

Soil Health and Nutrient Dynamics in Agroecosystems of the Midwestern US

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the  
Graduate School of The Ohio State University

By

Jordon Wade, M.S.

Graduate Program in Environment and Natural Resources

The Ohio State University

2019

Dissertation Committee

Steven W. Culman, Advisor

M. Scott Demyan

Kristin L. Mercer

Nicholas T. Basta

Jessica A.R. Logan

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## Abstract

Soil health is an emerging framework that seeks to integrate the physical, chemical, and biological components of soil. It is defined by the USDA as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans”. The breadth of this definition has allowed “soil health” to become a context-specific definition, letting soil health be defined in terms of the desired outcomes. In the context of agronomic nutrient management, the primary desired outcome is a tightening of the nutrient cycle to minimize losses to the environment. Here, I use the framework of soil health to understand how soil health indicators influence and are influenced by on-farm nutrient management practices. Three separate studies were conducted to: 1) understand the factors influencing the efficacy of the most widely used biological soil health metric, mineralizable carbon, 2) determine the effect of 12 years of phosphorus (P) restriction on biological and physical soil health in three Ohio sites, and 3) integrate biological soil health indicators into nitrogen (N) management strategies across the Corn Belt. The first study found that mineralizable C was variable across and within soil test labs. However, even after controlling for variations in methodology, a significant amount of the variability was soil-specific. The second study found very few effects of P restriction on soil biological and physical health. However, P restriction slightly increased organic P stocks and resulted in consistent shifts in the balance between the processed and easily-metabolized portions of the active C pool. In the third and final study, an increase in soil biological health was shown to increase the yields for a given N fertilization rate, as well as having slight predictive abilities

in predicting whether a site would be responsive to N fertilization. This study also showed that soil biological health may be slightly increased at moderate N fertilization rates. Collectively, these results show that biological soil health metrics can be used in nutrient management schemes, provided that a careful analysis and interpretation of the data is undertaken.

## Dedication

To caffeine and its metabolites paraxanthine, theobromine, and theophylline for keeping me alert, happy, and oxygenated (respectively) throughout my dissertation.

## Acknowledgments

I would never have been unable to complete this degree without the unwavering support of friends, family, and colleagues. I am eternally grateful to each and every one of you, although I'm sure I will forget some through my current dissertation fog.

First and foremost, I'd like to thank my advisor and mentor, Steve Culman. Your support—emotional, intellectual, and financial—has anchored me throughout this journey. I am so appreciative of the opportunities you have lavished upon me at each step. Working with you has truly been a joy and a privilege. To the remaining members of my committee—Scott Demyan, Kristin Mercer, Nick Basta, and Jessica Logan—I am so grateful for the time you have devoted to my personal and professional development. The insights and advice each of you have provided, both on my dissertation specifically and in the world of research more broadly, will continue to guide me for years to come. And to my unofficial committee member, Christine Sprunger, thank you for always having an open door and thoughtful advice to keep me on track.

To my OARDC coworkers: thank you for your support, your encouragement, and ultimately, your friendship. It has been a blessing to work side-by-side with you for the last few years. Specifically, to my labmates Bethany Herman, Nicole Hoekstra, Stuti Sharma, Meredith Mann, Mason Gingery, Madison Campbell, Otto Oppenheimer, and Nakian Kim: thank you for the countless hours of field work, sample prep, lab analyses, and data processing that you have

selflessly devoted to the projects contained here. And of course, the path would have been much rougher without my fellow grad students and comrades-in-soil: Matt Bright, Jennie Pugliese, Noelymar Gonzalez-Maldonado, Tania Burgos-Hernandez, Nall Moonilall, and Phoo Zone.

And finally, a heartfelt thank you to my family: the ones I was born with and the ones I have chosen. To my parents and relatives: thank you for your support and for being proud of me despite only vaguely grasping the weird world of academia I have immersed myself in. Your unwavering faith in me means more than I can say. And to Michael Manning, thank you for a ready ear, heartfelt praise and encouragement, and an easy laugh when I need it the most.

Somehow, you have supported me through a 3<sup>rd</sup> degree from thousands of miles away, a feat I will never understand. And of course, my best friend and partner Kelly Robyn Wilson, whose all-encompassing love and support springs eternal: I am forever indebted to and in awe of you.

## Vita

- 2005..... Capistrano Valley High School.
- 2012.....B.S. Soil & Water Science, University of California, Davis.
- 2016.....M.S. Soil & Biogeochemistry, University of California, Davis.

## Publications

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Field of Study

Major Field: Environment and Natural Resources

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## Chapter 1. Introduction

The history of agriculture is a consistent cycle of innovation and over-adoption, which is only slowed by ecological catastrophe. When the ancient Sumerians first began continuous cultivation of the Fertile Crescent c. 5000 B.C.E, they could never have anticipated the long-term effects of their irrigation management practices would be soil salinization and silt illuviation across the valley, ultimately contributing to the collapse of their civilization nearly 2000 years later (Hillel, 1992). Similarly, a direct line can be traced between John Deere's commercialization of the cast iron plow in the 1830s and the Dust Bowl of the 1930s that resulted from 100 years of excessive tillage (Baveye et al., 2011). While the Green Revolution has seen a nearly doubling of global food production, the intensive chemical fertilization that accompanied it has resulted in staggering losses of reactive nitrogen and phosphorus to the environment (MacDonald et al., 2011; Sattari et al., 2012; Lassaletta et al., 2014; Ladha et al., 2016).

The contemporary response to an over-reliance on chemical sources of fertility has been the development of a biologically-centered paradigm: soil quality or soil health. Although the earliest definition of soil quality in the scientific literature is “the ability of soils to yield corn, soybeans, and wheat” (Mausel, 1971), desirable soil health outcomes have since been broadened to include a diverse set of ecosystem services (Bünemann et al., 2018). In this way, soil health represents a substantial shift in the functionality of modern agriculture. It represents a bridge

between short-term thinking of yields and profit and the long-term thinking of land stewardship (Arbuckle, 2017). The desire for this bridging is evident in the farmer-reported concerns for the health of their soil, with short-term financial constraints being reported as the primary obstacle to achieving this goal (Carlisle, 2016; Arbuckle, 2017). While the paradigm of soil health largely centers biological activity, decades of work on indicators of soil biological health have produced little consensus in the scientific community (Doran and Zeiss, 2000). Those few indicators that have been proposed are underutilized and less well understood in terms of associated outcomes (Fierer et al., 2009; Bünemann et al., 2018).

In the second chapter, the most commonly used indicator of soil health—mineralizable C or respiration upon rewetting—is explored for its reliability across contexts. Reliability is determined by comparing values from across commercial labs, a comparison of several common methodological permutations, as well as an assessment of consistency of values obtained within a given lab. These results help bound the level of certainty we have in our current knowledge of soil biological health indicators.

Although biological soil health indicators are still largely understudied, understanding how they interact with fertilizer applications is a vital step in integrating soil biology into nutrient management. While N fertilizer applications can increase microbial biomass (Hartman and Richardson, 2013; Geisseler and Scow, 2014) and alter enzyme activity (Chen et al., 2018), P limitations can also constrain microbial metabolic activity (Ehlers et al., 2010; Hartman and Richardson, 2013). Therefore, a better understanding of how fertilization influences soil

biological health as well as how soil biological health influences fertilizer needs is a substantial gap remaining in the literature. The remainder of the dissertation explores these interactions.

The third chapter is an exploration of the potential impacts of phosphorus fertilizer restriction on soil physical and biological health. Consideration of agronomic productivity (e.g. grain yield and grain P content) is included in order to examine potential tradeoffs between soil health and maintaining yields. Results from this chapter show that 12 years without P fertilization will likely have no adverse impacts on soil health and very few impacts on crop yields.

In the fourth chapter, we have a multi-site study that looks to answer the basic question “does a healthier soil need less N fertilizer?” These sites include a variety of N rates and management practices that affect soil biological health, presenting a wide range of expected soil health levels. We use a combination of regression techniques, several common biological soil health indicators, and yield data to answer this basic question. Results show that increasing soil health—as indicated by our soil health variable—can help reduce, but not replace mineral N fertilizer application.

Improving the long-term sustainability of our agroecosystems is a monumental task, with many short-term considerations. Among these considerations is the ability to maintain agronomic efficiency or profitability. While the paradigm of soil health shows immense promise in endeavoring towards the long-term goal of sustainability, its short-term agronomic relevance has

been limited thus far. Collectively this dissertation strives to inch the paradigm of soil health along the path towards actionable sustainability for the Midwestern United States.

*The soil is the great connector of lives, the source and destination of all... It is alive itself. It is a grave, too, of course.... Given only the health of the soil, nothing that dies is dead for very long.*

*-Wendell Berry (The Unsettling of America)*

## Chapter 2. Sources of Variability that Compromise Mineralizable Carbon as a Soil Health Indicator

### Abstract

Mineralizable C, or respiration upon rewetting of dried soil, is a common soil health metric, but still lacks a widely-accepted and standardized protocol. A standardized protocol is an essential first step in quality control needed for a robust soil test. Here we examined numerous sources of laboratory variability associated with mineralizable C, with the overall goal of understanding the influence of each source on final values. Mineralizable C had 2-20 times greater inter-lab variability than other commonly-utilized soil tests, leading to a high degree of uncertainty associated with the interpretation of results. Procedural differences—such as sieve size and method of rewetting—significantly influenced mineralizable C measurements and underscore the need for the development of a standardized and universally adopted protocol. Capillary rewetting consistently suppressed mineralizable C relative to rewetting with a specific amount of water and is therefore not a recommended approach. However, the sensitivity of mineralizable C to changes in management did not differ between 6, 24, and 72 h incubation intervals. While these procedural effects may influence the inter-lab variability, there was also a considerable amount of analytical variability associated with mineralizable C measurements within a lab that is highly dependent upon soil type.

## Introduction

The development of commercially-viable soil health testing focused on biological properties is an essential step for improving the sustainability of our agricultural production systems (Kibblewhite et al., 2008). The burst of respiration upon rewetting of air-dried soil, commonly referred to as “the Birch effect” (Birch, 1959) or the “flush of CO<sub>2</sub> upon rewetting” (Franzluebbers et al., 2000), hereafter referred to as ‘mineralizable C’, is a potentially valuable tool in helping growers better understand the role that the microbial community plays in their soil (Franzluebbers, 2016). Mineralizable C has been widely accepted to be an important measure of the overall health and quality of a soil (Karlen et al., 1997; Moebius-Clune et al., 2016) and has been used as an integrated measurement of soil microbial biomass (Anderson and Domsch, 1978), microbial activity (Wang et al., 2003), and soil carbon availability (Franzluebbers et al., 2000; Wang et al., 2003).

The strong response from growers for currently available commercial tests of mineralizable C (e.g., Solvita ®) illustrates the demand for a rapid measure of soil biological activity and health. Additionally, governmental institutions have also begun to support the use of mineralizable C to measure improvements in soil quality and have established incentive programs for growers to use respiration measurements to track changes in their fields after improvements in management (NRCS, 2015). However, integrating biology—a central component of the framework of soil health (Doran and Zeiss, 2000)—results in increased complexity and, as with any new method, additional caution must be exercised upon the interpretation and use of the results. Mineralizable C, has been used extensively in research trials and although it has been shown to consistently differentiate between imposed treatment effects on a given soil type, there is no recognized standard operating procedure that has been utilized

across soil types. Standardization is one of many essential steps in the creation of a robust soil health indicator that can be translated across systems, soil type, and commercial test labs.

As with any laboratory metric, there are many potential sources of variation in mineralizable C measurements. One substantial source of variability is inter-lab variability, which is the basis for lab proficiency testing. However, proficiency testing assumes a standardization of methods between labs, which has not been the case thus far in multiple studies surrounding mineralizable C measurements. Variations in methodologies have included: sieve sizes ranging from 2mm to 6mm (Franzluebbers et al., 2000; Franzluebbers, 2016; Morrow et al., 2016; Castro Bustamante and Hartz, 2016), incubation intervals ranging from 6 to 72 h (Franzluebbers et al., 2000; Haney and Haney, 2010; Wade et al., 2016), as well as differences in the direction and final water content upon rewetting (Haney and Haney, 2010; Sherrod et al., 2012; Wade et al., 2016). Therefore, it is also necessary to investigate how any procedural or methodological differences may contribute to the variability of mineralizable C. Given that mineralizable C is a biologically-based metric, investigation of this variability is particularly salient when attempting to draw robust, accurate conclusions. Included in methodological considerations is the length of incubation for mineralizable C measurements, which has also differed between studies (Franzluebbers et al., 2000; Haney et al., 2008a; Wade et al., 2016). The ultimate goal of a universal protocol would be to minimize sources of unwanted variability so that the use of mineralizable C as a soil health metric would be as robust as possible. Therefore, this study seeks to: (1) assess inter-lab and analytical agreement for current commercially-available mineralizable C tests, (2) evaluate the effects of methodological differences—such as soil sieve size, water content, and direction of rewetting—on mineralizable

C values, and (3) determine the length of incubation that is most sensitive for detecting treatment and/or management differences.

## Materials and Methods

### Data Description

Our analysis included soil from eight studies on 72 agricultural cropland sites from across the United States (Table 2.1). In addition to traditional soil measurements (Table 2.2) mineralizable C was measured using permutations of soil processing and rewetting protocols ( $n=1142$  individual observations) to determine the sources of variation associated with these procedures (sieve size, water content, direction of rewetting). Additionally, selected studies were used to determine analytical and inter-lab variability associated with mineralizable C measurements. A description of methods and analyses performed on each study are shown in Table 2.3.

### Soil Analyses

Soil physiochemical characteristics, such as pH, textural characteristics, and soil C and N contents are listed in Table 2.2. To determine the effect of grinding or sieve size on mineralizable C, NY Grain soils were air-dried and either hand-sieved to <8mm, <2mm, or ground to <0.75 mm. ALP soils were either ground to <2mm or <0.8mm using an Agvise flail mill. Water-holding capacity (WHC) was calculated as the difference in weight between a saturated soil that was allowed to drain for an hour and the weight after the soil was oven-dried for 24 hours at 105°C.

## Mineralizable Carbon

Mineralizable C measurements were taken during incubations of 10-40g of air-dry soil ranging from 6h to 72h. The amount of soil used for each incubation was consistent within each study. For all studies, other than the Agricultural Proficiency Laboratory (ALP) study, gas samples were taken by extracting 1-5mL from the headspace of a 0.4 L Mason jar capped with a metal lid and a butyl rubber septum, and run on an infrared gas analyzer (model S-151, Qubit Systems Inc., Kingston, Canada). Mineralizable C was calculated as the difference between a sample and a control, using the total headspace and the ideal gas law (Zibilske, 1994) at a constant temperature of 22°C. In the ALP study, mineralizable C was measured using Solvita gel paddles at a constant temperature of 23°C. Rewetting of the air-dried soil was done either through capillary rewetting from below using the methods described in (Haney and Haney, 2010) or by adding a percentage (25, 50, 75, or 100%) of the calculated WHC with DI water dispensed directly on to the soil surface using a micropipette. In addition to rewetting from above, air-dried soil samples were also rewetted at 50% WHC from bottom to assess the effect of direction of rewetting on mineralizable C. For rewetting from the bottom, 50 mL polypropylene beakers with 4-5 6.5 mm diameter holes drilled in the bottom and glass microfiber filter were filled with soil and placed in the microcosm, which had been filled with the appropriate amount of DI water. All measurement methods (i.e. incubation length and instrumentation) are shown in Table 3. Depending on the study, soils were air-dried and stored at room temperature before mineralizable C analyses were performed. Storage time ranged generally clustered in three groups: <1 year (CA Grower Survey, WSREC, and RRSF studies), 2-4 years (TS, OUG, and WORT), or 9-11 years (NY Grain and select ALP Lab soils). While long-term storage of air-dried soil is well-known to increase the rewetting effect on mineralizable C measurements (De Nobile et al., 2006;

Kaiser et al., 2015), for this study we assumed that any artifacts due to sample storage would be influence all treatments within a study equally. For our data, we did not find any evidence that storage increased mineralizable C amounts ( $p=0.47$ ; data not shown) or variability. To further ensure that differences in storage time did not impact our results, all statistical analyses were constrained to individual studies or when comparing across multiple studies (e.g., Table 2.9), we used study was used as a covariate in the analysis.

Table 2.1 A description of study sites used for comparative analyses.

Study	Description	State (Location)	Sites (Plots/Site)	Reference(s)
Agricultural Lab Proficiency (ALP) Lab	Soils are collected from across the US and Canada and processed similarly to assess lab variability	Numerous <sup>†,‡</sup>	27 (1)	None
CA Grower Survey	Survey of grower fields across four growing regions of CA using mineral fertilizer with and without cover crops	California (38°37'-36°49' N, 121°51'-119°49' W)	21 (3-4)	(Wade et al., 2016)
CA Tomato Survey (TS)	Organically-managed tomato fields using compost and manures as fertilizer sources	California (38°33'-38°51' N, 121°48'-122°12' W)	13 (1)	(Bowles et al., 2014, 2015)
NY Grain	Grain farms across a management-induced soil fertility gradient	New York (42°36'-42°44' N and 76°42'-77°03' W)	7 (1-6)	(Schipanski et al., 2010; Schipanski and Drinkwater, 2011)
Ohio Urban Garden (OUG)	Urban garden using compost, compost + biochar, or compost + sudangrass cover crop	Ohio (41°04'49"N, 80°40'35"W)	1 (24)	(Beniston et al., 2015)
Russell Ranch Sustainable Agriculture Facility (RRSAF)	Long-term research trial involving corn-tomato rotations: mineral fertilizer w/ and w/o cover crops, or cover crops + compost/manure	California (38°32' N, 121°52' W)	1 (9)	(Wade et al., 2016)
West Side Research and Extension Center (WSREC)	Research plots with 15 years of minimal vs conventional tillage, with and without cover crops	California (36°20' N, 120°7' W)	1 (21)	(Mitchell et al., 2015)
Windsor Organic Research Trial (WORT)	Organic conversion trial with cropland converted from perennial ley, vegetable crops, or row crops, with compost, manure, or cover crop organic additions	Illinois (40°06' N, 88°16' W)	1 (36)	(Ugarte and Wander, 2013)

<sup>†</sup> ALP lab samples for inter-lab variability were from Arizona, British Columbia, Alabama, California, Connecticut, Florida, Idaho, Iowa, Kansas, Maine, Minnesota, Montana, Nebraska, Ontario, Quebec, South Carolina, South Dakota, Texas, and Wisconsin

<sup>‡</sup> ALP lab samples for sieve size, water content, direction of water addition, and analytical variability were from Iowa, Montana, Nebraska, North Dakota, Ohio, Saskatchewan, and Texas.

## Statistical Analyses

Table 2.2 Soil physical and chemical characteristics for each study. Values are mean (minimum, maximum).

Study	SOC (g kg <sup>-1</sup> soil)	TSN (g kg <sup>-1</sup> soil)	C:N	pH (1:1 water)	Clay (g kg <sup>-1</sup> soil)
Agricultural Lab Proficiency (ALP) Lab	18.1 (4.2, 55.7)	1.6 (0.3, 4.1)	11.1 (7.1, 13.5)	6.3 (4.6, 8.1)	195.1 (39.0, 320.0)
CA Grower Survey	9.4 (3.7, 19.7)	1.0 (0.4, 1.7)	9.0 (6.7, 13.5)	7.1 (5.1, 8.4)	329.6 (76.8, 608.0)
CA Tomato Survey (TS)	13.3 (5.9, 22.1)	1.5 (0.7, 2.2)	8.9 (7.6, 10.5)	6.7 (6.1, 7.3)	158.1 (88.7, 222.1)
NY Grain	19.0 (12.9, 26.8)	1.7 (1.2, 2.7)	11.1 (9.7, 12.9)	7.0 (6.2, 7.8)	273.9 (169.0, 369.5)
Ohio Urban Garden (OUG)	56.0 (10.4, 112.6)	4.1 (0.8, 8.3)	13.6 (12.2, 15.5)	7.7 (7.4, 8.0)	168.0
Russell Ranch Sustainable Agriculture Facility (RRSAF)	10.7 (5.6, 15.4)	1.2 (0.5, 2.0)	9.4 (7.3, 11.9)	7.2 (6.5, 8.2)	323.2 (147.2, 390.4)
West Side Research and Extension Center (WSREC)	6.2 (4.7, 8.0)	0.7 (0.5, 0.9)	8.9 (7.6, 10.5)	7.4 (6.7, 7.8)	358.8 (259.2, 531.2)
Windsor Organic Research Trial (WORT)	23.1 (13.4, 33.1)	1.8 (1.1, 2.3)	12.9 (11.4, 15.7)	ND <sup>†</sup>	ND

<sup>†</sup>ND = not determined

All statistical analyses were performed using RStudio (RStudio Team, 2016a). Linear regressions were run using the *lm()* command. To obtain F-values and p-values for associated differences, *Anova()* in the *car* package (Fox and Weisberg, 2011) was used to perform a type II ANOVA. This type of ANOVA only tests each effect after the other effects are accounted for, resulting in a more conservative attribution of significance than other methods of calculation (Langsrud, 2003). For all lettered differences, Tukey's HSD test was performed using the *HSD.test* command in the *agricolae* package (de Mendiburu, 2016). The sensitivity analysis for mineralizable C incubation length was performed using the *aov()* and the conservative type II

*Anova()* command in the *car* package, with the experimental factors (e.g. tillage, management, etc.) modeled as predictor variables. Three separate analyses were run using the incubation length (6, 24, or 72 h) as the response variable. The corresponding F-values are then representative of the magnitude of the effect exhibited by the predictor variable (experimental factors) on the response variable (incubation length).

