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How Does Phosphorus Restriction Impact Soil Health Parameters in Midwestern Corn–Soybean Systems?

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ABSTRACT

Limiting agricultural 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 yr of P restriction to measure impacts on soil P availability and 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.

Core Ideas

- Three sites showed no yield difference between control and P fertilized treatment after 11 yr.
- Tissue and grain P contents showed no sign of crop P stress associated with 11 yr of P restriction.
- Phosphorus fertilization rate was positively related to labile P pools and inversely related to organic P.
- No consistent effect of P fertilization on soil health parameters
- Greater proportion of active organic matter in unfertilized treatments, relative to fertilized

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The application of fertilizer-based 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 (i) increasing infiltration rates, (ii) improving soil structure, and (iii) 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

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Abbreviations: ACE protein, autoclaved citrate-extractable protein; CASH, Comprehensive Assessment of Soil Health; CEC, cation exchange capacity; DAP, diammonium phosphate; GWC, gravimetric water content; ICP–AES, inductively coupled plasma atomic emission spectroscopy; K_{sat}, saturated hydraulic conductivity; M3-P, Mehlich-3 extractable P; MLRA, major land resource area; PCA, principal component analysis; POXC, permanganate-oxidizable carbon; PR, penetration resistance; WSA, water stable aggregates; XRF, X-ray fluorescence.

Table 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)

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-yr) 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.

MATERIALS 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 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 yr 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 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 and 2.7 Mg ha^{-1}, respectively) by the estimated grain P removal rates (6.6 and 13.3 kg P_2O_5 Mg grain^{-1}, 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_2O_5 ha^{-1}, respectively, following corn and 35.9, 71.8, and 107.7 kg P_2O_5 ha^{-1}, 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^{-1} (as urea at planting and urea-ammonium-nitrate at sidedress) and fertilizer K rate was 43.7 kg ha^{-1} (as muriate of potash) was applied for corn following soybeans. Soybean crops received an additional 62.9 kg ha^{-1} of K fertilization to meet soybean demand, but no additional N fertilization. Specific dates for major field operations are outlined in Supplementary Table S1.

Immediately after planting in 2017, we took soil samples of 8–10 cores per plot to a depth of 25 cm using a 4 cm 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., 2018a).

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 50.0 g of air-dried soil and the hydrometer method (Bouyoucos, 1962). The results of these analyses are shown in Table 2.

We determined soil physical properties in-field approximately 1 wk after soil sampling. We measured penetration resistance (PR) using a SpotOn digital soil compaction meter from Innoquest, Inc. (Woodstock, IL) to a depth of 25 cm. 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):

Table 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^{-1} soil)	Total N (g N kg^{-1} soil)	Total C/N	Total P (mg P kg^{-1} soil)	CEC (mmol/kg)	Clay content (g kg^{-1} soil)	Sand content (g kg^{-1} 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)

$$PR_c = PR_0 + \frac{dPR_0}{dGWC_0}\left(GWC_c - GWC_0\right) \qquad [2]$$

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 Eq. [1], and PR_0 and GWC_0 are as denoted in Eq. [1]. The mean of all GWC_0 values (0.205 g H_2O g^{-1} 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_{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_{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 Phosphorus 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 h) with 20 mL each (in sequential order) of deionized water, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl. Then, we centrifuged each extract for 15 min at 8000 × 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 NaOH fractions, although both $NaHCO_3$ and HCl extract contained detectable organic P (He et al., 2006). Similar to Margenot et al. (2017b) the H_2O- and $NaHCO_3$-extractable P_i fractions will be considered Labile P_i.

Mineralizable Carbon

Mineralizable C, also known as respiration on rewetting or the flush of CO_2 on rewetting, was measured on triplicate 10.0-g samples using 24 h incubations in 50 mL microcosms. We rewetted soils from above to approximately 50% water-filled pore-space (Franzluebbers, 2016; Wade et al., 2018). 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_2 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 min in a 50 mL polypropylene centrifuge tube using 0.02 M $KMnO_4$ and allowed to settle for exactly 10 min. 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. (2018b) and the Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2016). In brief, 24 mL of 0.02 mol L^{-1} sodium citrate (pH = 7) was added to 3.0 g of air dried soil in a glass screw-top tube, shaken for 5 min, and autoclaved at 121°C (15 psi) for 30 min. After cooling, we shook the tubes for 3 min to resuspend soil particles and transferred 1.5 mL to microcentrifuge tubes for clarification of the extract (3 min at 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 1 h. After this 1 h incubation, we allowed the plate to cool for 5 min prior to reading the samples colorimetrically at 562 nm. 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 h. The

Table 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^{-1})	Corn tissue P‡ (%)	Corn yield (bu ac^{-1})	Corn grain P (%)
Site	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
Fertilization	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
ANOVA	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.
‡ Significant interaction is further explored in Supplementary Table 3.

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 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. Similar procedures were followed for soybean yields and plant tissue analyses in 2016, the results of which are shown in Supplementary Table 3.

