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Title: The Divergence and Significance of in Situ Soil pH in the Bacterial Ecology of Wisconsin's Soils Compared to Standard Soil pH.

Am I the first talk of the conference? I don't know, there were probably sessions before this, but if it is then it is quite an honor. Thank you so much, and thank you for being here.

My name is Michael Braus; I'm a soil scientist, a soil microbiologist/biogeochemist at the UW-Madison in Madison, Wisconsin, and I wanted to share with you some of things I've been working on the last year or two related to soil pH. As it happens, I was looking through one of my books -- I read many-many books -- about pH and chemistry from the last 100 years or so, and there's this fantastic cartoon that summarized sort of my Gestalt as I'm going around the lab. I would talk to people on and on about all kinds of pH metrology. Meanwhile, they have these really practical problems that I'm not seeing to, right? So one of my goals today is to help convey some of the major, key findings without getting too far into the weeds here.

Soil pH is a property that has been measured for a very-very long time, since 1900 or so. Around 1900 they developed the idea of hydrogen ion concentration, which ostensibly is what pH is supposed to be measuring. Soren Sorensen, this chemist -- he was actually a brewery chemist working in Europe -- is who came up with the pH concept; he got sick of writing 10 to the negative 7 every single time so he just said "the power, pH", right, thinking very practical. Then, right after that, Hoagland and Sharp picked up on this in the application to soil chemistry. Well, if you can find the pH of a solution, why not make a soil slurry? So you put some soil and water together and just measure the supernatant. Put one of these glass electrodes in, should give you a value.

An important change occurred between -- around 1930 to 1950, and they regarded hydrogen ion concentration as a bit fallacious. Instead called it "activity". This is something I'm going to come back to, just keep that in mind. And microbiologists picked up on this as well, finding that bacteria and soil bacteria as well, like we've recently found and validated, were very responsive to soil pH as one of the parameters that determines in large part but not in full part much of the diversity that we see in the wild. The smiley faces are something I added, by the way.

One of the predominant theories for why this is -- we always have to ask the question why is pH influential to bacteria -- turns out it has to do with the production of energy as ATP on their cell membranes. Just like our mitochondria, actually, if you're in the environment, the pH of the cell has a pH that is maintained energetically and the outside is variable but typically the cell will actually produce hydrogen to push out into the environment to acidify this area so that it flows back in it moves across this motor turning it and generating ATP. Now, when you take the context of pH in soil, this is what it looks like. There's this in situ pH that is the influential actor here in this equation, which creates energy, yes? And so, if you know this value, you know something about the energetics of bacteria in the soil.

The reason why I call it "in situ soil pH" is because I think that I found that the actual value -- the actual pH that bacteria are interacting with -- is different from the standard soil pH -- this method that was developed in the early 20th century is not actually what they're experiencing. Note also here that there's CO₂ being produced in this process quite often in aerobic conditions.

The theory of pH is quite important because the shift that has occurred over the last 100 years -- it being a concentration to it being an activity -- is something that is a little mercurial but is essential to understanding what is actually going on in soil bacteria. This on the left here, the typical definition that you find in your textbooks, is not correct. The reason why is because hydrogen ions are not exactly stable in water. There's a fleeting little moment when the hydrogen ion is moving from one molecule to another, and it's never at any point solvated, never sitting on its own, right? So this definition is not correct on a purely mechanistic level. But that's okay, because you can still apply the values that you get from pH you get back and instead call it the activity. And that's defined with these equations here. So you take the log 10 of the activity of hydrogen ion, which is just a shortened term for hydronium, for lack of a better term, and what it allows for are particular conditions that would change pH. So, a great example is here it says that at a pH of 7 at 25 degrees Celcius you actually, if you raised it by degrees you would change the pH. The "hydrogen ions" -- quote unquote -- would be more active. This shift, this autoionization, occurs more quickly in the water and becomes more acidic. This mechanism is called the Grotthuss mechanism when one proton moves to another.

