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Soil researchers dig deeper into dirt's complexity

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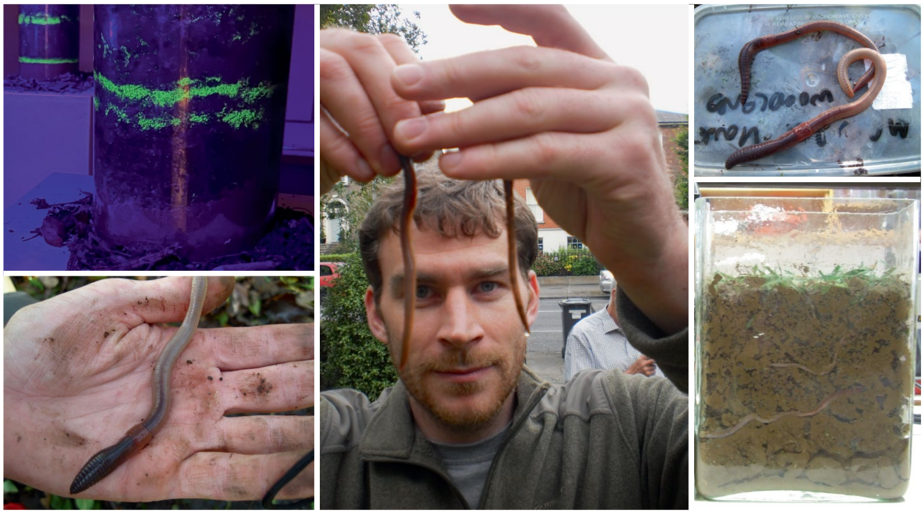
To study soil with a view to ecosystems, researchers iterate between experiments in the lab, the field and controlled environments.

By Vivien Marx

It was likely a rough day for Charles Darwin in 1881 when the satire magazine *Punch* published a cartoon of him looking morose, sitting outside, a book beside him and a giant earthworm hovering in front of him. As University of Colorado ecologist Noah Fierer notes[1], Darwin's book on earthworms became a best-seller just like *On the Origin of Species*. Burrowing, plowing earthworms, Darwin writes, have undermined monoliths and old walls, and "it may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organised creatures." A keen interest in earthworms is not solely a Victorian preoccupation. "I am endlessly fascinated by the impacts that earthworms can have on soils, their biophysical structure and functional processes," says soil ecologist Aidan Keith from the UK Centre for Ecology and Hydrology in Lancaster in northwest England, a non-profit research institute with centers across the UK. Along with nearly 150 researchers from 35 countries, Keith developed a global-scale atlas of earthworm distribution[2]. These apparently simple animals, he says, are largely responsible for dynamically shaping the soil of several ecosystems.

Soil harbors worlds of life: earthworms and nematodes, mites and ants, bacteria and fungi. A cup of soil presents around 5,000 taxa, says Wim van der Putten, a soil and ecosystems researcher at the Netherlands Institute of Ecology (NIOO-KNAW). Soil has physical and chemical traits, which are the classic domain of soil science, he says, while soil ecologists focus more on soil organisms. Facts on the ground are shifting these divisions[3]. Researchers bring a local and global mindset in the way they combine new and established approaches to characterize soil properties, plant–soil interactions, ecosystem dynamics and how to mitigate inflicted damage.

Climate change brings pressure from society and policymakers "to find solutions fast," says Alexandru Milcu, who directs the European Ecotron of Montpellier, a facility operated by the Centre National de la Recherche Scientifique (CNRS). He is also affiliated with Centre d'Ecologie Fonctionnelle et Evolutive. In the Netherlands and Belgium, intensive agriculture, car exhaust and factory runoff have heaped nitrogen onto soils, says Ciska Veen, soil and ecosystems researcher at NIOO-KNAW. Soils are acidifying, which kills forests. Birds have calcium deficiencies that make their eggs and bones fragile. The European Union is tasking its member states to address these issues. "Nature is really suffering," she says.