To assess analytical and inter-lab variability, each soil was run in triplicate for each lab × treatment combination. The analytical variability between these replicates, represented as the coefficient of variation (CV), was calculated using the standard deviation normalized by the mean. To investigate the effect of these methodological differences on analytical variability (e.g. does sieving alter the analytical variability of mineralizable C?), the CV values were used as a response variable and the treatments—study, site/field within a study, sieving, water content, and direction of water addition—were used as predictor variables. Analytical variability from the treatments was determined in two separate labs (Table 2.3) and the effects were nested within these labs to isolate from any inter-lab variation in these measurements. To calculate inter-lab variability, three replicates were averaged to obtain the mean mineralizable C values for a given soil from that lab. This value was then combined with the means obtained in other labs to calculate the inter-lab variability (expressed as a CV-value) for that soil. The CV for inter-lab variability was calculated using the median in place of the mean due to the high degree of skewness in the distribution. Similar to prior analyses, F-values and p-values were obtained using the *aov()* command and the conservative type II ANOVA using the *Anova()* command in the *car* package.

Source of Variability	Corresponding Table/Figure	Studies used	Sample size (n)	Measurement Method	Length(s) of Incubation
Inter-lab variability	Figure 2.1	CA Grower Survey	28	IRGA <sup>†</sup> (UC Davis), Solvita gel paddles (Commercial labs)	24h
Inter-lab variability	Table 2.4	ALP	480	Solvita gel paddles	24h
Sieve size	Table 2.5	ALP, NY Grain	585	Solvita gel paddles (ALP), IRGA (NY Grain)	24h
Water content	Table 2.6	RRSAF, WSREC	30	IRGA	6h, 24h, 72h
Direction of rewetting	Table 2.7	ALP	126	Solvita gel paddles	24h
Incubation length/ sensitivity analysis	Table 2.8	CA Grower Survey <sup>‡</sup> , TS <sup>‡</sup> , OUG <sup>‡</sup> , RRSFAF <sup>‡</sup> , WSREC <sup>‡</sup> , WORT <sup>‡</sup>	452	IRGA	6h, 24h <sup>‡</sup> , 72h
Analytical Variability	Table 2.9	ALP, NY Grain, OUG	219	Solvita gel paddles (ALP), IRGA (NY Grain, OUG)	24h

<sup>†</sup> IRGA = infrared gas analyzer  
<sup>‡</sup> data has been previously published in part or in full in (Hurisso et al., 2016)

## Results and Discussion

### Inter-lab Variability

Low inter-lab variability is a primary criterion for a robust soil health metric. If different labs return different values for the same soil sample, the efficacy of that data is greatly diminished. Although numerous studies have shown mineralizable C to be sensitive to management practices and outcomes (Fraser et al., 1988; Franzluebbers et al., 2000; Haney et al., 2001; Schomberg et al., 2009; Culman et al., 2013; Wade et al., 2016; Castro Bustamante and Hartz, 2016), these relative differences, expressed via linear correlations, are not enough to meet the criteria of repeatability necessary for a soil health metric. Therefore, assessing absolute differences between labs is essential. To study this difference, seven independent soil samples were analyzed for mineralizable C at three Solvita Partner Plus certified commercial labs and one university

analytical laboratory (Table 2.3). Comparison of these results show that although there were moderately strong linear relationships, there were significant absolute differences between labs (Figure 2.1). In 4 of the 6 comparisons, there were considerable absolute differences ( $p < 0.01$ ) between values obtained in different labs. However, there was no clear relationship between the strength of linear relationship ( $R^2$  values) and mean absolute differences between labs, with the greatest  $R^2$  value ( $R^2 = 0.77$ ) corresponding to a highly significant absolute difference ( $p < 0.0001$ ) and the lowest  $R^2$  value ( $R^2 = 0.50$ ) corresponding to a statistically insignificant absolute difference ( $p < 0.05$ ). Some differences in absolute mineralizable C values may be attributable to the equipment differences between the infrared gas analyzer (IRGA) used in the UC Davis lab and the Solvita gel paddles used in commercial labs (Table 2.3). This is supported by the similar slopes in the relationships between the UCD Lab and the commercial labs.

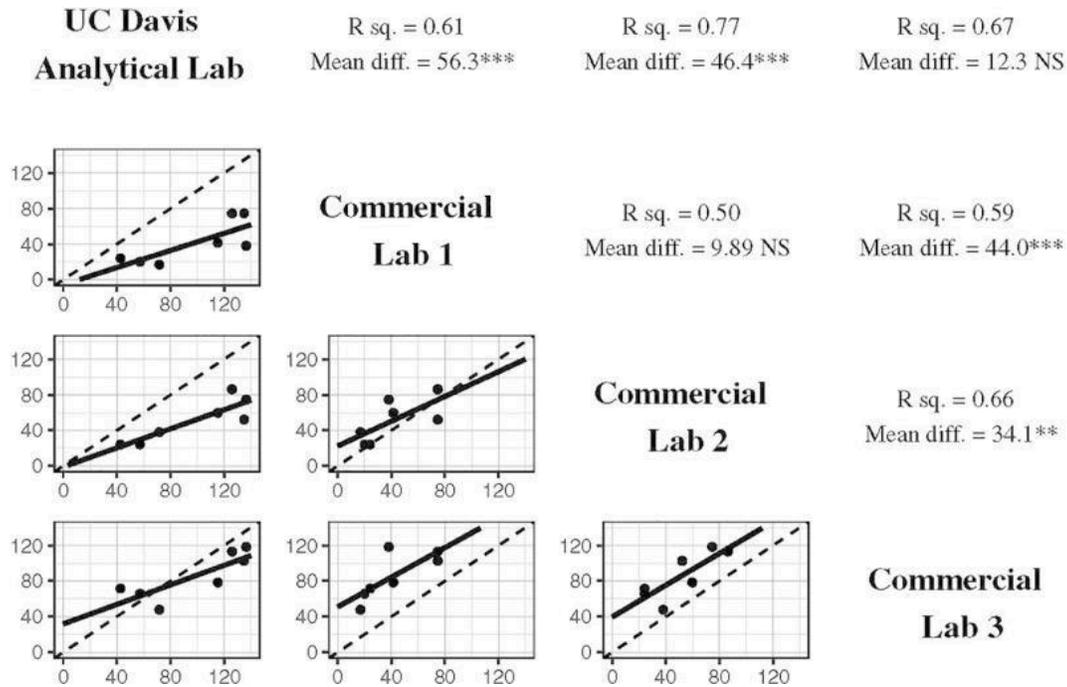


Figure 2.1 Differences in 24-hour mineralizable C values ( $\text{mg CO}_2\text{-C kg}^{-1}$  air dried soil) obtained in three commercial labs and one analytical lab. Dashed line represents a 1:1 relationship, indicating perfect agreement between values. “Mean diff.,” refers to the average difference between labs for a sample, with associated significance obtained by T-test.

\*, \*\*, and \*\*\* represent significance at the  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$  level, respectively. NS represents no significant differences.

However, the differences between regression lines and 1:1 lines that persist between commercial labs suggest that there is also significant amount of uncontrolled error associated with the use of Solvita gel paddles (Figure 2.1). Previous work has shown strong linear relationships ( $R^2 > 0.90$ ) between traditional methods of measuring mineralizable C, such as IRGA, gas chromatography (GC), and using a NaOH base trap (Haney et al., 2008b; Sherrod et al., 2012). However, these strong relationships are not shared in the relationships between the Solvita gel paddle and the traditional methods of measuring respiration, such as NaOH base trap ( $R^2 = 0.82$ ) and IRGA ( $R^2 = 0.79$ ) from (Haney et al., 2008b) and with NaOH base traps ( $R^2 = 0.84$ )

in (Haney et al., 2008a). Since the slopes and intercepts between NaOH and IRGA methods were comparable across studies (Haney et al., 2008a; 2008b; Sherrod et al., 2012), the IRGA-measured mineralizable C measurements should be considered the more consistent of the two methods presented (Table 2.3). In addition to the vetting and adoption of consistency in instrumentation, the focus of future study should be on establishing agreement in absolute rather than relative terms, which will be crucial in order to begin translating mineralizable C values from one lab to values obtained in another lab. Accordingly, the remainder of this paper will focus on variations and aberrations in absolute values of respiration rather than correlative values.

In order to determine the expected variation in mineralizable C values between labs and compare this variability to other traditional soil metrics (total C, total N, pH, clay, etc.), a set of 20 soils was sent to commercial labs, where each measurement was run in triplicate for each soil. The mean value of these three analytical reps was then compiled across labs for each soil to determine the inter-lab CV for each soil. A wide range of variability was shown between labs measuring 24-hour mineralizable C, with inter-lab CV values ranging from 4.21-53.17% across 20 soils. The mean inter-lab CV was greater than the median (Table 4), with a significant skewness value of 1.19 ( $p < 0.05$ ; data not shown) for the sample size (Pearson and Hartley, 1970), both of which indicate a long right tail on the distribution of CV values. This suggests that inter-lab variability is not evenly distributed across all of the 20 soils, but that most of the variability was actually less than the mean value in Table 4 and that median values may be a more appropriate measure. The median inter-lab CV value for mineralizable C (16.0%) is 2.8-19.3 times greater than the median inter-lab CV values for other commonly utilized soil test metrics, such as total C and N on combustion (2.93% and 5.63%, respectively), pH (0.83%), or

clay content (5.69%). Additionally, mineralizable C inter-lab CV was highly variable by soil, with CV values ranging from 4.2-53.2%. Taken together, this shows that mineralizable C measurements are much more variable between labs than other commercially available soil measurements and this variability is likely soil-specific.

Table 2.4 A comparison of the inter-lab coefficient of variability (%CV) for n=20 soils for 24h mineralizable C and other commonly-used lab procedures.

Metric <sup>†</sup>	# of Labs	Mean <sup>‡</sup>	Std Error	Median	Max	Min
Mineralizable C (Solvita)	8	19.8	3.1	16.0	53.17	4.21
Total C (combustion)	13	10.3	7.3	2.9	149.19	1.06
Total N (combustion)	15	11.8	4.5	5.6	86.36	2.00
pH (1:1 water)	59	0.9	0.1	0.8	1.59	0.54
Sand	26	4.4	0.8	3.6	16.48	0.80
Silt	26	5.7	0.8	4.9	17.10	2.28
Clay	26	7.6	1.1	5.7	20.33	2.26
Nitrate	42	5.0	0.4	4.4	10.19	2.93

<sup>†</sup> Each metric was run in triplicate in each lab, the mean of which was averaged with other labs to establish the inter-lab CV for each soil.

<sup>‡</sup> Mean refers to the mean inter-lab CV for all 20 soil types.

## Sieve Size

Soil processing (e.g. sieving of air-dried soils) can alter the results obtained in analyses. Given that the susceptibility of soil C to mineralization is largely controlled by physical protection rather than chemical recalcitrance (Kleber et al., 2011; Dungait et al., 2012), we hypothesized that sieving to smaller sizes would reduce the physical protection of soil C and result in higher mineralizable C values. Finely-ground (<0.75mm and <0.8mm in NY Grain and ALP studies, respectively) and <2mm soil had similar mineralizable C values in both NY Grain and ALP Lab studies, but soil that was sieved to <8mm had a significantly ( $p<0.05$ ) lower mineralizable C value relative to the other sieve sizes (Table 2.5). These results agree with previous findings by Franzluebbers (1999b) that the physical protection of mineralizable C reaches a threshold at

2mm, below which additional disturbances do not increase C mineralization. Additionally, our results show that the effect of sieve size on mineralizable C observed at 72h by Franzluebbbers (1999b) is also evident at 24h (Table 2.5). Thus, sieve or grinding size can influence mineralizable C values, and standardization for soil processing will be required for better comparison of treatment effects on soil health across studies.

Table 2.5 Mean values  $\pm$  standard error of 24h mineralizable C (mg CO<sub>2</sub>-C kg<sup>-1</sup> soil) for the NY Grain and ALP Lab studies. Within rows, means followed by the same letters indicate no significance at the  $p < 0.05$  level using Tukey's HSD test.

Study	n	Ground	2mm	8mm
ALP Lab	63	51.1 $\pm$ 3.7 a	50.6 $\pm$ 3.1 a	ND <sup>†</sup>
NY Grain	151	75.4 $\pm$ 2.3 a	76.5 $\pm$ 2.4 a	64.7 $\pm$ 2.3 b

<sup>†</sup>ND = not determined

## Water Content

The water content of incubated soil had a significant effect on soil mineralizable C (Table 2.6). We observed a bell-shaped response of mineralizable C to water content, similar to previous studies (Linn and Doran, 1984; Franzluebbbers, 2016), with a maximum response typically occurring between 50 and 75% WHC. The greatest mineralizable C value in the RRSF study at 72h was at 100%WHC, but this was not significantly different from 50 or 75% WHC ( $p < 0.05$ ; Table 2.6). The capillary method of rewetting bringing soil to 100% WHC had a distinct inhibitory effect on mineralizable C at both locations and all intervals, with the effect being more pronounced at the shorter intervals of 6 and 24 hours (Table 2.6). The glistening soil surface observed when using capillary rewetting and the further inhibition of mineralizable C measurements over 100% WHC suggest that the capillary rewetting method proposed by Haney

(2010) can result in supersaturated (>100% WHC) soils that do not optimize heterotrophic respiration incubations. This is in agreement with previous studies that have shown 50-60% of saturation simultaneously optimizes substrate transport and gas diffusivity across C contents (Linn and Doran, 1984; Hashimoto and Komatsu, 2006; Moyano et al., 2013).

Table 2.6 The interactive effects of water content and time interval on mineralizable C for two long-term research trials in California with n=3 fields for each value. Letters indicate significant differences ( $p<0.05$ ) within Location  $\times$  Mineralizable C interval using Tukey's HSD Test.

Location	Water Content	Mineralizable C (mg CO <sub>2</sub> -C kg <sup>-1</sup> soil)		
		6h	24h	72h
RRSAF	25% WHC	79.7 a	119.1 bc	144.0 c
	50% WHC	103.8 a	168.4 ab	267.2 ab
	75% WHC	105.8 a	197.1 a	349.6 a
	100% WHC	91.4 a	169.3 ab	353.4 a
	Capillary method	24.7 b	61.1 c	170.8 bc
WSREC	25% WHC	39.0 a	88.7 bc	284.0 ab
	50% WHC	51.2 a	141.7 a	444.2 a
	75% WHC	34.8 ab	126.2 ab	396.4 ab
	100% WHC	18.6 bc	90.2 abc	341.5 ab
	Capillary method	14.1 c	68.0 c	263.1 b

When directly comparing the two water contents that are currently utilized in commercial soil labs and in previous studies (50% WHC and the capillary method of rewetting), the 50% WHC had significantly greater mineralizable C values across all combinations of site and time interval, except at the 72h interval at RRSAF, which were statistically similar (Table 2.6). These trends show that the greater mineralizable C values measured at 50% WHC could allow for greater sensitivity of analysis (Wade et al., 2016; Castro Bustamante and Hartz, 2016) and therefore an increased ability to detect statistical differences due to management (Ladoni et al., 2015). Together, these results show that although the capillary method of rewetting represents a significant decrease in labor and analysis time, the decrease in sensitivity of response likely offsets the benefits. Both gravimetric (Culman et al., 2013; Wade et al., 2016; Castro Bustamante

and Hartz, 2016) and volumetric measurements (Franzluebbers, 1999a; Franzluebbers et al., 2000; Haney et al., 2001) have been utilized in previous studies and, while they have been found to be related to one another (Haney and Haney, 2010), the two approaches have not been comparatively evaluated.

Table 2.7 Differences in 24h mineralizable C by water content and direction of water addition. Letters indicate significant differences ( $p < 0.05$ ) using Tukey's HSD Test,  $n=42$  for each mean value obtained.

Water Content	Direction of Addition	24h Mineralizable C (mg CO <sub>2</sub> -C kg <sup>-1</sup> soil)
50% WHC	Top	70.66 a
	Bottom	49.90 b
Capillary	Bottom	31.95 c

#### Method of Rewetting

We assessed the effect of method of rewetting on mineralizable C by adding water (50% WHC) to air-dried soil (1) from the top as well as (2) from the bottom and then compared with (3) capillary rewetting from below. The absolute differences in 24h mineralizable C were greater between the directions of rewetting at 50% than between differing water contents when rewetted from below (Table 2.7). Thus, similar to the results found in Table 6, capillary rewetting inhibited respiration, relative to the 50% WHC, even when accounting for differences in direction of rewetting (Table 2.7). The difference between top- and bottom-wetted soils at 50% WHC is likely due to differences in water flow: wetting from above would fill all pores, followed by the draining of water from the macropores over a short time interval, whereas wetting from below is primarily driven by capillary action, which would result in slower and more unequal distribution of moisture towards the top of the soil column (McCoy et al., 1994).

This effect may be mitigated with incubation intervals longer than the 24h period investigated here, although currently, no studies have been conducted on the topic. Therefore, these results suggest that rewetting from above will optimize the sensitivity of the measurement for 24h incubations.

Table 2.8 A comparison of the relative sensitivity of mineralizable C measurement interval to detecting experimental factors associated with each study. F-values were generated using the mineralizable C interval as a response variable for each of the experimental factors (including significant interactions). Bolded F-values represent the interval that yielded the greatest sensitivity for a given experimental factor within a study.

Study Name	Experimental Factor	Mineralizable C F-Value					
		6h		24h		72h	
CA Grower Survey	Site	2.49	***	4.91	***	<b>5.49</b>	***
	Growing Region	<b>7.24</b>	***	6.06	***	7.01	***
	Cover crop use	0.84		0.83		<b>1.74</b>	
CA Tomato Survey	Site	<b>5.53</b>	***	1.71	†	1.39	
	Fertilizer Source	<b>4.69</b>	*	1.97		0.56	
OUG	Management	1.53		1.25		<b>2.40</b>	†
RRSAF	Management	7.10	**	<b>12.67</b>	***	6.89	**
WSREC	Cover crop use	7.67	**	8.89	**	<b>13.59</b>	***
	Tillage	1.50		1.81		<b>2.87</b>	
	Cover crop × tillage	0.87		1.08		<b>3.06</b>	†
WORT	Management	0.18		0.43		<b>0.62</b>	
	Fertilizer Source	<b>2.17</b>		1.56		1.50	
<b>Total instances where F-value was greatest</b>		4		1		7	
<b>Total instances where F-value was statistically significant (p&lt;0.10)</b>		6		5		6	
<b>Percentage where interval was most sensitive</b>		33%		8%		58%	
<b>Percentage where interval was statistically significant (p&lt;0.10)</b>		50%		42%		50%	

†, \*, \*\*, and \*\*\* represent significance at the p<0.10, p<0.05, p<0.01, and p<0.001 level, respectively.