In our lab, we turned an anaerobic chamber into a CO₂ chamber; you can essentially elevate the CO₂, and we put a pH electrode in there so you can have some fun experimenting with this. So if you allow CO₂ to rise in an environment, which is typical in soils, it's not like the air we're breathing here -- it's higher, maybe 2 or 3 percent CO₂. If you raise it up, you see a very interesting change for the basic solution. The red lines up here are a simple sodium bicarbonate solution. These yellow-ish-looking lines are pasta water, sodium chloride. And then there's an acidic solution made of regular vinegar, acetic acid of 4 percent. What you find is that if you dilute it by 10 all the way back, you find that the CO₂ becomes more influential in acidifying the solution. And, if you have something is very-very concentrated, the solute of acids and bases are influential. Now, the reason why this is important to soil is because when you're making the slurry for the pH, what you're essentially doing is diluting the soil down to the point that you don't really know if that's actually representative of the soil solution. Soil solution is what soil bacteria are actually interacting with, right? So, right in here, this is typically the ionic strength of the molality that you find in soil, is right at that point that you find factors like CO₂ and dilution being most influential. The rest of these plots, just to point out, are the identical plot to this, all the same data; all I've done is I've exponentiated and logged the values and all their different machinations. So, when you're looking at these types of plots, just as a "pro tip", be careful with these axes, they'll throw you off when you're making inferences because, if you start drawing lines willy-nilly, it's hard to make inferences that make sense as to what soil bacteria are actually experiencing.

If you dive in a little deeper and you actually ask the question "What is pH?", what you yield is something called the Bates-Guggenheim convention. This is essentially

saying, according to the IUPAC and NIST, which is the National Institute for Standards and Technology, the people who make pH standards and stuff. They use this inference system to go from something called a Harned cell to allow you calculate pH using standards that exist. The problem is that they're all based on a theory called the Debye-Hückel theory, and if you dig in the literature you'll find the paper it's from, pull from it the equation that is yielding this inference and what you find -- I don't have time to go into this in all its detail -- what you find is that it's highly reliant on the ionic strength as well as this a constant, which, for the life of me, I actually don't know how they determined. It's supposed to be the size of an ion, but if you have a hydrogen ion, which doesn't really exist in water, or only for the most briefest time, it doesn't really make sense for the situation, right? So essentially, all the factors that we have, all these assumptions that are built into the theory that pH is based on -- that soil pH is based on -- is "rocky"; it's extremely difficult to use, because it's like having old software running on new hardware, right? I think this is a big problem, that we need to solve this problem before we can move forward in even answering the question of why soil pH is so influential to soil bacterial community composition.

There are few options we can use. There's the first option, which is to use the Pitzer equations. These are essentially an extension of Debye-Hückel theory, if you see up here the function of I up there, that's essentially the ionic strength and the function is Debye-Hückel theory, right. What this does it is it takes the old theory, the one I was just talking about, and expands it. I'm not sure that's the right approach, but it's really all we have to work with right now, okay. Option two is something that I found in the older literature of Garrison Sposito in one of his books, where he presented this idea of proton cycling, I've relabeled it as "proton flow" as sort of a different view. It basically says that there are many parameters involved in yielding the soil solution's "proton condition" or "hydrogen ion concentration", but given that that concept is out of date, I've updated it to be mechanistically correct, right. I don't have a good way of measuring this, but this is I think how it works. So you have 5 different mechanisms in black here, protons doing sorption and desorption, carbonic acid dissolution, this chemiosmosis is what yields the protonmotive force, which I mentioned before, and proton-pumping just to regulate internal pH and then the Grotthuss mechanism that is just happening in solution. These are the five mechanisms; there are typically four pools: the atmosphere, the particle, solution, the cells or the life that we find in soil, and of course there are these pH values that you can gather. One is right on the surface of a particle, one is inside of a lot of cells -- I study bacteria but this applies to all different kinds of soil organisms -- and then of course there's this one in the middle, unstabilized external pH. This is the in situ soil pH that I've been working on figuring out. It's quite different from the paradigm where you just make a slurry and get a value.

