

Genome Insider S5 Episode 1: What Happens To a Rainforest When You Dial Up Drought? - Linnea Honeker and Malak Tfaily

Menaka: In southeastern Arizona, there's a town called Oracle. Mostly, getting there takes you through a bunch of desert.

Linnea Honeker: You drive like 45 minutes north of Tucson, and it's just, there's nothing anywhere. It's just, you know, desert, grasslands, cacti and stuff.

Menaka: This is Linnea Honeker, and she's a microbial ecologist. She's taken these trips out to Oracle, because she's done experiments there – but in a very different environment.

Linnea Honeker: And then you like, turn on this road, and all of a sudden, you drive down and? this like, odd, misplaced structure appears.

Menaka: It's a building called Biosphere 2. This structure looks like it fell out of outer space. It's all white steel, and shiny, reflective glass. It's a big conglomerate of a few different shapes -- a glass pyramid with its top cut off, a tower that looks like a giant white chess pawn, and a few domes covered in diamond-shaped windows.

Linnea Honeker: It's very, like futuristic looking. Yeah.

Menaka: Yeah. The photos I've seen are a little bit geodesic dome-y.

Linnea Honeker: Yes!

Menaka: These buildings have been there since the late 80's. At its start, Biosphere 2 was a pipe-dream come-true -- the billionaire and the engineer behind this project were constructing a giant, enclosed environment for a different kind of experiment than the ones Linnea has worked on.

The way they saw it, our planet was Biosphere 1. This new structure they'd designed -- it would be its own little enclosed copy of Earth -- Biosphere 2. It would exist independently from its surroundings -- so it was a chance to see if we could make a totally self-sufficient environment, one that could grow plants, supply oxygen, and cycle water and nutrients. If this could work, maybe we'd be able to recreate our biosphere on other planets, also.

So pretty soon, headlines about this Biosphere 2 popped up around the country. From the New York Times,

Announcer: ULTIMATE SURVIVAL: DESERT DREAMERS BUILD A MAN-MADE WORLD,

Menaka: Or, if you were reading the Arizona Republic at the time,

Announcer: DESTINATION MARS: The prototype of Biosphere II, a self-contained habitat that could be shipped to outer space, will be built on the 2,500 acre SunSpace Ranch in Oracle.

Menaka: As part of the original experiment, Biosphere 2's founders would stock their enclosed habitat with thousands of plants and animals, then recruit people to live there -- the biospherians. And for all of the complexity of that plan, the Chicago Tribune covered Biosphere 2 with a very tidy headline:

Announcer: SMALL WORLD.

Menaka: And that headline might just have been the first time Linnea Honeker heard about this.

Linnea Honeker: I grew up in Chicago. I was a little girl when they, when they built the Biosphere and then had the original biospherians spend the time enclosed in there. And I had remembered hearing about that in the paper and I was like, "Oh my gosh, it's so cool. I really want to go see the Biosphere 2 one day."

Menaka: And she would. Like, a lot of times. After she finished her PhD, she actually found a research position at the University of Arizona -- that's what landed her back at Biosphere 2.

Linnea Honeker: When I saw the job opportunity, at the Biosphere 2, I was like, "Oh my gosh, that's like, would be perfect."

Linnea Honeker and Elliana Tian Laton process soil samples from the Biosphere 2 drought experiment. (Laura Meredith)

Menaka: Wow. So you heard about it as a kid and then ended up working there as a researcher.

Linnea Honeker: I know, I know! It's really cool.

Menaka: Yeah.

Menaka: So in this episode: the work Linnea did at Biosphere 2, all those years after she first found out about it.

THEME

Menaka: 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.

We're spending this episode at Biosphere 2 -- a totally enclosed mini-Earth in Southeastern

Arizona. Biosphere 2 has quite a history, which I mostly have to leave to other documentaries.

These days, Biosphere 2 belongs to the University of Arizona. Inside this giant, sealed terrarium, scientists can tweak all kinds of environmental conditions, and study how that change affects an entire ecosystem. So in terms of climate change, they can do a kind of fast forwarding — seeing what happens when environments face the warmer, drier conditions that are coming. Hopefully, what they learn will let us prepare a little better for these shifts, to help ecosystems cope.

