Evaluating soil biology: Where do we stand?

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January 23, 2020

COLLEGE OF AGRICULTURE AND LIFE SCIENCES
SCHOOL OF PLANT AND ENVIRONMENTAL SCIENCES
VIRGINIA TECH™

Outline

Point #1: How has the state of the science changed related to measuring microorganisms in the environment?

Point #2: So what do we measure for biological soil health?

Point #3: What have we learned about soil health by using them?

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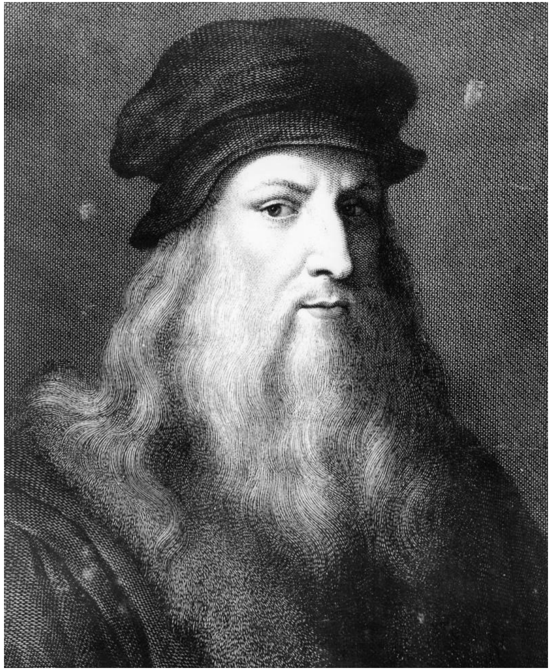
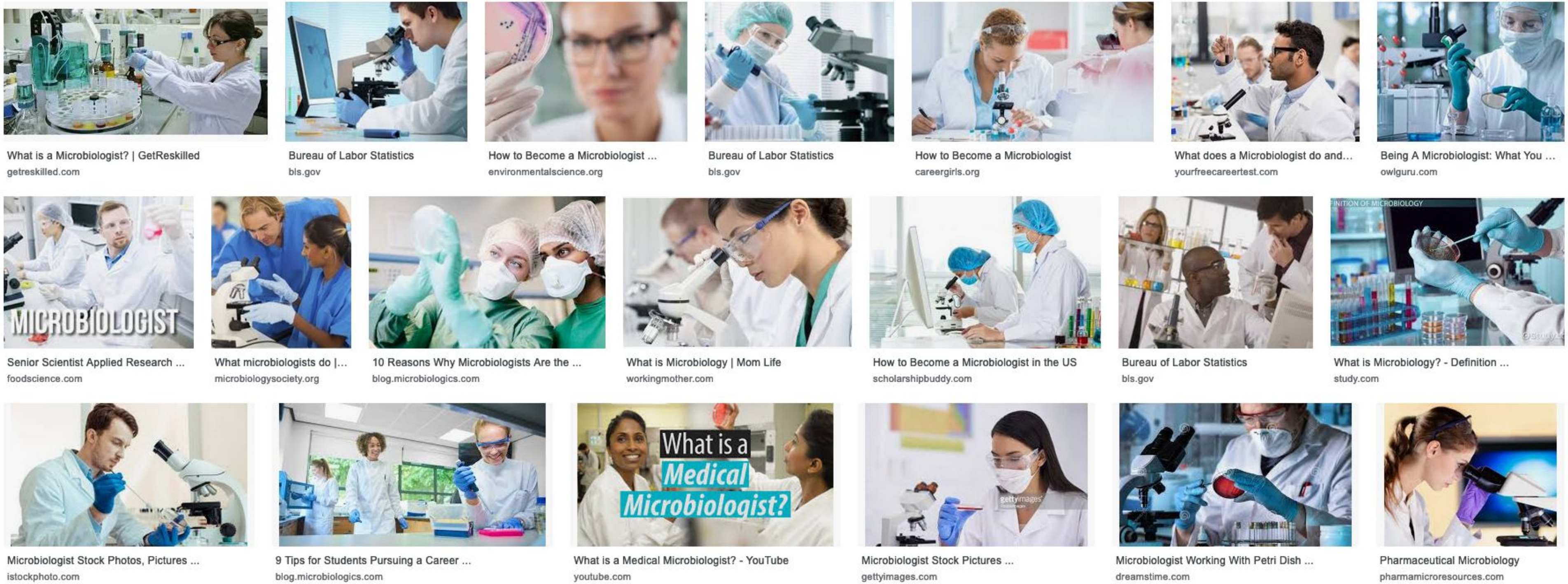
“We know more about the movement of celestial bodies than about the soil underfoot.”



-Leonardo Da Vinci, circa 1500’s



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”Everything is everywhere, but the environment selects”

