

Soil Health Testing: Status, Challenges & Future

Andrew Margenot

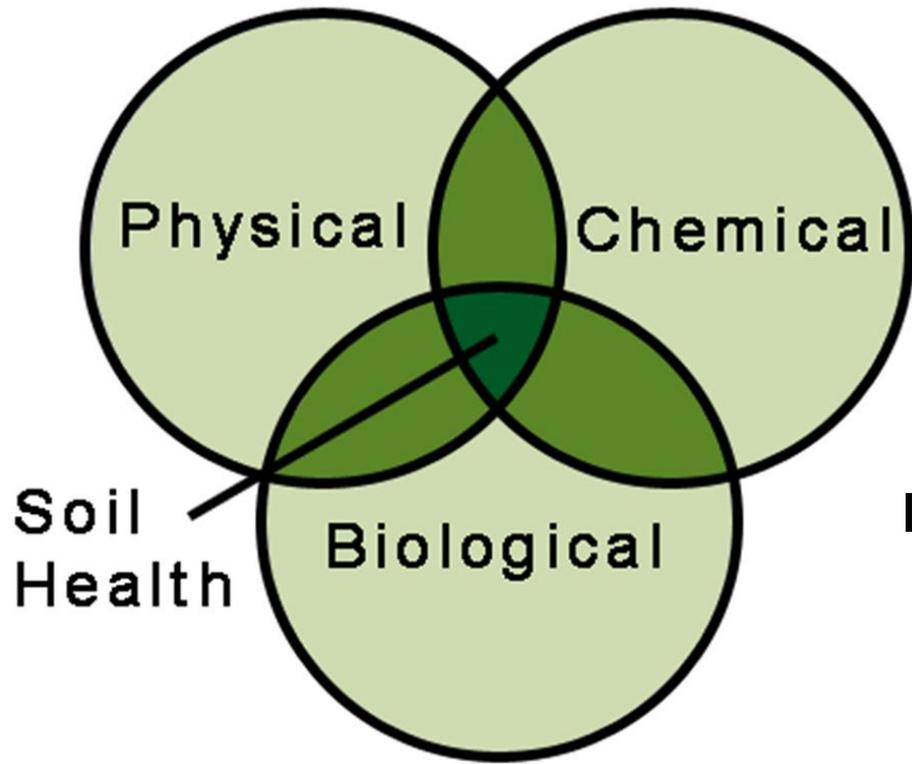
ALTA

Bloomington, IL

24 February 2026

<https://margenot.cropsciences.illinois.edu/>

Part I: Soil Health Conceptualizations



Indicators of soil health are classified as physical, chemical & biological

**Biological indicators are newest
– *and* least verified**



United States Department of Agriculture

Natural Resources Conservation Service

May 2019

Soil Health Technical Note No. 450-03

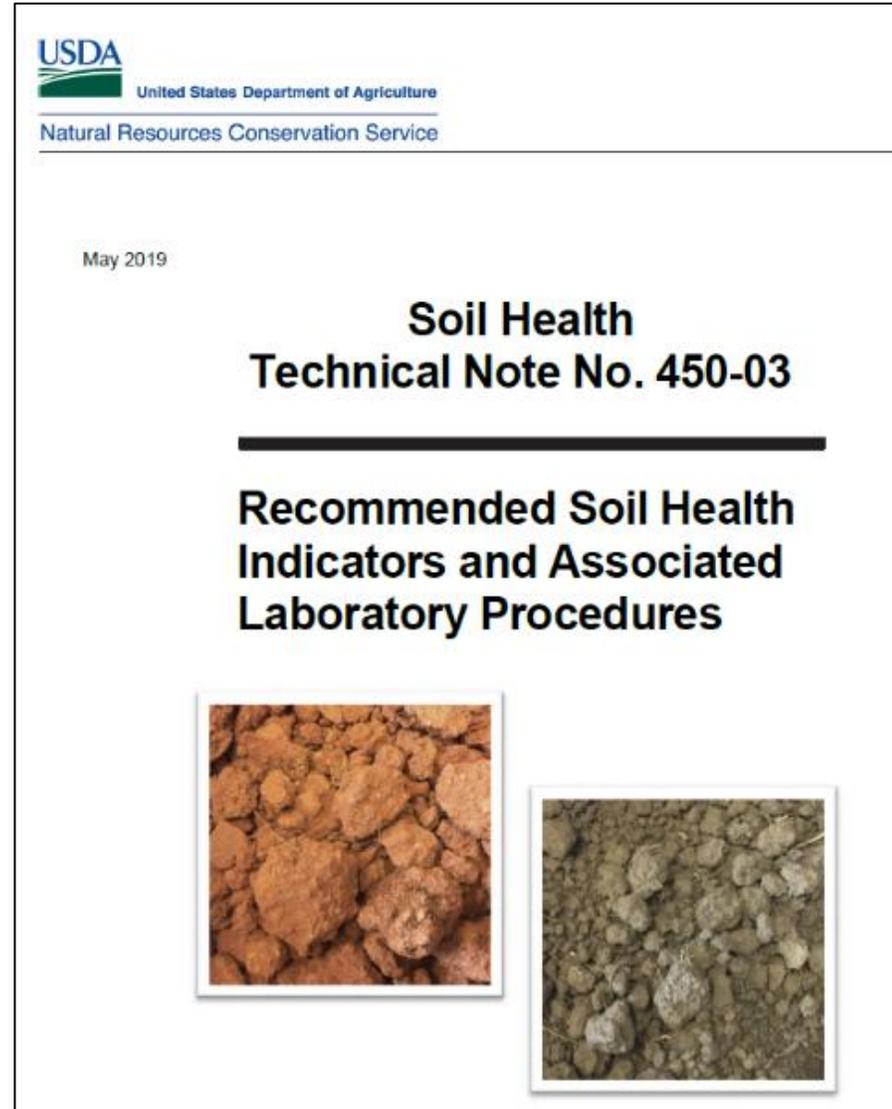
**Recommended Soil Health
Indicators and Associated
Laboratory Procedures**



Soil health “indicators”

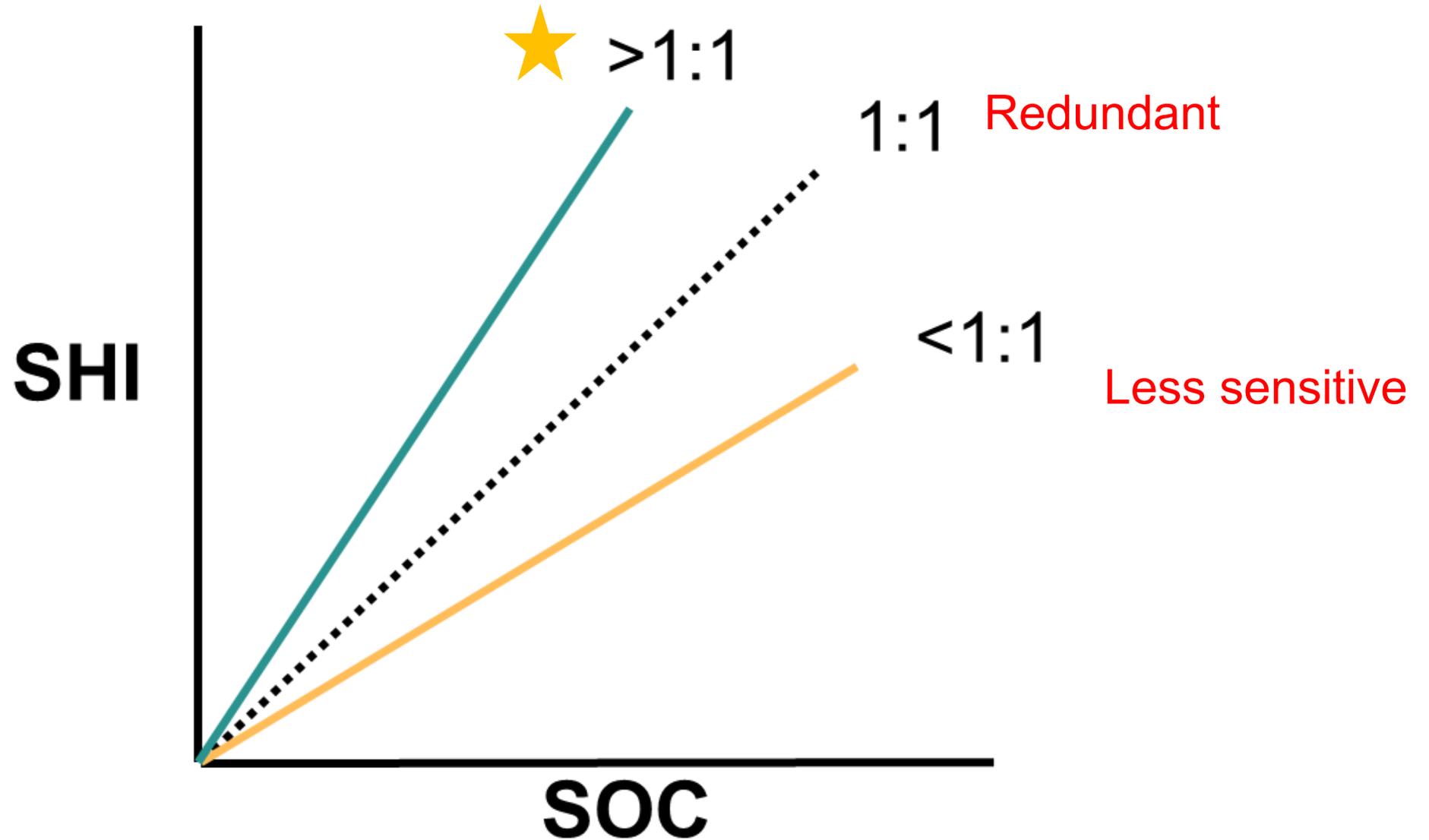
Biological indicators are newest – and *least* verified

- Permanganate oxidizable carbon* (POXC) / “active carbon”
- Enzyme activities
 - C-cycling: β -glucosidase
 - N-cycling: aminopeptidase, N-acetyl- β -glucosaminidase
 - P-cycling: phosphatase
- Aggregate stability
- Total soil organic carbon (SOC)
- Respiration



*Neither carbon, nor active or labile

The “sensitivity” criterion

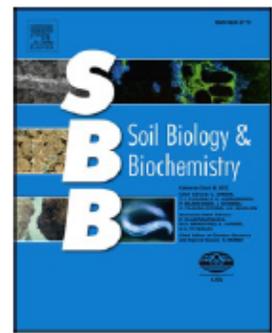




Contents lists available at [ScienceDirect](#)

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



Rigorous, empirical, and quantitative: a proposed pipeline for soil health assessments