Aidan Keith and researchers around the world built an earthworm distribution atlas. To gain a more systematic and quantitative understanding of soil bioturbation by earthworms, he is setting up a global research collective on new data syntheses and field methods. Top left: a photo from an exhibit in which he collaborated with artists.

CREDIT: A. KEITH, B. BOOTS

Soil biodiversity

Veen and colleagues experiment in lab vials, in greenhouses, in small and large controlled environments and in the field. Metagenomic analysis is applied to soil analysis as it is with ocean microbial communities. But it's harder with soil, says Milcu, given how much more heterogenous soil is than the ocean. Studying ecosystems takes long-term observation, perturbation and data collection, "because it's very dangerous to generalize from what happens on a short term as a perturbation."

A systematic quantification and understanding of bioturbation across ecosystems is needed. At this year's Global Soil Biodiversity conference, Keith ran a session on this knowledge gap, and he and others are setting up a research collective focused on this. One approach to capture soil biodiversity is to assess a large number of samples from many locations. In different biomes, researchers might tally typical and atypical ranges of organisms. Then, to compare and infer, "Context is key."

Functional indicators are also used, says Milcu. It's about "who is in the soil and what are they doing," says Veen. Findings can come from assessments at all scales and that can involve nutrient flux and water cycling, carbon storage and greenhouse gas emissions. Different indices of soil biodiversity exist because, Milcu says, scientists are still deciding what's best for predicting ecosystem function. Functional analysis is time-consuming. Species numbers can generally indicate health and functioning of an ecosystem.

The lack of standards in soil biology had been lamented for years, says Diana Wall, an ecologist at Colorado State University, and it's set an avalanche of activities in motion. She co-directs the Global Soil Biodiversity Initiative (GSBI), which seeks to bring together findings on soil biodiversity and ecosystems. GSBI connects to a group of observatories; the global Soil Biodiversity Observation Network (SoilBON) is one that Wall co-directs with Carlos Guerra of the German Centre for Integrative Biodiversity Research (iDiv).

One GSBI project is assessing soil at 900 sites around the world. From the many soil samples, iDiv teams extract nematodes and ship them to Colorado, where Wall and her team count the nematodes and figure out their role in the soil's food web. "We go through and separate them just morphologically," says Wall. Nematode mouthparts show their eating habits. Some eat bacteria, others fungi; some are predators, others omnivores. Mass spectrometry can be used to learn how much carbon or nitrogen passes through these animals to the soil. Add molecular data, other data from around the world and such soil analysis becomes global ecosystem analysis.

Lab, tiny pots, field

"We try to go through this circle from field to lab, greenhouse and then back to the field," says van der Putten. The smaller scale, says Veen, lends control to precisely manipulate specific factors. "That can be very nice if you want to untangle what the impact of certain factor is," she says. With soil samples taken from the field to the lab, soil's layered structure goes missing, and with it the option to study how the physics of soil shapes water transport within. It's better to work on soil with inhabitant species interacting across intact layers.

In soil science, says Michael Strickland of the University of Idaho, "we're making things oftentimes with PVC and duct tape to do experiments in the field." In controlled spaces called ecotrons, which can be open-air or sealed environments, sophisticated instruments continuously measure gases emitted from soil, such as CO_2, methane and nitrous oxide. Physics knows heavily instrumented facilities, but ecology less so, says Milcu. Quick experiments in a lab's climate chamber often suffice. Ecotrons are more expensive and time-consuming to use, but they yield ecosystem-level context for smaller-scale studies.