~Baas-Becking, 1936

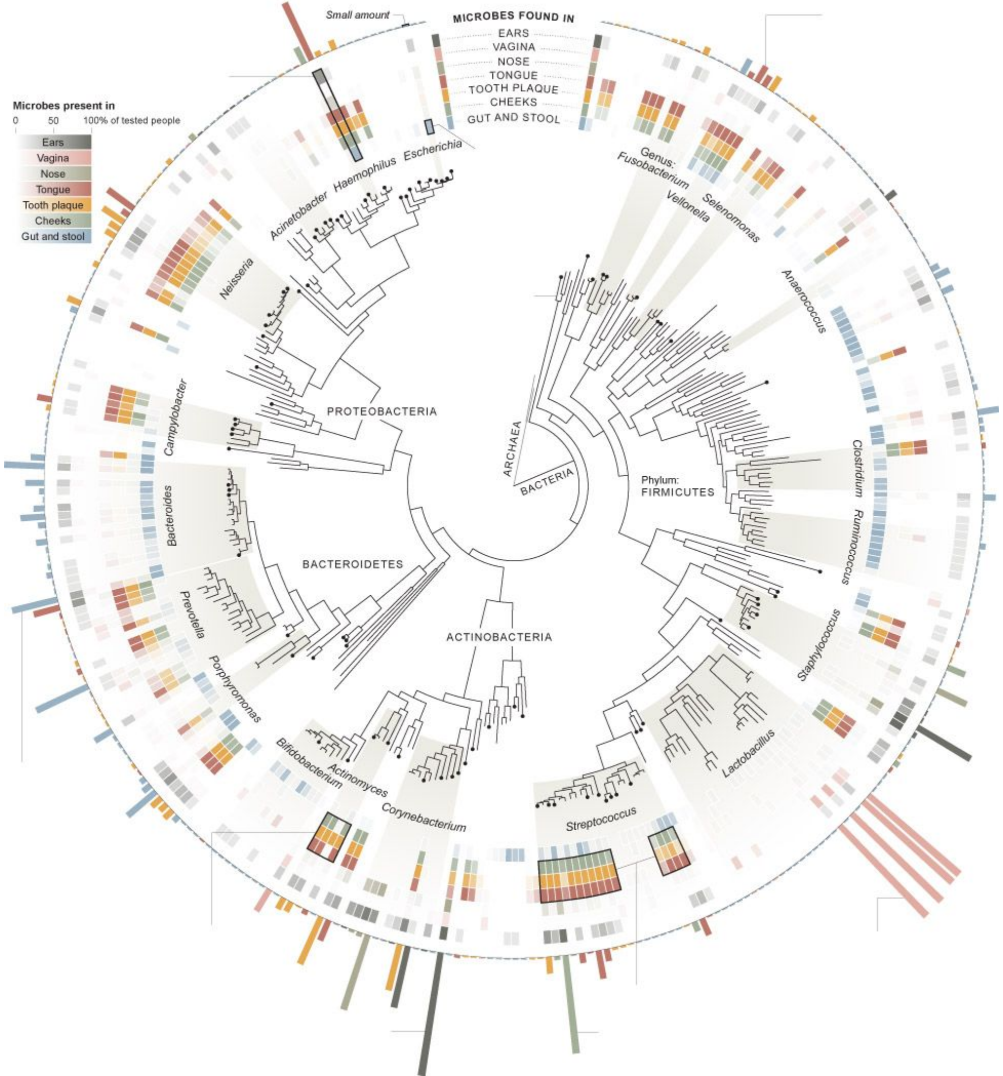


Photo credit: NY Times