## Length of Incubation

Several commercial implementations of mineralizable C have different lengths of incubations ranging from 24 to 96 hours. Although it is well documented that short-term mineralizable C

measurements correspond well to longer incubation intervals (Franzluebbers et al., 2000; Haney et al., 2008b), it is unclear if there is an incubation duration that is more sensitive to treatment differences within a trial. To assess the sensitivity of mineralizable C measurements at different incubation intervals to experimental factors, we used *F*-statistics generated from analysis of variance models in which incubation length (6, 24, or 72 h) was used to compare the degree of treatment effect across incubation times. Incubation duration served as a response variable with experimental factors (site, fertilizer source, tillage, etc) as predictor variables. The sensitivity of the incubation interval to differences in experimental factors was mixed (Table 2.8). The 6h and 72h intervals had the greatest sensitivity to experimental factors, being selected as the most sensitive indicator 33% and 58% of the time, respectively (Table 2.8). However, both were able to detect statistically significant differences in 50% of the studied factors, which was also similar to 24h mineralizable C, which was able to detect significant differences in 40% of the experimental factors studied here.

There were also no distinct trends in terms of types of managements that were better detected by mineralizable C measurements. In general, mineralizable C was sensitive to inputs of labile carbon and to determining differences between sites (Table 2.8). While many differences in labile C inputs were detected, such as cover crops in WSREC, management in both RRSAF and OUG, and fertilizer source in CA Tomato Survey, there were also many differences that respiration was not sensitive to, such as fertilizer source in the WORT study and cover crop use in the CA Grower Survey. Most of the effect of the management differences were shown across all three time intervals, with the exceptions of fertilizer source in the CA Tomato Survey and management in the OUG site, which were only detected by 6h and 72h mineralizable C, respectively. The ability to differentiate between sites was found in both of the multi-site studies

included, with 6h being most sensitive in the CA Tomato Survey and 72h being most sensitive in the Grower Survey. However, given that both of these were found within the Central Valley of California, it is unclear if these trends would be consistent in other climates and edaphic conditions. The current sensitivity analysis showed that no single mineralizable C interval was consistently more effective at detecting treatment differences, although a relaxing of the threshold of statistical significance may increase the efficacy of these metrics in an applied setting (Morrow et al., 2016).

Table 2.9 The effect of each factor on the precision of 24h mineralizable C, as measured by coefficients of variation among triplicate replications. F-values are based on a type II ANOVA with all factors included.

Factor	F-Value
Study	10.82**
Field	13.54***
Sieve Size	0.54 <sup>NS</sup>
Water content	2.25 <sup>NS</sup>
Direction of water addition	1.45 <sup>NS</sup>

\*, \*\*, and \*\*\* represent significance at the  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$  level, respectively. NS represents no significant effect.

### Analytical Variability

In order to determine the source and magnitude of analytical variability associated with mineralizable C measurements, samples that had been treated with procedural variations (e.g. sieve size and water additions) were run in triplicate to obtain a CV for a given procedure. These CV values were then used as response variables in a linear model to determine their effect, as well as study-specific treatment and edaphic effects, on analytical variability. The magnitude of

the effects associated with these variables, i.e. F-values, were then evaluated to determine their statistical effect on analytical variability (Table 2.9). A range of CV values from 0.5-84.4%, with a mean of 18.4% and a median of 12.4% were found (data not shown), which agree with the range of analytical variability found in the literature (Ahn et al., 2009; Zagal et al., 2009; Morrow et al., 2016). The examined soil processing sources of variability—sieve size, water content, and direction of rewetting—all showed that they did not significantly increase the analytical variability (Table 9) of 24h mineralizable C. This is not to say that these sources do not contribute to variation in the method, but rather how they are standardized (i.e, ground vs. sieved) has little influence on the repeatability of the measurement for a given field or soil type.

There were significant differences in the analytical variability between studies and between soils or fields within a study (Table 2.9), i.e. some soils within a study had higher intrinsic analytical variability than others and some studies had higher analytical variability than others. The analytical variability associated with these respective sources of variability was highly significant, although the soil- or field-level variability was slightly greater than the difference between studies ( $F_{\text{Study}} = 10.82$  and  $F_{\text{Field}} = 13.54$ ; Table 2.9).

The between-study variability can be attributed to both differences in instrumentation (Table 2.3) and climatic differences, although the relative importance of these factors is unclear. However, between-site variability is in agreement with the results obtained from the inter-lab variability tests (data not shown), in which some soils were more variable across labs than others.

This soil-specific variability is attributable many edaphic characteristics, such as carbon or O<sub>2</sub> availability, soil aggregation, and soil texture (Linn and Doran, 1984; Mikutta and Kaiser, 2011; Moyano et al., 2013; Angert et al., 2015; Yan et al., 2016), many of which would not be

addressed simply by sieving (Table 2.9). Additionally, the soil mineral composition can significantly alter the potential for hysteresis effects upon drying (Kaiser et al., 2015) and on the wettability upon rewetting (Woche et al., 2017), which has been previously shown to alter the mineralizable C measurements (Goebel et al., 2007). The relative importance of these potential confounding factors would likely be especially salient at lower concentrations of mineralizable C (Paterson and Sim, 2013) that are thought to indicate less “healthy” soils.

This study has examined several common method variations in mineralizable C procedures, but these variations are by no means exhaustive. Additional factors not examined here, such as soil column height, drying time, drying temperature, and incubation temperature (Creamer et al., 2014), have yet to be optimized, but may also contribute significantly to variability and should be investigated.

#### Broader Implications of Variability

Similar to other soil measurements, mineralizable C has multiple sources of variability: spatial, temporal and analytical. However, our findings that these sources of variability are soil-specific may be a substantial hurdle to a repeatable measurement of mineralizable C and to its utility as a robust soil health metric. Here we have used a conservative type II ANOVA to determine effect sizes, suggesting that the potentially confounding effects are even greater when more liberal analyses are performed. Several *in situ* studies of respiration have found that samples sizes of up to 75 separate samples are needed to achieve 95% confidence in values  $\pm 10\%$  of a population mean (Davidson et al., 2002; Adachi et al., 2005) in order to account for these multiple sources of variability. In the current study, the analytical variability is exemplified in the lack of statistical differences at the 95% confidence level between the means of 25% WHC (284.0 mg

CO<sub>2</sub>-C kg<sup>-1</sup> soil) and 50% WHC (444.2 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil) in the WSREC study (Table 2.6), despite a 56% increase in mean mineralizable C measured. In a commercial setting, this analytical variability can result in unreliable and/or inconsistent recommendations when using a single measurement. If additional analytical replicates were to be suggested, this would increase the cost of analysis and may serve as a financial barrier for growers (Carlisle, 2016).

## Conclusions

Mineralizable C is currently being used in multiple commercial tests as an indicator of soil health. Previous studies have often focused on a narrow range of soils in a given study or have examined linear relationships of mineralizable C with other variables, obscuring the potential discrepancies in absolute values that can be obtained using this metric. However, there are many sources of variation that contribute to differences in absolute mineralizable C measurements. Sieve size, water content, and direction of rewetting were all found to be significant sources of variability, underscoring the need for standardization of soil handling procedures in order to help minimize experimental error across locations. In particular, the capillary method of rewetting inhibited mineralizable C measurements, which would likely result in decreased analytical sensitivity and hence we recommend not using this method to rewet soils in mineralizable C analyses. Calculating water content to be added on a soil-by-soil basis will undoubtedly increase the analysis time and cost, but will improve the overall accuracy of the measurement. We found no evidence that flail grinding to pass through a 2mm sieve (as is commonly practiced in commercial labs) negatively impacts the measurement, nor that 6, 24, or 72h yielded results that were more sensitive to management differences. Therefore, we see no justification in modifying the most common approach of a 24h incubation on ground < 2mm soils. Even after controlling

for procedural variations, the repeatability of the metric varied widely across soils and studies. Until the sources of analytical variability are better understood, we recommend that mineralizable C measurements be run with analytical replication.

## Chapter 3. How Does Phosphorus Restriction Impact Soil Health Parameters in Midwestern Corn-Soybean Systems?

### Abstract

Limiting agricultural phosphorus (P) losses to surface waters is essential to overall ecological sustainability of agroecosystems. Recent studies have suggested that decreasing P fertilization rates decrease organic matter content, adversely impacting other mitigation strategies. Corn-soy cropping systems from three soil regions of Ohio were subjected to 11 years of P restriction to measure impacts on soil P availability, agronomic performance, as well as both physical and biological indicators of soil health. While both soil P availability and plant tissue P contents decreased with P fertilization rate, crops did not exhibit signs of P stress, such as consistent decreases in corn yield. Organic P levels increased in plots with no P fertilization. Both physical and biological indicators of soil health showed mixed responses to P fertilization, although trends suggested greater organic matter stabilization in unfertilized plots relative to the fertilized plots. This study suggests that reductions in P fertilization can result in more efficient nutrient cycling without adverse agronomic impacts, although it is unclear how long this effect would persist before P restriction would consistently impact grain yields.

### Introduction

The application of fertilizer-based phosphorus (P) is generally thought to be necessary for maximizing crop yields (Stewart et al., 2005). To ensure maximum yields are achieved, fertilizer

P is often applied in excess of crop P removal rates, a phenomenon that is ubiquitous across much of North American croplands (MacDonald et al., 2011). This over-application of both organic and mineral sources of P makes agriculture fertilizer a consistent contributor to nonpoint pollution of surface waters (Carpenter et al., 1998; Smith et al., 2015). The Western Lake Erie Basin is no exception to this surface water pollution, resulting in harmful algal blooms and significant risks to public health (Carmichael and Boyer, 2016; King et al., 2017).

Some strategies to mitigate agriculturally-based P losses focus on improving soil health and nutrient management, including: 1) increasing infiltration rates, 2) improving soil structure, and 3) decreasing P fertilization rates (Ohio EPA, 2013; Sharpley et al., 2015). Soil health has physical, chemical, and biological components to it, with organic matter acting as an intermediary between these three components (Magdoff and Weil, 2004). Therefore, the first two strategies to limit agricultural P losses to surface water are extensively linked to changes in soil organic matter content. On one hand, increases in organic matter lead to increases in aggregation (Tisdall and Oades, 1982; Franzluebbers, 2002; Six and Paustian, 2014), which in turn influences soil hydraulic conductivity and infiltration (Franzluebbers, 2002; Lado et al., 2004; Zeleke and Si, 2005). On the other hand, decreases in P fertilization rate may substantially alter the cycling of P through organic matter, with recent studies suggesting P constraints on soil organic matter accrual (Cai and Qin, 2006; van Groenigen et al., 2006; Khan et al., 2007; Manna et al., 2007; Reid, 2008; Kirkby et al., 2013; Poeplau et al., 2015; Tipping et al., 2016; Liang et al., 2016), possibly through plant exudation of low molecular weight organic acids in response to P stress (Clarholm et al., 2015; Keiluweit et al., 2015). To further support this mechanistic link, several field studies have also shown decreases in soil C content of temperate cropping systems with low P availability, further supporting this potential mechanism (Wuest and Reardon, 2016; Romanyà

et al., 2017). Collectively, these findings suggest that the differing dimensions of the overall strategy to limit P losses to surface water may be working in opposition to one another. Specifically, we hypothesize that decreases in P fertilization (dimension 3) could lead to decreases in organic matter, ultimately limiting improvements in soil health metrics (i.e. dimensions 1 and 2).

We used long-term (11-year) P restriction trials located in three of Ohio's major land resource areas (MLRAs) to investigate the effect of soil P availability on overall soil health and agronomic performance. Specifically, we sought to investigate the effect of P fertilization on: (i) agronomic performance in corn-soybean systems, (ii) soil P levels, (iii) biological indicators of soil health (i.e. active organic matter), and (iv) physical indicators of soil health, namely hydraulic conductivity, aggregate stability, and penetration resistance. These effects were examined across three sites that are representative of soil types commonly used in corn-soybean production in Ohio and across the Midwest. Therefore, any effects may have implications at greater spatial scales.

## Material and Methods

### Soil sampling and site description

We gathered soils in the spring of 2017 from three field trials across the state of Ohio (Table 3.1). These trials were established in 2005, the full details of which can be found in Fulford and Culman (2018). Each site was representative of a MLRA of Ohio extensively cropped in corn and soybean (NRCS, 2006). We averaged the site climatic data for the 20 years prior to sampling (1997-2017) using the Ohio Agricultural Research and Development Center Weather Network

(<http://www.oardc.ohio-state.edu/weather1/>). We described soil series and classification using the Web Soil Survey (NRCS, 2009) (Table 3.1).

All three field trials are a randomized complete block design with four replications (blocks). We established fertilization rates in a corn-soy or corn-corn-soy rotation using the estimated grain removal rates. To estimate the removal rates, we multiplied the average statewide corn and soy yields at the time of trial establishment (9.1 Mg ha<sup>-1</sup> and 2.7 Mg ha<sup>-1</sup>, respectively) by the estimated grain P removal rates (6.6 kg P<sub>2</sub>O<sub>5</sub> Mg grain<sup>-1</sup> and 13.3 kg P<sub>2</sub>O<sub>5</sub> Mg grain<sup>-1</sup>, respectively) (Vitosh et al., 1995). Fertilization rates were then applied at 0×, 1×, and 2× the estimated removal rate from 2005-2015. In 2016, we adjusted the highest rate from 2× to 3× grain removal and eliminated the previous corn-corn-soy rotation. In the current study, we only selected plots without rotation alterations, however the highest P rate did increase from 2× to 3×. This resulted in 1×, 2×, and 3× fertilization rates of 60, 120, and 180 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively, following corn and 35.9, 71.8, and 107.7 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively, following soybean. Fertilizer was surface broadcast following fall harvest of soybeans as diammonium phosphate (DAP) and incorporated via chisel tillage. To reflect this legacy of varying P fertilization rates in the highest fertilization treatment, this treatment will be referred to as the “2×/3×” treatment. Fertilizer P application rates and incorporation practices reflect the full range of farmer P application rates and are consistent with “best management practices” for the state (Smith et al., 2018). Total fertilizer N rate was 202 kg ha<sup>-1</sup> (as urea at planting and urea-ammonium-nitrate at sidedress) and fertilizer K rate was 43.7 kg ha<sup>-1</sup> (as muriate of potash) was applied for corn following soybeans. Soybean crops received an additional 62.9 kg ha<sup>-1</sup> of K fertilization to meet soybean demand, but no additional N fertilization.

Immediate after planting in 2017, we took soil samples of 8-10 cores per plot to a depth of 25cm using a 4cm diameter soil probe. Samples were composited, air-dried, hand-sieved to <2mm, and stored at room temperature pending analysis. To ensure precision, all analyses described below were conducted on <2mm sieved soil from the composite sample (Hurisso et al., 2018b).

Table 3.1 Site description and climatic data (1997-2017) for study sites. Values in parentheses indicate minimum and maximum, respectively.

Site	Major Land Resource Area	Dominant Soil Series	Classification	Mean Annual Precipitation (cm)	Mean Annual Air Temperature (°C)
Northwest	Erie-Huron Lake Plain	Hoytville clay loam	Fine, illitic, mesic Mollic Epiaqualf	86.2 (63.1, 126.1)	10.6 (8.9, 12.1)
Western	Indiana and Ohio Till Plain	Kokomo silty clay loam	Fine, mixed, superactive, mesic Typic Argiaquolls	97.2 (63.2, 138.3)	11.2 (9.7, 12.9)
Wooster	Lake Erie Glaciated Plateau	Canfield silt loam	Fine-loamy, mixed, active, mesic Aquic Fragiudalf	88.3 (69.1, 118.9)	10.2 (4.1, 11.7)

### Soil physical and chemical characterization

Soil chemical properties were determined for the pooled soil samples using the recommended analytical procedures for the region (NCR, 2011). In brief, pH was determined using a 1:1 soil:water mixture (Thomas, 1996) and soil organic C and N was determined via dry combustion. Soil nutrient concentration, including P, was determined via a Mehlich-3 extraction (Mehlich, 1984) and measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Total soil P was determined independently by x-ray fluorescence (XRF). Cation exchange capacity (CEC) was measured using the ammonium acetate method (Warncke and Brown, 1998).

Texture was determined using 50.0g of air-dried soil and the hydrometer method (Bouyoucos, 1962). The results of these analyses are shown in Table 3.2.

We determined soil physical properties in-field approximately one week after soil sampling. We measured penetration resistance (PR) using a SpotOn® digital soil compaction meter from Innoquest, Inc. (Woodstock, IL) to a depth of 25cm. Values from each plot were an average of 10 subsamples from across the plot. We adjusted these values for the gravimetric water content (GWC) using the following equation developed from Mielke et al. (1994):

$$PR_0 = aGWC_0^b \quad (1)$$

where  $PR_0$  is the original measured PR,  $GWC_0$  is the water content at the time  $PR_0$  was measured, and  $a$  and  $b$  are empirically-derived parameters for each site. Parameters  $a$  and  $b$  are derived for each site using a nonlinear least squares curve. The values were then adjusted for moisture content at sampling time using the following equation from Busscher et al. (1997):

$$PR_c = PR_0 + \frac{dPR_0}{dGWC_0} (GWC_c - GWC_0) \quad (2)$$

where  $PR_c$  is the corrected PR in MPa,  $GWC_c$  is the standardized gravimetric water content that measured values,  $\frac{dPR_0}{dGWC_0}$  is the first derivative of equation (1), and  $PR_0$  and  $GWC_0$  are as denoted in equation (1). The mean of all  $GWC_0$  values (0.205 g H<sub>2</sub>O g<sup>-1</sup> soil) was chosen for the standardized value of  $GWC_c$ . All references to “penetration resistance” (PR) will refer to  $PR_c$  values.

We measured hydraulic conductivity using Decagon Devices Mini Disc infiltrometers (Pullman, WA) at 2 cm of pressure head at two locations per plot. Hydraulic conductivity generally followed the stages of soil saturation proposed by Faybishenko (1995) where the second stage approaches the saturated hydraulic conductivity. Given the level of suction applied

(2 cm of pressure), the smaller pores are likely not saturated and this hydraulic conductivity is more appropriately termed “quasi-saturated hydraulic conductivity”. However, for the purposes of this study, we will use the term “saturated hydraulic conductivity” ( $K_{\text{sat}}$ ). We then calculated hydraulic conductivity according to Zhang (1997), which is expressed as the average of the two in situ measurements. At this scale and pressure head, the hydraulic conductivities likely overestimate  $K_{\text{sat}}$  relative to a truly saturated soil, where water flow through the smaller pores with pressure head  $> 2$  cm would decrease the measured hydraulic conductivity.

#### Hedley P fractionation

To assess the distribution of soil P fractions, we used a sequential chemical extraction that has been modified from Hedley et al. (1982). From the composited soil sample from each plot, we sequentially extracted two analytical replicates of 1.0g of air-dried soil, the means of which are reported for each fraction. Samples were extracted by shaking soil overnight (16 hr) with 20 mL each (in sequential order) of deionized water, 0.5 M  $\text{NaHCO}_3$ , 0.1 M  $\text{NaOH}$ , and 1 M  $\text{HCl}$ . Then, we centrifuged each extract for 15 minutes at  $8000 \times g$  and transferred the clear supernatant to new 50 mL polypropylene tubes. We determined inorganic P ( $P_i$ ) using molybdate colorimetry (Murphy and Riley, 1962) by reading on a 96-well plate reader at 880 nm. We analyzed all extracts in duplicate wells on the plate and used the average of those duplicates for each analytical replicate. To minimize analytical variability associated with sample processing, we determined total P in each extract by ICP-AES on unfiltered, unacidified, undigested extracts (Do Nascimento et al., 2015). Organic P was determined by difference between total P and  $P_i$  in each fraction. Total organic P was considered the sum of organic P from all fractions, which was predominantly ( $>98\%$ ) in the  $\text{NaOH}$  fractions, although both  $\text{NaHCO}_3$  and  $\text{HCl}$  extract contained

detectable organic P (He et al., 2006). Similar to Margenot et al. (2017b) the H<sub>2</sub>O- and NaHCO<sub>3</sub>-extractable P<sub>i</sub> fractions will be considered Labile P<sub>i</sub>.

#### Mineralizable carbon

Mineralizable C, also known as respiration upon rewetting or the flush of CO<sub>2</sub> upon rewetting, was measured on triplicate 10.0 g samples using 24 hour incubations in 50 mL microcosms. We rewetted soils from above to approximately 50% water-filled pore-space (Franzluebbers, 2016; Wade et al., 2018a). Mineralizable C was calculated as the difference between a sample and a blank control, using the headspace and the ideal gas law (Bottomley et al., 1994; Zibilske, 1994) and a constant temperature of 25°C. We measured the concentration of CO<sub>2</sub> by analyzing 1.0 mL of sample from the headspace on a LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE).

