

[1] Hi, my name is Michael Braus. I'm going to be talking today a little about a property that you're all quite familiar with called "soil pH". It's something I've been studying for the last year or two, and I'm just going to share some of the findings that I've gathered for my doctoral research.

[2] The first thing I'd like to do is thank the people who have made my participation in this amazing conference possible in lovely Helsinki, so kiitos to my department at the UW-Madison, USA, as well as my doctoral advisor, Dr. Thea Whitman, who has just started her lab and it's going really well. As well as the USDA that's funding part of my doctoral research, and of course FEMS for the Early Career Scientist Grant, which has been very useful and helpful. So thank you very much.

[3] What I've noticed in the literature is that there is a very large gap in this observation that we're all familiar that soil pH is what influences soil microbial communities, but that there is also something going on on the microbial level that is causing this, right, there is this mechanism happening in between, but it's kind of unclear what that is. What I'm suggesting is that we incorporate a more methodological approach to bridge this gap, and that's in the form of this property that I call "in situ soil pH".

[4] Just to review some things you've seen before, Delgado-Baquerizo and others just this year confirmed that pH, this property soil pH, is determining, sort of, setting up ecological clusters, showing what type of structure you find, diversity of what microbes, soil bacteria, and other microbes are found in the community.

[5] This is actually confirming another study, from the Fierer lab, that basically says that different bacteria have different optimal soil pH, but the extreme-living bacteria or organisms in this case are less diverse, and this is something we've seen quite often.

[6] However, there's a problem where I often find myself when I read these kinds of papers where I have more questions than answers, right, like why is this happening? How does it happen? How much? What exactly is soil pH if you're a microbe, right? And why should anyone care?

[7] So, there are a number of reasons why it's very important to understand pH, because most bacterial physiology, as well as your physiology and the physiology of pretty much everything, runs on proton gradients. So, if you want to generate ATP, you need to create what's called a protonmotive force and that involves creating a gradient between an acidic exterior and a very basic interior. So, when you have this gradient protons will want to move across and that can do work, right, so for bacteria it's good for making ATP, running flagellar motors, active transport. It's extremely important. And this soil pH property right here, this external pH, which is technically the periplasm right here, for people familiar with the anatomy of a microbe or of a bacterium, but it's found that the exterior solution and the periplasm are quite similar. So, that's what we're trying to get at, because it's very important to their physiology.

[8] So, how is standard soil pH measured? It's very simple: you take equal parts soil and water and mix them together and let them settle, and you take this instrument, which I'm sure people have seen before and used, called a glass pH electrode, and at the bottom here it's actually a brilliant design, a small thing silica bulb that's hydrated, so the silica is hydrated glass. And when it interacts with your test solution it's basically saying here's the test and here's the

reference with liquid inside that tells you what the potential is between those two. It's really not an electrode but rather what they call a potentiometer; the little needle will go back and forth, and when calibrated it will tell you the pH of the test solution over here. And over course the sediment below is soil. Very interestingly, you'll find that--what's actually inspired some of my work--there's thing called the "suspension effect" whereby if you take this electrode and dip it into the sediment, the pH changes. The potentiometer actually ticks a little more acidic, which is a little confusing, right? If this is a pH and that is a pH and they're all in the same jar, why isn't it the same? So this suspension effect is still a little bit up for debate, but it probably has to do with something called the salt bridge. There's a tiny glass frit in most of these electrodes, and if you have salts that are exchanging in part with particles of the sediment, it'll actually confuse the electrode. And this is a very slow process (the measurement) as well, so you can imagine using this is not exactly as efficient and is prone to error.

[9] Now, I really want to mention some quickly that was rather baffling to me, but hydrogen ions--which are technically part of the definition of pH, the negative log of hydrogen ions--they don't exist in water. I think that this is something we need to revisit if we really want to get to the bottom of this process. So I just wanted to point out that when you see this in some of these textbooks as concentrations, they're not exactly accurate to the phenomena of pH. It's more like this, where a proton can jump from its attachments to a hydronium ion, which is essentially a cation of water, and it'll jump to water in these sort of wires, and that's where this acidity comes from. That's where this exchange comes from. It's not a concentration; it's an activity. This is going to come up.

[10] So, most soil thermodynamics boils down to having these 6 properties of all the different solutes and particles and essentially elements you find in soil, and the one I want to talk about here quickly is chemical potential because in the current convention of soil thermodynamics and biogeochemistry, protons have a chemical potential of zero. I want you to take a moment and contrast that with what I've just said about protonmotive force. So all the ATP that you're generating and all the ATP that bacteria are generating use a protonmotive force, so there's got to be some sort of potential of the proton, right? But in biogeochemistry, it's zero. I think that's a contradiction, but I'm still trying to figure out if I'm getting lost in some semantic detail.

[11] Anyways, I referred to the CRC Handbook and basically it confirmed what I was thinking and learning about the physical chemistry of pH, saying that pH is not exactly a concentration but this activity, and this activity is based around essentially the activity coefficient here, γ_{H} or rather $\gamma_{\text{big-H}}$. This is an extremely important property that I'm trying to figure out right now as well because I think it's based on the Debye-Huckel theory of activity, which only applies to like spherical, stable anions and cations. So if you're a sodium molecule or a sodium ion, you follow the Debye-Huckel theory of activity. However, if you're a proton, and you're hopping from molecule to molecule, it doesn't really apply. So, it would like the whole foundation of pH is a little rocky, which causes a lot of problems for people like me, who want to know the actual in situ pH of soil.