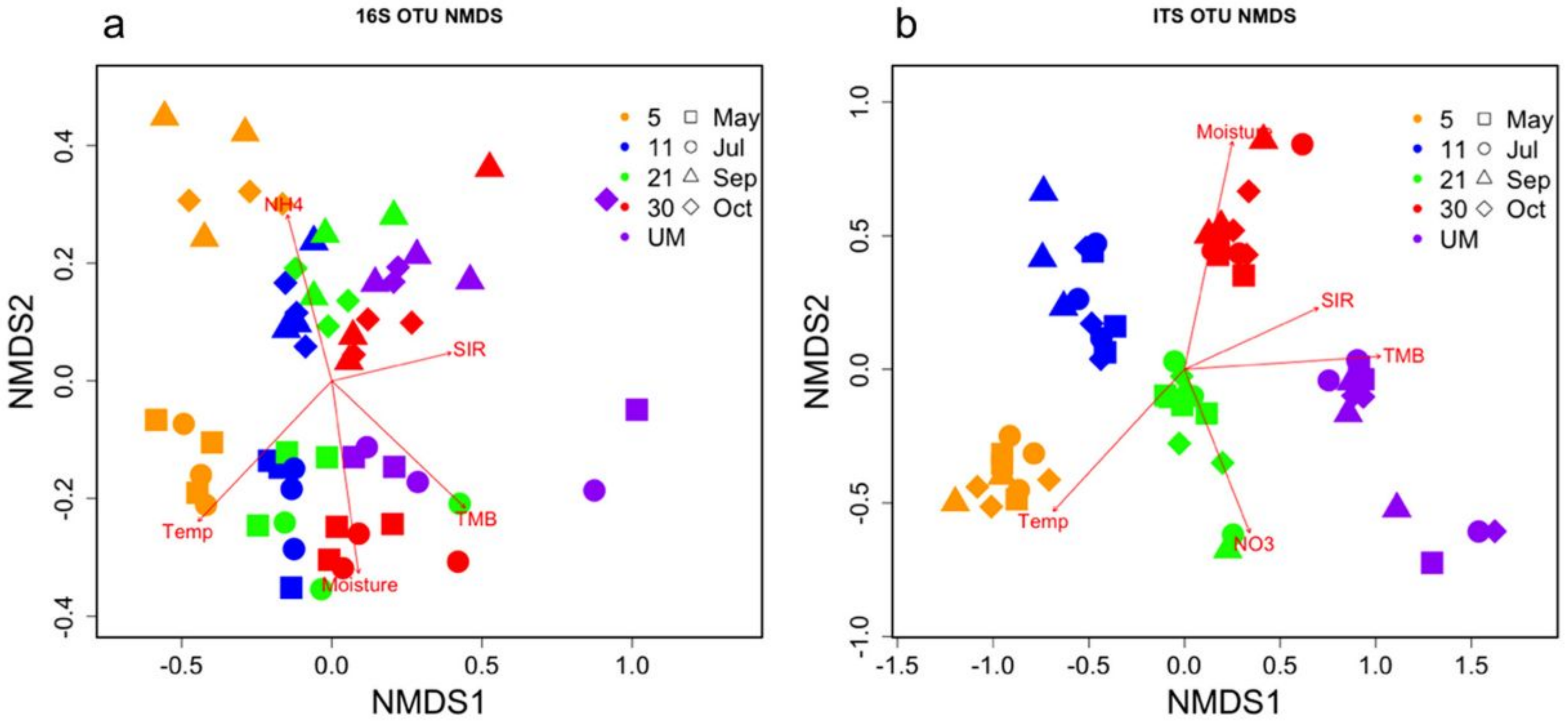
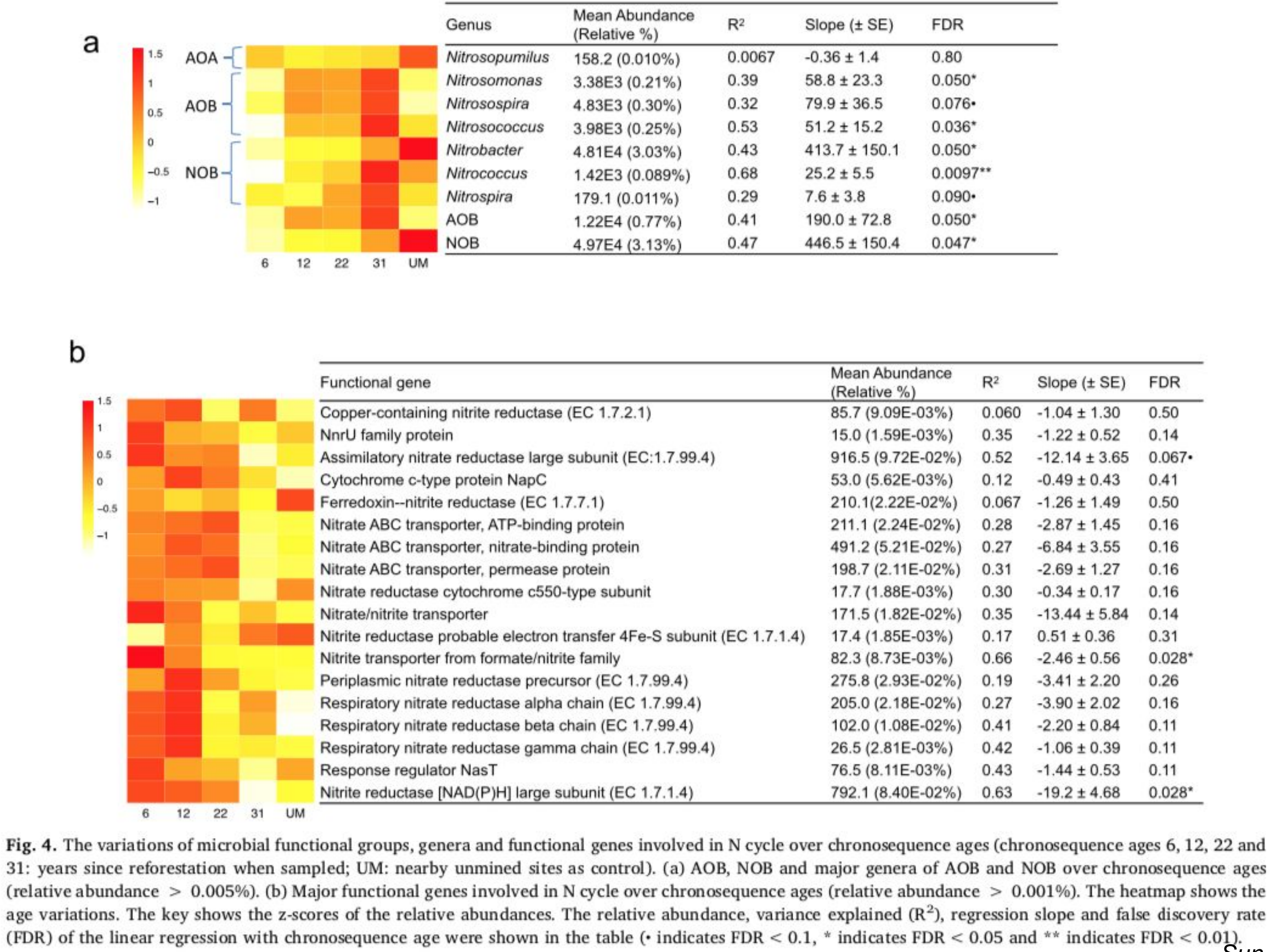