#### Permanganate oxidizable carbon

We measured permanganate oxidizable carbon (POXC)—also referred to as “active C” in some soil health tests (Moebius-Clune et al., 2016; Fine et al., 2017)—based on the methods of Weil et al. (2003), using the modifications proposed by Culman et al. (2012). In brief, 2.5 g was shaken for exactly 2 minutes in a 50 mL polypropylene centrifuge tube using 0.02 M KMnO<sub>4</sub> and allowed to settle for exactly 10 minutes. Next, we immediately transferred 0.5 mL of the supernatant to a new 50 mL centrifuge tube with 49.5 mL of deionized water to create a 100× dilution. We then measured sample absorbance on a 96-well spectrophotometer at 550 nm.

### Autoclaved citrate-extractable protein

Autoclaved citrate-extractable (ACE) protein was measured using the methods of Hurisso et al. (2018c) and the Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2016). In brief, 24 mL of 0.02 mol L<sup>-1</sup> sodium citrate (pH =7) was added to 3.0 g of air dried soil in a glass screw-top tube, shaken for five minutes, and autoclaved at 121°C (15 psi) for 30 minutes. After cooling, we shook the tubes for three minutes to resuspend soil particles and transferred 1.5 mL to microcentrifuge tubes for clarification of the extract (three min @ 10,000 × g). After cooling, we added a bicinchoninic acid reagent to soil extracts. Soil extracts were then transferred to a 96-well plate, sealed, and heated on a block heater at 60°C for one hour. After this one hour incubation, we allowed the plate to cool for 5 minutes prior to reading the samples colorimetrically at 562nm. We quantified ACE protein levels using bovine serum albumin standards fit to a second-order standard curve.

### Water stable aggregates

To measure water stable aggregates (WSA), we used the methods of the CASH (Moebius-Clune et al., 2016). Briefly, we dry sieved air-dried soil to collect aggregates between 0.25 to 2.00 mm. Next, we evenly distributed approximately 30 g of soil on a 0.25 mm sieve and subjected it to a simulated heavy rainfall event. Any soil that slaked and passed through the sieve was collected on a filter and air-dried at 105°C for at least 24 hours. The amount of slaked soil was subtracted from the total weight to determine the proportion of the total that was “water-stable”.

## Yield and plant tissue analyses

Plant tissue was gathered at the onset of the reproductive phase (R1). Approximately 10-12 corn ear leaves were collected per plot. Tissue was then air-dried, ground, and digested with nitric-perchloric acid before measuring P content on ICP-AES (Jones Jr and Case, 1990). Yield from each plot was determined by harvesting ears from the two central rows of corn plants in each plot using a plot combine and adjusting each sample to 15.5% moisture content. After harvest, grain was separated, processed, and analyzed for P content similarly to tissue.

## Statistical analyses

All statistical analyses were performed in JMP Pro 13 (JMP, Version 13, 2017). Linear regressions were run as a linear mixed model with site, fertilization rate, and their interaction considered fixed effects and block considered a random variable nested within site. Response variables (e.g. Yield, POXC, etc.) were log transformed as needed to meet assumptions of normality. Planned orthogonal contrasts between specific combinations of treatments were run on these mixed models. Principal components analysis (PCA) was performed in RStudio (RStudio Team, 2016b) on a standardized correlation matrix using the *rda()* command in the *vegan* package (Oksanen et al., 2016) following the method of Borcard et al. (2011). All biological and physical soil health indicators were included in the PCA, as well as clay content, corn tissue P, and corn yield data.

All plots were constructed on transformed data using the *geom\_boxplot()* command in the *ggplot2* package (Wickham, 2016). Mean values are denoted using a white diamond.

To analyze organic matter trends using soil health indicators, the method of Hurisso et al. (2016b) was used where residuals are examined by treatment. A simple linear regression was run

for each site using the *lm()* command with POXC as the response variable and mineralizable C as the predictor variable. Residuals were extracted and positive residuals (i.e. trending towards POXC) were considered organic matter building while negative residuals (i.e. trending towards mineralizable C) was considered organic matter use.

Table 3.2 Soil physiochemical characteristics for each site. All measurements are n=12 for each site, with standard errors in parentheses.

Site	pH (1:1 water)	Total C (g C kg <sup>-1</sup> soil)	Total N (g N kg <sup>-1</sup> soil)	Total C:N	Total P (mg P kg <sup>-1</sup> soil)	CEC (mmol/kg)	Clay content (g kg <sup>-1</sup> soil)	Sand Content (g kg <sup>-1</sup> soil)
Northwest	6.23 (0.12)	25.1 (0.41)	2.56 (0.06)	9.84 (0.11)	846.1 (16.4)	16.8 (0.83)	415 (4)	272 (4)
Western	5.88 (0.03)	19.2 (0.45)	2.02 (0.05)	9.52 (0.10)	621.2 (17.7)	10.2 (0.38)	216 (6)	276 (14)
Wooster	6.71 (0.08)	19.0 (0.35)	2.01 (0.05)	9.47 (0.13)	708.3 (9.6)	6.0 (0.37)	182 (4)	253 (7)

Table 3.3 Site and fertilization main effects for crop and soil agronomic parameters. All values are estimated least-square means with associated standard errors in parentheses. Letters represent significant differences within a main effect at the  $p < 0.05$  level using Tukey's HSD means separation. Results from analysis of variance (ANOVA) are from mixed effect model with block as random variable.

Factor	Treatment	Mehlich-3 P (mg kg <sup>-1</sup> )	Corn Tissue P <sup>†</sup> (%)	Corn Yield (bu ac <sup>-1</sup> )	Corn Grain P (%)
<b>Site</b>	Northwest	51.7 (2.4) a	0.41 (0.01)	154 (8) b	0.27 (0.01) a
	Western	32.3 (2.4) b	0.41 (0.01)	205 (8) a	0.24 (0.01) b
	Wooster	35.6 (2.4) b	0.42 (0.01)	164 (8) b	0.26 (0.01) ab
<b>Fertilization</b>	0×	21.3 (3.0) c	0.36 (0.01) c	171 (8)	0.23 (0.01) b
	1×	36.7 (3.0) b	0.42 (0.01) b	169 (8)	0.26 (0.01) a
	2×/3×	61.6 (3.0) a	0.46 (0.01) a	183 (8)	0.28 (0.01) a
<b>ANOVA</b>	Site (S)	**	ns	**	**
	Fertilization (F)	****	****	ns	****
	S × F	ns	**	ns	ns

†, \*, \*\*, \*\*\*, and \*\*\*\* correspond to p-values of <0.10, <0.05, <0.01, <0.001, and <0.0001, respectively. ns indicates no significance at the  $p < 0.10$  level.

<sup>a</sup> Significant interaction is further explored in Table 3.6.

## Results

### Agronomic response to P

Corn crop yields were not responsive to P fertilization across all sites (Table 3.3). Even non-significant general trends emphasized a lack of response to fertilizer, with least-square means in the order of  $2\times/3\times > 0\times > 1\times$ . While fertilization rate showed no effect on yield, there were marked differences between sites ( $p=0.008$ ), with Western having higher yields than either the Northwest ( $p=0.004$ ) or Wooster sites ( $p=0.009$ ).

Overall, plant indicators of P status—such as tissue P content at R1 and grain P content at harvest—showed consistently strong effects of P fertilization ( $p<0.0001$ ; Table 3.3). The effect of fertilization on tissue P and grain P followed the expected trend of  $0\times<1\times<2\times/3\times$ . Corn crops showed no site effects on tissue P and strong site effects on grain P. Among the three sites, Northwest had the highest grain P contents. The site  $\times$  fertilization interaction for tissue was due to the lower tissue P content in the  $0\times$  rate ( $p=0.003$ ) at Western, relative to the other sites.

### Soil phosphorus fractions

Both Labile  $P_i$  and M3-P increased with increasing fertilization rates (Figure 3.1;  $p<0.0001$ ) and differed strongly by site ( $p=0.02$  and  $p<0.0001$ , respectively). A significant interaction term between site and fertilization rate for Labile  $P_i$  ( $p=0.07$ ) indicates that this effect size differed between sites, with Northwest having the largest differentiation between fertilization treatments. Generally speaking, the M3-P values for  $0\times$  treatments were in the “buildup” or the lower end of the “maintenance” range, the  $1\times$  treatments in the “maintenance” range or lower end of the “drawdown” range, and the  $2\times/3\times$  in the “drawdown” range (Figure 3.1). The exception to this

trend was the Northwest site, which had 0x in the “maintenance” range and both 1x and 2x/3x in the “drawdown” range.

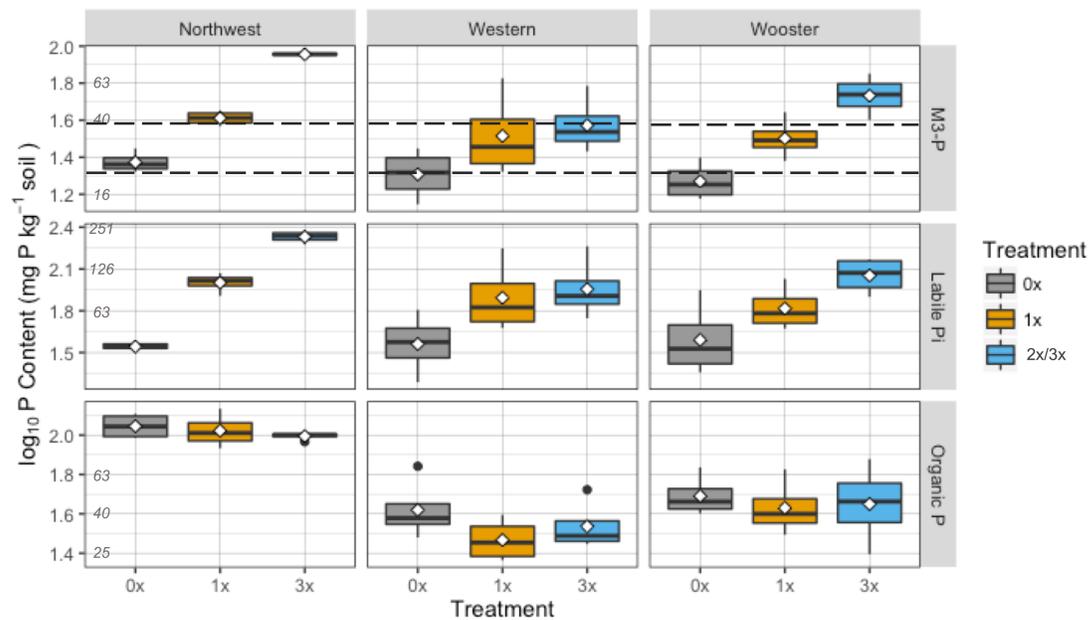


Figure 3.1 Soil phosphorus fractions by site and treatment. Italicized numbers on y-axis show backtransformed values. Dotted lines for M3-P indicate limits of the “maintenance range” of 20-40ppm from Tri-State Fertilizer recommendations (Vitosh et al. 1995; Culman et al., unpublished data).

Organic P levels differentiated strongly by site (Figure 3.1;  $p < 0.0001$ ): Northwest had higher levels of Organic P than Western or Wooster, which were not different from one another ( $p = 0.17$ ). There was a slight treatment effect, which showed an inverse relationship between fertilization rate and Organic P content ( $p = 0.068$ ). Thus, the ratio between Labile P<sub>i</sub> and Organic P increased as fertilization rates increased (Figure 3.1). Orthogonal contrasts showed that the 0x treatment had an overall much higher level of Organic P than the fertilized treatments ( $p = 0.029$ , data not shown). However, this relationship was only significant in the Western site ( $p < 0.001$ ).

### Biological soil health indicators: active organic matter fractions

All three indicators of active organic matter differentiated by site ( $p < 0.10$  for all, Table 3.4). Northwest had higher levels of both ACE protein and POXC than the other sites ( $p < 0.0001$  for both). Conversely, Northwest had lower values of mineralizable C than the other two sites ( $p < 0.05$ ), which did not differ. Between the remaining two sites, the Wooster site had higher ACE protein than Western ( $p = 0.002$ ) and no difference for mineralizable C ( $p = 0.31$ ) or POXC ( $p = 0.25$ ). Only ACE protein showed a response to P fertilization treatment across sites. There were no differences between the 0x and the 2x/3x treatments ( $p = 0.39$ , data not shown), which had the highest ACE protein levels. However, the 1x was significantly lower than both the 0x ( $p = 0.015$ ) and the 2x/3x ( $p = 0.087$ ) treatments across sites.

### Physical soil health indicators

Average  $K_{\text{sat}}$  values for each site generally agreed with the  $K_{\text{sat}}$  values of Dohnal et al. (2010) for their respective textural classes (data not shown). Accordingly,  $K_{\text{sat}}$  was much greater at Western than either Northwest or Wooster sites (Table 3.4;  $p < 0.0001$ ). Overall,  $K_{\text{sat}}$  had a negative relationship with fertilization rate ( $p = 0.032$ ). However, this relationship was site-dependent, with fertilization effects observed at Northwest ( $p = 0.004$ ), but not at Western ( $p = 0.787$ ) or Wooster ( $p = 0.430$ ).

Overall, penetration resistance is considered a “lower is better” metric (Moebius-Clune et al., 2016). In the current study, penetration resistance values ranged from 2.32 to 4.69 MPa, which is considered a high PR value (Ditzler et al., 2017). PR showed a strong site effect ( $p < 0.0001$ ) and a slight fertilization effect (Table 4;  $p = 0.006$ ). Generally, the 1x treatments resulted in similar PR to the 0x ( $p = 0.18$ ) and lower PR than the 2x/3x treatments ( $p = 0.027$ ).

However, within any given site, the only significant fertilization effect was at Western, where the 1× had a lower PR value than the 2×/3× ( $p=0.094$ ).

Table 3.4 Site and fertilization main effects on biological and physical soil health indicators for 0-30cm depth. All values are estimated least-square means with associated standard errors in parentheses. Letters represent significant differences within a main effect at the  $p<0.05$  level using Tukey's HSD means separation. Results from analysis of variance (ANOVA) are from mixed effect model with block as random variable.

Factor	Treatment	Biological			Physical		
		Mineralizable C (mg CO <sub>2</sub> -C kg <sup>-1</sup> soil)	POXC (mg kg <sup>-1</sup> soil)	ACE Protein (g kg <sup>-1</sup> soil)	K <sub>sat</sub> <sup>a</sup> (mm hr <sup>-1</sup> )	Penetration Resistance (MPa)	Water stable Aggregates (%)
Site <sup>b</sup>	Northwest	36.6 (3.1)	510 (20) a	5.14 (0.14) a	1.16 (0.93, 1.44) b	2.77 (0.05) c	70.8 (5.5) a
	Western	47.9 (3.1)	319 (20) b	3.19 (0.14) c	5.17 (4.17, 6.40) a	3.24 (0.05) b	60.8 (5.5) ab
	Wooster	44.9 (3.1)	284 (20) b	3.95 (0.14) b	1.22 (0.98, 1.51) b	4.15 (0.05) a	42.7 (5.5) b
Fert. <sup>b</sup>	0×	42.3 (2.7)	381 (14)	4.23 (0.11) a	2.37 (1.94, 2.88) a	3.40 (0.08)	58.0 (3.9)
	1×	41.1 (2.7)	359 (14)	3.92 (0.11) b	1.89 (1.57, 2.28) ab	3.21 (0.08)	53.8 (3.9)
	2×/3×	46.1 (2.7)	373 (14)	4.13 (0.11) ab	1.63 (1.35, 1.96) b	3.54 (0.08)	62.4 (3.9)
ANOVA	Site (S)	†	****	****	****	****	*
	Fertilization (F)	ns	ns	*	*	*	ns
	S × F	ns	ns	ns	ns	ns	ns

†, \*, \*\*, \*\*\*, and \*\*\*\* correspond to p-values of <0.10, <0.05, <0.01, <0.001, and <0.0001, respectively. ns indicates no significance at the  $p<0.10$  level.

<sup>a</sup> analysis was performed on transformed data, values here represent backtransformed data and values in parentheses are 95% confidence intervals; <sup>b</sup> Values for each S × F combination can be found in Table 3.6.

Water stable aggregates varied predominantly by site (Table 3.4). The Wooster site had lower WSA than Western ( $p=0.043$ ) or Northwest ( $p<0.001$ ). While Western and Northwest were not significantly different from one another ( $p=0.228$ ), the values at Northwest were generally higher. Across these sites, there was no significant treatment effect ( $p=0.119$ ), although the 1× had lower WSA than the 2×/3× ( $p=0.042$ ). The difference between the 1× and the 2×/3×

only occurred at Western ( $p=0.022$ ), not at Wooster ( $p=0.824$ ) or Northwest ( $p=0.145$ ). Overall, the 0× treatment showed no difference in WSA from the 2×/3× ( $p=0.270$ ) or the 1× ( $p=0.306$ ).

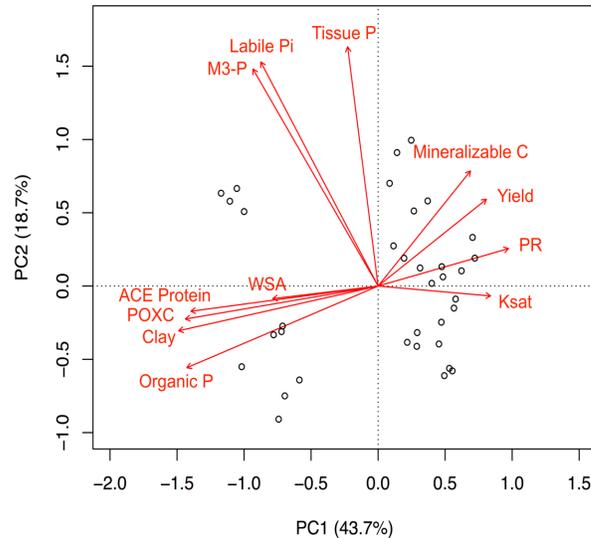


Figure 3.2 Biplot for principal component analysis (PCA) of soil health metrics, soil physiochemical characteristics, and agronomic performance indicators.

### Patterns of variation amongst agronomic and soil health variables

The first principal component (PC1) described 43.7% of the variance amongst our variables, primarily soil health indicators and yield (Figure 2). ACE protein, POXC, WSA, clay, and organic P were all negatively associated with PC1. Conversely, K<sub>sat</sub> and PR were positively associated with PC1. The second principal component (PC2) described 18.7% of the variance. PC2 was primarily associated with soil available P fractions and tissue P content. Mineralizable C and yield were associated with both PC1 and PC2 and were closely associated with one another. Interestingly, yield was nearly orthogonal to available soil P and tissue P content.

Table 3.5 Organic matter trends from residuals of linear regressions of POXC vs. mineralizable C. Positive residuals indicate treatment trending towards POXC (stabilization) and negative residuals indicate trends towards mineralizable C (oxidation).

Treatment	Site		
	Northwest	Western	Wooster
0×	(+)	(+)	(+)
1×	(-)	(-)	(+)
2×/3×	(-)	(-)	(-)

### Organic matter trends: building vs. breakdown

The residuals from linear regressions of POXC and mineralizable C showed fairly consistent treatment effects across all sites. All 0× treatments had positive residuals (i.e. tending towards POXC) and all 2×/3× treatments had negative residuals (i.e. tending towards mineralizable C) (Table 3.5). The 1× treatment showed mixed results, exhibiting negative residuals for both Northwest and Western sites, but positive residuals at the Wooster site. Thus, according to the framework laid out by Hurisso et al (2016), the 0× was building organic matter while the majority of the 1× and 2×/3×—with the exception of the 1× at Wooster—were breaking down organic matter.

