Characterizing the Relationship between Soil Phosphorus and Land Management on Working Farms in the Western Lake Erie Basin

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Abstract
Persistent harmful algal blooms in Lake Erie caused by non-point source nutrient losses from surrounding agricultural regions have driven an increased interest in tightening nutrient management in the basin. Phosphorus is a key nutrient for plant growth, but applications in excess of crop needs have built large legacy pools of this nutrient in soils in the watershed, and farmers continue to apply phosphorus to maximize yields. This research applies a modified Hedley sequential extraction protocol to elucidate how land management practices influence the distribution of phosphorus to pools of varying accessibility to plants and loss to leaching. Functional diversity and species richness are found to increase Pi availability in NaOH Pi and more plant available pools, while the practice of harvesting perennials is found to draw-down plant resin and bicarb Pi. Paired agriculture and forest plots allow for soil-type matched comparisons, and the NaOH Pi pool is found to be larger on agricultural fields than forests identifying a pool of interest for further research on legacy P. Heterogeneity is found between pairs, and more research is needed to understand how land management practices impact deviation from native soil characteristics.

Introduction
Phosphorus (P) losses from intensive agriculture are a leading cause of eutrophication, with negative impacts on drinking water, recreation, fisheries, and natural ecosystems. In the Laurentian Great Lakes, Lake Erie in particular has struggled with extensive nutrient pollution leading to harmful algal blooms (HABs) over the years. The first round of these blooms was largely mitigated beginning in the 1970s through reductions in point-source emissions, especially from municipal waste facilities (Charlton, 2008). By the late 1980s, annual bloom sizes had diminished considerably. There was a general sense of optimism in the scientific community about future reductions of so-called "nuisance algae" in the lake and potential for improvement of invertebrate population conditions (Ludsin et al 2001, Makarawicz & Bertram 1991, De Pinto et al 1986). However, HABs returned in full force by the late 2000s with a large bloom in 2014 which necessitated the closure of the Toledo water treatment facility for almost 3 days and directly affected approximately 500,000 people (Jetoo et al. 2015, Michalak et al. 2013). Streamflow concentrations of dissolved reactive phosphorus (DRP, also called soluble reactive phosphorus or SRP) from agriculturally intensive watersheds have risen, indicating that an increase in non-point source phosphorus from agricultural areas is the probable primary cause of the return of large HABs (Baker et al. 2014, Ludsin et al. 2025, Scavia et al. 2014, Matisoff et al. 2016). Ongoing streamflow analysis has confirmed the significance of agricultural fertilizer applications to inputs to the watershed, as have retrospective modeling estimates (Guo et al. 2020, Daloglu et al. 2012).

Both biological and geochemical processes mediate P cycling and the distribution of P forms in soil. Generally, P is found in organic and inorganic forms in soil, across a gradient of chemical recalcitrance or rapidity of cycling. 30-65% of P is found in organic forms, with highly organic soils containing up to 90% organic P (abbreviated as Po) (Harrison 1987). Organic forms are compounds formed by biological processes and include both a rapid-cycling pool which is mostly composed of microbial biomass and a slower-cycling pool composed of plant detritus.

Inorganic forms include a rapid-cycling pool which is loosely adsorbed to soil surfaces and is very small, and two occluded pools which represent most of the total P in soils. The first of these is a primary mineral pool, mostly consisting of apatite minerals originating in the parent material of the soil, which have a wide range of chemistries but, by definition,contain phosphate anions in a hexagonal framework with Ca^{2+} cations. The second is a secondary mineral pool, consisting of iron, manganese, or aluminum oxyhydroxides, which adsorb phosphate as it is released from apatite minerals over time by biochemical weathering processes (Tian et al. 2021, Filippelli 2002, Schlesinger 1997, Condron et al. 2005)

The microbial community plays a critical role in cycling of Po as well as weathering mineral forms of P. Phosphorus solubilizing microbes (PSMs) come in a wide variety of forms, primarily bacteria and fungi. These microorganisms frequently appear as free-living organisms in bulk soil and in the rhizosphere and include some members that form associations with plants. PSMs produce a wide range of organic acids and enzymes, which either increase acidity to drive redox detachment or enzymatically detach P into soil solution. Plants that form mycorrhizal associations have a significant advantage in scavenging soil P, primarily because fungal hyphae decrease diffusion distances and increase the volume of soil that plants can access. Mycorrhizae can also mobilize P from more complex organic compounds in soil (Bolan 1991, Smith & Read 2008, Tian et al 2021, Dodd & Sharpley 2015).

Phosphorus accumulates in agricultural soils when manure or fertilizer inputs consistently exceed harvested exports (i.e., a positive P mass balance). Farmers tend to overapply fertilizer relative to recommended rates for reasons ranging from a lack of confidence in model estimates to general risk aversion and fear of reduced yields (Sherriff 2005). Over time, consistent P surpluses saturate the soil's capacity to store P in organic forms and on mineral surfaces. "Residual" P - defined as an annual excess - adsorbs to soil complexes. "Legacy" P, which is defined as the interannual accumulation of residual P (i.e., sustained P surpluses over time) can take long periods of time to draw down to natural or native levels, depending on soil characteristics, crop selection, and hydrology, among other factors (Zhou & Margenot 2023). Typically this process occurs on decadal time scales once fertilization has ceased; Zhang et al. (2020) reported 11 years with no yield decrease, while Sharpley et al (2013) reported 8-30 years for soil test P to draw down on fields kept in agricultural use. Some fields may take even longer to reduce legacy P, especially organically rich soils in areas with a long history of agricultural production. Sun et al. (2022) describe a set of fields in Iowa with a high manure input legacy which had only lost 12-30% of P after a 36-year experiment without fertilization. Legacy P can leave fields by export in harvested crops, by soil erosion in the form of sediment P, or by surface or subsurface leaching in the form of soluble P (Hussain et al. 2021).