FIG 2 Bacterial (a) and fungal (b) β-diversity. Microbial β-diversity was visualized with NMDS based on OTU abundance data. Environmental factors (Temp, moisture, available NH_4^+, and NO_3^-; gas fluxes of N_2O, CO_2, and CH_4; and TMB and SIR) are fit to the ordination with function envfit in R vegan package. Only significant factors are shown in the figure.

Sun et al. (2017)

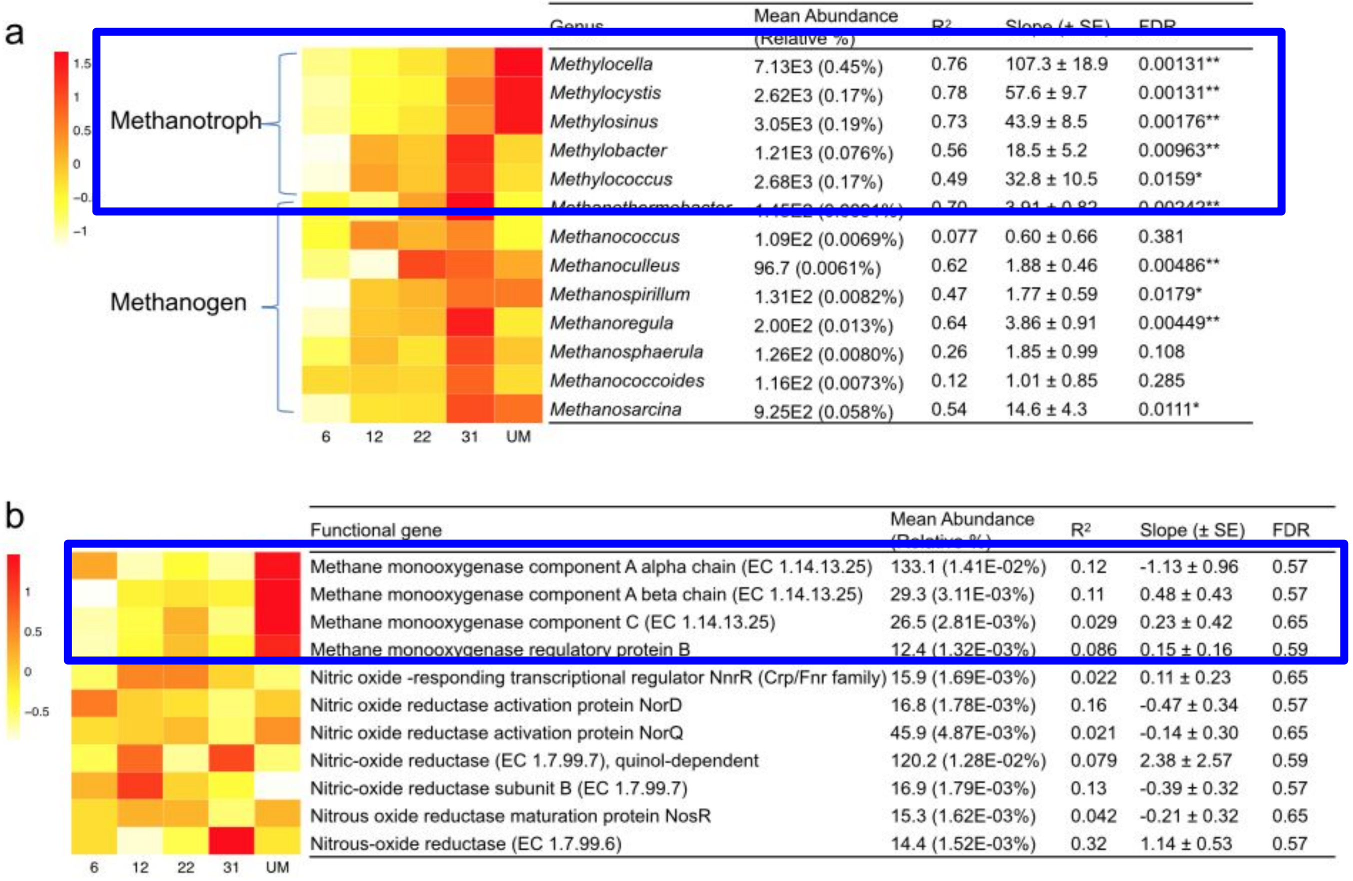


Genus	Mean Abundance (Relative %)	R²	Slope (± SE)	FDR
Nitrosopumilus	158.2 (0.010%)	0.0067	-0.36 ± 1.4	0.80
Nitrosomonas	3.38E3 (0.21%)	0.39	58.8 ± 23.3	0.050*
Nitrosospira	4.83E3 (0.30%)	0.32	79.9 ± 36.5	0.076•
Nitrosococcus	3.98E3 (0.25%)	0.53	51.2 ± 15.2	0.036*
Nitrobacter	4.81E4 (3.03%)	0.43	413.7 ± 150.1	0.050*
Nitrococcus	1.42E3 (0.089%)	0.68	25.2 ± 5.5	0.0097**
Nitrospira	179.1 (0.011%)	0.29	7.6 ± 3.8	0.090•
AOB	1.22E4 (0.77%)	0.41	190.0 ± 72.8	0.050*
NOB	4.97E4 (3.13%)	0.47	446.5 ± 150.4	0.047*

Functional gene	Mean Abundance (Relative %)	R²	Slope (± SE)	FDR
Copper-containing nitrite reductase (EC 1.7.2.1)	85.7 (9.09E-03%)	0.060	-1.04 ± 1.30	0.50
NnrU family protein	15.0 (1.59E-03%)	0.35	-1.22 ± 0.52	0.14
Assimilatory nitrate reductase large subunit (EC:1.7.99.4)	916.5 (9.72E-02%)	0.52	-12.14 ± 3.65	0.067•
Cytochrome c-type protein NapC	53.0 (5.62E-03%)	0.12	-0.49 ± 0.43	0.41
Ferredoxin--nitrite reductase (EC 1.7.7.1)	210.1(2.22E-02%)	0.067	-1.26 ± 1.49	0.50
Nitrate ABC transporter, ATP-binding protein	211.1 (2.24E-02%)	0.28	-2.87 ± 1.45	0.16
Nitrate ABC transporter, nitrate-binding protein	491.2 (5.21E-02%)	0.27	-6.84 ± 3.55	0.16
Nitrate ABC transporter, permease protein	198.7 (2.11E-02%)	0.31	-2.69 ± 1.27	0.16
Nitrate reductase cytochrome c550-type subunit	17.7 (1.88E-03%)	0.30	-0.34 ± 0.17	0.16
Nitrate/nitrite transporter	171.5 (1.82E-02%)	0.35	-13.44 ± 5.84	0.14
Nitrite reductase probable electron transfer 4Fe-S subunit (EC 1.7.1.4)	17.4 (1.85E-03%)	0.17	0.51 ± 0.36	0.31
Nitrite transporter from formate/nitrite family	82.3 (8.73E-03%)	0.66	-2.46 ± 0.56	0.028*
Periplasmic nitrate reductase precursor (EC 1.7.99.4)	275.8 (2.93E-02%)	0.19	-3.41 ± 2.20	0.26
Respiratory nitrate reductase alpha chain (EC 1.7.99.4)	205.0 (2.18E-02%)	0.27	-3.90 ± 2.02	0.16
Respiratory nitrate reductase beta chain (EC 1.7.99.4)	102.0 (1.08E-02%)	0.41	-2.20 ± 0.84	0.11
Respiratory nitrate reductase gamma chain (EC 1.7.99.4)	26.5 (2.81E-03%)	0.42	-1.06 ± 0.39	0.11
Response regulator NasT	76.5 (8.11E-03%)	0.43	-1.44 ± 0.53	0.11
Nitrite reductase [NAD(P)H] large subunit (EC 1.7.1.4)	792.1 (8.40E-02%)	0.63	-19.2 ± 4.68	0.028*

Fig. 4. The variations of microbial functional groups, genera and functional genes involved in N cycle over chronosequence ages (chronosequence ages 6, 12, 22 and 31: years since reforestation when sampled; UM: nearby unmined sites as control). (a) AOB, NOB and major genera of AOB and NOB over chronosequence ages (relative abundance > 0.005%). (b) Major functional genes involved in N cycle over chronosequence ages (relative abundance > 0.001%). The heatmap shows the age variations. The key shows the z-scores of the relative abundances. The relative abundance, variance explained (R^2), regression slope and false discovery rate (FDR) of the linear regression with chronosequence age were shown in the table (• indicates FDR < 0.1, * indicates FDR < 0.05 and ** indicates FDR < 0.01).

Sun and Badgley (2019)



Genus	Mean Abundance (Relative %)	R^2	Slope (± SE)	FDR
Methylocella	7.13E3 (0.45%)	0.76	107.3 ± 18.9	0.00131**
Methylocystis	2.62E3 (0.17%)	0.78	57.6 ± 9.7	0.00131**
Methylosinus	3.05E3 (0.19%)	0.73	43.9 ± 8.5	0.00176**
Methylobacter	1.21E3 (0.076%)	0.56	18.5 ± 5.2	0.00963**
Methylococcus	2.68E3 (0.17%)	0.49	32.8 ± 10.5	0.0159*
Methanothermobacter	1.45E2 (0.0091%)	0.70	3.91 ± 0.82	0.00242**
Methanococcus	1.09E2 (0.0069%)	0.077	0.60 ± 0.66	0.381
Methanoculleus	96.7 (0.0061%)	0.62	1.88 ± 0.46	0.00486**
Methanospirillum	1.31E2 (0.0082%)	0.47	1.77 ± 0.59	0.0179*
Methanoregula	2.00E2 (0.013%)	0.64	3.86 ± 0.91	0.00449**
Methanosphaerula	1.26E2 (0.0080%)	0.26	1.85 ± 0.99	0.108
Methanococcoides	1.16E2 (0.0073%)	0.12	1.01 ± 0.85	0.285
Methanosarcina	9.25E2 (0.058%)	0.54	14.6 ± 4.3	0.0111*