### Discussion

#### Relationships between physical and biological indicators of soil health

Biological indicators of soil health differentiated predominantly by site, rather than by fertilization. Consistent with previous work (Fine et al., 2017), POXC and ACE protein showed a

strong relationship with one another ( $r=0.82$ ;  $p<0.0001$ ). Additionally, POXC—which is considered a readily-stabilized soil C fraction (Culman et al., 2012; Awale et al., 2013; Hurisso et al., 2016b; Margenot et al., 2017a)—showed similar trend between sites as ACE protein. Although ACE protein is a relatively new soil health indicator, the essential role of proteins as a “base” for further stabilization of organic matter (Rillig et al., 2007; Kleber et al., 2007; Sollins et al., 2009) suggests that covariation of these two metrics could serve as an indicator of long-term organic matter trends. The close association of increased organic matter—namely proteins and their similarly bound organic matter—with water stable aggregates (Rillig et al., 2007; Wilson et al., 2009; Fokom et al., 2012) is also observed in the covariation of POXC, ACE protein, and WSA (Figure 3.2). Mineralizable C—which is considered an indicator of processes inverse to these processes (Hurisso et al., 2016b)—was inversely associated with these metrics. However, in agreement with previous work (Culman et al., 2013; Hurisso et al., 2016b; Fine et al., 2017), POXC and mineralizable C were positively related to one another for each site (data not shown), indicating greater overall C cycling at higher POXC and mineralizable C values. Although not significant across dozens of sites within the Midwest and Northeastern US (Fine et al., 2017), the positive association between clay and POXC and negative association between clay and mineralizable C indicates that texture could be influencing this relationship amongst our current sites (Figure 2). The increased aggregation of illitic clays typical of the Northwest site is likely further contributing to the effect of clay in differentiating soil health parameters between sites (Blevins and Wilding, 1968; Deneff et al., 2002; Deneff and Six, 2005).

Fertilization effects on biological indicators of soil health were mixed. Absolute values of both POXC and mineralizable C were unresponsive to P fertilization ( $p=0.341$  and  $p=0.259$ , respectively). Previous work in temperate systems has shown that C mineralization was

unchanged across many levels of inorganic P (Spohn and Widdig, 2017). However, at a global scale, inorganic P availability has been shown to constrain the mineralized C per unit of microbial biomass (i.e. the metabolic quotient) (Hartman and Richardson, 2013). While these studies point to complex linkages between C and P dynamics, the sensitivity of C mineralization to methodological variation make direct comparisons to the current study difficult (Curiel Yuste et al., 2007; Franzluebbers and Haney, 2018; Wade et al., 2018a). As such, there is a dearth of information regarding effects of long-term P fertilization on the mineralizable C soil health metric. Similarly, relationships between soil P status and POXC have been largely unexamined. Margenot et al. (2017b) saw increases in POXC after 13 years of P restriction in a tropical Oxisol. However, differences in soil type, pH, and clay content imply much different C dynamics than the current study (Rasmussen et al., 2018). Unlike mineralizable C and POXC, ACE protein was responsive to P fertilization. However, our results of sometimes decreasing soil protein differ from previous work which has shown increased contents with P fertilization (Wu et al., 2011; Dai et al., 2013). Considering that these studies were simply presence/absence of P fertilization, rather than multiple rates of P, it is possible that the response of soil protein may be non-linear across P fertilization rates.

Similar to biological soil health indicators, the effect of P fertilization on physical soil health was mixed. Some of the clearest effects were within  $K_{sat}$  values, which were greater in 0x treatment than either the 1x ( $p=0.092$ ) or 2x/3x ( $p=0.010$ ). This effect was largest at the Northwest site, although the trend was still evident at the Wooster site. Increased root growth used for topsoil foraging of soil P is one potential explanation (Lynch and Brown, 2001; Zhu et al., 2005). Root-derived C is considered more stable in soils (Jackson et al., 2017; Shahbaz et al., 2017) and therefore has been associated with increased porosity (Basche and DeLonge, 2017)

and increased aggregation (Tisdall and Oades, 1982; Six et al., 2000; Vidal et al., 2018).

Although biological soil health indicators showed limited fertilization effects (Table 5), the complex relationship between organic matter, aggregation, and  $K_{sat}$  (Lado et al., 2004) makes it difficult to draw causal links between fertilization effects on aggregation and  $K_{sat}$  (Table 3.4).

Effects of phosphorus fertilization on organic matter trends

Although fertilization effects on individual soil health indicators were weak, the effects of P fertilization on overall organic matter trends were much clearer. The indication of organic matter stabilization in the 0× treatment of all sites was unexpected (Table 3.5). Though not reflected in total C content (data not shown), this finding runs counter to many studies that have found P fertilization to be required for increases in organic matter (van Groenigen et al., 2006; Kirkby et al., 2013). However, Keller et al. (2012) suggested that increases in soil C content associated with P fertilization only occur in environments rich in labile soil C. Agricultural soils in full tillage corn-soy cropping systems are expected to be depleted in labile C (i.e. they are sources rather than sinks (Karlen et al., 2006), therefore the agroecosystems in the current study would not be expected to exhibit a soil C response to P fertilization. However, there are two non-exclusive explanations for the consistency of the observed effects.

The first potential mechanism for trends inferred from biological soil health indicators could be indicative of a greater proportion of C being held in microbial biomass. Recent stable isotope studies have shown that increases in soil C content are attributable to the retention of microbially-processed compounds (Kallenbach et al., 2015, 2016; Liang et al., 2017). A high degree of plasticity in microbial biomass C:P ratio has been shown (Ehlers et al., 2010; Hartman and Richardson, 2013; Horwath, 2017), suggesting that microbial biomass C levels could be maintained under low P availability (e.g. 0× treatment). However, these microbes still are highly

competitive with plants to meet metabolic P requirements, likely resulting in P immobilization in the microbial biomass (Richardson and Simpson, 2011). Microbial immobilization of P is common across ecosystems (Bünemann et al., 2012; Heuck et al., 2015; Spohn and Widdig, 2017), with 20-35% of inorganic P availability in agricultural systems mediated by microbial processes (Bünemann, 2015). Immobilization of P by the microbial community could therefore result in increases in organic P (Figure 1) without concomitant increases in overall soil C levels. At low levels of soil P, organic P is also mineralized to satisfy both plant and microbial P demand (Spohn et al., 2013; Bünemann, 2015; Heuck et al., 2015; Spohn and Widdig, 2017). Thus, our observed changes in organic P (Figure 3.1) should be considered a balance between these two competing processes.

Increased root growth under low P availability is a second potential mechanism driving observed organic matter trends. Under conditions of reduced soil P availability, both corn and soybean forage for soil P by increasing root growth in the P-rich topsoil (Lynch and Brown, 2001; Rubio et al., 2003; Zhu et al., 2005). Root-derived carbon is considered more stable than shoot-derived C (Rasse et al., 2005; Jackson et al., 2017; Shahbaz et al., 2017), which could explain the projected trend of stabilization (Table 3.5). Additional root growth could also reconcile the concurrent trends in organic P (Figure 3.1). Liu et al. (2017b) showed an increase in organic P in the form of phosphomonoesters after 27 years of no P fertilization, relative to both baseline and P fertilized samples. They attributed these increases to maize root-derived organic P. The hypothesis of increased root growth is also supported by several lines of evidence from physical indicators of soil health. Colombi et al. (2018) showed that for compaction values similar to values observed in the current study (Table 3.4), corn showed a marked increase in lateral root growth. If root growth was concentrated in the upper layers, this could also explain

the accompanying increases in  $K_{\text{sat}}$ . Additionally, this increased root growth would explain the association of  $K_{\text{sat}}$  values with yield (Figure 3.2). Therefore, this potential mechanism should also be considered, although we do not have any data to specifically support or refute this hypothesis.

Are the crops experiencing P stress?

Corn crops showed a considerable response of both tissue and grain P content to fertilization rate (Table 3.3). However, the values were generally between 0.30-0.50%, which is considered the “sufficiency” range for tissue P concentrations in the Tri-State area (Vitosh et al., 1995). While it could be argued that hybrids developed since the formulation of the Tri-State recommendations would exhibit improved internal P cycling (Calderón-Vázquez et al., 2011), recent studies in the region have generally corroborated the sufficiency range for corn ear leaf P concentrations (Kovács and Vyn, 2017). Additionally, recent work in Iowa has suggested that P sufficiency could be indicated at values closer to 0.25% (Mallarino, 2011). Although tissue P at R1 was associated with plant available P pools (labile  $P_i$  and M3-P), tissue P and yield were largely unrelated to one another (Figure 3.2). Infrequent yield responses to P fertilization have been previously documented for these sites dating back to 2006 (Fulford and Culman, 2018). This lack of yield response after 11 years without P fertilization is notable when considering profound differences in both labile  $P_i$  and M3-P between treatments. As previously discussed, increased root growth is potentially mediating decreases in available P—as indicated by labile  $P_i$  and M3-P—and yield. However, this effect would only be possible at moderate levels of P deficiency (Erel et al., 2017). Thus, although there are differentiations in tissue and grain P between

treatments, there is no indication of P deficiency and/or stress. This, in addition to the lack of yield response, suggests that the plants are not experiencing P stress.

## Implications

The implications of a lack of yield response after 11 years without P fertilization cannot be understated. This represents the substantial legacy effects of long-term P fertilization. In agricultural soils, continued application of fertilizer P results not only in increases in the labile, plant-available forms of P, but also increases the less labile pools (Zhang et al., 2004; Negassa and Leinweber, 2009; Shi et al., 2013; Soltangheisi et al., 2018). The “drawdown” of residual labile phosphorus is essential for maintaining surface water quality (King et al., 2017). A recent analysis of isotopically-exchangeable  $^{32}\text{P}$  and  $^{33}\text{P}$  across a global dataset of 217 studies has shown that plant-available phosphorus in solution is highly buffered by phosphorus sorbed to the mineral phase (Helfenstein et al., 2018). They found that even though differences in solution P may be evident at a given time point, the total amount of exchangeable P available for biological uptake remains largely unchanged over a 3-month period. This suggests that as legacy phosphorus is drawn down in high-P agricultural soils, physiochemical constraints on P availability can give way to a greater contribution of biological processes (Bünemann, 2015). In the current study, the relationship between soil P levels and corn yield was lacking (Figure 3.1; Figure 3.2). Additionally, we have very little evidence that indicators of biological soil health are 1) altered by low solution P or 2) mediating the relationship between soil P availability and crop yield. However, it is possible that as legacy P effects diminish and available P starts to become limiting, the transition to a more biologically-based model of P availability will produce effects on biological soil health indicators.

## Conclusions

Eleven years of P restriction predictably lowered labile P fractions across all three surveyed sites. However, these changes in labile P did not reduce yields or provide evidence of crop P stress. Unexpected increases in organic P content also occurred in unfertilized plots. Although absolute values of most biological soil health indicators did not change with P fertilization, their relative trends showed increases in organic matter stabilization in P restriction (unfertilized) plots. Changes in physical indicators of soil health were mixed and the causal mechanisms unclear. However, unfertilized plots generally did not exhibit any decreases in soil health or yields associated with P restriction. Therefore, substantial reductions in long-term P fertilization rate could be implemented, although it is unclear how long this effect of residual P would continue.

Table 3.6. Soil test P and soil health parameters for all sites and treatments. All values are means with standard errors in parentheses (n=4). Results from analysis of variance (ANOVA) are from mixed effect model with block as random variable.

Site	Fertilization Treatment	Mehlich-3 P (mg kg <sup>-1</sup> )	Biological Soil Health Indicators			Physical Soil Health Indicators		
			Mineralizable C (mg CO <sub>2</sub> -C kg <sup>-1</sup> soil)	POXC (mg kg <sup>-1</sup> soil)	ACE Protein (g kg <sup>-1</sup> soil)	K <sub>sat</sub> (mm hr <sup>-1</sup> )	PR (MPa)	WSA (%)
Northwest	0×	23.8 (1.5)	40.7 (3.8)	524 (6)	5.32 (0.11)	4.54 (2.60)	2.88 (0.08)	73.9 (7.8)
	1×	41.0 (1.8)	33.6 (0.3)	502 (19)	5.05 (0.15)	0.93 (0.10)	2.66 (0.09)	64.0 (3.0)
	2×/3×	90.3 (0.9)	35.5 (0.9)	506 (17)	5.06 (0.10)	0.94 (0.10)	2.77 (0.11)	74.4 (5.7)
Western	0×	21.0 (3.1)	47.1 (5.8)	332 (22)	3.39 (0.18)	5.54 (0.87)	3.16 (0.06)	61.4 (8.2)
	1×	36.5 (10.5)	46.7 (2.1)	295 (38)	2.95 (0.05)	6.12 (1.20)	3.01 (0.23)	52.0 (7.7)
	2×/3×	39.3 (7.5)	49.9 (9.4)	328 (26)	3.23 (0.27)	4.69 (0.74)	3.54 (0.04)	69.0 (9.5)
Wooster	0×	19.0 (2.3)	39.2 (0.6)	288 (16)	3.97 (0.10)	1.38 (0.17)	4.16 (0.25)	38.6 (6.7)
	1×	32.5 (4.2)	42.9 (5.7)	280 (33)	3.76 (0.29)	1.32 (0.14)	3.97 (0.15)	45.4 (2.4)
	2×/3×	55.3 (6.7)	52.8 (4.1)	285 (22)	4.09 (0.22)	1.09 (0.20)	4.33 (0.14)	43.9 (6.1)
ANOVA	Site (S)	**	†	****	****	****	****	*
	Fertilization (F)	****	ns	ns	*	*	*	ns
	S × F	ns	ns	ns	ns	ns	ns	ns

## Chapter 4. Healthier Soils Increase Corn Response to N Fertilizer Across the Corn Belt

### Introduction

Beginning in the early 1960s, the increased use of nitrogenous fertilizers has nearly doubled grain crop yields across the world (Lassaletta et al., 2014). While this yield increase may be a boon for global food security, the losses of reactive nitrogen have also doubled, resulting in a myriad of adverse environmental effects (Vitousek et al., 1997; Van Groenigen et al., 2010; Bowles et al., 2018). Despite promising developments in crop breeding (Han et al., 2015; Li et al., 2018b), yields have generally plateaued with increasing N fertilization rates, ultimately decreasing nitrogen use efficiency (NUE) (Lassaletta et al., 2014; Bouwman et al., 2017). Recent estimates have suggested that between 1961 and 2010, approximately 44% of the N fertilizer applied globally to maize systems was lost to the environment (Ladha et al., 2016). The Corn Belt of the midwestern United States is no exception to this trend, with NUE stagnating at approximately 60% or even decreasing as N fertilization rates increase in recent decades (Swaney et al., 2018).

There are many strategies to address N losses from cropping systems, including the “4Rs” of fertilizer management (Christianson and Harmel, 2015; Venterea et al., 2016): right rate, right time, right form, and right placement. These strategies are largely centered on the management of N fertilizer—which comprises less than 50% of the plant N uptake in a given growing season (Kramer et al., 2002; Gardner and Drinkwater, 2009)—neglecting the role of soil biology in supplying plant N. This neglect of soil biology reflects the inherent complexity and

uncertainty associated with the prediction of N mineralization rates (Ros et al., 2011; Liu et al., 2016a; Li et al., 2018a).

The framework of soil health seeks to highlight the role of soil biology, ultimately integrating soil biologically with the historically-emphasized chemical and physical soil components (Doran, 2002; Kibblewhite et al., 2008). Soil health has been widely embraced by farmers, researchers, and private industry alike (Romig et al., 1995; Idowu et al., 2009; Arbuckle, 2016; Soil Health Institute, 2017). Buy-in from these stakeholders represents a nexus of several of the most influential sources of information growers use in making nutrient management decisions (Stuart et al., 2014, 2015, 2018), making soil health uniquely positioned to address agricultural N losses.

To date, there has been little convergence in which biological indicators of soil health represent vital soil functions (Bünemann et al., 2018). While many studies have described management-induced changes in biological soil health indicators (Culman et al., 2012; Lucas and Weil, 2012; Mitchell et al., 2017; Wang et al., 2017; Karlen and Obrycki, 2018; Obrycki et al., 2018), these indicators have only been loosely correlated with crop productivity (Dick and Culman, 2016; Hurisso et al., 2016b; Bongiorno et al., 2019). Recent work has begun to connect changes in biological soil health to potential changes in crop fertilization rates (Franzluebbers, 2018a; Yost et al., 2018). However, these studies 1) used a limited number of measurements of soil health and fertility and 2) were conducted under a limited range of pedoclimatic conditions. The clustering of data within a given pedoclimatic context can overestimate the strength of the relationships between the biological soil health indicators and crop productivity by confounding context effects with biological phenomena, increasing the potential for type I errors (Clarke, 2008). The use of multilevel models helps to reduce bias from data clustering, allowing us to

differentiate between effects exerted on a site-by-site basis and effects exerted within a site. Therefore, while previous studies have established strong correlations, the lack of accounting for other potentially confounding factors undermines the ability to establish a causal link between soil biological health and crop productivity measures (Feller and Gelman, 2015).

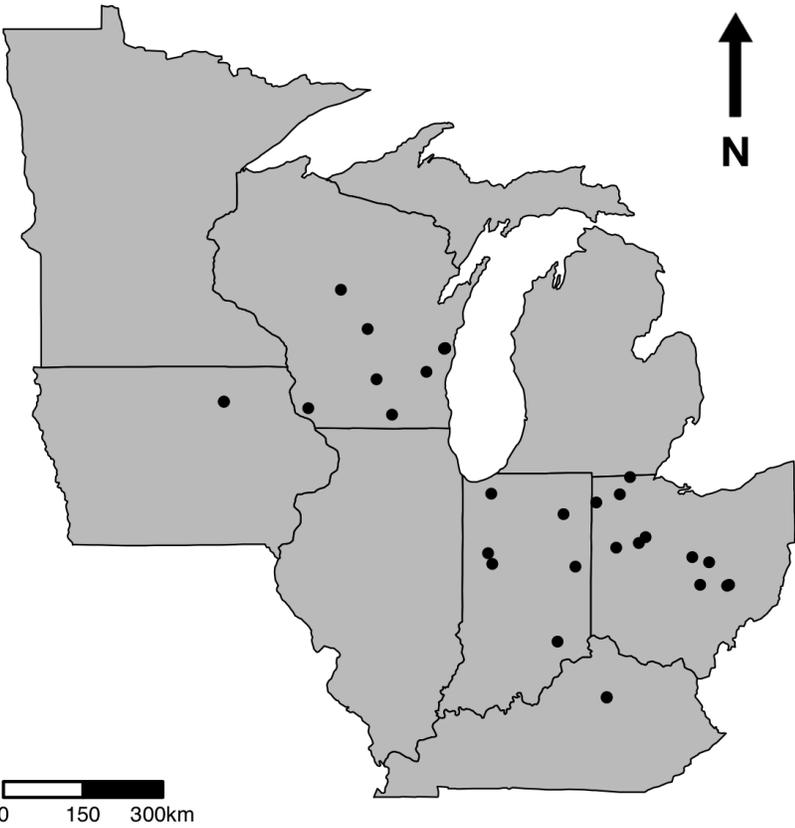


Figure 4.1 Locations of all sites included in the current study.

In this study we use replicated fertilizer N rate trials across the Corn Belt of the Midwestern United States to better elucidate the link between soil biological health and crop productivity. These potential linkages will seek to answer the general question “does a healthy

soil need less fertilizer?” Although there are many methods of expressing crop-soil N dynamics to inform N fertilization decisions (Morris et al., 2018), here we are looking to answer two basic questions. Considering that agronomic optimum grain yields are often achieved in unfertilized treatments, our first question is “can biological soil health indicators predict if N fertilization is needed?” Secondly, we seek to answer the more general question “do biologically active soils produce greater yields than less biologically active soils, given similar N fertilization rates?”. Due to the wide range of climatic and edaphic influences on relative yield with N fertilization, these primary research questions incorporated site-level effects of climate and texture to better elucidate potential relationships between soil health and plant-soil N dynamics.. Trials included in this study represented a variety of soil health-building managements and pedoclimatic zones, allowing for interpretation across a breadth of contexts.

Table 4.1 Location and primary management information for all study sites. All sites had a minimum of four N fertilization rates. Additional management information can be found in Table 4.9 or in references cited in-table.