Jordon Wade^{a,b,*}, Steve W. Culman^c, Caley K. Gasch^d, Cristina Lazcano^e,
Gabriel Maltais-Landry^f, Andrew J. Margenot^g, Tvisha K. Martin^{c,h,i}, Teal S. Potter^j,
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What we have (it's a mess)

a

Current state of soil health assessment

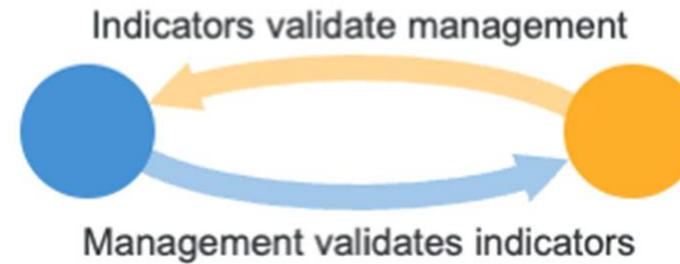


b Differing conceptualizations and approaches

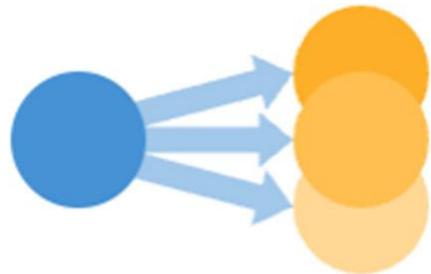


c

Circular logic



d Redundancy of indicators

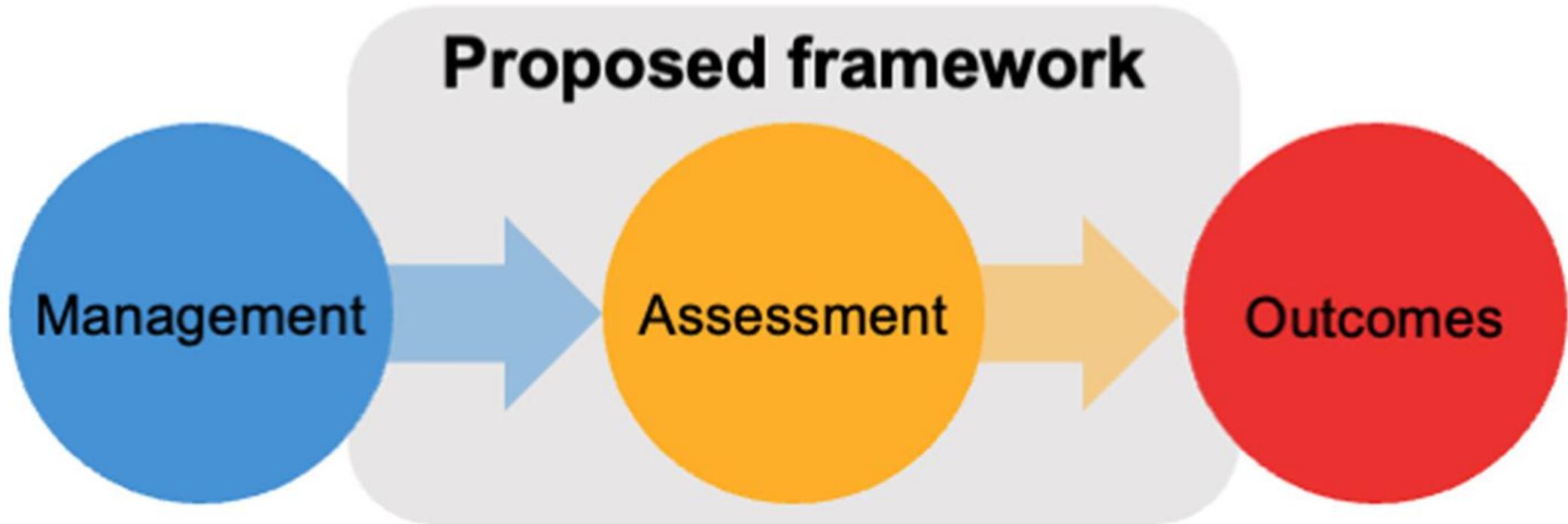


e

Management effects differ by environment



What we need



ECONOMICS OF SOIL HEALTH SYSTEMS
IN ILLINOIS



BUILD SOIL, BUILD LEGACY:

The Profitable Power of Year-Round Soil Health



HOLGANIX

ASK THE SCIENTIST:

SOIL HEALTH, FERTILIZER
REDUCTIONS, AND FARM
PROFITABILITY



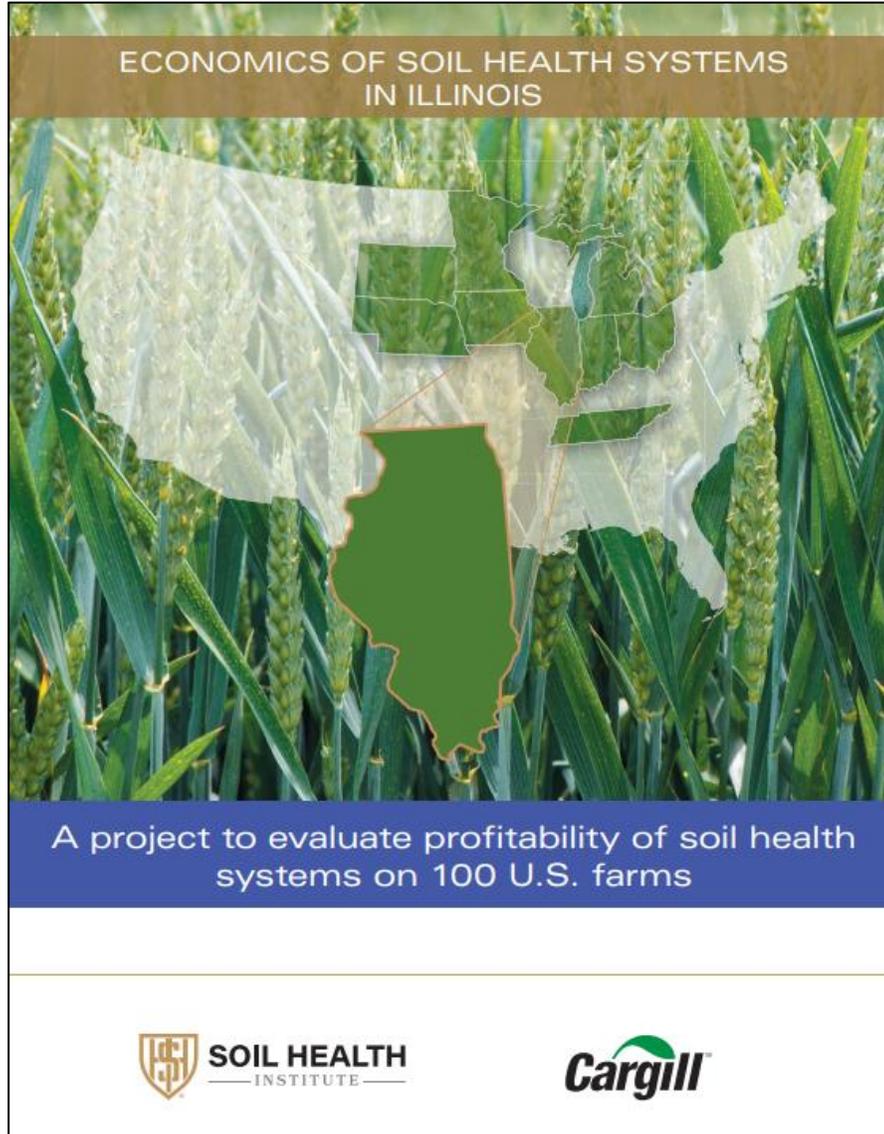
WATCH NOW

SOIL VS. INPUTS

OPTIMIZE SOIL
HEALTH, MAXIMIZE
FARM PROFITS



Economics of soil health: reduced inputs & increased yields?



Case study on 11 farms across Illinois

- 934 acres average
- 29% no-till and 43% reduced till
- 3% cover crops

Figure 1. *Geographic distribution of the 11 farms in Illinois used for economic analysis of soil health management systems.*



Economics of soil health: reduced inputs & increased yields?

CORN		
	Benefits	Costs
Expense Category	Reduced Expense	Additional Expense
Seed	8.64	22.30
Fertilizer & Amendments	28.91	7.47
Pesticides	13.26	6.65
Fuel & Electricity	3.27	2.63
Labor & Services	10.99	7.61
Post-harvest Expenses	0.00	2.37
Equipment Ownership	15.09	14.25
Total Expense Change	80.16	63.28
	Additional Revenue	Reduced Revenue
Yield, bu.	5.27	0.00
Price Received ² , \$/bu.	4.22	4.20
Revenue Change	22.24	0.00
	Total Benefits	Total Costs
Total Change	102.40	63.28
Change in Net Farm Income	39.12	

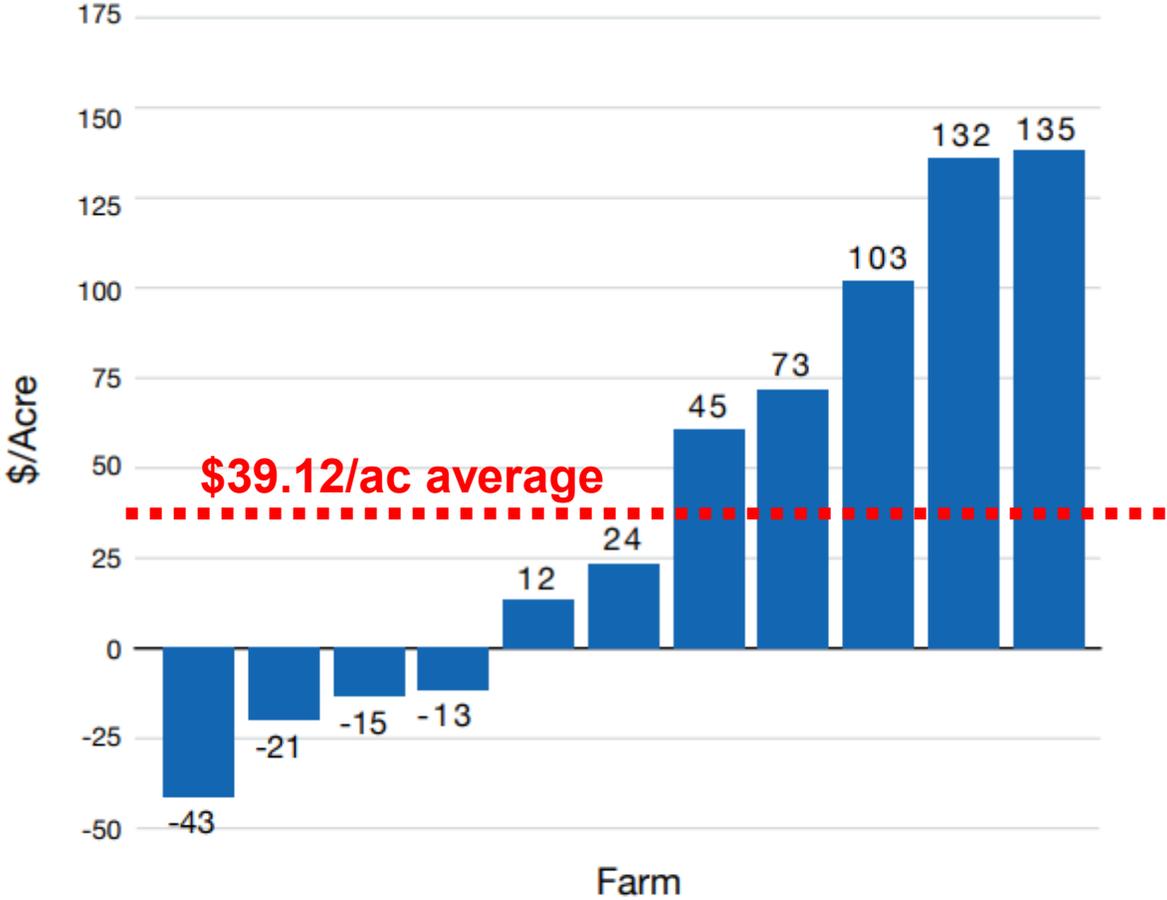


Figure 3. Change in net farm income for 11 farms after adopting a soil health management system compared to a conventional system, corn, \$/Acre.

What about soils functioning “better”?

ECONOMICS OF SOIL HEALTH SYSTEMS IN ILLINOIS

Fertilizer and amendment expenses were reduced by an average of \$28.91/acre for corn and \$12.20/acre for soybean, with a majority of farmers implementing nutrient management practices such as grid soil sampling (91%), variable rate fertilizer application (82%), and split application of nitrogen (100%) as part of their overall soil health management system.

None of the 11 Illinois farms reported a yield decline from adopting a soil health management system. In fact, 73% reported increased yield, averaging 5.27 bu./acre for corn and 2.18 bu./acre for soybean (Table 2).

Decreasing fertilization costs by grid soil sampling and VRT and split N application are solid agronomic practices

....but that isn't really “soil health” from the perspective of soils doing more for the crop

What about the *nutrient benefits* of healthy soils?