My approach has been to take a core, make a good mixture of it, put it into a small tube, and then lower the amount of water that you add to it, right, so I have one that is a regular 1-to-1 solution and then I work down to maybe a 1-to-4 solution. 1-to-4 is a bit more like soil that you would find, maybe at field capacity rather than a slurry. Centrifugation is very useful because it spins out the water, or at least it makes the water into a supernatant, that I then pipet off into a very-very tiny tube and then use a microelectrode to measure. I also elevated the CO₂ using a "volcano reaction" -- never thought you'd use that again, right, remember that from

the old science fairs as a kid -- very useful if you have a chamber -- this is what it looks like. If you have an anaerobic chamber, what you can do is (I don't have a pointer) you can have a computer running a CO₂ meter, so that measures how much CO₂ is in the atmosphere. You have a little vinegar and baking soda reactions going with little aliquots of baking soda so it doesn't overflow. And it elevates the CO₂ in such a way that your samples have been equilibrated. It essentially turns an anaerobic chamber into "a big macropore"; it resembles soil in the strangest way but it works really well.

What soils do you work with? This last summer I traveled all over Wisconsin, which fortunately has a huge gradient of soil pH -- these are standard soil pH values. So if you go the southeast it's a little more on the basic side; if you go the far northwest and the north, it's very acidic. So I went and dug 23 or so and I got about 65 horizons that I submitted to our local agricultural research station for analysis, and that gave us the standard soil pH values that we can work with. One of the goals was to get a big variety so that we can start testing different methods on these soils. And there's a great variety here, right. This the depth and the different sites of soil pH; it goes from anything about at 4 all the way up to almost 8, so perfect for validating a new type of method getting to the bottom of what's actually controlling this relationship between standard soil pH and in situ soil pH.

There was another paper that I found that sort of inspired some of this work, right at the development of this soil pH method that we still use today. In 1958, Jackson wrote an interesting book about soil chemistry. They essentially did the same thing where they were reducing the amount of water that they would put in and then measuring soil pH. They're a little unclear about how they did that, so I replicated it and this is just a preliminary study that I did last year where I found that soils actually responded differently. They didn't all become immediately more acidic than normal soil water content. They actually were all over the place, so it's not like there's just one equation that you can put all the soil pH values into and get out [the in situ soil pH] at the end of the day. Then if you add in this final step of changing the CO₂, you get an even more interesting picture, right. So I have 65 soils to work with, as well as -- I should have mentioned this -- in Spooner, Wisconsin, there's a special icon there because it has a pH trial that has a series of plots that have been treated to be a different soil pH, everything from about 4.7 to 7, right. It's well-replicated, it's been maintained for a number of years, and I went up there and I sampled three cores from each one of the plots; I'll also be doing an analysis of those in addition to all the soils that I got from Wisconsin. So if there are confounding factors with the soils of Wisconsin, because it's a huge place, that's okay because there's an experiment here that's identical in its location and nature and it's fully controlled because it's all the same soil, from the same site, and it's really well-documented.

The last thing I wanted to mention is CO₂ is a non-trivial factor. It is probably the most important factor in making any conversion from standard soil pH to in situ soil pH. Remember, knowing in situ soil pH is extremely valuable because this is the *real* pH that soil bacteria are using to generate ATP. Any little shift will throw off their energetics, which could be the actual selective pressure that they're undergoing. If you only take the standard soil pH values, which are represented by something like this -- see this red line here, that's a normal CO₂ concentration that you'd find in the lab. If you elevate the CO₂ and change it quite drastically and if

you lower the amount of water, you see that it changes in a variety of ways, right, and it's not really predictable yet if we don't look at a huge variety of soils. Thankfully I have a bunch of those sitting in a freezer at the UW-Madison.

Just a quick reference, too, that if you exponentiate pH it actually looks quite different to this, and I'm actually on the fence about whether I might just exponentiate pH permanently and just keep doing that from now on because having everything logged is a little confusing when you're making lines, like trying to make regression lines. You're allowed to make lines through logged values, but it's difficult to interpret. It might be time to kind of roll back what Søren Sørensen in 1909 did out of convenience, right; it might be inconvenient to put all the values down but it's more correct.

The sequencing that we have paired with this is very useful, which is just completed, actually in the last couple days. What we're going to do is we're going to take the value that a laboratory CO₂ of a 1-to-1 mixture, which is the standard pH, and we're going to compare that in terms of its predictive ability of soil microbial communities with elevated CO₂ -- normal soil CO₂ and normal soil water content. We're going to see which one is more predictive. My hypothesis is that the in situ pH is going to be the better predictor not a worse predictor, and I'm looking forward to see that [unfold]; hopefully we'll be publishing it soon.

Great, that's what I wanted to talk about, and I'll be happy to take any questions.