Keith wants to understand behavior, activity and impacts of earthworms in the field, where there's less control over environmental conditions. "We can't resample the same location without having altered it," he says. Thus, fieldwork should be complemented by ex situ experimental systems so a researcher can quantify and model events and change conditions. He is happy about emerging methods that characterize earthworm burrowing and soil porosity. It's still early days for non-invasive measurement approaches such as tomography-based techniques to assess soil microfauna, says Milcu. But such assessments can shape findings about how earthworms might be changing soil porosity. "There is a big debate, actually, in the literature at the moment," he says. Perhaps, according to a meta-analysis, earthworm activity stimulates emission of greenhouse gases such as carbon dioxide and nitrous oxide. He generally likes meta-analyses, says Milcu, but available data can introduce bias. He and colleagues drilled into this finding[4] and found that the analyzed studies had mainly involved small pots kept in darkness, without plants, at constant soil moisture levels. "That is extremely artificial," he says. Ecotron experiments completed over longer time frames showed that earthworm activity might actually decrease soil emissions. That may be due to soil moisture changes over those time frames, he says, which "you cannot recreate in a realistic way in a tiny little pot."

Enter the 'tron'

Especially with longer-term studies, changing field conditions can hinder finding patterns and disentangling mechanisms, says Milcu. The controlled environments of ecotrons are not the only way to go; simplified model systems can scale up well. Yet reality can't be oversimplified, "because what we observe may not be generalizable, or it may not scale up very well."

Ecotrons are "still not the real world, but they're already quite close to it," says Veen. In ecotron soils, scientists can measure and manipulate species interactions and get soil science experiments done with replicates. In their close-to-reality conditions, says Wall, ecotrons offer ways to avoid fieldwork's many confounding factors. Many researchers in the Global South may lack access to an ecotron, but a grant can help them access one, she says, with collaboration and information exchange helping all to benefit from experiments done in large and small contexts.

Globally, ecotron facilities are limited. They are expensive to build and maintain. Not all scientists can avail themselves of ecotrons; it's logistically challenging to travel to one, and it takes time and resources, says Veen. "Every approach has its advantages and disadvantages; that's why we also use all of them," she says. NIOO-KNAW has set up a 60-unit soil ecotron with clay, sand and peat, the three dominant soil types in the Netherlands. From different regions, the researchers removed 60 soil cores one meter in diameter and one meter deep with layers intact. "Now we do all kinds of manipulations and measurements on them," she says.

Alexandru Milcu (upper left) directs the European Ecotron of Montpellier. Scientists can do various types of experiments in the controlled environments that micro-, meso- and macrocosms provide. All units have automated measurement of gases from soil. One project underway is to compare the carbon sequestration effects of adding of biochar or basalt to soil.

CREDIT: EUROPEAN ECOTRON OF MONTPELLIER

In a review[5], Milcu and colleagues describe 13 ecotrons in Europe, Australia and the United States. At the Montpellier facility he directs, fluxes of oxygen and major greenhouse gases, such as carbon dioxide, methane and nitrous oxide, are measured automatically. The macrocosms are 12 units of 20 m^3 with controlled atmospheric conditions and soil up to 2 meters deep; 18 mesocosms are 4 m^3 each; and 13 microcosms are 2 m^3 each. The transparent domes of the macro and mesocosms let in daylight. The microcosms have plasma lamps or LEDs. At the facility, experimenters can track events such as photosynthesis, respiration or microbial activity and use optical lasers, infrared systems and portable gas exchange systems to capture ecophysiological data. Using ^{13}C labeled CO_2, they can track carbon as it cycles into plants, organisms and soil. It's a method the community has been using for a while, "but we're using it at a different scale," he says.

Each ecotron has its own tradition and focus, says Milcu. At the Montpellier ecotron, researchers from anywhere, not just France or Europe, can submit a project proposal, but it cannot involve invasive species or genetically modified organisms. He and a colleague at a different ecotron are setting up an experiment related to carbon sequestration. They will assess whether biochar, which is pyrolyzed plant residues that decompose slowly, or basalt enhance soil-based carbon sequestration and how soil biodiversity and soil fauna are affected.

Plant–soil interaction

Invasive species displace others by growing fast and usurping resources from native species. Drought seems to hit invaders harder than natives, but a closer look reveals complexity, says Yanjie Liu from the Northeast Institute of Geography and Agroecology of the Chinese Academy of Sciences in Changchun, China.