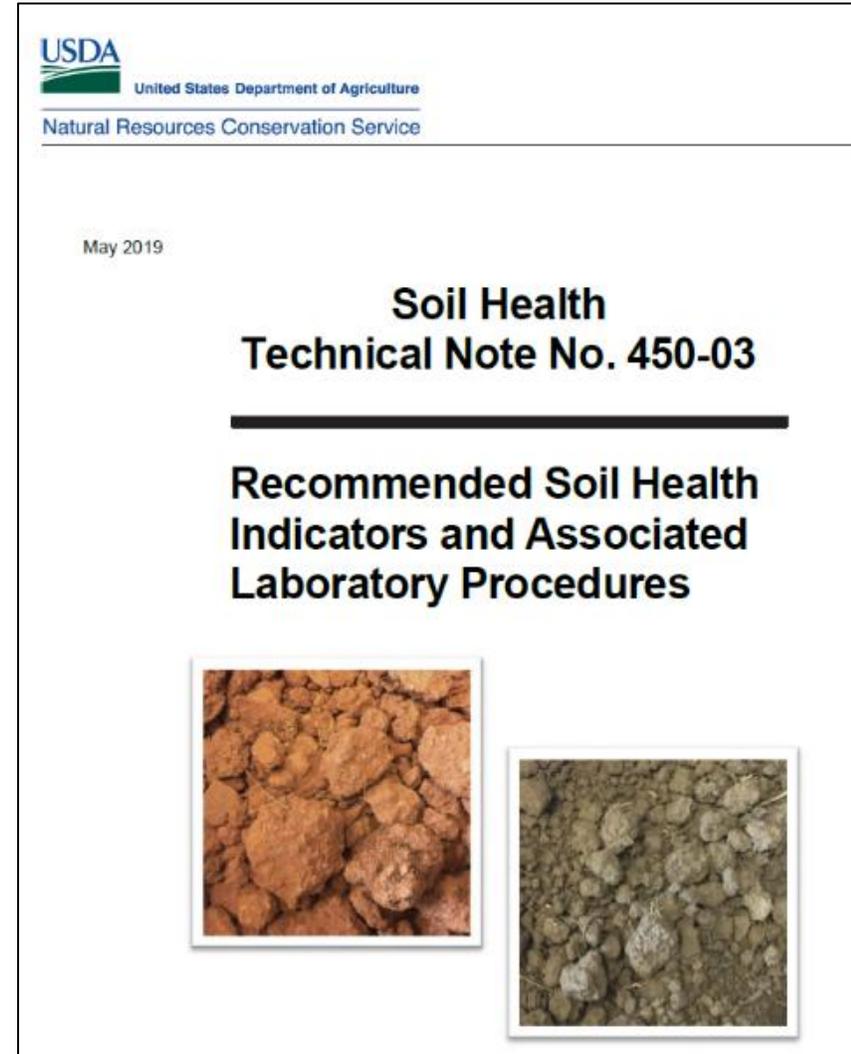
- In theory, greater soil health should translate to “doing more”
- Related but different from soil health practice (e.g., reduced tillage, cover cropping) effects

Part II: Soil health testing: indicators

Biological indicators are newest – and *least* verified

Common biological soil health indicators

- Permanganate oxidizable carbon* (POXC) / “active carbon” / “reactive carbon”
- Enzyme activities
 - C-cycling: β -glucosidase
 - N-cycling: aminopeptidase, N-acetyl- β -glucosaminidase
 - P-cycling: phosphatase
- Aggregate stability
- Total soil organic carbon (SOC)
- Respiration



*Neither carbon, nor active or labile

Soil respiration: CO₂ release

- Arguably the 'oldest' and most widely used today to monitor soil health
- Recognized as a soil health indicator by USDA NRCS

PARALLEL FORMATION OF CARBON DIOXIDE, AMMONIA AND NITRATE IN SOIL

P. L. GAINEY

Bacteriological Laboratories, Kansas Agricultural Experiment Station

Received for publication April 28, 1919

1919

The biological significance of carbon-dioxide formation in the soil has long been recognized and its measurement under varying conditions has attracted the attention of many students of soil biology. Potter and Snyder (2) have recently reviewed the literature relative to this subject. Similarly, the vast amount of literature relative to ammonia and nitrate formation in soils attests the importance attached by students of soil biology, to a better understanding of the factors controlling these very important processes.¹ It has long been recognized that to a large extent the processes through which carbon dioxide, ammonia and nitrates are formed in soil are biological in nature. The fact that the formation of any one of these compounds may take place independently of the others, has resulted in each process being studied more or less independently of the others. There are few instances on record where the parallel formation of carbon dioxide, ammonia and nitrates in a soil has been studied.

MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: VII. CARBON DIOXIDE EVOLUTION¹

SELMAN A. WAKSMAN AND ROBERT L. STARKEY

New Jersey Agricultural Experiment Stations

Received for publication, December 12, 1923

1923

HISTORICAL

The evolution of carbon dioxide has been used by a number of investigators as an index of the decomposition of organic matter in the soil.

Wollny (38) found in 1880 that the carbon dioxide content of the soil rises and falls with the amount of organic matter present in the soil. Kiszling and Fleischer (8) used the production of carbon dioxide in peat soils as an index of the rapidity of the decomposition going on in the soil; the addition of sand was found to stimulate oxidation, while the temperature was among the most important factors affecting oxidation.

Déhérain and Déroussy (2) placed the soil under examination in a closed tube of 100 cc. capacity and kept it at constant temperature. At the end of a certain period of incubation, the gas was extracted and the carbon dioxide present determined. It was found that the formation of carbon dioxide was due almost entirely to the action of microorganisms and that the carbon dioxide content increased with temperature to about 65°C., then decreased, and at 90° another increase took place due to chemical agencies. There is an optimum moisture content for the formation of carbon dioxide, which is also influenced by the state of division of the soil and its aeration. Although sterile soils were found to produce small amounts of carbon dioxide, the latter increased twenty-five times when soil infusion was added [Severin (23)]. Sterilized and inoculated soil gave two to five times as much carbon dioxide as unsterilized and uninoculated soil.

Microbial burps and exhalation: CO₂ release

Principle: CO₂ released by soil microbial activity 24 h after the soil has been dried and rewetted. Expressed as mg CO₂-C per kg soil (ppm)

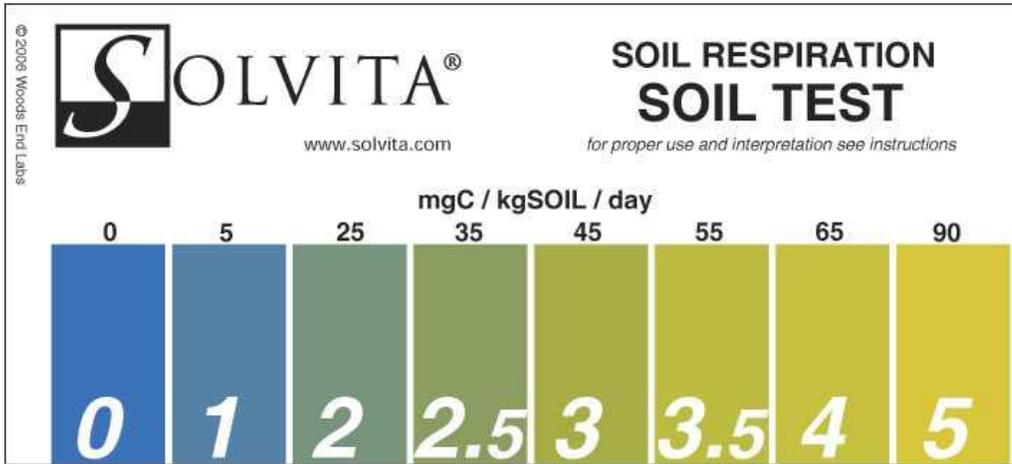


Pros

- + Simple
- + *Relatively* cheaper
- + Clear what we are measuring
- + Related to OM mineralization (N?)
- + Can be highly sensitive

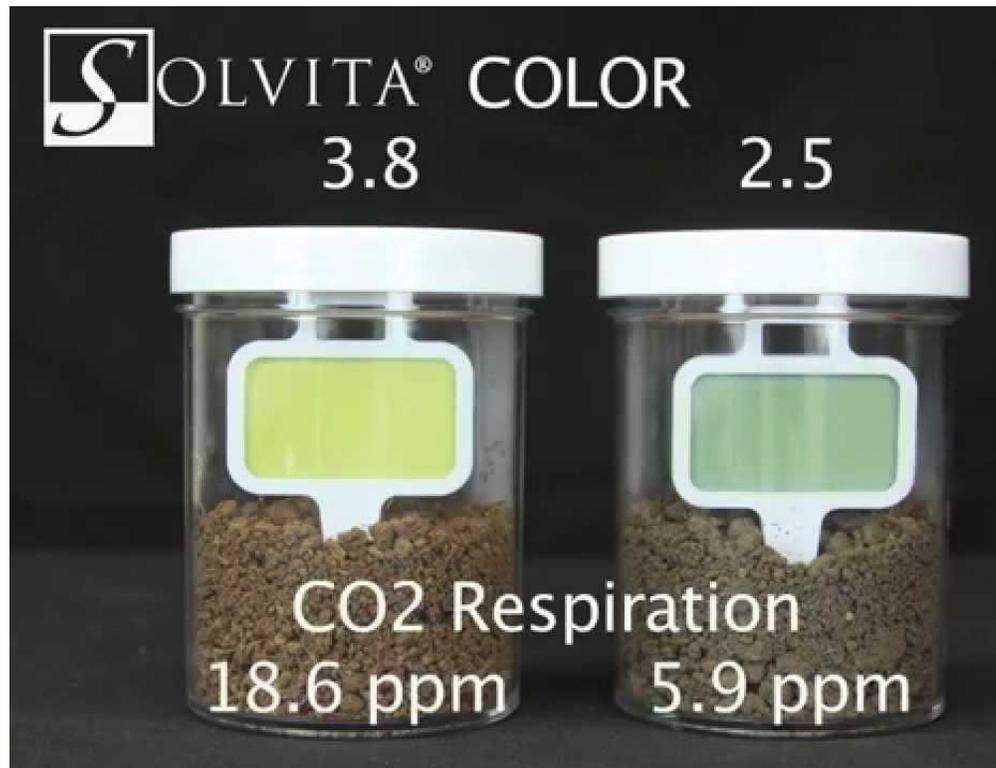
Cons

- Not fully standardized
- High lab variability
- Unclear how to interpret for nutrients
- Likely high seasonal variability
- Can be highly sensitive



Synonyms

- Respiration
- 24 hour respiration
- Solvita test
- Mineralization C
- C_{\min}
- CO_2 burst
- CO_2 evolution



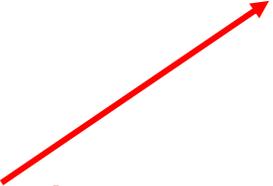
What is a “good” value?