Functional gene	Mean Abundance (Relative %)	R^2	Slope (± SE)	FDR
Methane monooxygenase component A alpha chain (EC 1.14.13.25)	133.1 (1.41E-02%)	0.12	-1.13 ± 0.96	0.57
Methane monooxygenase component A beta chain (EC 1.14.13.25)	29.3 (3.11E-03%)	0.11	0.48 ± 0.43	0.57
Methane monooxygenase component C (EC 1.14.13.25)	26.5 (2.81E-03%)	0.029	0.23 ± 0.42	0.65
Methane monooxygenase regulatory protein B	12.4 (1.32E-03%)	0.086	0.15 ± 0.16	0.59
Nitric oxide -responding transcriptional regulator NnrR (Crp/Fnr family)	15.9 (1.69E-03%)	0.022	0.11 ± 0.23	0.65
Nitric oxide reductase activation protein NorD	16.8 (1.78E-03%)	0.16	-0.47 ± 0.34	0.57
Nitric oxide reductase activation protein NorQ	45.9 (4.87E-03%)	0.021	-0.14 ± 0.30	0.65
Nitric-oxide reductase (EC 1.7.99.7), quinol-dependent	120.2 (1.28E-02%)	0.079	2.38 ± 2.57	0.59
Nitric-oxide reductase subunit B (EC 1.7.99.7)	16.9 (1.79E-03%)	0.13	-0.39 ± 0.32	0.57
Nitrous oxide reductase maturation protein NosR	15.3 (1.62E-03%)	0.042	-0.21 ± 0.32	0.65
Nitrous-oxide reductase (EC 1.7.99.6)	14.4 (1.52E-03%)	0.32	1.14 ± 0.53	0.57

Fig. 5. The variations of microbial genera and functional genes involved in greenhouse gas emission over chronosequence ages (chronosequence ages 6, 12, 22 and 31: years since reforestation when sampled; UM: nearby unmined sites as control). (a) Major methanotrophs and methanogens over chronosequence ages (relative abundance > 0.005%). (b) Major functional genes involved in methane and nitrous oxide production over chronosequence ages (relative abundance > 0.001%). The heatmap shows the age variations. The key shows the z-scores of the relative abundances. The relative abundance, variance explained (R^2), regression slope and false discovery rate (FDR) of the linear regression with chronosequence age were shown in the table (• indicates FDR < 0.1, * indicates FDR < 0.05 and ** indicates FDR < 0.01).

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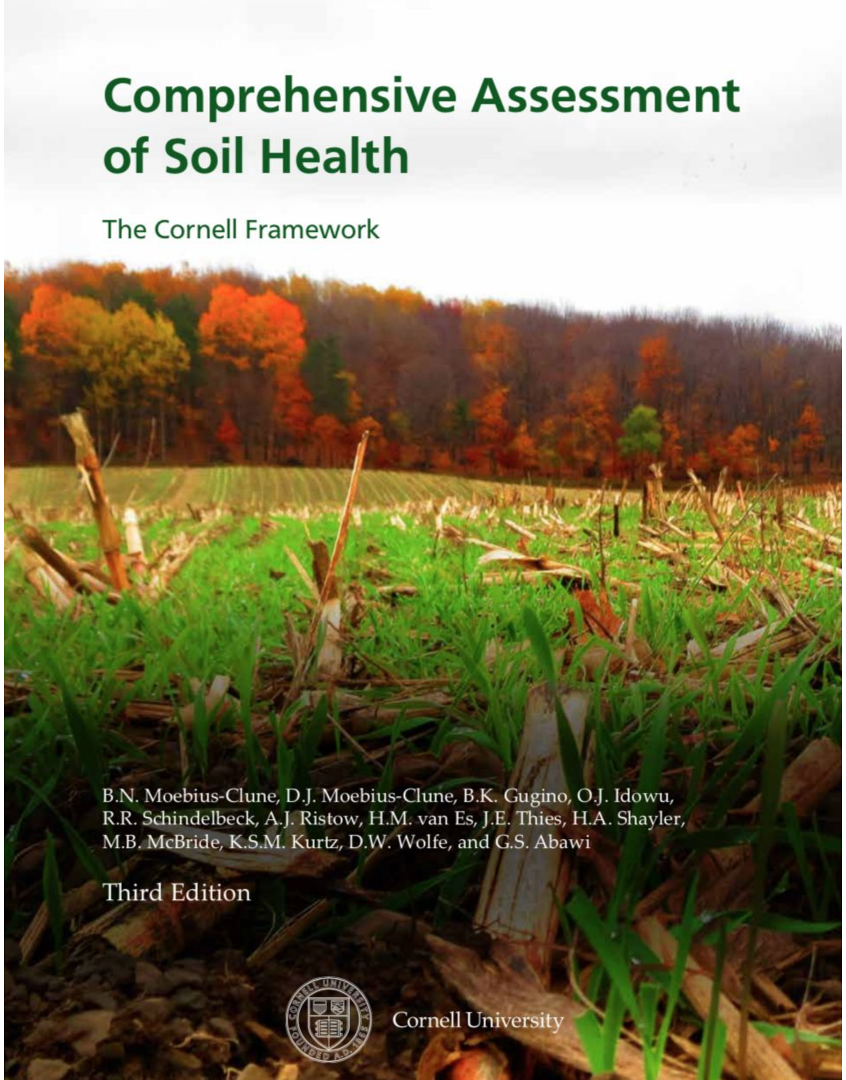