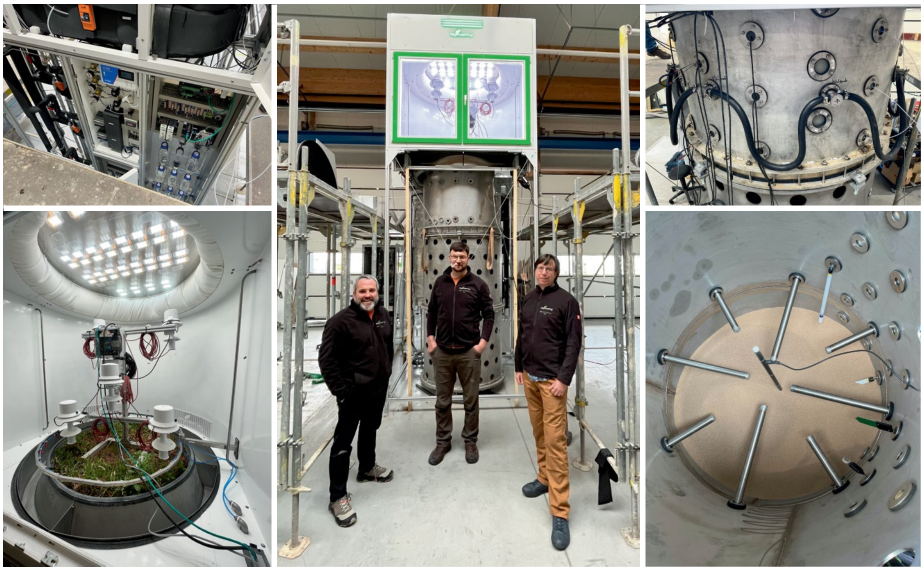
"Although ecotrons are an excellent alternative for simulating environmental and biota changes, we do not have them in our lab," he says. Liu and team do experiments in greenhouse pots and in field tests. In controlled field experiments they assess roles of soil fauna and microbes in plant community dynamics. One experiment still underway has plots with different conditions, such as soil lacking herbivores or pathogens. Most studies, says Liu, focus just on drought and neglect rewetting, which frequently follows natural drought. When drought doesn't kill invaders, subsequent rewetting actually gives them an edge over native plants, he says.

Studies on invasive plants have long ignored the influence of soil biota. He and his team compared growth in live soil and in sterile soil, which has been treated to lack microfauna and microbes[6]. In live soil after drought and rewetting, invasive plants recover faster, and that indicates this importance of soil biota[7]. The team is also combining controlled experiments and metagenomic analysis to tease out the roles of specific taxonomic groups and how individual soil biota factors underpin plant invasions. This can deliver insight about global environmental changes, too, says Liu.

Approaches are needed that look at ecosystem impact and intermingling factors, such as, in this case, drought and rewetting, says Veen. Factors can't all be studied at once, and when you start adding them to experiments, this complicates setup. Higher sample numbers are needed, and the combinations of variables to consider rise exponentially. Says Veen, "this is a challenge for the field."

Deep soil experiments

Zachary Kayler from the University of Idaho, who was interviewed jointly with his colleague Strickland, has focused on soil's biogeochemistry and is intrigued by plant–soil interactions and the way plants pump carbon below ground to drive nutrient cycles. "The pivotal piece to that is microbial," says Kayler.



The Deep Soil Ecotron is under construction on the University of Idaho campus. Michael Strickland (left) and Zachary Kayler (right) are the two principal investigators of the project, which is a collaboration of several universities and PNNL. Ben Harlow (middle) is technical engineer. The facility, with 24 units, is slated to open in 2026 and will focus on studying soil up to three meters deep and apply many types of instrumentation.