Study Name	Treatment(s)	State	Number of Sites	Number of total plots	Treatment Duration	References
N Rotation	Four rotations: continuous corn (grain), corn-soybean, and corn-corn-corn-soybean	Iowa	1	66	36 years	(Karlen et al., 2006; Russell et al., 2006)
Manure × CC <sup>a</sup>	Non-manured control, manure only, manure + barley CC	Wisconsin	4	48	2 years	None
Radish CC	No CC control, radish CC, radish CC + pre-plant N <sup>b</sup>	Wisconsin	3	28	3 years	(Ruark et al., 2018)
Purdue N Trials	None	Indiana	6	90	4 years	(Moser, 2016)

Blevins	Conventional tillage and continuous no-till	Kentucky	1	32	48 years	(Liu et al., 2017a)
F05	Continuous corn (grain), corn-soybean rotations	Kentucky	1	48	35 years	
Legume CC	No CC control, clover CC, barley CC	Wisconsin	3	36	1 year	None
Ohio N Trials	On-farm trials (varies)	Ohio	12	36	1 year	None

<sup>a</sup> CC= cover crop; <sup>b</sup> 67 kg N ha<sup>-1</sup> was added at during radish planting

## Materials and Methods

### Study Details

For this study, we compiled soil and yield data from replicated N-rate studies from across the Corn Belt (n=31 sites; n=389 total soil samples). Trials had a minimum of four N rates imposed, each of which was replicated 3-6 times across a site. Each replication had an unfertilized control plot without applied N as well as a minimum of three additional N rates, the highest of which was at least 180 kg ha<sup>-1</sup> N. The one exception was the Purdue N Trials, in which the check plots had an N rate of 19-27 kg ha<sup>-1</sup> N applied at planting. Layered within these N rate trials were additional soil health-building management strategies: varying rotation lengths and diversities, manure application, cover crops, and decreases in tillage intensity (Table 4.1). One site with extremely high organic matter content (>20% by mass) was eliminated due the unique fertility properties of muck soils (Silva, 2012). Therefore, the final analysis included 29 sites with a total of 386 soil samples.

These studies represented both on-farm and research plots covering a broad range of pedoclimatic conditions. Using GPS coordinates for each site, we extracted climatic data for each site from the WorldClim2 database (Fick and Hijmans, 2017) using RStudio (RStudio Team, 2016b) and the *raster* package (Hijmans et al., 2015). This climatic data was then used to describe climatic influences on relative yield across site. We determined the representativeness of our selected sites using technological extrapolation domains (TEDs) (Cassman, 2017; Edreira et al., 2018). TEDs are constructed using a combination of climatic and edaphic factors and delineate regions of varying yield potential for rainfed cropping systems. Namely, these factors are 1) annual growing degree-days, 2) aridity index, 3) annual temperature seasonality, and 4) plant-available water holding capacity of the soil. Using the GPS coordinates and the “corn mask” in the TED framework (<http://nutrientstar.org/ted-framework/>), our sites represented 52% of the total rainfed corn production area in the central/eastern United States.

Soil samples for each study were gathered in the spring prior to or immediately following planting of the corn grain crop. We took multiple soil subsamples to a depth of 6-12”, which were then composited and homogenized to comprise one representative sample for each plot. Samples were then air-dried and stored pending analysis. The specific sampling protocols for each study can be found within references cited in Table 4.1 or in Table 4.9.

#### Soil Nutrient Status

We determined soil inorganic N content as the sum of both nitrate and ammonium. Both were determined using a 1:5 soil:solution extract using 1 or 2M KCl. Extracts were shaken for 1 hour, clarified, and measured colorimetrically (Mulvaney, 1996). Ammonium was determined using the salicylate method (Verdouw et al., 1978). Nitrate was determined using the cadmium

reduction method (Dorich and Nelson, 1984; NCR, 2011) or by reduction with vanadium (III) chloride (Doane and Horwath, 2003). Both nitrate reduction methods typically yield consistent nitrate values (Doane and Horwath, 2003).

Table 4.2 Site data for each study field.

Study Name	Field Number	Clay (%)	MAT <sup>b</sup> (°C)	MAP <sup>c</sup> (mm)	Temperature seasonality <sup>d</sup> (°C)	Precipitation seasonality <sup>e</sup> (%)
N Rotation	1	22.5	7.6	858	11.2	4.8
Manure × CC <sup>a</sup>	2	22.9	7.4	806	10.6	3.9
	3	12.8	5.5	837	11.0	4.5
	4	4.3	6.4	809	10.8	4.2
	5	22.7	7.9	846	10.6	4.2
Radish CC <sup>a</sup>	6	14.7	7.1	812	10.4	3.7
	7	24.7	7.3	833	10.2	3.2
	8	22.5	8.0	850	10.5	3.6
Purdue N Trials	9	36.9	10.1	943	9.7	2.5
	10	43.6	9.8	944	9.5	2.2
	11	29.6	11.9	1102	8.9	1.7
	12	32.3	9.5	973	9.7	2.5
	13	28.0	10.3	962	9.6	2.3
	14	27.3	9.5	947	9.6	2.2
Blevins F05	15	18.0	12.5	1148	8.5	1.5
Legume CC <sup>a</sup>	16	17.9	12.5	1140	8.6	1.5
	17	22.9	7.4	824	10.1	3.2
	18	22.1	7.4	824	10.1	3.2
Ohio N Trials	19	22.3	7.3	833	10.2	3.2
	20	24.0	10.0	899	9.6	2.1
	21	14.0	10.6	974	8.7	2.0
	22	14.1	10.5	978	8.8	2.0
	23	28.6	9.9	909	9.5	2.2
	24	19.1	9.5	1016	9.0	2.0
	25	17.8	9.2	1014	9.1	2.1
	26	28.8	9.3	867	9.6	2.1
	27	19.5	10.4	1012	9.0	2.0
	28	25.3	9.2	853	9.5	2.0
	29	38.9	9.4	872	9.6	2.0

<sup>a</sup> cover crop; <sup>b</sup> mean annual temperature; <sup>c</sup> mean annual precipitation; <sup>d</sup> calculated as the standard deviation of the average monthly temperature; <sup>e</sup> coefficient of variation for monthly total precipitation within a year

## Soil Health Metrics

As an aggregated measure of soil health, we selected three commonly-utilized biological soil health metrics. Permanganate oxidizable carbon (POXC) (Weil et al., 2003; Culman et al., 2012), autoclave citrate-extractable protein (ACE protein) (Hurisso et al., 2018c), and mineralizable C (Franzluebbers et al., 2000; Franzluebbers, 2016; Wade et al., 2018a) were determined for each sample. We measured mineralizable C by rewetting 10g of air-dried soil in 50mL microcosms to 50% water-filled pore space, incubating for 24 hours at a constant temperature of 25°C, and measured CO<sub>2</sub> concentrations on a 1mL aliquot of headspace on a LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE). We calculated mineralizable C as the difference in CO<sub>2</sub> concentration between a sample and a blank control using the ideal gas law (Bottomley et al., 1994; Zibilske, 1994). In the statistical models, the mean value of duplicate measurements was used to account for potential measurement variability (Wade et al., 2018a). We measured POXC using 2.5g of air-dried soil, which was shaken for precisely 2 minutes in a 0.02 mol L<sup>-1</sup> KMnO<sub>4</sub> solution, allowed to settle for 10 minutes, diluted 1:100 with water, and then measured spectrophotometrically at 550 nm on a 96-well plate. We measured ACE protein using 3.0g of air-dried soil that was extracted with 0.02 mol L<sup>-1</sup> sodium citrate solution (pH=7.0), shaken for 5 minutes, and autoclaved at 121°C (15 psi) for 30 minutes. Cooled extracts were then clarified (3 minutes @ 10,000 × g) and reheated (60°C for 1 hour) after adding a bicinchoninic acid reagent. After a second cooling for 5 minutes, we quantified ACE protein colorimetrically at 562 nm using a bovine serum albumin standard.

In addition to the biological soil health indicators, we also measured organic matter content, as it is the most commonly measured metric associated with soil health (Bünemann et

al., 2018). Organic matter content was measured using the NCERA-13 recommended method for the region (NCR, 2011): loss-on-ignition (LOI) (Cambardella et al., 2001). In brief, 10-15g of soil was dried overnight at 110°C, placed in a dessicator, and then combusted at 360°C for 2 hours, with the difference being representative of total organic matter content. While calibrations between LOI and total C on combustion have proved quite successful for the region (Konen et al., 2002), the total C on combustion method is generally preferred (Nelson and Sommers, 1996). Therefore, wherever both combustion and LOI values were available, total C values were used. Where only LOI values were available, LOI was converted to total C, or soil organic carbon (SOC), using the conversion factor of 1.74 (Pribyl, 2010) to facilitate comparisons with the broader literature.

#### Relative Yield and Relative Fertilization Rate Calculations

Relative yield (RY) was calculated as the ratio between yield in a manipulated plot and the calculated yield at the agronomic optimum N fertilization rate (AONR). In the N responsiveness model, check plots were used as the numerator. In the N fertilizer efficiency model, yields from all applied N rates were used. AONR was calculated using the linear, linear plateau, quadratic, and quadratic plateau methods of Cerrato and Blackmer (1990). Model fit ( $R^2$ ) was used to determine the appropriate response curve, with an increase of  $>0.05$  being needed to justify any variations from the preferred quadratic plateau model. Yield was then calculated using the AONR and resulting yield response curve. If calculated AONR exceeded the highest fertilization rate, then yield at the highest fertilization rate was used. AONR was calculated independently for each treatment  $\times$  block combination to account for: 1) potential long-term effects of treatment on AONR (Poffenbarger et al., 2018) and 2) within-field variation in yield potential (Mamo et al.,

2003). RY was then determined for each treatment  $\times$  block combination and expressed as a ratio, where non-responsive sites have a value of RY=1.0. In the long-term studies (N Rotation, Blevins, and F05), yields were averaged across years (up to 5 years) to minimize year-to-year weather effects on N response (Tremblay et al., 2012). Due to high variability in optimum N rate between sites (Dhital and Raun, 2016), the relative fertilization rate was used to compare N rate across sites. Relative fertilization rate (RFR) was calculated as the applied N rate divided by the AONR. Thus, some plots had values of RFR  $>$  1.0, indicating excess N fertilization.

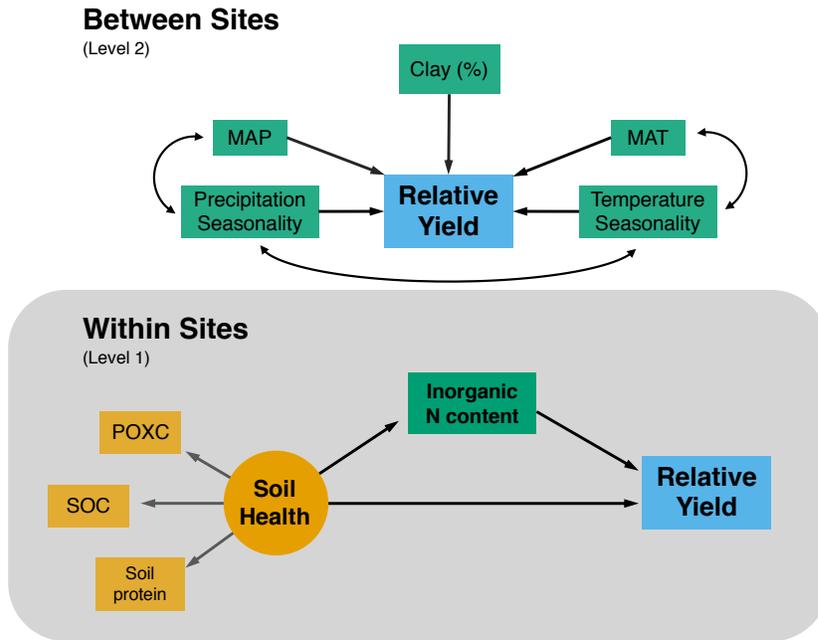
### Statistical Analyses

Our overall statistical approach consisted of several steps. First, we used a factor analysis to determine the appropriate number of “soil health” factors, as well as which measured variables were to be considered. Next, these factors were integrated into a theoretical model describing the relationships between soil health, soil inorganic N content, and relative yield. This theoretical model (represented in the lower level of our model) was then fit into a multilevel context, wherein site-specific effects (i.e. texture and climate) were used to more accurately constrain and quantify these relationships.

We used exploratory factor analysis (EFA), or common factor analysis, to determine if the biological soil health indicators describe similar underlying processes. EFA uses the pattern of correlations (i.e. covariance structure) of a set of measured variables to infer underlying process(es) or constructs (Thurstone, 1947; Fabrigar and Wegener, 2011). The goal of EFA is distinctly different from other data reduction processes (e.g. principal components analysis) in that EFA is attempting to describe unmeasurable latent constructs. The classical example of this is the concept of “general intelligence” being represented by a blending of measured test scores

(Spearman, 1904). EFA was performed using a total of four common indicators of biological soil health and fertility—POXC, mineralizable C, ACE protein, and soil organic C content—to determine if these measured variables are describing similar latent constructs. To determine the appropriate number of factors, we used the package *nFactors* (Raiche and Magis, 2014) in RStudio (RStudio Team, 2016b) to conduct several quantitative assessments for factor retention: eigenvalues, parallel analysis, optimal coordinates, and the acceleration factor. All four of these methods suggested a best fit of one factor to describe soil health, i.e. these four indicators are all describing some portion of the underlying construct of “soil health”. However, these four indicators differed in their ability to describe this underlying soil health construct; factor loading  $<0.50$  and correlation with other measured variables  $<0.50$  (Table 4.5) led us to exclude mineralizable C from the final latent factor.

(a)



(b)

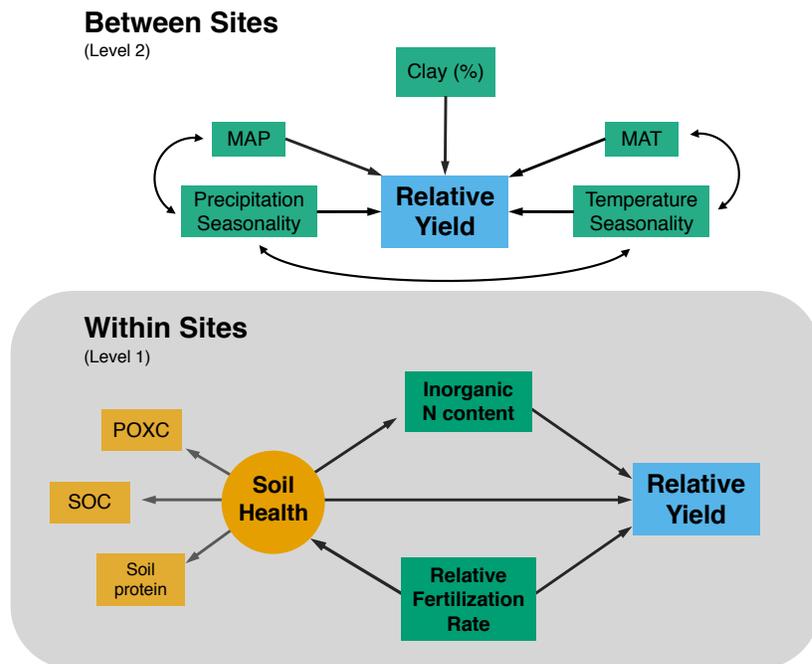


Figure 4.2 Baseline structural equation models for (a) N responsiveness model and (b) N fertilizer efficiency model.

MAT = mean annual temperature; MAP = mean annual precipitation; POXC = permanganate oxidizable C; SOC = soil organic carbon

We used multilevel structural equation models to determine the effects of soil health on crop N responses. Multilevel models—also referred to as hierarchical models—offer many advantages over classical regression models that are relevant to broad-scale studies such as the current study (Gelman and Hill, 2006). First, the multilevel structure accounts for varying effects to be simultaneously quantified across several scales of analysis. The hierarchically lower level in our current model was the “within field” portion of the model and the hierarchically higher level was the “between fields” (or “across field”) portion of the model. Within-field models are comprised of components that inform N management decisions (e.g. inorganic N content). In order to allow for variations in yield potential for each field, we allowed the intercept to vary (as a random effect), but included measured model predictors as fixed effects. The upper level of our model—the between- or across-field effects—were parameters that would exert their influence on a field-by-field basis (e.g. climate conditions). Thus, we could estimate within-field changes in N dynamics while holding between-field climatic or edaphic parameters constant. This alludes to a second advantage of multilevel modeling over classical regression: prediction to new groups drawn from the same sampling population. In accounting for variations between/across fields, prediction in new contexts is more robust and reliable. A third salient advantage of multilevel models is the balancing of type I and type II errors in multi-site studies. Oftentimes, multi-site studies will include all observations in a single predictive regression from which to draw conclusions, often referred to as “complete pooling”. While this results in a larger sample size, clustering within fields overestimates the strength of that relationship, increasing the potential for Type I errors (false positives), (e.g. Figure 2 in Haney et al. (2004)). Alternatively, many multi-site studies will average parameters across replicates within a site and then use that average in

regression-based evaluations. This approach decreases the overall sample size, resulting in an underpowered analysis and an increase in the probability of a type II error (see: Yost et al. (2018) and Franzluebbbers (2018b) for relevant examples using multi-site N analyses). The partial pooling method of multilevel analysis balances these two considerations, producing a more accurate error estimate while maintaining an appropriate balance of type I vs type II errors.

To answer the two primary questions surrounding crop response to N fertilization, we built two similar, but separate models (Figure 4.1). Our first model was constructed to address the question “can biological soil health indicators predict if N fertilization is needed?”. This model, referred to hereafter as the “N responsiveness model”, was conducted using only soil data from our check plots. Associated yields from those check plots were then used as the numerator and yields at AONR as the denominator in our relative yield (RY) calculations. We constructed our second model to answer the question “do biologically active soils produce greater yields for similar N fertilization rates?”. This model, which we will refer to as the “N fertilizer efficiency model”, included all soil and yield data available. We analyzed our models using the *sem()* command in the *lavaan* package (Rosseel et al., 2018) of RStudio with our data clustered at the field level. Using our constructed baseline model (see below for justification), paths were iteratively eliminated based on their level of significance until each individual path was significant at  $p < 0.10$ . Overall model fit was assessed using the standardized root-mean-square of the residuals (SRMR) as a measure of absolute fit and the Comparative Fit Index (CFI) and Akaike’s Information Criteria (AIC) as measures of relative fit (Akaike, 1974; Hu and Bentler, 1999). Combining CFI and SRMR, we used the cutoff criteria of Hu and Bentler (1999), wherein either a  $CFI > 0.95$  or a  $SRMR < 0.05$  was considered a close model fit. For  $CFI < 0.95$  and  $SRMR > 0.05$ , we would expect a combined type I and type II error rate of  $\sim 1\%$  for  $n \approx 200$  and

$\leq 0.0\%$  for  $n \approx 400$ . We determined fit separately at each level using SRMR (Hsu et al., 2015) with a similar criteria of  $SRMR < 0.05$  being considered a close fit and  $SRMR < 0.08$  being an acceptable fit. Values of  $SRMR > 0.10$  are considered to be a mediocre model fit (Browne and Cudeck, 1993). To ensure the robustness of our results, we used bias-corrected and accelerated (BCa) bootstrapping to construct parameter confidence intervals during model development and validation (Efron, 1987), as well as to assess mediation (i.e. indirect effects) (Hayes, 2009).

#### Statistical model justification

The N responsiveness model and the N fertilizer efficiency model shared similar general model structures. At the lower (within-field) level of our model we included the long-established relationship between pre-plant inorganic N content and relative corn yield (Magdoff et al., 1984; Bundy and Malone, 1988). One of the primary relationships of interest—the relationship between the soil health factor comprised of biological soil health indicators—also was hypothesized to have a direct effect on relative yield. While inorganic N content could be considered an indicator of soil health itself, the large body of literature relating inorganic N content to active organic matter fractions (Franzluebbers et al., 2000; Haney et al., 2001; Russell et al., 2006; Culman et al., 2013; Osterholz et al., 2017; Hurisso et al., 2018c), led us to hypothesize that inorganic N would be influenced by the biologically active fractions described by our soil health factor. These structural components were identical across models. However, the integration of fertilizer rate allowed for the interactive effects of fertilizer application and soil health on relative yield to be determined. Given the influence of nitrogenous fertilizer on soil microbial biomass and activity (Geisseler and Scow, 2014; Geisseler et al., 2016a) and its effects on carbon dynamics (Poffenbarger et al., 2018), we hypothesized that our soil health factor

would also be influenced by N fertilization rates. At the upper level of our model, we included both edaphic (e.g. clay content) and climatic variables that been shown to influence crop N responses (Puntel et al., 2018; Spackman et al., 2019).

## Results

### Soil health and physiochemical properties

Soil physiochemical properties were largely within expected value for Corn Belt fields (Table 4.3). CEC values ranged from 2.0-25.9, encapsulating a wide range of soil fertility status. Similarly, pH for each study was comparable to the median values for each state (Murrell et al., 2015), with the exception of F05, which was below the state median of pH=6.1. Notably, nearly all of the SOC values fell below the ~2% SOC threshold that Oldfield et al. (2019) estimated as a plateau for yield increases with N fertilization.