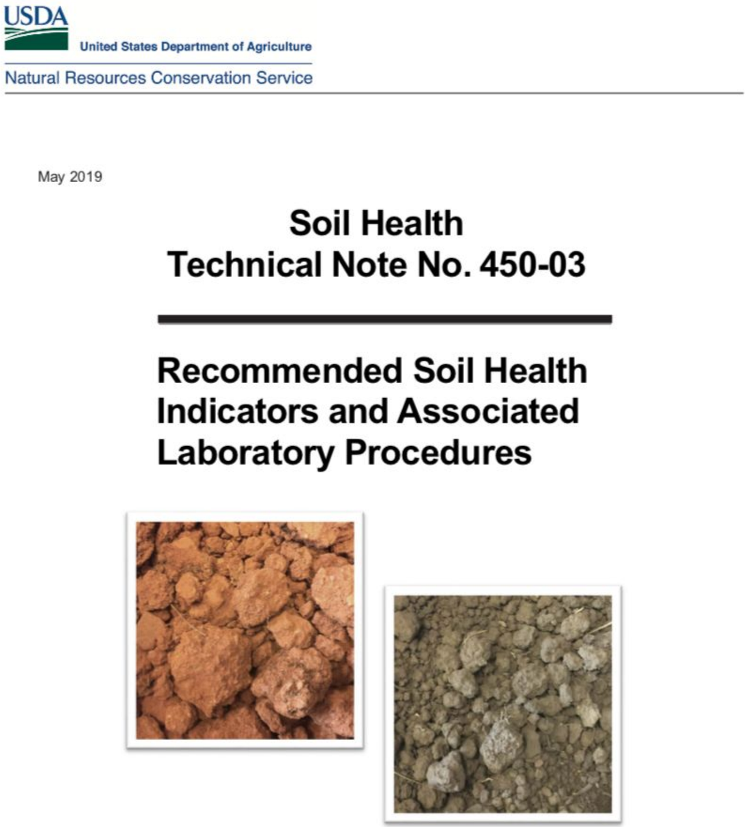
Comprehensive Assessment of Soil Health

The Cornell Framework

B.N. Moebius-Clune, D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu,
R.R. Schindelbeck, A.J. Ristow, H.M. van Es, J.E. Thies, H.A. Shayler,
M.B. McBride, K.S.M. Kurtz, D.W. Wolfe, and G.S. Abawi

Third Edition

Cornell University



USDA
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

Common Biological Indicators

“Food” Sources:

- Soil organic matter/carbon
- permanganate oxidizable carbon (readily available C)
- soil protein (readily available N)

General Microbial Activity:

- Carbon mineralization
- Nitrogen mineralization
- Respiration assays

Moebius-Clune et al. (2016), Stott et al. (2019)

Common Biological Indicators

Enzymatic assays:

- β-glucosidase: cellulose degradation
- N-acetyl-β-D-glucosaminidase: chitin degradation
- Phosphomonoesterases: P mineralization
- Arylsulfatase: S mineralization

Diversity:

- Phospholipid fatty acid analysis - recommended now
- DNA sequencing - recommended for archiving if possible

Moebius-Clune et al. (2016), Stott et al. (2019)