And inside Biosphere 2, there are actually several different environments for these experiments. So for example, there's a patch of Floridian mangrove trees, an ocean for studying coral reefs — and there's a tropical rainforest. That's where we're headed.

This tropical rainforest is about the size of a hockey rink, but it's tall — inside, some of the trees hit 80 feet. Hundreds of rainforest plants live there, growing and existing the way they would in the wild. But remember -- this is a completely controlled environment. Temperature, rainfall -- all of that is up to researchers.

And the experiments we're talking about today focus on drought, for a rainforest. Because as the outdoor rainforests of our planet see more drought than they used to -- the way they handle those droughts matters. It matters for the plants and animals and people living near those forests. But it also matters for the rest of the world, too.

Malak Tfaily: In reality, it's going to impact the whole earth, it's going to influence the atmosphere everywhere. So it's really special.

Menaka: Malak Tfaily is a professor at the University of Arizona. So she's referencing the fact that rainforests are giant carbon reservoirs for the whole planet. We want that carbon stored so it doesn't head to the atmosphere, since atmospheric carbon drives climate change.

If you think about the Amazon rainforest alone — it stores 150 billion metric tons of carbon — a pretty big fraction of all the carbon stored on Earth. Half of that carbon is in the trees and plants of the Amazon, and the other half — is in the soil, where plants' roots, and microbes, and fungi hang onto it.

So rainforest soil is a major focus for the researchers we're hearing from today. They wanted to see — when a rainforest hits a drought, could its soil hang onto its carbon stores? Or would organisms shift, releasing more carbon from soil up to the sky?

Malak Tfaily: And we were lucky in Arizona that we didn't even have to travel to South America. It would have been cool to do that, but actually we have the tropical rainforest at Biosphere 2, which gave us an extra advantage of being able to do that while being at home still.

Menaka: Malak led this project with another professor at the University of Arizona, Laura

Meredith. And Linnea Honeker, who heard about Biosphere 2 as a kid -- she worked with them as a postdoc.

This project was part of a big campaign in 2019, where researchers hit Biosphere 2's rainforest with a severe drought. Normally, the rainforest in Biosphere 2 gets a little over an inch of rain per week. For this field campaign, it got zero rain. For 65 days.

Before and after that, these scientists headed into the Biosphere 2 rainforest to collect samples. And both Linnea and Malak told me -- that is quite an experience.

A panoramic view of the rainforest ecosystem within Biosphere 2. (Laura Meredith)

Malak Tfaily: It is! First of all, you're gonna be bombarded with the humidity.

Menaka: Usually, between 60 and 90% relative humidity, and that's on top of pretty warm temperatures -- from 70 to 100 degrees Fahrenheit. But anyone who went in to sample was essentially entering their experiment -- and they had to be mindful of that. Any chemicals that come into Biosphere 2 are pretty much stuck there.

Malak Tfaily: And that's why when we were doing the experiment, it was important that people do not put too much perfume or cologne and other kinds of things we do on a daily basis. We don't think about it -- because all of that will be trapped and then it will influence your signal. So people had to go in with that high humidity with little to minimal any products on so that they did not mess with the measurements, too, because that's one of the unique aspects of it. It's all trapped, so you could control what comes in and out, so you also have to be careful of that thing, what you bring in with you.

Menaka: Oh my gosh, I hadn't thought about the no deodorant or no hairspray part at all.

Malak Tfaily: Yeah, especially in a very humid and hot environment, you think about that.

Menaka: So, the humans working in Biosphere 2 might have gotten a little smelly while they sampled -- but the enclosed rainforest was also making plenty of smells of its own.

Malak Tfaily: You know, how sometimes when it rains for the first time after a while you go outside and some people say, "Oh, go smell the soil outside." You feel like that earthy smell. A Tropical Rainforest smells like that all the time. But also as you walk through the Tropical Rainforest, there are different vegetation and each vegetation has a distinctive smell. And that smell is all related to the compounds they release.