There are few studies with regionally-representative values for biological soil health indicators, so determining “high” vs. “low” values is often difficult. However, the samples in the current study exhibited a generally wide range of values. POXC ranged from 160 to 1054 mg kg<sup>-1</sup> soil; around one order of magnitude. Similarly, mineralizable C ranged from 5.8 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil to 167.1 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil. This represents a broader range of mineralizable C values than was recorded by Franzluebbers et al. (2000), despite the narrower range of climatic conditions in the current study. Soil protein values ranged from 1.7 to 7.1 g kg<sup>-1</sup> soil, which was generally within expected values for medium or fine-textured soils (Fine et al., 2017).

Table 4.3 Mean values of soil fertility, soil health, and physiochemical characteristics for each study. Values in parentheses indicate the minimum and maximum values within each study, respectively.

Study Name	pH	SOC (%)	CEC (meq/100g soil)	Inorganic N (mg N/kg soil)	POXC (mg/kg soil)	Mineralizable C	
						(mg CO <sub>2</sub> -C/kg soil)	Soil Protein (g/kg soil)
N Rotation	5.7 (4.6, 6.7)	1.5 (0.8, 2.2)	14.2 (10.0, 18.8)	55.2 (8.7, 149.2)	533 (431, 623)	48.7 (26.2, 79.1)	3.8 (2.8, 5.4)
Manure × CC <sup>a</sup>	6.6 (5.9, 7.2)	1.2 (0.1, 2.1)	9.8 (2.0, 17.8)	11.0 (4.6, 23.4)	450 (160, 810)	35.2 (5.9, 92.7)	3.3 (1.7, 7.1)
Radish CC	7.0 (6.4, 7.5)	1.4 (1.1, 1.8)	12.4 (9.8, 16.1)	7.6 (4.3, 18.1)	518 (347, 710)	47.3 (27.0, 69.0)	3.0 (2.3, 4.2)
Purdue N Trials	6.2 (5.4, 7.3)	1.9 (1.1, 3.1)	14.6 (7.3, 25.9)	14.2 (3.1, 32.2)	570 (291, 821)	59.4 (22.8, 167.1)	3.9 (2.9, 5.9)
Blevins	5.5 (4.9, 6.3)	1.5 (1.0, 2.1)	11.6 (10.3, 13.4)	6.1 (3.6, 14.7)	620 (398, 921)	40.8 (13.4, 75.5)	4.7 (3.5, 6.5)
F05	5.0 (4.0, 5.8)	1.5 (1.3, 1.9)	12.7 (10.1, 16.7)	8.4 (4.9, 32.5)	565 (347, 1054)	45.0 (11.3, 73.5)	4.2 (3.3, 5.8)
Legume CC	7.4 (6.8, 7.6)	1.1 (0.8, 1.9)	13.2 (11.1, 16.7)	7.4 (3.7, 13.3)	527 (370, 761)	50.0 (22.4, 81.8)	2.7 (1.8, 4.2)
Ohio N Trials	6.1 (4.8, 6.9)	2.2 (1.6, 6.4)	11.7 (5.3, 19.3)	21.8 (9.7, 47.9)	473 (309, 761)	33.0 (5.8, 55.4)	4.6 (3.5, 6.3)

<sup>a</sup> CC = cover crop

## Relationships between soil health, agronomic, and climatic variables

In both the reduced dataset (N responsiveness) and the full dataset (N fertilizer efficiency), many of the soil health and agronomic variables were significantly linearly correlated to one another (Table 4.4 and 4.5). Interestingly, mineralizable C was largely unrelated to many of the climatic variables and only weakly related to other soil health indicators in both datasets. However, it was moderately related to clay ( $r \approx 0.50$ ). Climatic variables were largely well-correlated with one another, with many of the relationships  $r \geq 0.90$ . Both MAT and MAP were inversely related to their seasonality components. Thus, our warmer sites had less variability in their temperature between seasons and our wetter sites had more consistent monthly precipitation. One area of significant divergence between the datasets was the relationship between POXC and the climatic

variables. In the check plots in the N responsiveness model, POXC was largely unrelated to climatic variables. However, in the full dataset (N fertilizer efficiency model), POXC was significantly related to all four. Across datasets, very few of the soil health or climatic variables exhibited strong relationships with relative yield, except relative fertilizer rate in the full dataset. Collectively, these interrelationships represent substantial multi-collinearity within both the reduced and full datasets.

Table 4.4 Correlation matrix of all measured variables in the N responsiveness model (n=186).

	<b>Inorg. N</b>	<b>Min.C</b>	<b>POXC</b>	<b>Soil protein</b>	<b>SOC</b>	<b>Clay</b>	<b>MAT</b>	<b>TS</b>	<b>MAP</b>	<b>PS<sup>i</sup></b>
<b>RY<sup>a</sup></b>	0.23**	0.10	0.12 <sup>†</sup>	0.16*	0.07	0.13 <sup>†</sup>	-0.17*	0.19**	-0.18*	0.15*
<b>Inorg. N<sup>b</sup></b>		-0.06	0.02	0.39***	0.36***	0.08	0.13 <sup>†</sup>	-0.05	0.15*	-0.03
<b>Min. C<sup>c</sup></b>			0.67***	0.24***	0.25***	0.45***	-0.04	0.10	-0.01	0.07
<b>POXC<sup>d</sup></b>				0.44***	0.46***	0.39***	0.05	-0.04	0.06	-0.08
<b>Soil protein</b>					0.66***	0.20**	0.34***	-0.33***	0.38***	-0.34***
<b>SOC<sup>e</sup></b>						0.60***	0.40***	-0.34***	0.31***	-0.40***
<b>Clay</b>							0.34***	-0.21**	0.12 <sup>†</sup>	-0.32***
<b>MAT<sup>f</sup></b>								-0.87***	0.92***	-0.83***
<b>TS<sup>g</sup></b>									-0.82***	0.98***
<b>MAP<sup>h</sup></b>										-0.74***

<sup>†</sup>, \*, \*\*, and \*\*\* correspond to p-values of <0.10, <0.05, <0.01, and <0.001, respectively. Italicized values are not significant at p<0.10.

<sup>a</sup> relative yield; <sup>b</sup> inorganic N; <sup>c</sup> mineralizable C; <sup>d</sup> permanganate oxidizable C; <sup>e</sup> soil organic carbon; <sup>f</sup> mean annual temperature; <sup>g</sup> temperature seasonality; <sup>h</sup> mean annual precipitation; <sup>i</sup> precipitation seasonality

### Soil health and fertilizer N responsiveness

Within fields, only inorganic N content exhibited a positive direct effect on relative yield ( $\beta=0.27$ ,  $p=0.049$ ) (Figure 4.3). However, soil health exhibited a positive direct effect on inorganic N content ( $\beta=0.44$ ,  $p<0.0001$ ). Thus, soil health exerted an overall positive indirect effect on relative yield that was mediated by inorganic N content ( $\beta=0.12$ ,  $p=0.062$ ). Between

sites, there were considerable impacts of the seasonality of temperature ( $\beta=1.68$ ,  $p=0.014$ ) and precipitation ( $\beta=-1.40$ ,  $p=0.037$ ) on RY. Bootstrapped 95% confidence intervals for all of these regression coefficients did not include zero, indicating that our estimates were consistent in the direction of effect across the range of observations in our dataset. Overall model fit—as indicated by the combination of the relative fit index CFI (comparative fit index) and the absolute fit index SRMR (standardized root-mean residual)—was adequate (Hu and Bentler, 1999). A greater amount of model error was found at the lower level ( $SRMR_w=0.079$ ) than at the higher level ( $SRMR_B=0.028$ ). This indicates that the estimated values for the lower level structural model are moderately variable from field to field, while the effects at the higher level were more consistent.

#### Soil health and N fertilizer efficiency

At the within-field level, the relative fertilization rate (RFR) exerted a positive direct effect on relative yield ( $\beta=0.74$ ,  $p<0.0001$ ) as well as on the soil health latent variable ( $\beta=0.39$ ,  $p<0.0001$ ) (Figure 4.4). Soil health had a smaller, positive direct effect on relative yield ( $\beta=0.13$ ,  $p=0.083$ ). The overall indirect effect of RFR on relative yield was mediated by soil health ( $\beta=0.05$ ,  $p=0.097$ ), resulting in a total standardized effect of RFR on relative yield of  $\beta=0.79$  ( $p<0.0001$ ). At the site level, mean annual precipitation (MAP) exerted a negative direct effect on relative yield ( $\beta=-0.35$ ,  $p=0.087$ ). The overall model was a generally close fit (CFI=0.995 and SRMR=0.038). Similar to the N responsiveness model, the majority of the variability was exhibited at the field level ( $SRMR_w=0.029$ ) rather than across sites ( $SRMR_B=0.010$ ).

Table 4.5 Correlation matrix of all measured variables in the N fertilizer efficiency model (n=290).

	<b>RFR</b>	<b>Inorg. N</b>	<b>Min. C</b>	<b>POXC</b>	<b>Soil protein</b>	<b>SOC</b>	<b>Clay</b>	<b>MAT</b>	<b>TS</b>	<b>MAP</b>	<b>PS<sup>j</sup></b>
<b>RY<sup>a</sup></b>	0.69***	0.19***	0.18*	0.25***	0.32***	0.18***	0.21***	0.23***	-0.13*	0.23***	-0.15**
<b>RFR<sup>b</sup></b>		0.20***	0.12*	0.29***	0.48***	0.18**	0.15**	0.49***	-0.35***	0.49***	-0.35***
<b>Inorg. N<sup>c</sup></b>			-0.12*	-0.03	0.20***	0.07	0.02	-0.24***	0.42***	-0.22***	0.45***
<b>Min. C<sup>d</sup></b>				0.45***	0.13*	0.33***	0.51***	0.02	0.01	0.00	-0.03
<b>POXC<sup>e</sup></b>					0.43***	0.45***	0.22***	0.18**	-0.15**	0.19**	-0.17***
<b>Soil protein</b>						0.50***	0.06	0.46***	-0.39***	0.49***	-0.37***
<b>SOC<sup>f</sup></b>							0.59***	0.28***	-0.22***	0.19***	-0.28***
<b>Clay</b>								0.12*	-0.05	-0.04	-0.17**
<b>MAT<sup>g</sup></b>									-0.92***	0.96***	-0.88***
<b>TS<sup>h</sup></b>										-0.89***	0.98***
<b>MAP<sup>i</sup></b>											-0.82***

†, \*, \*\*, and \*\*\* correspond to p-values of <0.10, <0.05, <0.01, and <0.001, respectively. Italicized values are not significant at p<0.10.

<sup>a</sup> relative yield; <sup>b</sup> relative fertilizer rate; <sup>c</sup> inorganic N; <sup>d</sup> mineralizable C; <sup>e</sup> permanganate oxidizable C; <sup>f</sup> soil organic carbon; <sup>g</sup> mean annual temperature; <sup>h</sup> temperature seasonality; <sup>i</sup> mean annual precipitation; <sup>j</sup> precipitation seasonality

#### The effect of mineralizable C on model fit

The initial exclusion of mineralizable c (or flush of CO<sub>2</sub> upon rewetting) from the latent variable of soil health represents a considerable deviation from a broad range of previous soil health literature. The inclusion of mineralizable C as an additional (exogenous) predictor of relative yield resulted in a decrease in overall model fit and parsimony for both final models. For the N responsiveness model, the inclusion of mineralizable C doubled the RMSEA and the SRMR<sub>w</sub> value (Table 4.8) without changing the SRMR<sub>B</sub> values. Similarly, for the N fertilizer efficiency model, the RMSEA and SRMR<sub>w</sub> values increased 300% and 400%, respectively, with a negligible decrease in SRMR<sub>B</sub> (0.001). Thus, the inclusion of mineralizable C resulted in considerable increases in the absolute error associated with both model fits, with concomitant decreases in CFI values of 0.500 and 0.194 for the N responsiveness and N fertilizer efficiency

models, respectively. Accordingly, AIC values increased with the inclusion of mineralizable C, indicating a much less parsimonious model.

Table 4.6 Regression coefficients and bootstrapped confidence interval for each relationship in the N responsiveness final model ( $n_B=29$  sites,  $n_W=186$  samples).

Level	Regression <sup>a</sup>	Standardized estimate ( $\beta$ )	Unstandardized estimate (B)	95% CI (unstandardized)	p-value
Between/	(1)	-1.40	-21.2	(-41.1, -1.27)	0.037
Across	(2)	1.68	32.7	(6.75, 58.7)	0.014
Fields	(3)	0.95	0.64	(0.30, 0.98)	<0.001
Within	(4)	0.27	0.69	(0.02, 1.38)	0.049
Fields	(5)	0.44	3.56	(2.40, 4.71)	<0.0001
	(6)	0.49	66.2	(47.0, 85.3)	<0.0001
	(7)	0.94	0.47	(0.39, 0.54)	<0.0001
	(8)	0.75	0.73	(0.58, 0.88)	<0.0001

<sup>a</sup> regression reference numbers correspond to Figure 4.2a;  $n_B$  and  $n_W$  indicate sample sizes for the between and within field levels of the model, respectively

## Discussion

### Soil health and responsiveness to N fertilization

The importance of pre-plant inorganic N in predicting crop response to N has resulted in its inclusion in many N recommendation frameworks (Morris et al., 2018). Here, we show that its importance is ubiquitous across a substantial portion of the Corn Belt (Figure 4.3). However, the incorporation of soil health measurements can contribute substantially to the prediction of responsiveness to N. Here, the indirect effect of soil health ( $\beta=0.05$ , Table 4.6) contributed substantially to the capability of pre-plant inorganic N ( $\beta=0.27$ ) to predict N responsiveness. It is somewhat surprising that soil health did not have a stronger, direct effect on relative yield, considering that previous work has shown that biologically-supplied N exerts an increasingly

strong role when mineral fertilizer is not applied (Swaney et al., 2018; Mahal et al., 2019). However, the elevated error of model estimation ( $SRMR_w=0.079$ ) indicates considerable site-to-site variability. Thus, adjustment for other soil characteristics and constraints (Tedersoo et al., 2014; Docherty et al., 2015; Delgado-Baquerizo et al., 2018; Jilling et al., 2018; Wade et al., 2018b) may be necessary to improve the accuracy of relationships between soil health and N responsiveness. Nevertheless, an accurate measure of N responsiveness is an important and separate component of N fertilization frameworks (Raun et al., 2011; Arnall et al., 2013)

Table 4.7 Regression coefficients and bootstrapped confidence interval for each relationship in the N fertilization efficiency final model ( $n_B=29$  sites,  $n_W=290$  samples<sup>b</sup>).

Level	Regression <sup>a</sup>	Standardized estimate ( $\beta$ )	Unstandardized estimate (B)	95% CI (unstandardized)	p-value
Between/ Across Fields	(1)	-0.35	-0.37	(-0.79, 0.05)	0.086
Within Fields	(2)	0.13	2.99	(-0.24, 6.21)	0.069
	(3)	0.74	40.13	(35.0, 45.3)	<0.0001
	(4)	0.39	0.90	(0.56, 1.25)	<0.0001
	(5)	0.58	70.4	(55.8, 84.9)	<0.0001
	(6)	0.79	0.33	(0.27, 0.39)	<0.0001
	(7)	0.64	0.57	(0.46, 0.67)	<0.0001

<sup>a</sup> regression reference numbers correspond to Figure 4.3;  $n_B$  and  $n_W$  indicate sample sizes for the between and within field levels of the model, respectively

Although soil health and pre-plant inorganic N content influenced N responsiveness, the strongest influences were climatic variables (Figure 4.3; Table 4.6). The within-year variability (i.e. seasonality) of precipitation increased the relative yield in check plots, whereas the seasonality of temperature decreased the relative yields. Higher precipitation seasonality typical of temperate climates—lower rainfall in the spring and summer, relative to the winter and fall—

would spur relatively deeper root development due to less in-season rainfall (Lynch, 2013) and greater retention of inorganic N within the rooting zone. Together, these would likely lead to a more efficient use of available inorganic N and a higher relative yield in the check plots. Less clear is how temperature variability reduced N responsiveness. The lower winter temperatures associated with greater temperature seasonality (Table 4.4) would be expected to reduce microbial activity during the winter, especially in unfertilized plots (Contosta et al., 2011). This would allow for an accumulation of readily-oxidizable organic matter (Davidson and Janssens, 2006) that would presumably increase N availability and subsequently increase relative yields. However, here we observe the opposite trend, where temperature seasonality decreased relative yields. Nevertheless, the small amount of model error across sites ( $SRMR_B=0.028$ ) suggests that these climatic factors are strongly predictive of crop responsiveness to N fertilization.

Table 4.8 Comparison of model fit indices with and without the inclusion of mineralizable C as an exogenous predictor variable for relative yield.

	N responsiveness		N fertilizer efficiency	
	With	Without	With	Without
CFI <sup>a</sup>	0.416	0.911	0.800	0.995
SRMR <sub>w</sub> <sup>b</sup>	0.197	0.079	0.148	0.028
SRMR <sub>B</sub> <sup>c</sup>	0.028	0.028	0.010	0.010
RMSEA <sup>d</sup>	0.308	0.162	0.219	0.052
AIC <sup>e</sup>	7635.1	6013.0	10140.7	7598.3

<sup>a</sup> CFI= comparative fit index; <sup>b</sup> SRMR<sub>w</sub>=standardized root mean square residual within fields; <sup>c</sup> SRMR<sub>B</sub>=standardized root mean square residual between fields; <sup>d</sup> RMSEA=root mean square error of approximation; <sup>e</sup> AIC=Akaike Information Criteria

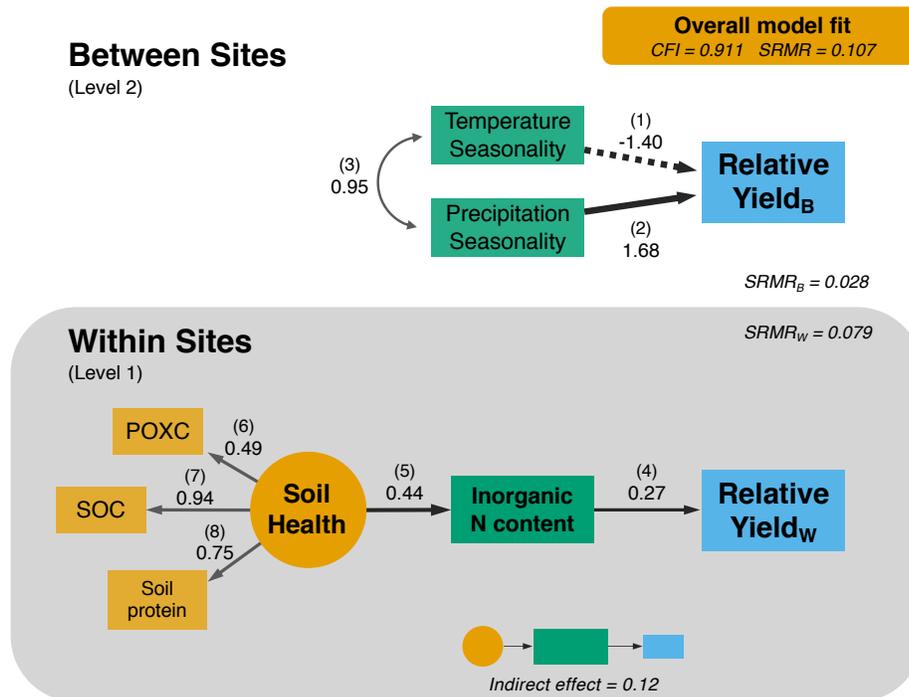


Figure 4.3 Final model for N responsiveness. All path coefficients are standardized. Numbers in parentheses refer to regression coefficients in Table 4.6.

MAT = mean annual temperature; MAP = mean annual precipitation; POXC = permanganate oxidizable C; SOC = soil organic carbon; CFI = comparative fit index; RMSEA = root-mean-square error of approximation; SRMR = standardized root-mean residual; SRMR<sub>B</sub> = standardized root-mean residual between sites; SRMR<sub>W</sub> = standardized root-mean residual within sites; Relative Yield<sub>B</sub> = relative yield between sites; Relative Yield<sub>W</sub> = relative yield within sites

### N fertilizer efficiency and soil health

Improvements in soil health increased the efficiency of applied fertilizer (Figure 4.4). While the magnitude of this effect ( $\beta=0.12$ , Table 4.7) was relatively modest in comparison to the effect of fertilization ( $\beta=0.75$ ), this represents an opportunity for reductions in N fertilization through increased soil health. This finding is largely in agreement with a recent global meta-regression showed that SOC and N fertilization rate both have positive relationships with crop yield, as well

as a synergistic effect (Oldfield et al., 2019). In the current study, the low model error at the within-field level ( $SRMR_w=0.029$ ) illustrates the robustness of this relationship in a broad range of pedoclimatic contexts. Additionally, it demonstrates that biologically-based soil health indicators capture the beneficial effects of improved management on N fertility (Tonitto et al., 2006; Blanco-Canqui et al., 2012; Gaudin et al., 2015; Osterholz et al., 2018). This validates the claims that: 1) these metrics are appropriate indicators of biological soil health, and 2) that a healthy soil supplies a greater amount of nitrogen to crops.