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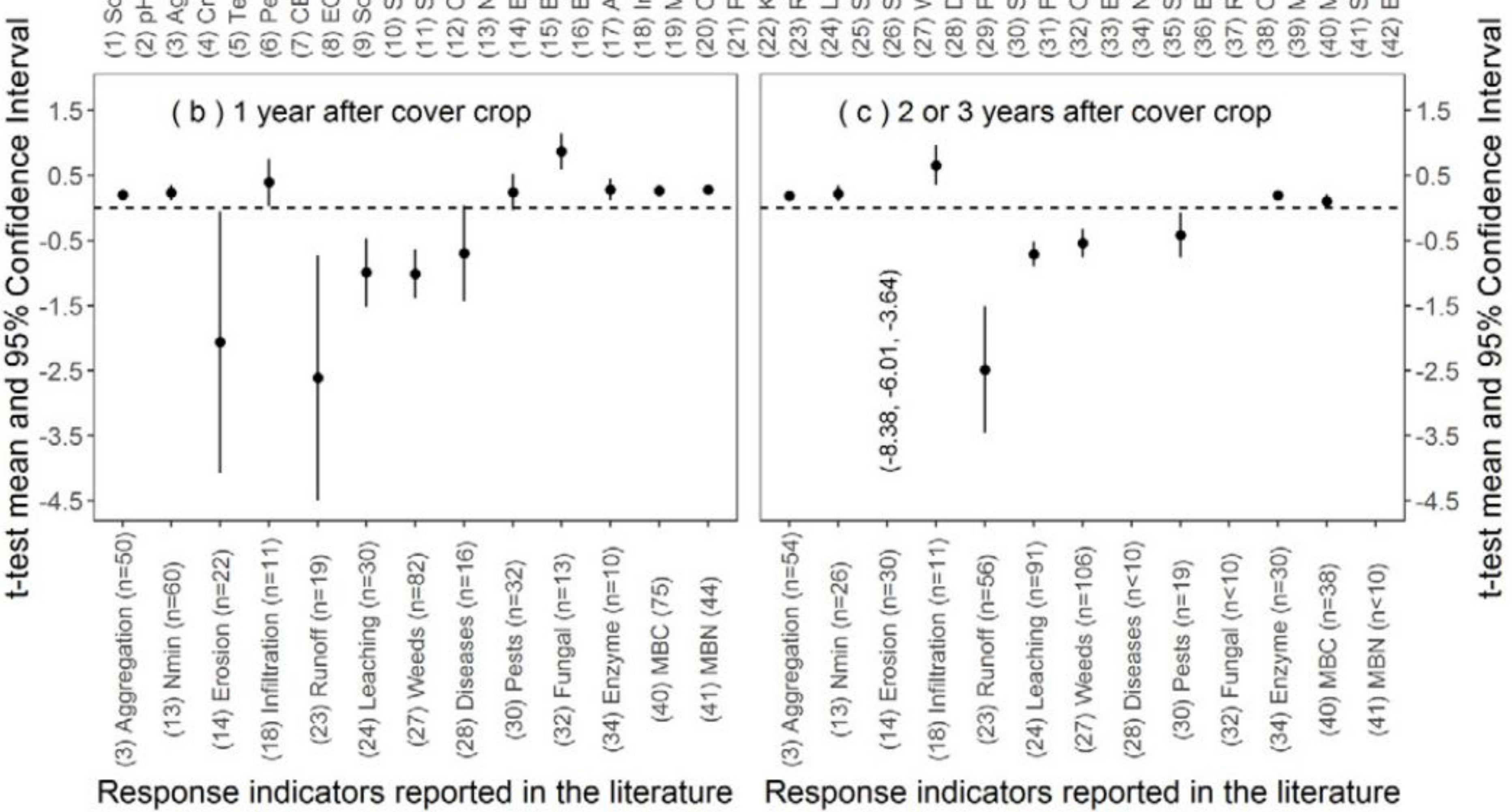
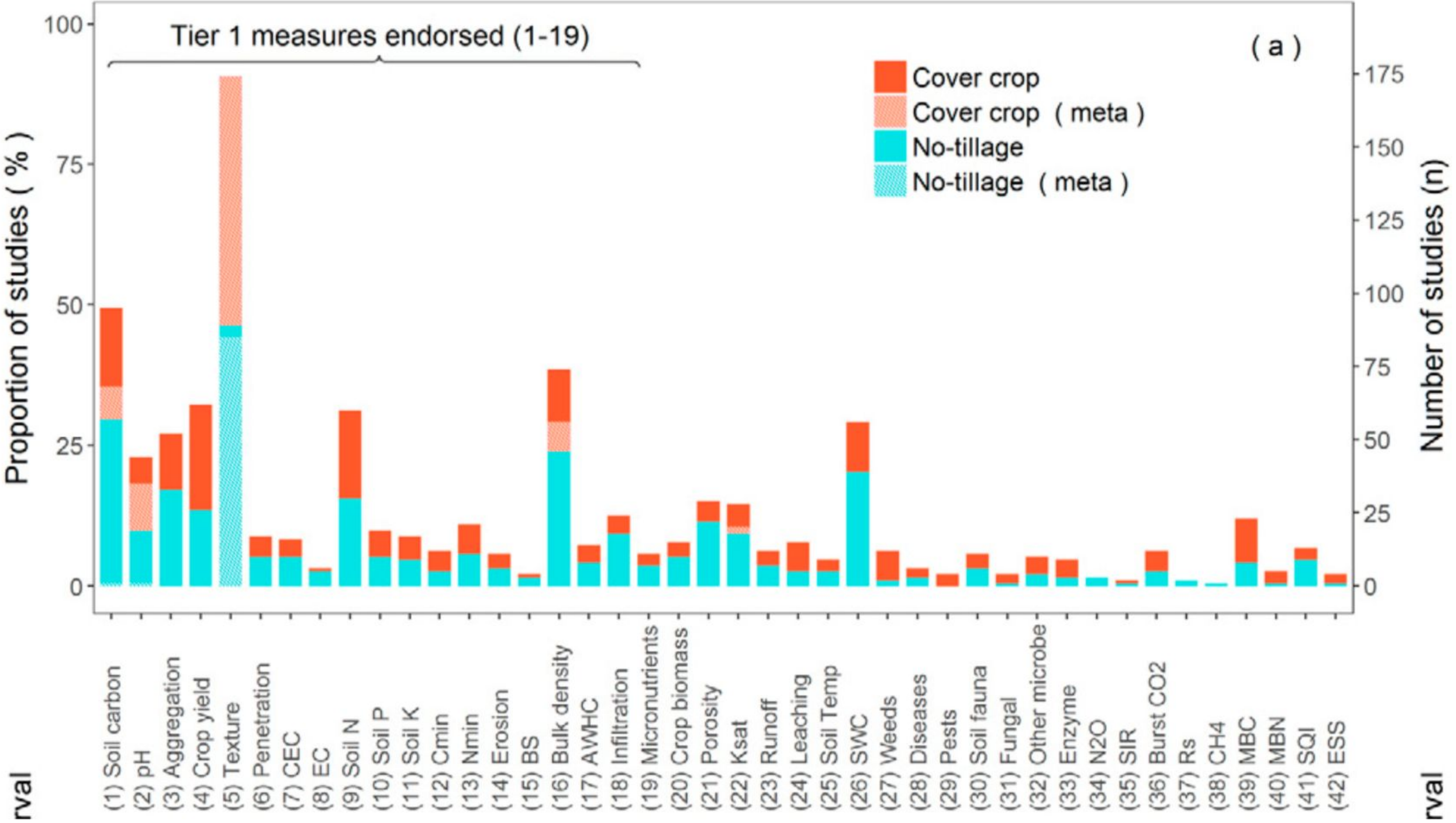
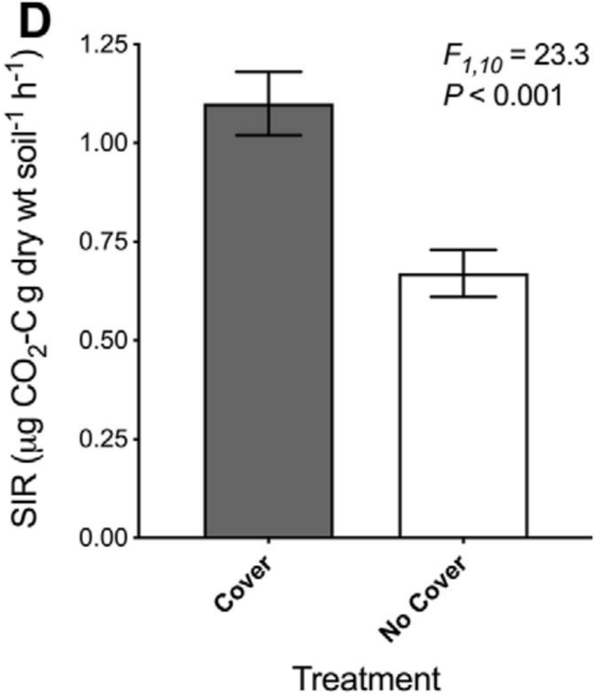
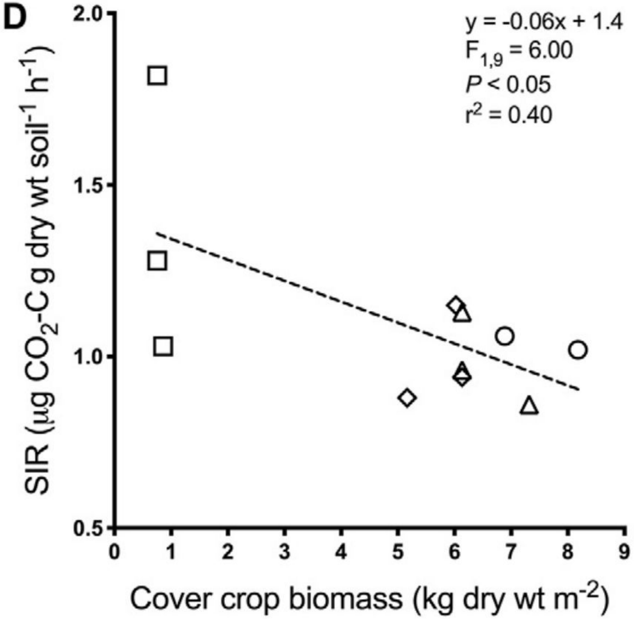