- Not (yet?) calibrated to outcomes of interest
- Will differ markedly by soil type, in particular texture and total OM
- Within a given clay/OM range, then differences are likely to be meaningful

Example: Urbana crop rotation trial respiration values

Crop phase	Respiration (mg kg ⁻¹)
C S	45.3
C -W/S	52.1
S -C	37.0
W/ S -C	44.7

Average
≈45 mg kg⁻¹



*Example of non-objective **claims** on value ranking of respiration*

Soil Respiration Ranking Table

CO ₂ -C in ppm	Ranking	Implications
0-10	Very Low	Very little potential for microbial activity; slow nutrient cycling and residue decomposition; high carbon residue may last >2-3 yrs. with limited moisture; Nearly no N credit given; Additional N may be required due to microbial immobilization
11-20	Low	Minimal potential for nutrient cycling; residue management can still be a problem; Very little to no N credit given
21-30	Below Average	Some potential for nutrient cycling; residue management can still be a problem with prolonged use of high carbon crops: Little N credit given
31-50	Slightly Below Average	Low to moderate potential for microbial activity; Some N credit may be given
51-70	Slightly Above Average	Moderate potential for microbial activity; Moderate N credit may be given; May be able to start reducing some N fertilizer application
71-100	Above Average	Good potential for microbial activity; Moderate N credit may be given depending on size of organic N pool; Can typically reduce N application rates
101-200	High	High potential for microbial activity; more carbon inputs may be needed to sustain microbial biomass; moderate to high N credit from available organic N pools may be given; N fertilizer reduction can be substantial
>201	Very High	High to very high potential for microbial activity; residue decomposition may be <1 yr.; keeping the soil covered could be a problem in some systems; high potential for N mineralization and N credits from available organic N pools may be given; N fertilizer reduction can be substantial

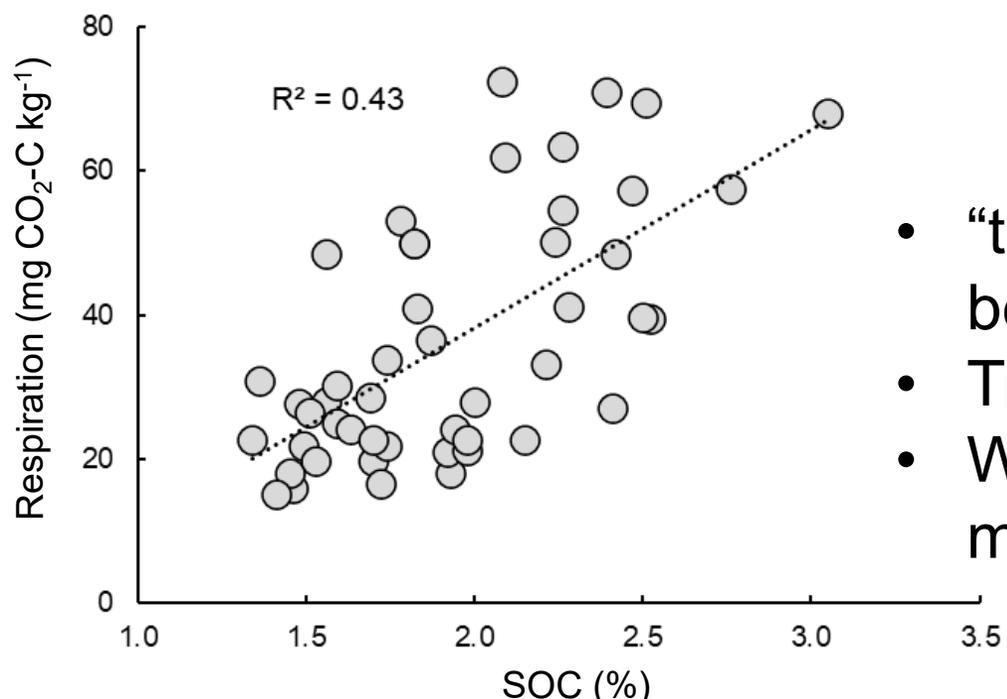
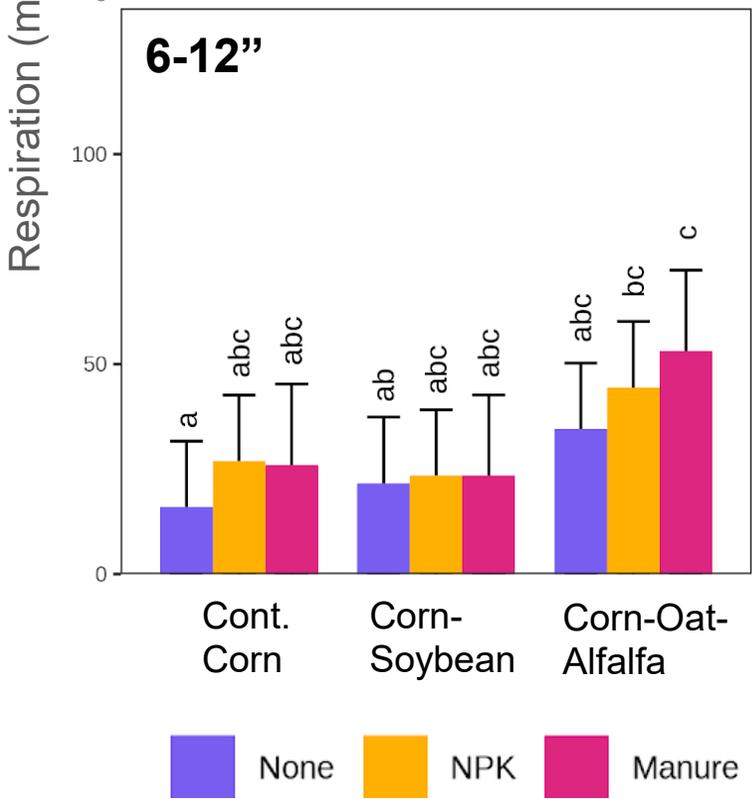
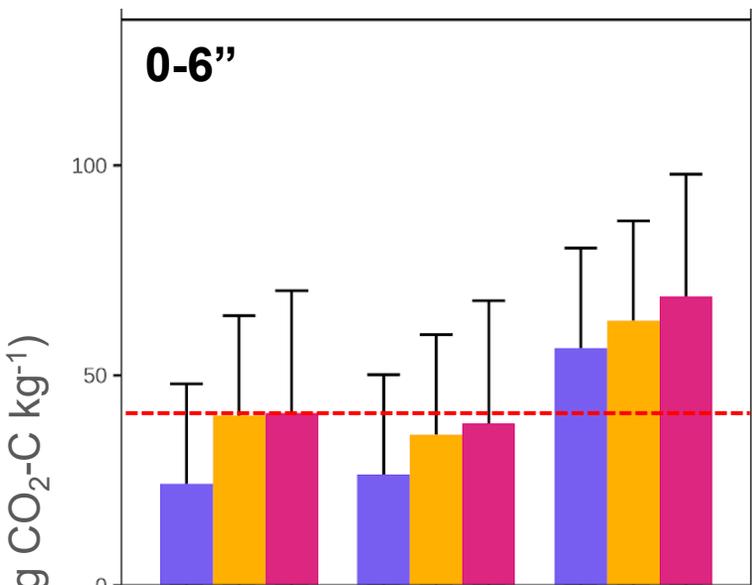
Respiration values after 145 years of crop rotation × fertilization

- Morrow Plots (est. 1876)



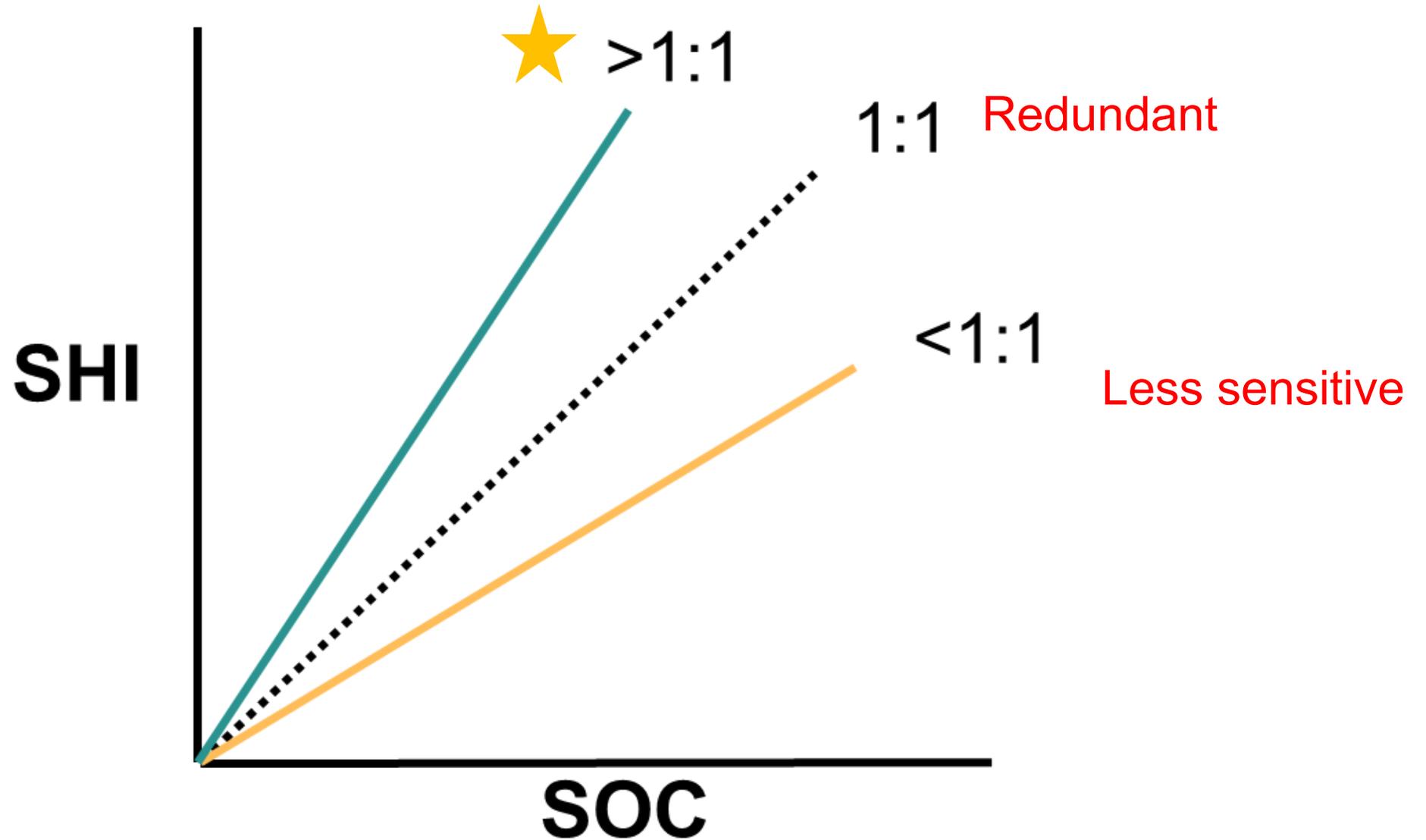
Respiration values after 145 years of crop rotation × fertilization

- Morrow Plots (est. 1876)
- No difference in respiration values at 0-6" depth after nearly 1.5 century of crop rotation and fertility treatments



- “trends” appear to be different
- Tracks with OM
- Why not just measure OM?

The “sensitivity” criterion



Example: sensitivity of indicators vs SOC to tillage

- Long-term (>15 y) tillage experiments of varying mineralogy
- Conventional tillage (CT) → chisel or moldboard
- No-tillage (NT)
- Maize-based cropping systems
- Synchronized in the maize-phase in the preceding year
- 0-6" depth