Menaka: And we're partly talking about these smells so you get a picture of what it's like to go into a rainforest. But more than that, these compounds are really important to the experiments that Malak and Linnea worked on -- these smells are a class of chemicals called volatile organic compounds. Which is a mouthful. They mostly call them VOCs. And even without visiting the

tropical rainforest, we've all smelled VOCs, because organisms all over the world release these chemicals.

VOCs are part of what make pine trees smell piney — that's a compound called pinene — and oranges and lemons smell the way they do, too — that's another called limonene. Humans even make VOCs, too -- we exhale them along with the carbon dioxide we create by breathing.

And there are lots and lots of different VOCs, but they're all chemicals that include carbon, and easily evaporate into air. They matter, because they're a very specific slice of the carbon in our atmosphere. They're not as warming as carbon dioxide or methane, but they could build up and contribute to warming. VOCs can also cause other effects -- they're reactive, and they can mix with other emissions. When that happens, some VOCs cause smog. But when forests release these chemicals, it's part of a response they've evolved -- here's Linnea Honeker, again.

Linnea Honeker: some VOCs can actually be cloud condensation nuclei, so they can actually help cloud formation, so like, for instance, trees can release instance isoprene as like a stress response and some of those VOCs can actually kind of be like a SOS call to draw rain to the system, so it's not always like a bad thing, but some of them like have reactions that lead to more climate warming.

Menaka: But the biggest thing about VOCs is that we're still figuring out exactly what they do, and what makes organisms release them.

Malak Tfaily: It turned out until recently, people did not realize that plants and microbes communicate not just through metabolites in the solid or in the liquid phase, but they actually emit things.

And think about it, when they emit gaseous substances, they are able to get them to different places. Because if you release something only in the soil, it's trapped within the soil, and depending on how much water is in the soil, it will dictate how far that compound being released by the root of a plant could reach.

But when you release something in the air, within a few seconds, you're gonna smell it somewhere else. So that's, to me, it's something if I have to think about from an evolutionary perspective, it's a way that plants and microbes realize it's a more effective communication style. But to me, again, because of the lack of the tools as well to measure these compounds, nobody focused much on them.

Menaka: It's tricky to measure a chemical in the air, in a small amount, that travels so quickly.

Linnea Honeker: A lot of research kind of skips looking at VOCs. They, they look at metabolites, but they're looking at metabolites that are more soluble in the soil. Like: we're collecting samples from the soil, taking 'em to the lab – any metabolites that are volatile are gonna have lots of opportunities to escape from the system.

Menaka: So historically, VOCs have been easy to miss. But they're a key way of understanding our ecosystems, and they're also important for getting an accurate tally of carbon cycling. So these researchers were interested in new ways of looking at VOCs as they put their rainforest through drought. And lately, they've published some results.

Linnea Honeker: One of the unique things about this paper is just having this, like, real time VOC data.

And so next we'll get to these experiments, and those data – but first, a quick break.

Allison: The JGI supported this project via the FICUS program. That stands for "Facilities Integrating Collaborations for User Science". Through this collaboration, users can submit a single proposal to access the capabilities of multiple user facilities in the same project. Here, Malak Tfaily wrote a proposal where she and a team of collaborators would work with both the JGI and EMSL, the Environmental Molecular Sciences Laboratory. This access to such a wide breadth of resources across facilities helped take the team's work to the next level. But you don't have to take it from us. Here's Malak:

Malak Tfaily: We wanted to do the science, we have the ecosystem, but we want the tools to do that. And to me, JGI, as well as other DOE facilities, really provide these tools that make it possible for us. So to me, it was like a perfect match, you know?

Now, yeah, you might argue that you could get some of these tools available through other companies. But to me, there's something unique to working with JGI among other DOE facilities because it's not just about the tool, I really value the expertise of the staff there.

As somebody who was at a — I was at another DOE facility, I used to work at a DOE facility, I really, really, used to see how people worked really hard to actually really help other scientists from other facilities and other researchers. So I really feel with them. So I'm really thankful for that. But basically JGI provided not just the tools, the expertise.

