotic relationship with big benefits for crops and soil. So researchers want an up close view of what's going on here. I spoke to Karen Serrano, from UC Berkeley and the Joint BioEnergy Institute, and Benjamin Cole from the Joint Genome Institute about their work on this.

Menaka: And so when these organisms are in symbiosis, what's the right thing to picture? Like, what does it look like for them to interact?

Karen Serrano: Yeah, that's a great question. So, it looks like a mess.

Menaka: Turns out, this isn't like, a neighborly exchange that's happening across a firmly delineated picket fence.

Karen Serrano: So in the soil the fungi will travel to the root, and the root's actually like secreting chemicals that recruits the fungi. And so, once they get to the root surface, they then begin to penetrate root cells, they actually sort of manipulate the plant into housing them.

Benjamin Cole: You could think of it like, as a new roommate. That kind of moves into the cell and kind of sets up shop so it reorganizes the cell to suit its needs. And it's actually really beneficial.

Menaka: So, yeah. This is a fungus that comes into a plant cell like a new roommate, from a different kingdom of life. A roommate that does a ton of redecorating, but also shares food and always pays rent on time. And these researchers are working out new ways of understanding this kind of partnership.

Menaka: Karen, Ben and their team have been working with one fungus in particular,

Karen Serrano: Which is called *Rhizophagus irregularis*. It's a really broadly used fungus, in agriculture. A lot of farmers will, like, spray this one on their fields.

Menaka: and these fungi easily form a relationship with a model plant that looks a little like clover -- that one is called *Medicago truncatula*. They've been looking at this for a few years.

Benjamin Cole: Going into it, we were thinking, you know, we could figure out pathways that we can tinker with, that would improve the association, make the association more productive, maybe push the fungi to get more carbon from the plant, as a way of carbon sequestration.

Menaka: So that would be a boost on the soil side -- but there are also plant benefits above the ground, of course.

Karen Serrano: At JBEI, we focus on biofuel production, a lot of the biofuel land is marginal. So it's like really horrible soil, and these are, this is one interaction that allows plants to better survive in those environments. So our thoughts were that if we could understand this interaction a little bit better, we could then engineer plants to better manipulate the interaction and take advantage of these fungi instead of trying to engineer plants with, like, all of the improved benefits that these fungi provide.

Menaka: In other words, this is a complicated symbiosis, but it might lead to simpler ways of boosting biofuel feedstock crops. And because this relationship is a two-way exchange, Ben, Karen and their team want information from both sides of this messy, plant-fungi roommate-relationship. Their work goes beyond what this move-in process looks like, into the genes behind how this symbiosis starts and succeeds.

But remember -- this plant-fungi roommate-relationship only happens in specific plant root cells -- so they've used a technique called single nuclei RNA sequencing to look at individual plant cells involved in these relationships.

Karen Serrano: That way you can track the genes, not just as root genes, you can track them as like — cortical cell genes or epidermal cell genes. So that's what we applied here because we have this interaction that only happens in very specific cells. What we didn't get from that technique was fungal nuclei,

Menaka: And for that, they've used another technique called spatial transcriptomics -- where they can make a little map of gene expression, within a sphere that's about as wide as a human hair.

Benjamin Cole: The really nice thing about the spatial transcriptomics approach is — we get signal not only from the plant, but also from the fungus. So we quantified the expression of about 12,000 transcripts from the fungus.

Menaka: This kind of study is still pretty rare -- especially getting this many genes, at this kind of resolution, is a big deal. So this work doesn't stop here. Across their datasets, they've found dozens of genes that are upregulated

Karen Serrano: And those are really great candidates for anyone who wants to do functional characterization, actually looking at those genes individually, for their role in the symbiosis. Those are great candidates for genetic engineering. So our hope is that, you know, like the community at large will follow that up.

Menaka: And really, there are lots of follow-up studies that this work could lead to. Ben is

curious about all kinds of improvements to these relationships – for example, if certain nutrients or conditions foster good symbiosis between fungi and plants. He’s also looking to train these techniques on specific plants — like plants grown for biofuels, sorghum and switchgrass. And eventually, he’d like to take this work even broader -- beyond studying one fungus and one plant.

Benjamin Cole: Maybe the holy grail would be looking at a very complex community, and one — understanding who's in that community in this one very, narrow window of, of space, but also what everybody is doing. That would be very neat,

Menaka: Like an ant farm view of what's happening underground.

Menaka: So, eventually, we could know quite a bit more about the messy world of how plants and microbes cooperate.

That was Karen Serrano from UC Berkeley and the Joint BioEnergy Institute, and Benjamin Cole from the Joint Genome Institute.

And that’s just about a wrap on this episode – we’ve found our way through some of the fungi that cycle nutrients in our planet’s forests, the seagrasses that store carbon around the world, and this symbiosis between fungi and plants below ground.

Thanks for coming along for these stories. You can find more like them in this podcast feed, and online at JGI.doe.gov.

We heard from Francis Martin and Lucas Auer, from the French National Research Institute for Agriculture, Food and Environment. Then Adam Healey from the HudsonAlpha Institute for Biotechnology, and Xiao Ma from Ghent University. Finally Benjamin Cole from the JGI, and Karen Serrano from the Joint BioEnergy Institute at Lawrence Berkeley National Lab. We’ve got links to all of their work wherever you’re listening to this episode, and you can find out more about each of their work on our website as well.

This episode was written and produced by me, Menaka Wilhelm. I had production help from Allison Joy, Massie Ballon, and Graham Rutherford.

If you liked this episode, hit subscribe or follow in your podcast app! And tell someone else about Genome Insider – leave a review, send a friend a link, or share this episode online.

Genome Insider is a production of the Joint Genome Institute, a user facility of the US Department of Energy Office of Science located at Lawrence Berkeley National Lab in Berkeley, California.

Thanks for tuning in – until next time!