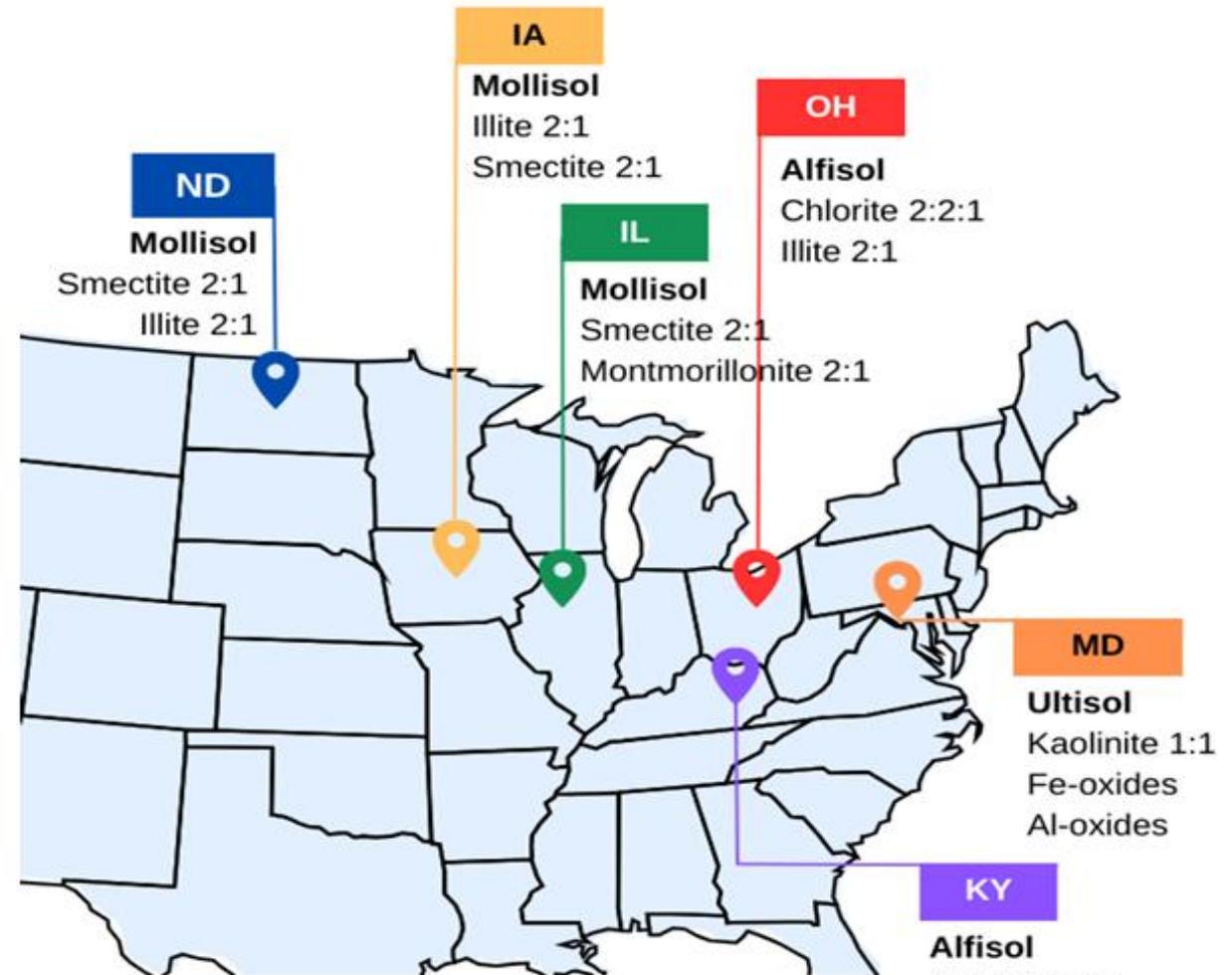
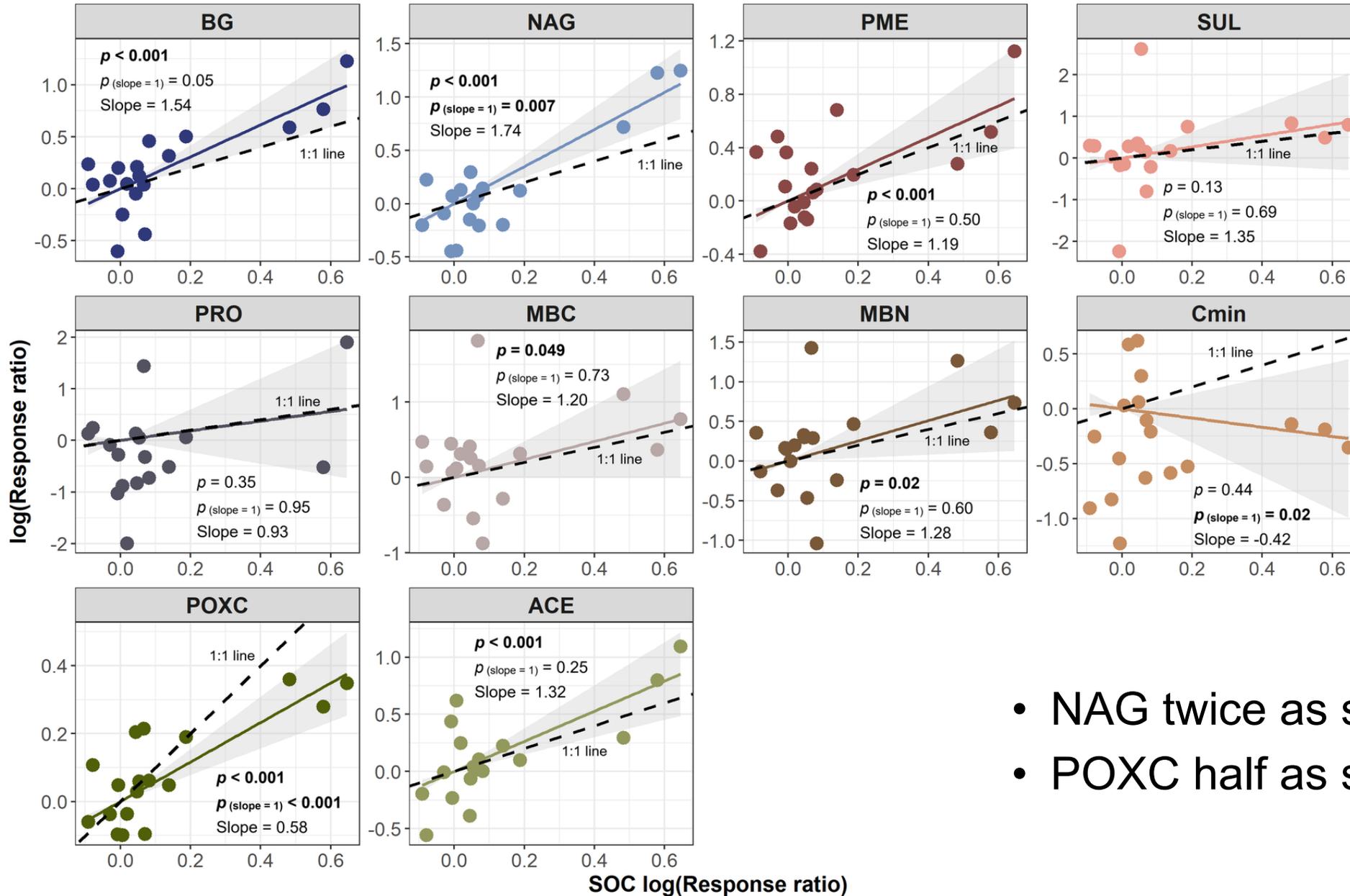


Figure 2. Location, soil order and dominant soil minerals of each long-term (>15 y) tillage trial site.

Only one SHI more sensitive than SOC to tillage



All SHI but NAG and POXC were equally sensitive to tillage as SOC

- NAG twice as sensitive (2:1)
- POXC half as sensitive (1:2)

Permanganate-oxidizable "C" (POXC)



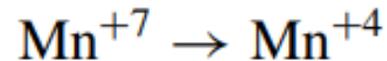
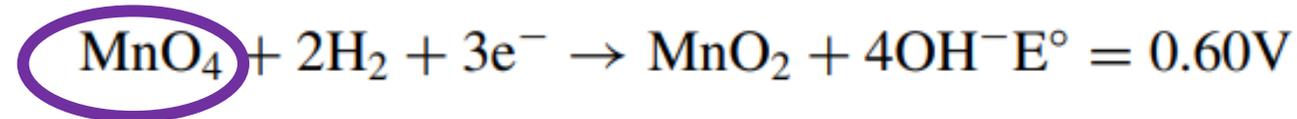
—————→
Lower POXC (mg/kg) = more purple permanganate remains unreacted

Principles of permanganate: reduction-oxidation (redox)

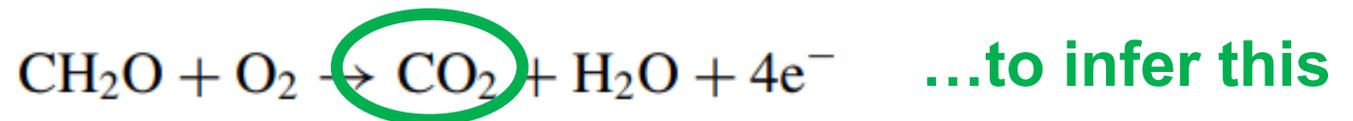
- Measure permanganate ($\text{MnO}_4^- = \text{Mn}^{7+}$) reduction
- Critical assumption for converting what we measure to what we think we are measuring: **1 mol permanganate reduced = 9,000 mg C oxidized**

Reduction half reaction

Measure
disappearance of
this...



Oxidation half reaction



How is POXC measured?

- Diversity of methods: at first, 0.33 M KMnO_4
- Recently, 20 mL of 0.02 M KMnO_4 is used per 2.5 g *or* 5.0 g soil
- Combine 2.5 g soil + 0.4 mmol permanganate
- Shake 2 min, let sit for 10 min
- Centrifuge (or not?)
- Absorbance of permanganate remaining measured at 550 nm

25

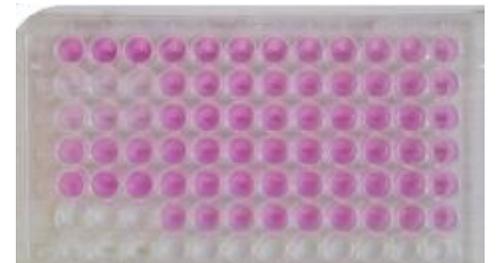
2.5 g soil
+ 2.0 mL KMnO_4
+ 18.0 mL H_2O



Shake for 2
minutes

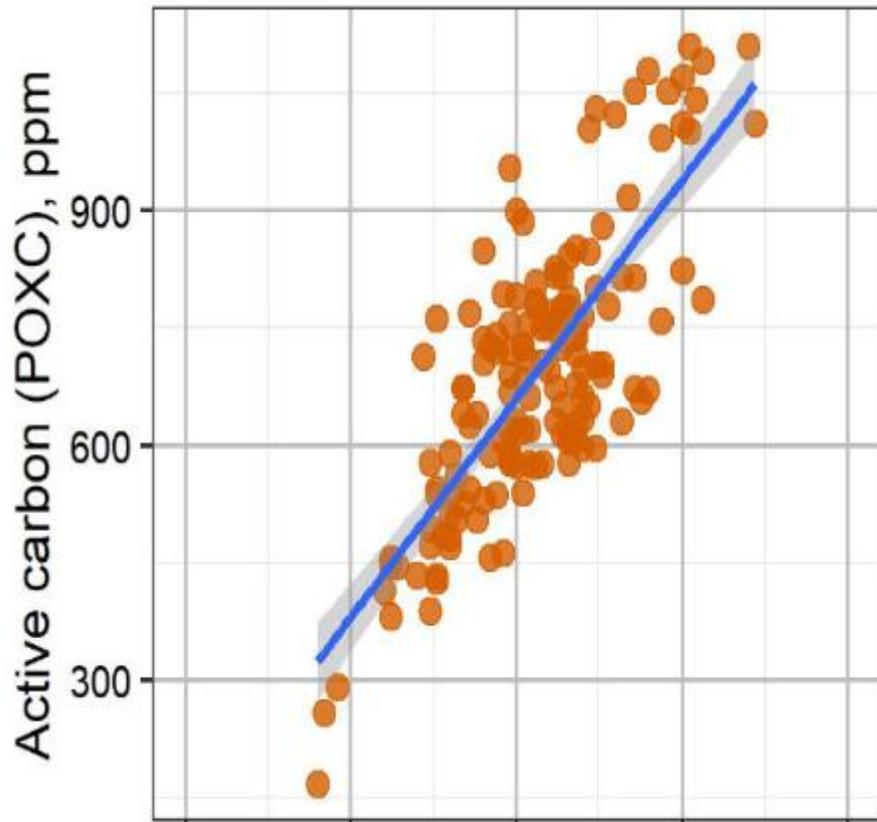


Settle for
10 minutes



Popularization of permanganate oxidation for “POXC”

- Proposed in 2003 as an early indicator of total soil OM change
- Positively correlated with total soil organic matter
- Rapid, inexpensive measure of OM?
- Can show differences by practices