Statistical Analyses

All statistical analyses were performed in JMP Pro 13 (JMP, 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, 2016) 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. Loadings for each variable and proportion explained for each principal component are shown in Supplementary Table 4.

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. (2016) 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 toward POXC) were considered organic matter building while negative residuals (i.e., trending toward mineralizable C) was considered organic matter use.

Table 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.

		Biological			Physical		
Factor	Treatment	Mineralizable C (mg CO_2–C kg^{-1} soil)	POXC (mg kg^{-1} soil)	ACE protein (g kg^{-1} soil)	K_{sat}† (mm h^{-1})	Penetration resistance (MPa)	Water stable aggregates (%)
Site§	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
Fertilization§	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.
‡ Analysis was performed on transformed data, values here represent backtransformed data and values in parentheses are 95% confidence intervals.
§ Values for each S × F combination can be found in Supplementary Table 2.

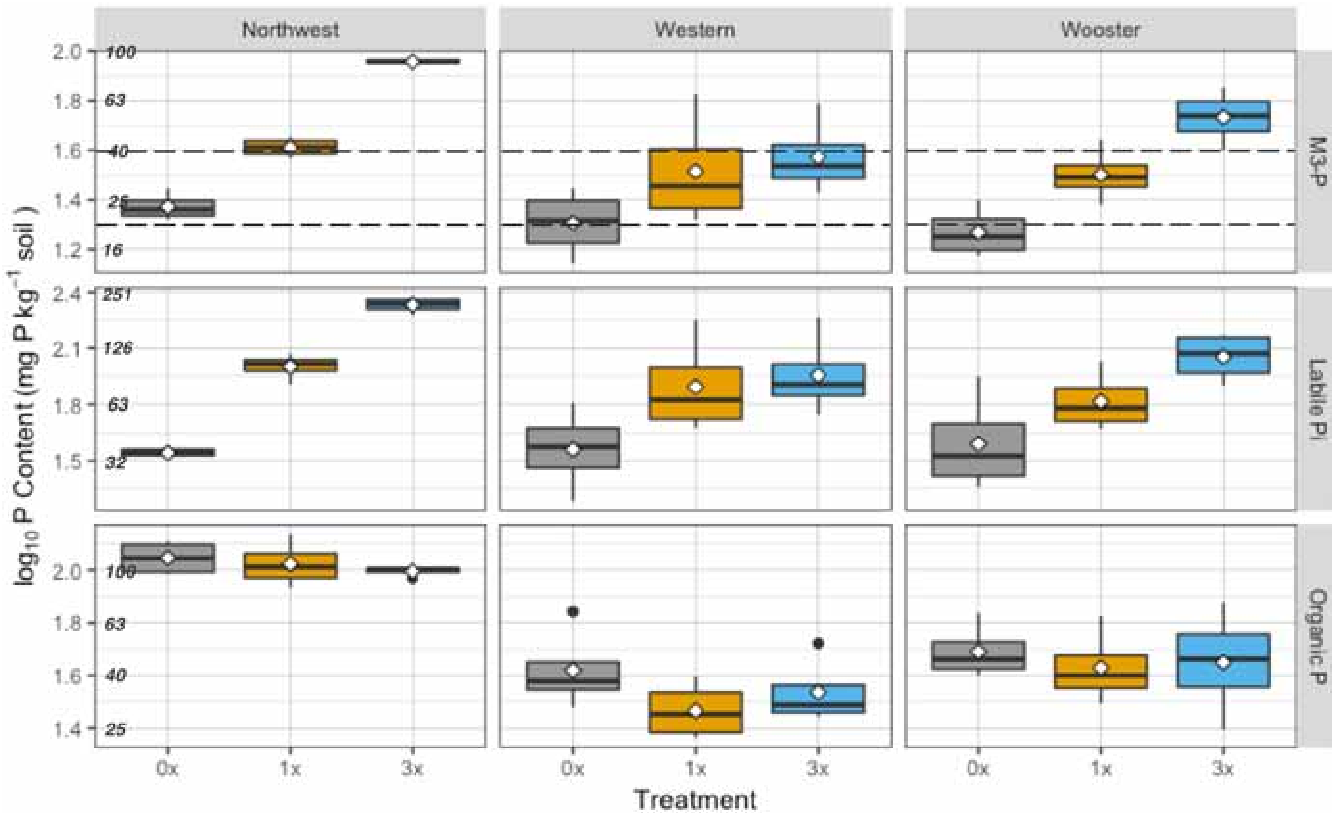

Fig. 1. Soil phosphorus fractions by site and treatment. Italicized numbers on y-axis show backtransformed values. Dotted lines for M3-P indicate limits of the “maintenance range” of 20–40 mg P kg^{-1} soil from Tri-State Fertilizer recommendations (Vitosh et al. 1995; S.W. Culman et al., unpublished data, 2019).