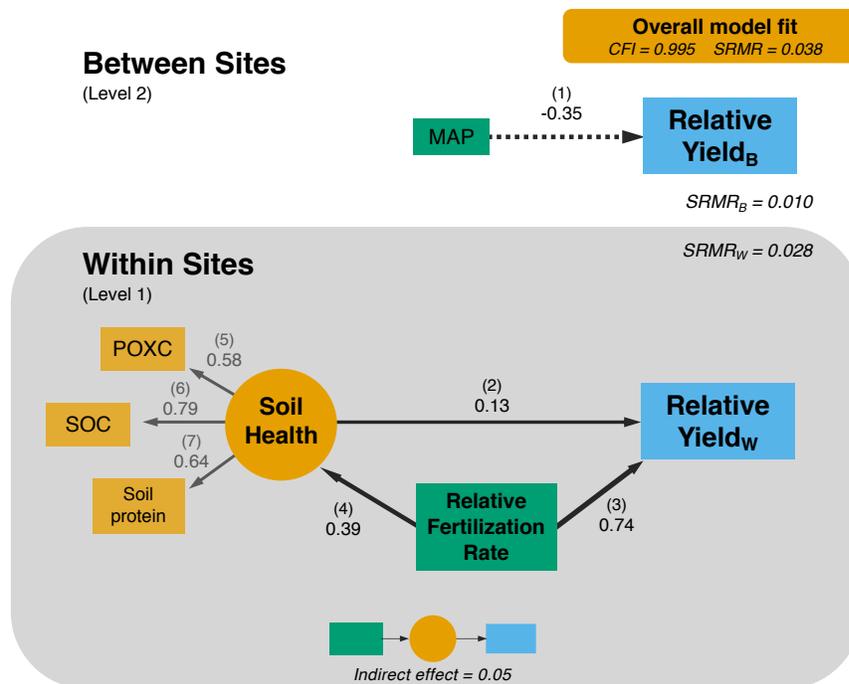


Figure 4.4 Final model for N fertilizer efficiency. All path coefficients are standardized. Numbers in parentheses refer to regression coefficients in Table 4.7.

MAT = mean annual temperature; MAP = mean annual precipitation; POXC = permanganate oxidizable C; SOC = soil organic carbon; CFI = comparative fit index; RMSEA = root-mean-square error of approximation; SRMR = standardized root-mean residual;  $SRMR_B$  = standardized root-mean residual between sites;  $SRMR_w$  = standardized root-mean residual within sites;  $Relative Yield_B$  = relative yield between sites;  $Relative Yield_w$  = relative yield within sites

Interestingly, higher fertilization rates also increased soil health ( $\beta=0.39$ , Figure 4.4). While there is a large literature debating how or if mineral fertilizers influence soil C stocks and fluxes (McGill and Cole, 1981; van Groenigen et al., 2006; Khan et al., 2007; Reid, 2008; Mulvaney et al., 2009; Ladha et al., 2011; Lu et al., 2011; Kirkby et al., 2013; Tipping et al., 2016; Poffenbarger et al., 2017), here we see evidence that it significantly improves soil health ( $p<0.0001$ ). While this may be due to increases in SOC (Table 4.5;  $r=0.18$ ,  $p=0.002$ ), N fertilization can also influence microbial biomass (Geisseler and Scow, 2014), plant residue composition (Liu et al., 2016b), and ligninolytic activity (Chen et al., 2018). While these parameters are not explicitly measured in our soil health latent variable, they are indicative of alterations in C and N cycling that are highly related to our biological soil health indicators (Grandy et al., 2013; Tiemann and Grandy, 2015; Hurisso et al., 2016b, 2018c; Margenot et al., 2017a). This linkage between soil health and relative N fertilization rate implies that improvements in soil health don't necessarily equate to drastic decreases in fertilizer application rate. Rather, this suggests that more modest decreases in N fertilization may be preferable for optimizing both yield and soil health (Poffenbarger et al., 2017). However, the broad applicability of our results—more than half of the rainfed corn acreage in the US—suggests that even these modest fertilizer reductions could have profound effects on grower profitability and reactive N losses to the environment. Previous work has estimated a 12% reduction in N fertilization can reduce nitrate fluxes to Gulf of Mexico by 33% (McIsaac et al., 2001).

At the upper level of our model, the negative effect of MAP on relative yield (i.e. increased responsiveness to N fertilization) was unexpected in rainfed maize-based systems

(Figure 4.4). Given the coarseness of this metric, this effect is likely attributable to an overall higher probability of N losses throughout the corn growing season (Randall and Mulla, 2001). This includes crucial periods of the growing season where N supply exceeds crop demand, such as in the spring before crop establishment or in the fall after crop physiological maturity (Meisinger and Delgado, 2002; Zhou and Butterbach-Bahl, 2014). These N losses would decrease the amount of plant-available N—residual or from mineralized organic sources—ultimately increasing crop reliance on fertilizer N.

#### Lack of predictive ability with inclusion of mineralizable C

Mineralizable C is one of the most widely-adopted measurements of biological soil health (Bünemann et al., 2018), yet universally lacked utility in our current study. Mineralizable C had little shared variance with other biological soil health indicators—leading to its exclusion from our factor model—and made our N response models less accurate and parsimonious (Table 4.8). Stated differently, after accounting for confounding site-specific effects, mineralizable C offered almost no information on N responsiveness that was not already described by our other biological soil health indicators. This was a surprising finding given the large body of literature supporting its relationship to N mineralization (Haney et al., 2001; Franzluebbers, 2018c), crop response to N fertilization (Franzluebbers, 2018b; Yost et al., 2018), and overall agronomic productivity (Culman et al., 2013; Hurisso et al., 2016b). This discrepancy between previous studies and the current one is almost entirely attributable to site-level variability in mineralizable C (Table 4.10). Aerobic respiration in surface soils is largely influenced by soil physiochemical characteristics, such as texture or bulk density (Moyano et al., 2012, 2013; Ghezzehei et al., 2019) and climatic variables (Engelhardt et al., 2018). These effects would operate at the

between- or across-field level, rather than the within-field level, rendering the information provided by mineralizable C largely redundant in the context of a multilevel model. This result alone underscores the value of multi-level modeling approaches that control for site-to-site variability.

## Conclusions

While N management is an inherently multidisciplinary endeavor (Stuart et al., 2015; Bowles et al., 2018), the widespread embrace of soil health allows for it to play a key role in mediating the biophysical and the socioeconomic components of this challenge. The push to decrease mineral N inputs must be accompanied by an increasing reliance on biologically-mediated organic N sources. Thus, the utility of biological soil health indicators is acutely of interest in guiding N management decisions. Here, we have used rainfed corn N trials representative of over half of US corn acres to show that: 1) selected biological indicators can be used in conjunction with inorganic N content to predict if a field will be responsive to N fertilization, 2) improved biological soil health increases the efficiency of applied fertilizer N, and 3) that appropriate applications of N fertilizer can improve soil health. Additionally, our results suggest that the use of mineralizable C in N fertilization decisions is not robust across much of the US corn production acreage. While these effects are able to be extrapolated widely across sites, climatic influences exerted on a site-by-site basis are also highly influential on soil-plant N dynamics.

Supplementary

Table 4.9 Location, soil classification, and management information for each site.

Study Name	Site #	Location	Soil Series	Taxonomic description	Textural		Rotation	Cover crop	Sampling depth (in)	State
					class	Tillage				
N Rotation	1	42.93661, -92.57011	Kenyon	Fine-loamy, mixed, superactive, mesic Typic Hapludolls	loam	conventional-till	Continuous corn (grain), corn-soybean, corn-corn-corn-soybean	None	0-6	IA
Manure × CC	2	43.30129, -89.34742	Plano	Fine-silty, mixed, superactive, mesic Typic Argiudolls	silt loam	conventional-till	Corn (grain)	Barley, none	0-12	WI
	3	44.75818, -90.09975	Withee	Fine-loamy, mixed, superactive, frigid Aquic Glossudalfs	silt loam	conventional-till	Corn (grain)	Barley, none	0-12	WI
	4	44.1198, -89.53573	Plainfield	Mixed, mesic Typic Udipsammments	sand	conventional-till	Corn (grain)	Barley, none	0-12	WI
	5	42.83066, -90.78855	Fayette	Fine-silty, mixed, superactive, mesic Typic Hapludalfs	silt loam	conventional-till	Corn (grain)	Barley, none	0-12	WI
Radish CC	6	43.42253, -88.29314	Theresa	Fine-loamy, mixed, superactive, mesic Typic Hapludalfs	silt loam	no-till	Corn-soybean-wheat	Radish, none	0-12	WI
	7	43.79907, -87.91845	Kewaunee	Fine, mixed, active, mesic Typic Hapludalfs	silt loam	no-till	Corn-soybean-wheat	Radish, none	0-12	WI
	8	42.72563, -89.02026	Plano	Fine-silty, mixed, superactive, mesic Typic Argiudolls	silt loam	no-till	Corn-soybean-wheat	Radish, none	0-12	WI
Purdue N Trials	9	40.47114, -86.99223	Chalmers	Fine-silty, mixed, superactive, mesic Typic Endoaquolls	silty clay loam	conventional-till	Corn-soybean	None	0-8	IN
	10	40.25335, -85.14803	Pewamo	Fine, mixed, active, mesic Typic Argiaquolls	clay loam	strip tillage	Corn-soybean	None	0-8	IN
	11	39.03314, -85.52582	Cobbsfork	Fine-silty, mixed, active, mesic Fragic Glossaqualfs	silt loam	no-till	Corn-soybean	None	0-8	IN
	12	41.43992, -86.92521	Tracy	Coarse-loamy, mixed, active, mesic Ultic Hapludalfs	sandy loam	conventional-till	Corn-soybean	None	0-8	IN
	13	40.29690, -86.90358	Toronto	Fine-silty, mixed, superactive, mesic Udollic Epiaqualfs	silt loam	conventional-till	Corn-soybean	None	0-8	IN
	14	41.10729, -85.39953	Morley	Fine, illitic, mesic Oxyaquic Hapludalfs	silty clay loam	no-till	Corn-soybean	None	0-8	IN

Blevins	15	38.12194, -84.48638	Maury	Fine, mixed, active, mesic Typic Paleudalfs	silt loam	conventional- till and no-till	Continuous corn	None	0-8	KY
F05	16	38.12944, -84.48611	Lanton	Fine-silty, mixed, superactive, thermic Cumulic Epiaquolls	silty clay loam	no-till	Continuous corn, corn-soybean	None	0-8	KY
Legume CC	17	43.8065, - 87.89767	Manawa	Fine, mixed, active, mesic Aquollic Hapludalfs	silt loam	conventional- till	Corn-wheat	Barley, clover, none	0-12	WI
	18	43.80939, -87.8976	Kewaunee	Fine, mixed, active, mesic Typic Hapludalfs	silt loam	conventional- till	Corn-wheat	Barley, clover, none	0-12	WI
	19	43.80057, -87.91917	Kewaunee	Fine, mixed, active, mesic Typic Hapludalfs	silt loam	conventional- till	Corn-wheat	Barley, clover, none	0-12	WI
Ohio N Trials	20	40.56152, -84.28631	Pewamo	Fine, mixed, active, mesic Typic Argiaquolls	clay loam	conventional- till	Corn-soybean-wheat	Oats- cereal rye	0-8	OH
	21	39.95756, -81.90373	Zanesville	Fine-silty, mixed, active, mesic Oxyaquic Fragiudalfs	silt loam	conventional- till	Corn-soybean	Rye	0-8	OH
	22	39.94164, -81.94457	Zanesville	Fine-silty, mixed, active, mesic Oxyaquic Fragiudalfs	silt loam	conventional- till	Corn-soybean	Cereal rye	0-8	OH
	23	40.73274, -83.66592	Blount	Fine, illitic, mesic Aeric Epiaqualfs	silt loam	no-till	Corn-soybean	Cereal rye	0-8	OH
	24	40.32361, -82.32527	Titusville	Fine-loamy, mixed, active, mesic Aquic Fragiudalfs	silt loam	no-till	Corn-soybean	None	0-8	OH
	25	40.40753, -82.68032	Centerburg	Fine-loamy, mixed, active, mesic Aquic Hapludalfs	silt loam	no-till	Corn-soybean	None	0-8	OH
	26	41.29715, -84.70729	Lenawee	Fine, mixed, semiactive, nonacid, mesic Mollic Epiaquepts	silty clay loam	conventional- till	Corn-soybean	None	0-8	OH
	27	39.95722, -82.51416	Bennington	Fine, illitic, mesic Aeric Epiaqualfs	silt loam	no-till	Corn-soybean	None	0-8	OH
	28	41.70961, -83.99495	Brady	Coarse-loamy, mixed, active, mesic Aquollic Hapludalfs	sandy loam	conventional- till	Corn-soybean	None	0-8	OH
	29	41.42986, -84.21012	Hoytville	Fine, illitic, mesic Mollic Epiaqualfs	clay loam	no-till	Corn-soybean-wheat	Cereal rye, radish	0-8	OH

Table 4.10 The relationships between mineralizable C and relative yield in a simple regression and a mixed effects model.

	Regression coefficient	F-value	Variance explained (R <sup>2</sup> )		
			Mineralizable C	Field (random)	Total
Simple regression	0.24	10.8**	0.031	--	0.031
Mixed effects model	0.07	0.6 <sup>ns</sup>	0.002	0.362	0.364

## Chapter 5: Synthesis and Broader Impacts

The soil health framework has been increasingly gaining traction within both the academic literature (Figure 5.1) and in farmer peer-to-peer networks (Arbuckle, 2017). While this framework has been increasingly popular, there have also been numerous criticisms of the soil health concept. Most notably, critiques have been raised about the value-laden nature of the term, as well as the inability to connect to specific (potentially-conflicting) outcomes (e.g. multiple ecosystem services) (Sojka and Upchurch, 1999; Bünemann et al., 2018). While these critiques are well-received, the opportunity for a framework that unifies grower needs with the ecologically-focused concerns of researchers (primarily at land-grant universities) (Romig et al., 1995; Ingram et al., 2010) is a unique opportunity that merits consideration beyond a strict scientific framework. Soil health has the potential to extend beyond strict scientific scrutiny into the domain of decision science and agricultural extension (Ingram, 2008; Shepherd, 2015), a necessary component to facilitating adoption of any practices. Accordingly, recommendations and management prescriptions from soil health researchers should be both reliable and robust across contexts, as well as being actionable for growers.

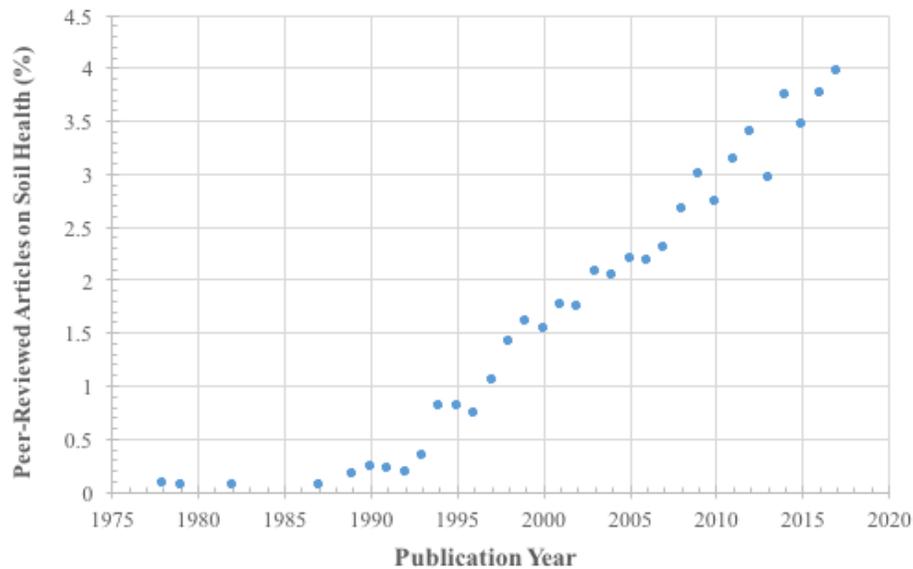


Figure 5.1 Percentage of total yearly peer-reviewed agricultural publications that are on the topic of “soil health” from 1978-2017 ( $n=6,989$ ). Source: ISI Web of Knowledge using topics of “soil science”, “agronomy”, and “interdisciplinary agriculture”.

The most commonly-used biological soil health metric is mineralizable C, or respiration upon rewetting (Bünemann et al., 2018). Here, chapters 2 and 4 show that even though this is the most commonly-used measurement, it has yet to meet the desired conditions of reliability and robustness. Its lack of reliability across labs or even between the subsamples used in analytical replication (chapter 2) is cause for concern. Its use could be justified—given considerable caveats on the appropriate levels of certainty—if it were to provide data that is easily integrated into on-farm management decisions. However, we have shown that much of the biological “information” it contains (at least in the context of N management) is better-described by other soil biological metrics (chapter 4). These measurements have the added benefit of being more analytically repeatable and, in the case of soil protein, less temporally variable as well (Hurisso

et al., 2018a). However, that is not to say that mineralizable C is without value when making management decisions. Hurisso et al. (2016b) have shown that mineralizable C can be used in conjunction with POXC to infer management-related effects on organic matter dynamics.

As soil health transitions from a theoretical framework into a more applied, agronomic context, the nature of the research questions will also shift. One of the foremost questions surrounding soil health indicators is the risk associated with reductions in fertilizer applications, a claim that growers have long been skeptical of (Andrews et al., 2003). Recent work has begun to determine the relationships between biological soil health indicators and N fertilizer response (Franzluebbers, 2018b; Yost et al., 2018; Haney et al., 2018). Here, chapter 4 shows that while these biological soil health indicators have agronomic utility across much of the Corn Belt, the effects are likely more modest than previous studies have shown. Within chapter 4 we also see that N fertilization can actually increase our biological soil health indicators, whereas chapter 3 illustrates that P fertilization does not increase biological soil health. Collectively, these findings are compatible with the work illustrating that soil N content was a significant constraint on microbial biomass, whereas microbial biomass showed a greater degree of plasticity with soil P content (Hartman and Richardson, 2013; Geisseler and Scow, 2014; Geisseler et al., 2016b). This agreement with previous work supports the claim that the chosen indicators are biologically-based.

Another critique of using the term “soil health” in an agronomic setting has been the wide range of measurement methods. There are many official suites of soil health tests, including the Haney Soil Health Test (Yost et al., 2018; Haney et al., 2018), the Comprehensive Assessment of Soil Health (Moebius-Clune et al., 2016), the Soil Management Assessment Framework (Andrews et al., 2004), or even the use of a single indicator in lieu of a suite of tests

(Franzluebbers, 2018c). These efforts are then often further consolidation in search of a “minimum data set” for a given context (Govaerts et al., 2006; Rezaei et al., 2006; Lima et al., 2013; Fine et al., 2017). However, these studies are often disconnected from a specific desired outcome and instead intended as an all-encompassing description of soil health. This reductionism fails to recognize the heterogeneity of soils and their limitations. In chapter 4, we use an approach that has a strong potential to address the arbitrary nature of quantifying soil health and its lack of connection to specific outcomes. Factor analysis provides a less subjective way of assessing which measurement(s) are necessary. The output—a single value—meets the request by growers for less complexity in terms of soil health evaluations (Andrews et al., 2003) as well as being amenable to connection with outcomes via linear regression. Integration into a multilevel model also allows for extrapolation to fields beyond a given study, an essential component to providing growers with management prescriptions.

As soil health transitions into an increasingly applied context, a greater understanding of its role in nutrient management will be central to its utility in providing ecosystem services (one of the primary concerns raised regarding soil health). Here, we have begun to quantify uncertainties associated with measuring soil health and have provided an initial exploration into interactions between soil health and N and P fertilizer management.

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