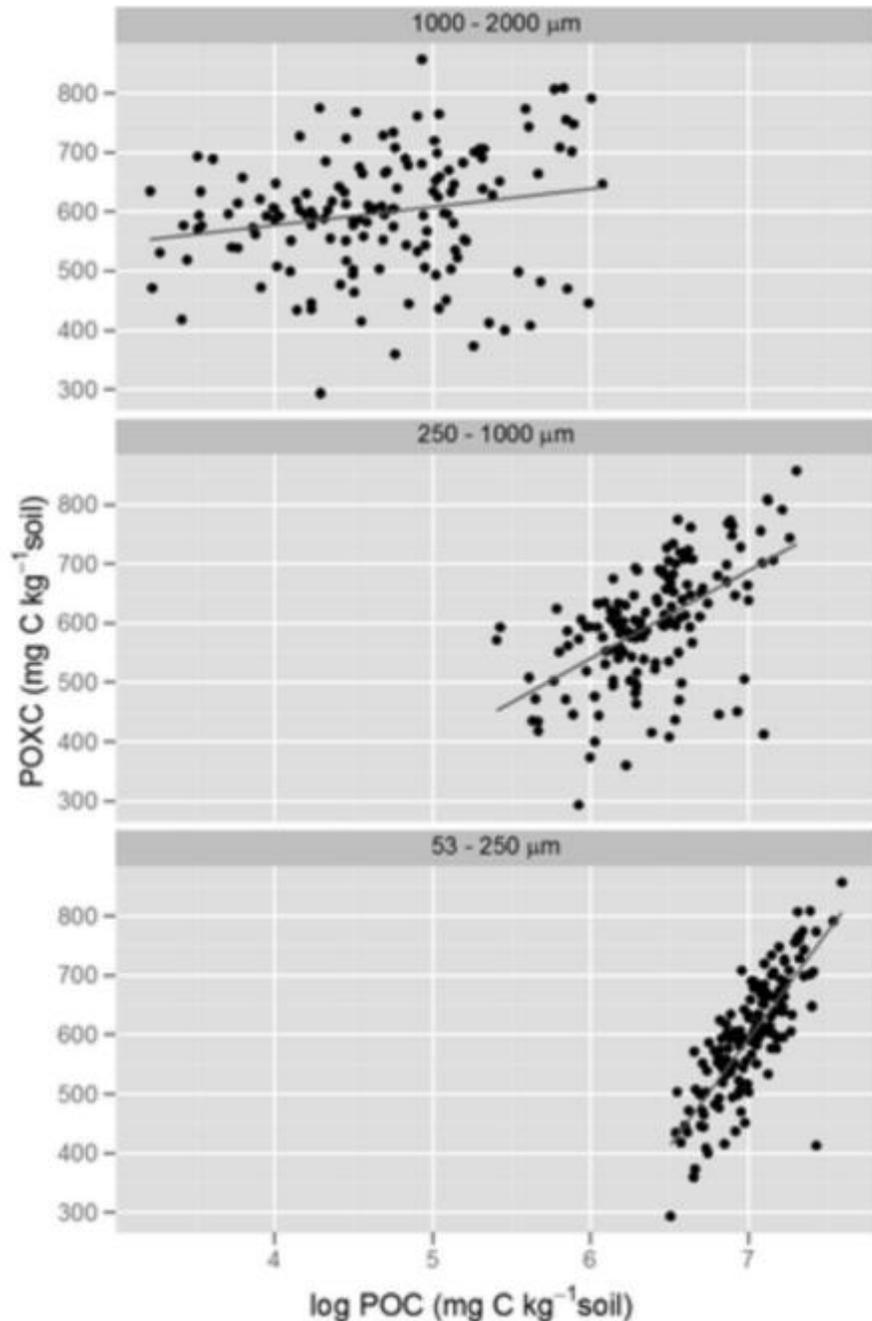


Weil et al 2003. *Am. J. Altern. Agric.* 18(1):3



The first claim of 'labile' carbon

- Used wide range of soil types, ecosystems, and geographic areas (53 sites, $n = 1379$)
- POXC more closely related to smaller (53–250 μm) than larger POC fractions (250–2000 μm)
- “indicating that it reflects a relatively processed pool of labile soil C”



Relationship of permanganate oxidizable C (POXC) and three particulate organic C (POC) sizes in the Winters study. Coefficients of determination are 0.04, 0.31, and 0.63 for the largest, medium and smallest size class, respectively.

2019 USDA NRCS Soil health “indicator”

- Recommended method for US national monitoring as an indicator of “**microbially active soil organic carbon**” (2019 No. 450-03)
- However, POXC is
 - Operationally defined
 - Sensitive to methodological parameters, many of which are not standardized
 - Difficult to interpret
 - Literally: what *is* it?
 - No direct evidence of lability
 - “more is better”

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May 2019

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How reproducible is POXC across labs?

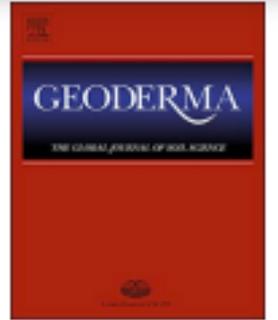


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Geoderma

journal homepage: www.elsevier.com/locate/geoderma



Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: An interlab comparison across soils



Jordon Wade^{a,*}, Gabriel Maltais-Landry^b, Dawn E. Lucas^b, Giulia Bongiorno^{c,d},
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Andrew J. Margenot^{a,*}

2019 USDA NRCS Soil health “indicator”

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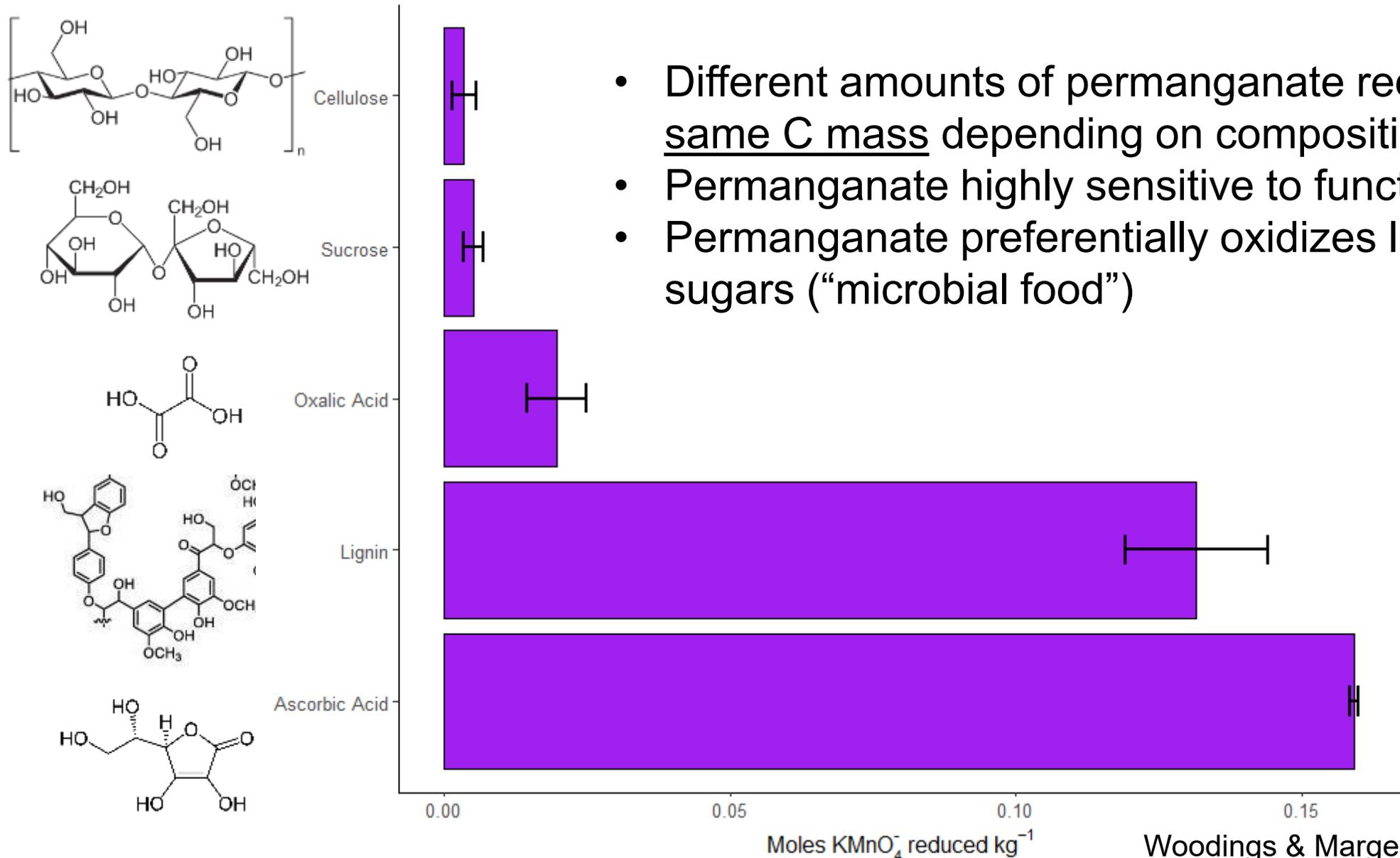
May 2019

**Soil Health
Technical Note No. 450-03**

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Problem 1: POXC is not “labile” or “active” carbon



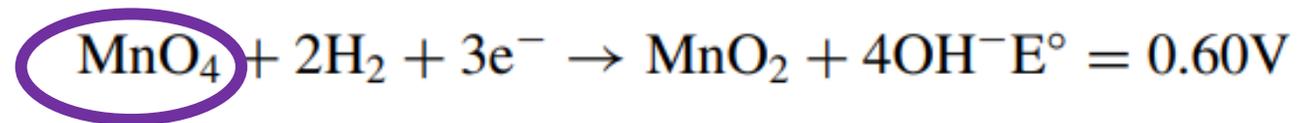
- Different amounts of permanganate reduced by the same C mass depending on composition
- Permanganate highly sensitive to functional groups
- Permanganate preferentially oxidizes lignin, but not sugars (“microbial food”)

Back to basics: redox reactions

- We are not measuring **C oxidized**
- We are measuring **permanganate reduced**
- Critical assumption for converting what we measure to what we think we are measuring: **1 mol permanganate reduced = 9,000 mg C oxidized**

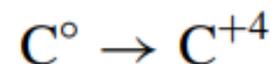
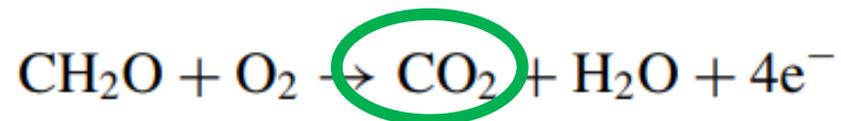
Reduction half reaction