RESULTS

Agronomic Response to Phosphorus

Corn crop yields were not responsive to P fertilization across all sites (Table 3). Even nonsignificant general trends emphasized a lack of response to fertilizer, with least-square means in the order of 2×/3× > 0× > 1×. 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). The effect of fertilization on tissue P and grain P followed the expected trend of 0× < 1× < 2×/3×. 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 × fertilization interaction for tissue was due to the lower tissue P content in the 0× 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 (Fig. 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× treatments were in the “buildup” or the lower end of the “maintenance” range, the 1× treatments in the “maintenance” range or lower end of the “drawdown” range, and the 2×/3× in the “drawdown” range (Fig. 1). The exception to this trend was the Northwest site, which had 0× in the “maintenance” range and both 1× and 2×/3× in the “drawdown” range.

Organic P levels differentiated strongly by site (Fig. 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_i and organic P increased as fertilization rates increased (Fig. 1). Orthogonal contrasts showed that the 0× 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 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 0× and the 2×/3× treatments ($p = 0.39$, data not shown), which had the highest ACE protein levels.

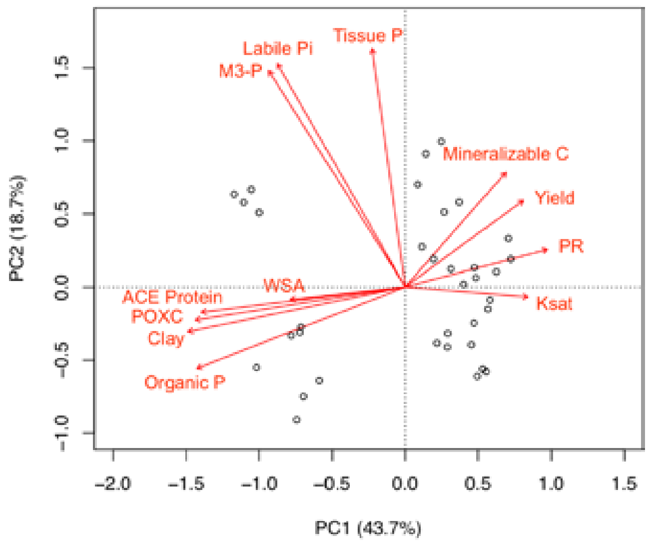

Fig. 2. Biplot for principal component analysis (PCA) of soil health metrics, soil physiochemical characteristics, and agronomic performance indicators.

However, the 1× was significantly lower than both the 0× (p = 0.015) and the 2×/3× (p = 0.087) treatments across sites.

Physical Soil Health Indicators

Average K_{sat} values for each site generally agreed with the K_{sat} values of Dohnal et al. (2010) for their respective textural classes (data not shown). Accordingly, K_{sat} was much greater at Western than either Northwest or Wooster sites (Table 4; $p < 0.0001$). Overall, K_{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 1× treatments resulted in similar PR to the 0× (p = 0.18) and lower PR than the 2×/3× 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).

Water stable aggregates varied predominantly by site (Table 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).

Patterns of Variation among Agronomic and Soil Health Variables

The first principal component (PC1) described 43.7% of the variance among our variables, primarily soil health indicators and yield (Fig. 2). The ACE protein, POXC, WSA, clay, and organic P were all negatively associated with PC1. Conversely, K_{sat} 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 5. Organic matter trends from residuals of linear regressions of POXC vs. mineralizable C. Positive residuals indicate treatment trending toward POXC (stabilization) and negative residuals indicate trends toward mineralizable C (oxidation).

	Site		
Treatment	Northwest	Western	Wooster
0×	+	+	+
1×	–	–	+
2×/3×	–	–	–

Organic Matter Trends: Building Versus 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 toward POXC) and all 2×/3× treatments had negative residuals (i.e., trending toward mineralizable C) (Table 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., 2016; 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 (Fig. 2). Mineralizable C (which is considered an indicator of processes inverse to these processes) (Hurisso et al., 2016) was inversely associated with these metrics. However, in agreement with previous work (Culman et al., 2013; Hurisso et al., 2016; 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 United States (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 among our current sites (Fig. 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; Denef et al., 2002; Denef 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., 2018). 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 yr 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 nonlinear 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 0× treatment than either the 1× ($p = 0.092$) or 2×/3× ($p = 0.010$). 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 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 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 (Fig. 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 (Fig. 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 5). Additional root growth could also reconcile the concurrent trends in organic P (Fig. 1). Liu et al. (2017) showed an increase in organic P in the form of phosphomonoesters after 27 yr 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 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_{sat}. Additionally, this increased root growth would explain the association of K_{sat}

values with yield (Fig. 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 Phosphorus Stress?

Corn crops showed a considerable response of both tissue and grain P content to fertilization rate (Table 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 (Fig. 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 yr 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 yr 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}P and ^{33}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-mo 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 (Fig. 1; Fig. 2). Additionally, we have very little evidence that indicators of biological soil health are (i) altered by low solution P or (ii) 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.

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