And it was really simple, once your proposal got accepted, it's a matter of submitting your samples and they could provide any help you want. So you can't ask for more than that. I mean as a scientist — that's basically, it's cutting half of the work. None of the work and the papers we put out, it wouldn't have been possible if we did not work with JGI. Honestly, because it allowed us access to the metatranscriptome, metagenomes, basically looking at which microbial communities are there, what they're doing and also from the respect to the plant — what's going on there?

So to me, it was a partner, a really, a partner that allows us to take science to the next level. And the more I was thinking about it, the more I realized how thankful I am for these user facilities.

Allison: You can find out more about submitting proposals to the JGI on our website. Head to jointgeno.me/proposals. We've also got a link to our website waiting for you, wherever you're listening to this episode — either in the episode description, or the show notes.

Menaka: All right -- back to Biosphere 2, our fully enclosed rainforest environment. For the experiments we're talking about, Malak Tfaily, Linnea Honeker, and a team of other researchers have pushed this indoor rainforest into a drought, because they want to see how that changes the rainforest's carbon cycle. And they're focused on soil microbes – a major part of carbon cycling happens underground.

So first — a quick look at where soil microbes fit into the global carbon cycle. Microbes initially get carbon from the environment – from plants, or fungi, or other microbes. These carbon-containing organic compounds are like food for them. As they process an organic compound, three different things can happen. Here's Malak Tfaily:

Malak Tfaily: Microbes will take some organic compounds to build biomass. It's kind of like us eating. We have to grow. At the same time, they release some byproducts back into the soil

Menaka: So carbon sent back to soil would be stored, or passed off to other organisms. Then there's the third thing microbes do with carbon —

Malak Tfaily: And they release back into the atmosphere. And the balance of that is actually what maintains carbon cycle on, on Earth.

Menaka: And because carbon compounds in the atmosphere contribute to climate change, it's useful to know more about what flips this switch. When do soil microbes store carbon underground, and when do they send carbon out, as a gas, like carbon dioxide, since that CO₂ will hit the atmosphere. Here's Linnea Honeker:

Linnea Honeker: If we want less carbon in the atmosphere and more carbon in the soil, we want, we want to see less CO₂ emissions. But you really need to take a look at, like, all the carbon.

Menaka: So this is why Malak and Linnea set out to measure the volatile organic compounds they did – the VOCs, because they're some of the other gasses these microbes release to the atmosphere.

Malak Tfaily: People used to think, they only released CO₂ and methane. Now we realize there's so many other VOCs.

Menaka: So Linnea headed into these experiments with a specific question about what was

happening between the soil and the sky of the rainforest.

Linnea Honeker: My question was really like, How does drought impact carbon allocation into CO₂ versus VOCs?

Menaka: And remember, VOCs are compounds that, in lots of cases, we can smell. So these experiments are sort of measuring the smells that come out of soil, in real time. If that sounds wild, it's because it is.

At left, soil samples receive a pyruvate label, so researchers can trace the path of carbon throughout the rainforest ecosystem. These samples are connected to the shed at right, where analysis equipment takes real-time VOC measurements. (Laura Meredith)

The setup is like this -- they've got specific patches of soil, where plants aren't growing, so they'll capture data from microbes only. Those patches are about the size of a coaster, the kind you'd put a drink on, so 5 inches by 5 inches, to be exact. And then there's a white metal tray that sits over that soil patch, to close in a little chamber. So this is a much smaller chamber around a patch of soil, within the big chamber of the whole Biosphere 2 rainforest ecosystem.

And then of course, they had to connect these soil patches to the machine that would measure VOCs -- that machine sat in a shed within the rainforest.

Menaka: I think I saw in a video about Biosphere 2, I saw a lot of tubing.

Linnea Honeker: There were so many tubes everywhere! It looked trees, leaves, everywhere -- there were tubes, soil. It was like a highly instrumented rainforest.