We measure
disappearance of this



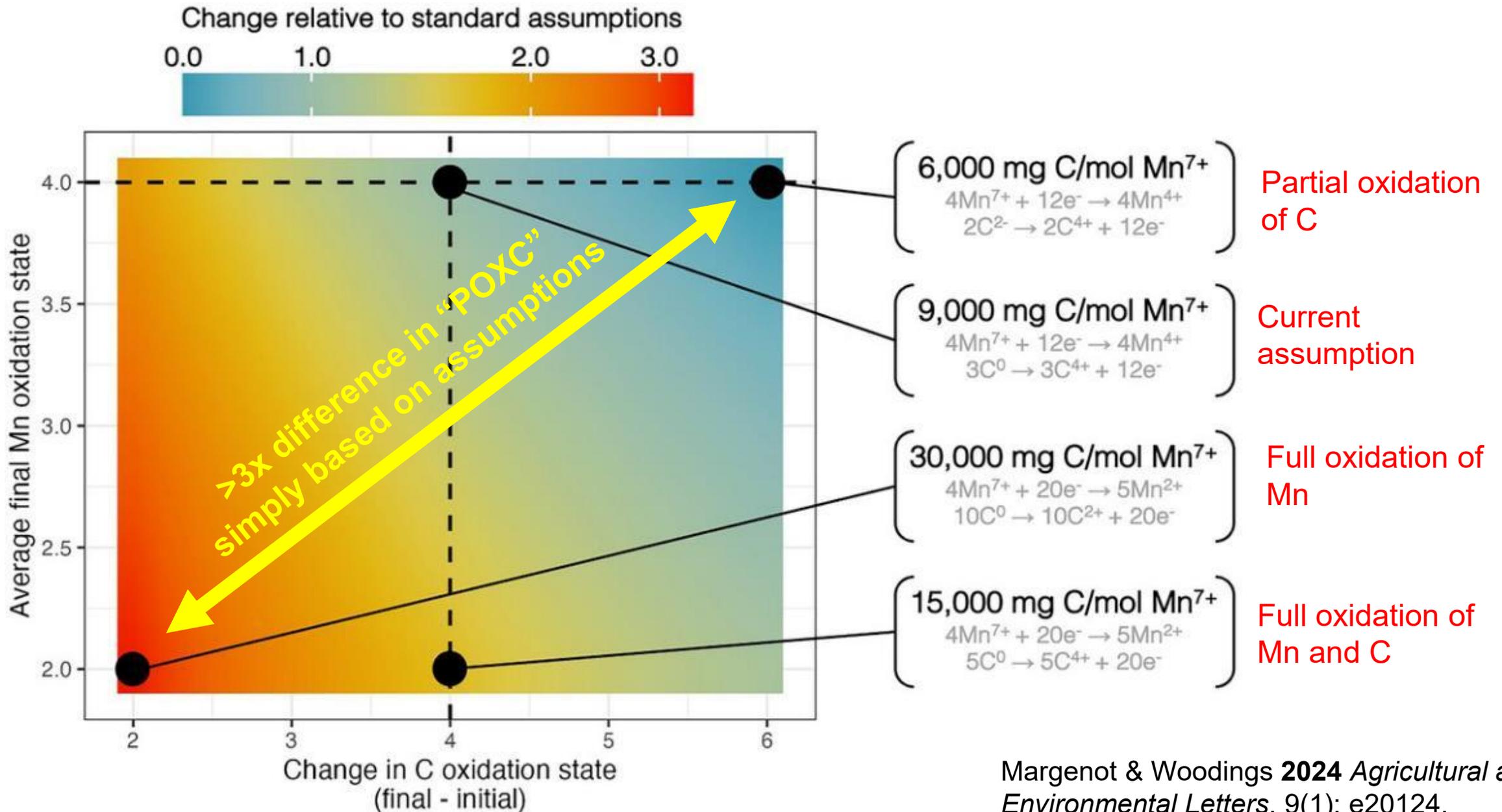
Mn⁴⁺ may not be the end state (may be Mn²⁺)

Oxidation half reaction



Do we see CO₂ produced?

Problem 2: POXC is not even “carbon”: we cannot calculate it



Implications of redox

- “POX C ” is not C
- **Cannot be expressed as an amount of C** oxidized (mg C kg^{-1} soil)
- Should be expressed operationally (which it is): MnO_4^- reduced kg^{-1} soil

The image shows a screenshot of a Geoderma journal article page. The page header includes the Elsevier logo and the journal title "Geoderma". The article title is "The misuse of permanganate as a quantitative measure of soil organic carbon". The authors listed are Andrew J. Margenot^{1,2}, Jordon Wade³, and Finnleigh S. Woodings¹. The article is categorized as a "RESEARCH LETTER" and is available as an "Agricultural & Environmental Letters" article, which is open access. The article was received on 4 February 2024 and accepted on 21 March 2024. The DOI is 10.1002/acl2.20124. The article is part of a special issue titled "Revisiting the permanganate oxidizable carbon (POXC) assay assumptions: POXC is lignin sensitive", edited by Finnleigh S. Woodings and Andrew J. Margenot. The article abstract discusses the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric across different soils.

Received: 4 February 2024 | Accepted: 21 March 2024
DOI: 10.1002/acl2.20124

RESEARCH LETTER

The misuse of permanganate as a quantitative measure of soil organic carbon

Andrew J. Margenot^{1,2} | Jordon Wade³ | Finnleigh S. Woodings¹

Revisiting the permanganate oxidizable carbon (POXC) assay assumptions: POXC is lignin sensitive

Finnleigh S. Woodings¹ | Andrew J. Margenot^{1,2}

Soil mass and grind size used for permanganate-oxidizable carbon use as a national soil health indicator

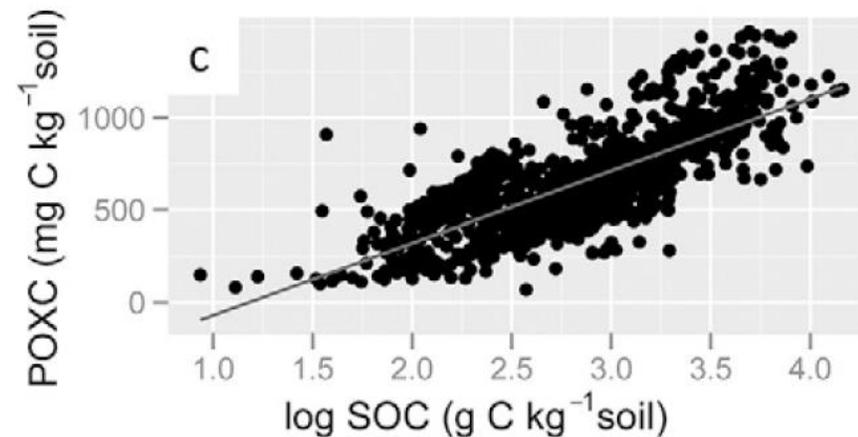
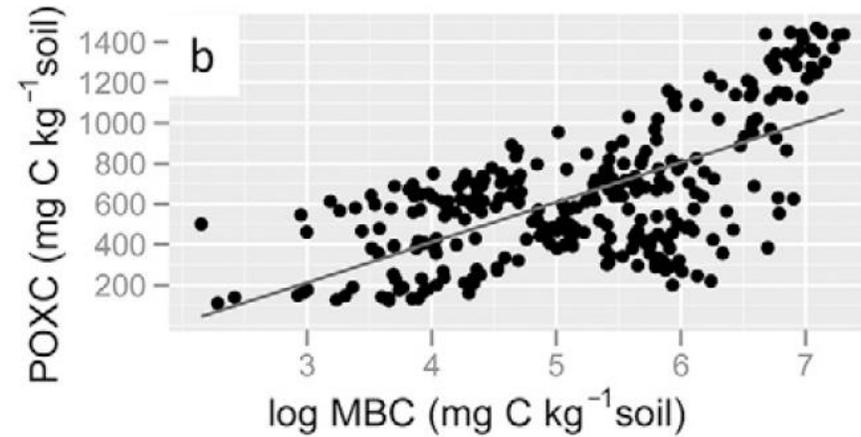
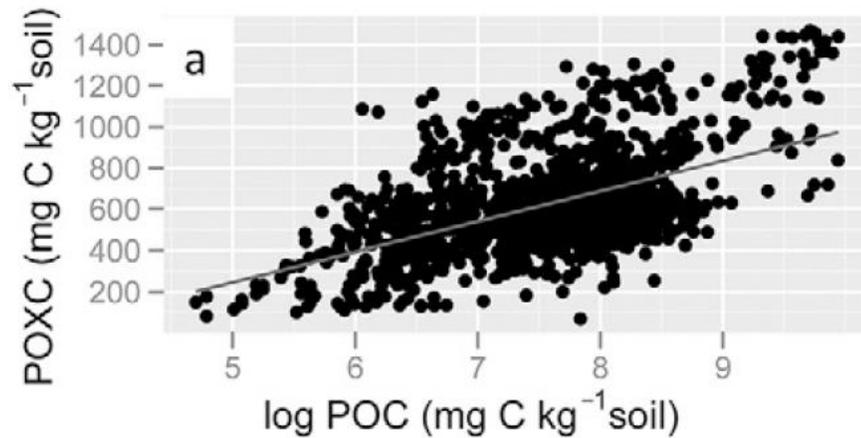
Mirjam Pulleman^{a,b,c}, Skye Wills^c, Rachel Diane Hooper^c, Candiss Williams^c, Andrew J. Margenot^a

Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: An interlab comparison across soils

Jordon Wade^a, Gabriel Maltais-Landry^b, Dawn E. Lucas^b, Giulia Bongiorno^{c,d}, Timothy M. Bowles^e, Francisco J. Calderón^f, Steve W. Culman^g, Rachel Daughtridge^h, Jessica G. Ernakovichⁱ, Steven J. Fonte^j, Dinh Giang^k, Bethany L. Herman^l, Lindsey Guan^m, Julie D. Jastrowⁿ, Bryan H.H. Loh^o, Courtland Kelly^p, Meredith E. Mann^q, Roser Matamala^r, Elizabeth A. Miernicki^s, Brandon Peterson^t, Mirjam M. Pulleman^{u,v}, Kate M. Scow^w, Sieglinde S. Snapp^{x,y}, Vanessa Thomas^z, Xinyi Tu^{aa}, Daoyuan Wang^{ab}, Nicolas A. Jelinski^{ac}, Garrett C. Liles^{ad}, Felipe H. Barrios-Masias^{ae}, Devin A. Rippner^{af}, Maria L. Silveira^{ag}, Andrew J. Margenot^{ah}

Problem 3: What “is” POXC?

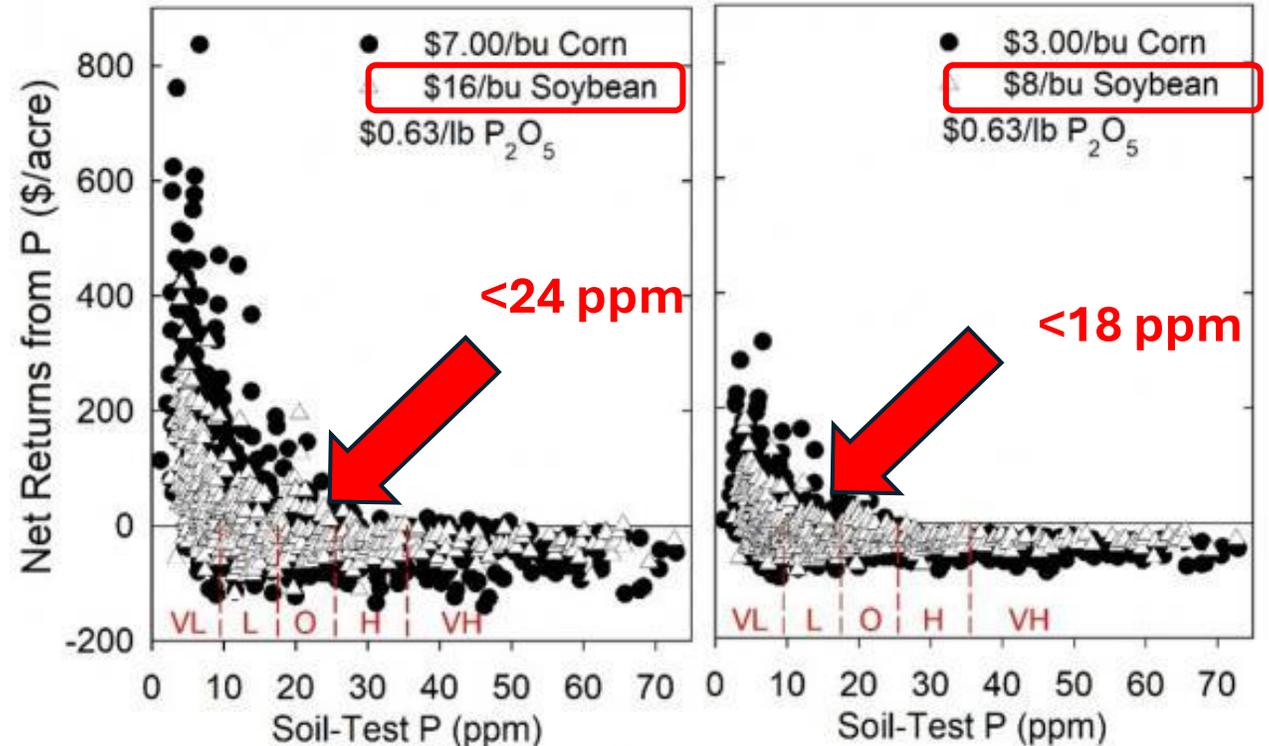
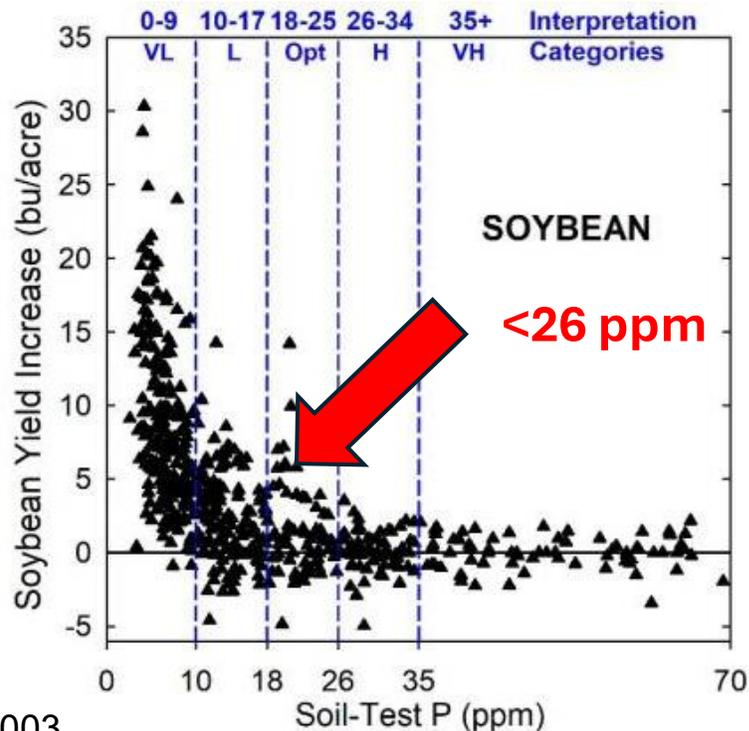
- **Zero** direct, causal evidence (e.g., ^{13}C isotopic labeling) of POXC
- Assumptions based on correlations