Stewart et al. (2018)



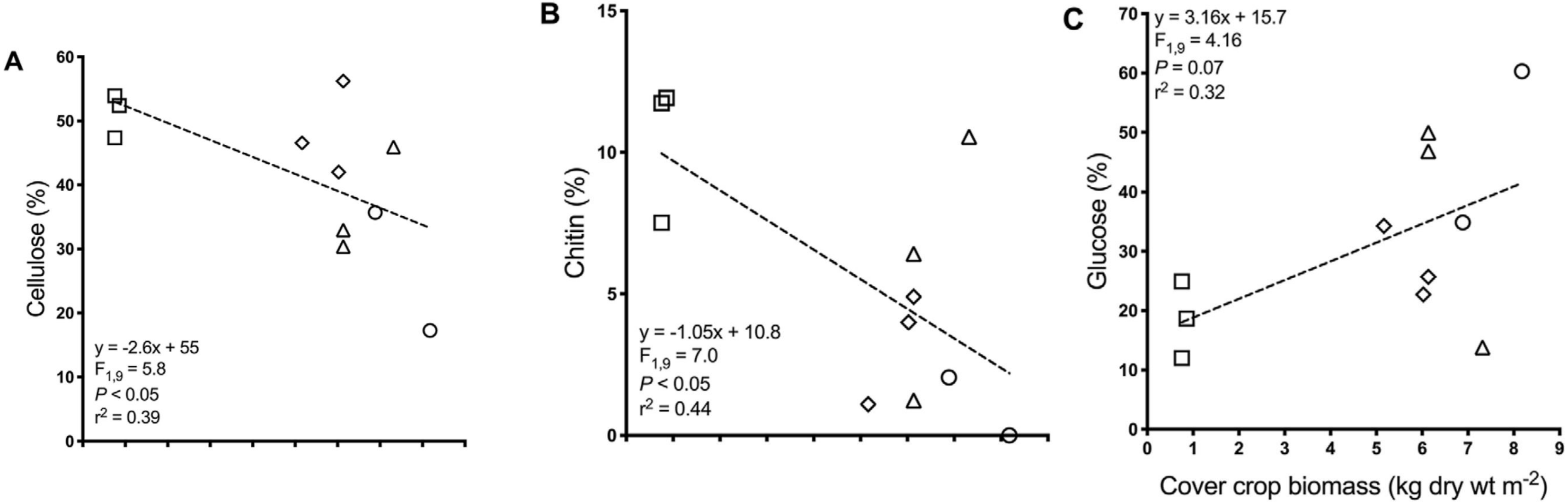
Stewart et al. (2018)





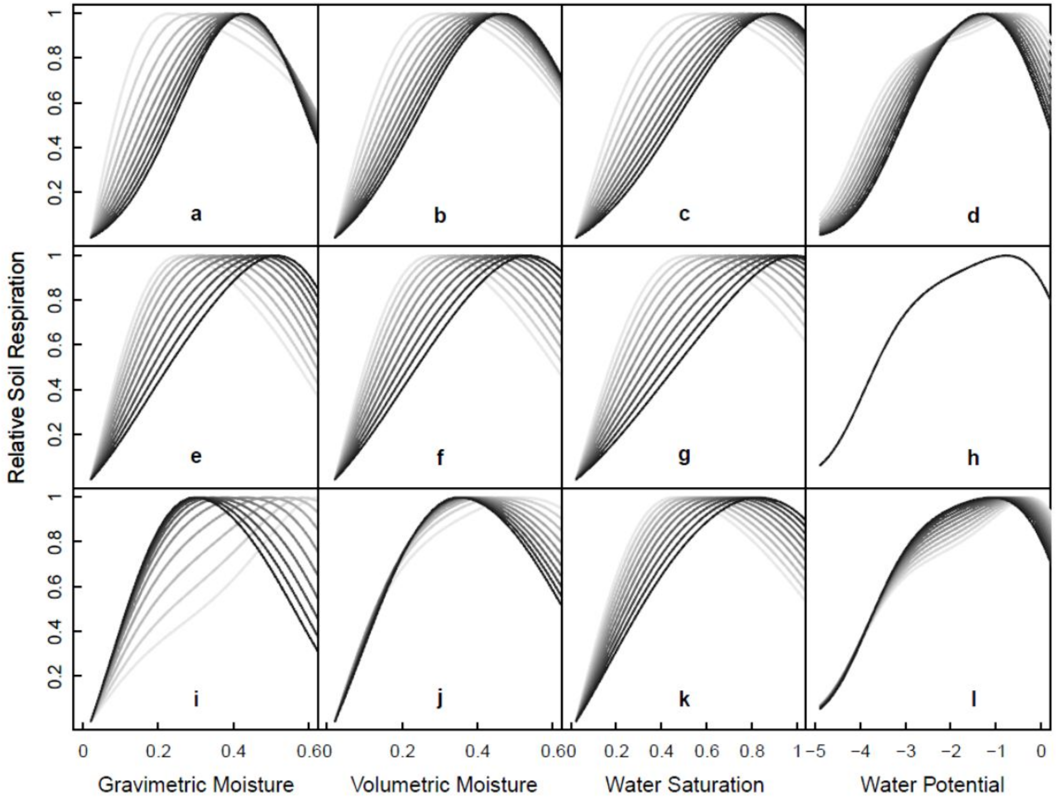
*Neither *total* microbial biomass nor fungal:bacterial ratios were significantly different with and without cover crops

Strickland et al. 2019



Strickland et al. (2019)

What about water availability?



Varying clay content

Varying organic matter

Varying bulk density

Moyano et al. (2012)

What about water availability?

- Soil moisture availability is a key determinant of microbial activity
  - Too little = moisture stress
  - Too much = decreased oxygen availability

- Most microbial activity assays are determined at the bench scale on dried soils re-wetted to controlled moisture conditions
  - 50-60% of field capacity
  - Saturation for enzyme assays

Conclusions

Point #1: DNA sequencing has revolutionized soil microbiology but the information provided has not been linked to soil health metrics

Point #2: Commonly recommended indicators are primarily related to SOM content and activity, but methods still vary

Point #1: Biological indicators can certainly be responsive to soil management changes it's generally assumed that "more is better"; but consistency and benchmarking is tricky across studies and regions

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Sun, S., & Badgley, B. D. (2019). Changes in microbial functional genes within the soil metagenome during forest ecosystem restoration. *Soil Biology and Biochemistry*, *135*, 163-172.

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