Laura Meredith and the highly instrumented rainforest of Biosphere 2 -- these tubes lead to analysis machinery that sits in a shed within the ecosystem. (Courtesy of Laura Meredith)

Menaka: So whatever gasses the soil microbes release, these tubes -- and then the connected machinery -- would pick up. And these experiments included multiple soil chambers.

Linnea Honeker: And so, and then that machine is, is continuously measuring the VOCs from each of the chambers.

Menaka: And to get even more detail about Linnea's question -- about how microbes shift between producing carbon dioxide vs. VOCs, they gave soil patches a labeled carbon source -- that label acted like a little tag that they could trace through the carbon cycle.

Linnea Honeker: we used a ¹³C pyruvate label to really trace how the microbes were taking the ¹³C from pyruvate, which is a central metabolite, and allocating it into CO₂ or VOCs.

Menaka: So all of these measurements, the pyruvate tracing and the VOC tallying -- that's all

happening in real time, in the rainforest. And then during that data collection, this team also took periodic soil samples. These are the samples they sent to the JGI and EMSL to understand microbial gene expression.

So all together, they had a bunch of dots to connect. Which gasses the soil microbes released, and from the JGI, which genes drove what they were producing. On top of that, EMSL sent metabolomics data that showed all of this in context of what microbes were producing overall.

And what they saw — was that in drought conditions, carbon cycling from these soil microbes changed. With less water, these microbes released less carbon dioxide to air. But that didn't translate to more carbon in soil -- which would be the ideal outcome. Instead, the microbes created more VOCs that function as stress signals, and VOCs built up more in the air.

Malak Tfaily: and these organisms, especially the bacteria that is living alone in the soil, not correct, not, uh, not like doing any interaction with plant, they seem to completely change their metabolic pathways, rather than investing in carbon allocation and building biomass or releasing metabolites into the soil, what they ended up doing, they converted everything to VOC, they really wanted help.

Linnea Honeker: And so in this case, the metabolic intermediates being produced aren't being recoup— immediately recouped, and so, the VOCs, the volatile metabolites, intermediate metabolites are building up and escaping to the system.

Menaka: Got it. They're basically escaping the soil because no one is picking them up and using them,

Linnea Honeker: Yeah. That's kind of in my mind how I'm picturing what's going on. So I think it's kind of a combination of increased production, decreased consumption of these VOCs.

Menaka: Overall, during a drought, soil microbes pushed more carbon molecules out of the soil, and at the same time pulled less carbon in. And so Malak and Linnea want to keep looking into this.

Malak Tfaily: We believe that those compounds are becoming more and more important for us to better understand what the plants are going through, what the microbes are going through, and if we understand what they're going through, then we might be able to help mitigate some of these impacts on them.

Linnea Honeker: We have to pay attention to the volatile compounds too, because it's a subset of the complete soil metabolome. And so, in the research in the future, just taking that into consideration as part of the soil metabolome, and part of the carbon cycle.

Menaka: Yeah, definitely. But what a unique opportunity to actually get to capture them all in the biosphere.

Linnea Honeker: Yeah, I know. They don't have anywhere to escape, so.

Especially at sunrise and sunset, the Biosphere 2 structure stands out against its desert environment. (Laura Meredith)

Menaka: And even outside Biosphere 2 — the carbon cycle of our bigger environment — our Biosphere 1 — is full of interconnected processes. The more we know about them, the better. Because eventually, a shift in the carbon cycle of rainforest soil will have effects much farther away — and ideally understanding things like this will help us predict and prepare for those kinds of changes.

Menaka: So again, that was Linnea Honeker and Malak Tfaily, who published this work in *Nature Microbiology*. We'll link to their paper in our show notes. Malak is a professor at the University of Arizona, and Linnea is now a postdoc at Lawrence Livermore National Laboratory.

The JGI and EMSL enabled this work through the FICUS program. You can find out more about that program, at the JGI website – there will also be a link in the show notes! And, the transcript will be posted online.

This episode was written, produced and hosted by Menaka Wilhelm, with production help from Allison Joy, Massie Ballon, and Graham Rutherford. You heard music in the middle of this episode by Cliff Bueno de Mesquita.

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Thanks for tuning in. Until next time!