Part III: Developing actionable soil health testing: what do we need?

Do we need to test for a practice that we 'know' is good?

- Yes and no
- Soil health testing is “new” in at least four ways compared to traditional soil fertility testing
 1. Not yet calibrated to an outcome of interest



Do we need to test for a practice that we 'know' is good?

- Yes and no
- Soil health testing is “new” in at least four ways compared to traditional soil fertility testing
 1. Not yet calibrated to an outcome of interest
 2. Lower confidence in what we are actually measuring
 3. Lower degree of standardization – and high variability

The image shows a collage of screenshots from the Geoderma journal website. The top row displays the journal's homepage, which includes the Elsevier logo, the journal title 'Geoderma', and the URL 'www.elsevier.com/locate/geoderma'. The middle row shows a specific article page for 'Soil mass and grind size used for permanganate-oxidizable carbon use as a national soil health indicator'. The bottom row shows another article page for 'To standardize by mass of soil or organic carbon? A comparison of permanganate oxidizable carbon (POXC) assay methods'. The author 'Finnleigh S. Woodings' is highlighted in a box in the bottom left and bottom right corners.

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

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Geoderma 404 (2021) 115392

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

ELSEVIER

Soil mass and grind size used for permanganate-oxidizable carbon use as a national soil health indicator

Mirjam Pulleman^{a,b,*}, Skye Wills^c, Rachele Hooper^c, Candiss Williams^c, Andrew J. Margenot^{b,*}

Finnleigh S. Woodings¹

and repeatability of permanganate oxidizable carbon (POXC) assay methods: An interlab comparison across soils

is-Landry^b, Dawn E. Lucas^b, Giulia Bongiorno^{c,d}, J. Calderón^f, Steve W. Culman^g, Rachel Daughtridge^h, J. Fonteⁱ, Dinh Giang^k, Bethany L. Herman^e, Lindsey Guan^l, Lohⁱ, Courtland Kelly^j, Meredith E. Mann^g, Roser Matamala^l, Ron Peterson^f, Mirjam M. Pulleman^{c,m}, Kate M. Scow^k, Thomasⁿ, Xinyi Tuⁿ, Daoyuan Wang^k, Nicolas A. Jelinski^p, Prios-Masias^r, Devin A. Rippner^k, Maria L. Silveira^{b,s}

Finnleigh S. Woodings¹

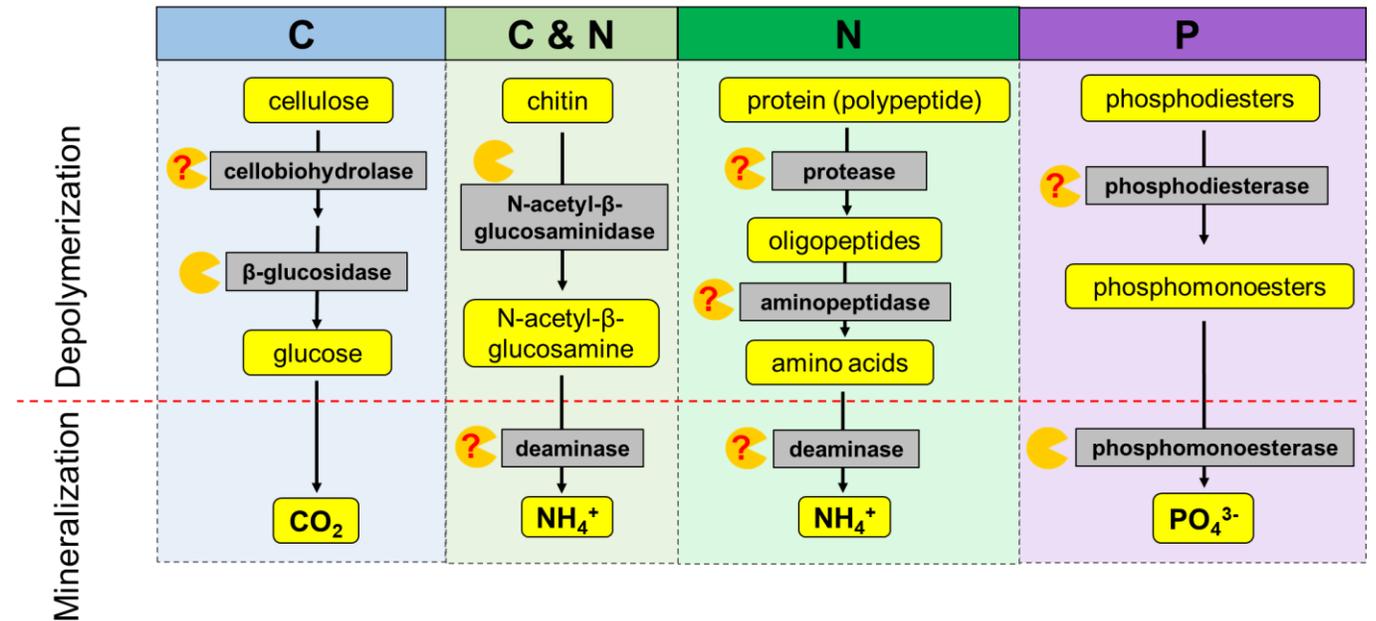
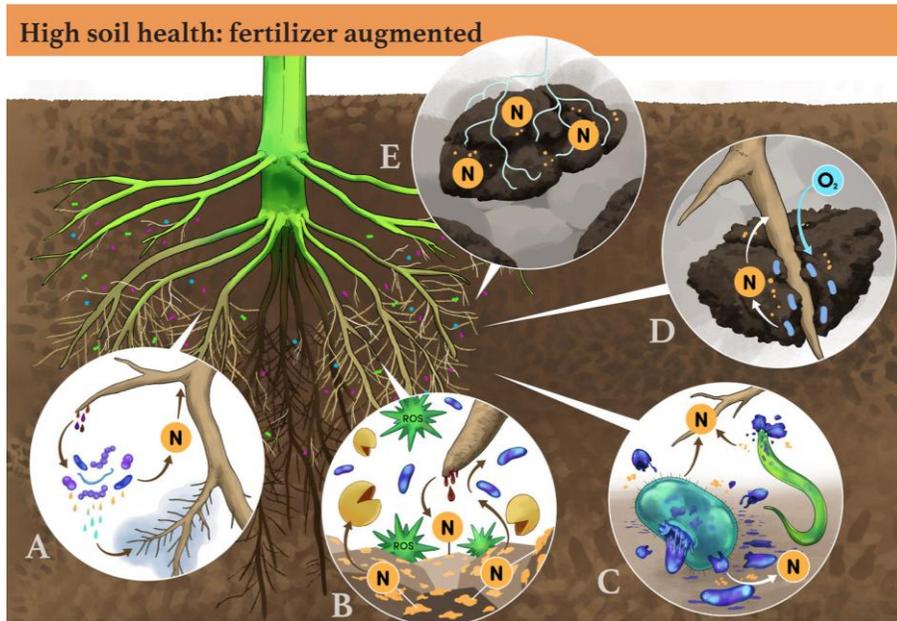
To standardize by mass of soil or organic carbon? A comparison of permanganate oxidizable carbon (POXC) assay methods

Jordan Wade^{a,b,*}, Chongyang Li^b, Mirjam M. Pulleman^{c,d}, Grace Trankina^b, Skye A. Wills^e, Andrew J. Margenot^{b,*}

Check for updates

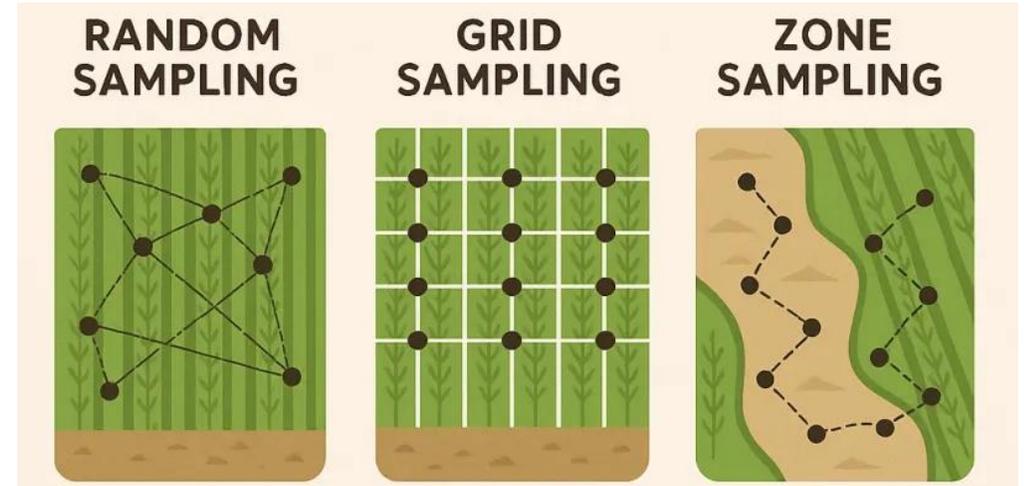
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- Soil health testing is “new” in at least four ways compared to traditional soil fertility testing
 1. Not yet calibrated to an outcome of interest
 2. Lower confidence in what we are actually measuring
 3. Lower degree of standardization – and high variability
 4. One metric may be insufficient to capture the “thing of interest”



Do we need to test for a practice that we 'know' is good?

- **Risk:** eat up ROI with expensive tests
 - At \$17-40/test, at 2 ac-grid, **\$8.50/ac** to **\$20/ac** cost
- Consider: how would you change practice(s) based on results?
 - What is “your number”?
 - What is your direction?
 - Do you understand the variability enough to tell apart signal from noise?
- **Benefit:**
 - Track progress following practice change(s)
 - Identify thresholds of change
 - If linked to nutrient functions, inform fertilization decisions to save on inputs



V: Summary

- Most soil health tests (“indicators”) are relabeled soil fertility/property tests
- Soil biology is a key aspect of the nutrient functions of soil health
- Growing interest with federal support of soil health indicators
- An **evidence-based** and **actionable** soil health testing approach **remains lacking**
- Defining soil health is first needed before tests can be performed
 - Key outcomes