[12] They also mention a very important thing about pH in that it's not a physical

property, right, it's all derived -- or sorry, it's not even a derived property, it's an operational definition. So there's a standard electrode, and that's the thing that's used to create traceable calibration solution for all the pH electrodes that are used around the world. This is largely produced by the National Institute for Standards and Technology in the US, and there are other traceable solutions around the world. Anyways, it's not a property like length or mass or something. I think it'd be nice to kind of improve that, maybe give activity some kind of SI units to make it useful to us.

[13] What this boils down to, and where the connection is for what we work on, is this causality cycle. If you look at all the properties of soil, they kind of cycle around each other: one property change, then another, then back to another and back and forth. Right here the properties of the solution has a lot to do with its ionic strength. This is a property that's basically how many ions or how many active things are in solution: clays, organic matter, it's all very important. And if you raise the ionic strength too high, your pH electrode isn't going to work.

[14] If you do some of the math, some of the back-of-the-envelope calculations of what's actually in soil at normal water concentrations, so not a slurry, not like the standard way of measuring soil pH, just regular old water in hydrofilms in soil, you'll find that it's more like a brine. It's not exactly water and that really violates the ionic strength threshold for measuring pH. All these things are very important, right?

[15] Add that the fact that CO₂ is an extremely important factor in the acidification of water, right, so if I had a glass of water and the atmosphere in here is probably higher in CO₂ than the regular atmosphere, probably like 600 or 700 parts per million, maybe a thousand, it's actually going to acidify the water. If you have a water, it's not a pH of 7 unless you boil it or bring it down to a CO₂ that's more normal, and this is apparent in the actual literature when they were developing methods for soil pH. If you would have a high CO₂ environment, the in situ or fresh sample is probably going to have a different pH than what you measure with your pH electrode under the standard protocol.

[16] Then add this to the fact that dilution effects are non-linear. If you put a bunch of water in soil, it's not like it's going to dilute the way you make an isotherm of something like ammonium or calcium or sodium or something, right? It's very, very dynamic, and it's responsive to temperature. This kind of goes back to the fact that it's an activity, not a concentration, right? So if you get it colder it gets more basic, I think. That's a little strange.

[17] So I took all these factors and I put them into one diagram. It's looks a little complicated, but really the only thing that's new is you have these major bodies interacting with the solution. Soil atmosphere is depositing CO₂, bacterial cells are doing both chemiosmotic protonmotive force, which I'd mentioned, as well as just regular proton-pumping to maintain a neutral internal environment, and there's also exchanges of protons on soil particle surfaces, and these wires, what they call water nanowires occurring inside the solution itself. All of this is completely lost when you dump water onto soil to do a standard soil pH measurement. But this is actually what the bacterium is experiencing.

[18] I want you to zoom out a second--we zoomed way in and when you zoom way out--you can say to yourself, what would big studies like this--this is a study by Eric Slessarev, really fantastic work gathering a lot of the data from around the

world to correlate things like mean annual precipitation and soil pH--what if they used in situ pH? Given the problems I've mentioned about standard soil pH? That's a big question that I'd like to start answering.

[19] There's very few papers that have actually tried this. I've only found I think 3 or 4 of them in the last 20 years. But mostly what they find is that the in situ pH is generally more acidic than the standard soil pH, and sometimes it's just super variable, depending on the texture.

[20] A lot of this work has been done in archaeology as well. They really want to know what pH their artifacts are actually experiencing, but if you excavate--like in this paper by Matthiessen, 2006--if you excavate you actually just degas all the CO₂. So when you take your measurement, you're not getting the exact pH that you really want. If you have soil at 2 or 3% CO₂, that can really change the pH, up to a unit.

[21] I found a book actually about this, or in part, and this was a book written during the development of the standard soil pH measurement method itself. What they did was they took a lot of soils and they started measuring soil pH at different soil water content, to get an idea of where they want to put the standard. They chose 1-to-1, but it's actually [wincing] it's not really representative of what a soil microbe, for instance, would experience under unsaturated conditions, which most soils are. Here (left) this is the really interesting spot where the pH dips down, depending on the soil.

[22] I conducted an experiment in a soil from northern Wisconsin, and what I found was very interesting:

[23] The A horizon dropped quite a bit, so the actual pH, the more the in situ pH, was much lower, but, interestingly, the B horizon stayed the same. No single model is going to solve all your problems if you want in situ soil pH calculated from that.

[24] I'm going to continue this across Wisconsin, which is in central United States. What's cool is there's a pH gradient of standard soil pH, so I can collect from many places.

[25] And then I'm also very excited about developing new methods of pH measurements using fluorophores, which are widely used in microbiology but not widely used in biogeochemistry. This gets around a lot of the glass electrode problems.

[26] (ORGANIZERS: "We have to stop here.") You guys got it.