Microbes might be involved in organo-mineral interactions, but the question is, "How much does it matter? And we're always looking for that impact," he says. That also entails working with earth system modelers. Strickland and Kayler hope the Deep Soil Ecotron — slated to open in 2026 and currently under construction on the University of Idaho campus — can help with such questions. To their knowledge, no current facility studies deep soil, which is three meters deep. They are principal investigators of the ecotron, run jointly by the University of Idaho, Universities of Hawaii, Delaware, Alaska and Wyoming, University of Colorado Boulder and Pacific Northwest National Laboratory (PNNL).

Says Kayler, soil science, field system science and ecosystem ecology are rather data poor. He recalls how ecologist Alan Knapp of Colorado State University once pointed to the Large Hadron Collider's enormous data output and contrasted it with a five-year drought experiment, which might have just five decisive data points. These data shape next scientific steps and conversations with policymakers and those who apply findings. The Deep Soil Ecotron, says Strickland, with its 24 controlled environments, gives researchers replicates and a way to assess multiple factors. Its custom-engineered units are metal sleeves, slated for delivery this fall, with sensor ports running all along the sleeve's length. Strickland and Kayler have been working with companies on instruments to slot into these ports, such as X-ray technologies, ground-penetrating radar, acoustic approaches and different types of gas sensors.

Among the experiments scientists might do, says Kayler, are ones that measure soil porosity and assess soil fauna. They might track gas emissions and, using ^{18}O and ^{15}N, track carbon and nitrogen as they travel through the food web. They might apply tomography to assess roots branching into the soil. They can dial in soil moisture and temperature conditions. They might set a five-degree temperature increase at depth or a drought and then continuously monitor and measure. They can set a contained fire and explore impact on vegetation and soil, measure microbial transport during pyrolysis and combustion, and see how much of the pyrogenic material, or black carbon, ends up in the soil.

Planning and then running a facility will take up plenty of Strickland's and Kayler's time, but they will keep doing their own research, too. Strickland plans to use the Deep Soil Ecotron to study the dynamic processes of soil microbes at depth, beyond the typically studied depth of ten centimeters. Microbial communities are known to be more sparse at depth, but their roles are not well understood. How might a perturbation at 10 or 30 centimeters depth shape deeper events? Might below-ground systems lend resilience to an ecosystem affected by drought, heat or other shifts? For metagenomic analysis and transcriptomic profiling, University of Colorado's Fierer is developing data analysis pipelines to be deployed at the ecotron. When the facility is ready, scientists will be able to submit proposals to use it.

Scientists tend to be careful, says Veen, "sometimes maybe too careful, because we know everything's context-dependent." But there's urgency to fix problems. "We do have a lot of knowledge already," she says, which can inform ways to steer systems that mitigate damage. Experiments will continue to keep addressing the many remaining unknowns. Much can be measured but plenty still cannot.

Ecologists have long assessed plant community composition, says Veen, and there's been a shift toward exploring how these assemblies function. Soil science is also pursuing function questions by, for example, using techniques to trace elements in the soil. It will be exciting, she says, to link isotope-based element tracing with metagenomics and in situ techniques to continuously measure soil processes and overall soil biodiversity. It's about "not only seeing who's there, but what are they actually doing, because for me, that in the end is the most interesting part." And it will help to assess which carbon from above ground is entering the soil and staying there.

With the many experimental opportunities and a variety of tools, says Wall, researchers can now characterize larger effects of climate change such as how carbon and nitrogen are processed through soil animals and microbes or how water filtration in soil changes, among many other ecosystems aspects. Soil scientists and biogeochemists have long played together, she says; now the collaboration includes those who study soil life, such as soil biologists and soil ecologists. "Finally we've gotten to the place where we are just like above-ground ecologists and terrestrial ecosystem people or ocean ecosystem people," says Wall. "That's why I think it's such a rapidly moving science: it's that everybody has to play together."

Vivien Marx ✉
Nature Methods.
✉e-mail: v.marx@us.nature.com

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CREDIT: DEEP SOIL ECOTRON