While the costly negative consequences of excess P are evident in HABs, sufficiency of this nutrient is critical for maintaining productivity and yield in agricultural systems. P is a key constituent of important biological substrates including nucleic acids (gene transfer), sugar phosphates (energy transfer), and phospholipids (cell membranes), and deficiency can require adaptation in the production and maintenance of these tissues or simply limit the capacity of plants to grow (Shen et al 2011, Raghothama 2005). There is significant variation across

cultivars and species to the growth limitations imposed by low P availability, and while P deficiency is generally not fatal it does have negative consequences for crop yields and development (Halsted and Lynch 1995, Zheng et al. 2021, Iqbal et al. 2023, Atkinson 1973). It is understood to be globally one of the most frequent limiting nutrients to crop yields (Lott et al. 2011), although P limitation is rare in Michigan soils.

Understanding how soil P is cycled and stored - i.e., which pools it is in and how available it is for plant uptake or losses - is critical to decreasing pollution to downstream waterways while maintaining agricultural production. Many of the most commonly used soil tests focus on inorganic P (abbreviated to Pi, orthophosphates) and correlate with yield maintenance. However, these tests do not accurately quantify the total portion of soil P that may be accessible to plants and microbes (Dodd & Sharpley 2015). Bray-1, Mehlich-3, and Olsen are among the most commonly used chemical soil extractions for determination of soil P and have been used to identify a nonlinear (logarithmic) response of maize and soy yields to P availability (Dodd & Mallarino 2005). Additionally, $CaCl_2$ and Morgan's P tests are used to inform P fertilizer application recommendations on soils in the Northeastern United States (Kleinemann et al. 2000). All of these tests are single extractions, where a set of chemical extractants are shaken once with a soil sample for 5-10 minutes. The measurements are generally interpreted by farmers against standard screening recommendations for their soil type and the crop they seek to grow. In the Western Lake Erie Basin, Mehlich-3 soil P results are placed in “buildup-maintenance-drawdown” categories in the Tri-State Fertilizer Recommendations (Culman et al 2020). These thresholds are based on 95% yield optimum, assuming only P limitation, and tend to be higher than observed critical requirements in field trials (Dodd & Mallarino 2005, Rutan & Steinke 2021). While most of these tests are correlated with annual yield, much yield variability is unexplained by these test results. Furthermore, the most commonly used soil P tests do not provide information on the full suite of ecologically-relevant pools of P required to pursue a more sustainable management strategy.

By contrast, sequential extraction protocols allow for the differentiation of ecologically relevant pools of P. One of the most widely used sequential extraction tests was initially developed by Hedley (1982) and modified by Tiessen & Moir (1993) to address this challenge. It consists of a series of extractions with increasing P extraction capacity mimicking ecologically relevant extracting agents. The P extracted in each fraction can then be separated into organic and inorganic pools. P is first extracted using DI water and an anion exchange membrane (or resin) to assess P that is readily available to plants. 0.5 M sodium bicarbonate is then used to mimic plant rhizospheric conditions and extracts organic and inorganic constituents.Together, these fractions - resin and bicarbonate - are representative of short-term plant-available P. 0.1 M sodium hydroxide is then used to extract “intermediate” P; typically found in the iron, manganese and aluminium hydroxides or secondary minerals as well as more recalcitrant organic material. Some portion of this fraction can be accessed via microbial activity. Secondary P is abundant in highly weathered soils but can also form as a result of accumulation with agricultural inputs and in some cases may increase in the form of legacy P (Zhang et al. 2004). This fraction can also be separated into organic and inorganic constituents. Finally, extraction with 1M HCl is used to assess the primary mineral P, representing calcium-bonded phosphate in

the form of apatite minerals present in the parent material or calcium carbonate complexes. This fraction is thought to be determined by parent material and geologic weathering rates (Levy & Schlesinger 1999, Cross & Schlesinger 1995, Yang & Post 2011).

In addition to soil type and mineralogy, management factors also influence P distribution in soils. Fertilizer type, input rates, and inter-season balances play a role in total P and in fractionation between pools. Zhang & Mackenzie (1997) examined the different impacts of high and low manure and inorganic fertilizer additions in a 4-year continuous maize system, finding increases in sodium bicarbonate and NaOH with high P inputs and generally larger increases to the bicarbonate fractions when soluble fertilizers were used. In an 18-year subtropical system, Beck & Sanchez (1994) considered the impact of any fertilization and found that NaOH Po had a particularly important impact on maintaining Pi levels, as did the generally recalcitrant HCl and residual pools. Nitrogen fertilization generally increases P transformation from primary to secondary forms, possibly as a result of increased acidification or increased production of organic material (Yu et al. 2024). In low-fertility soils, like those studied by Crespo et al. (2024) and Chen et al (2023), cover crops increase Po across fractions - but in high fertility or high-input soils (like the heavily-manured soils in Takeda et al.(2009)), the P cycling impacts of cover crops were minimal compared to the high soil levels of P.

Traditional nutrient management has focused on increasing immediately-available Pi exclusively to ensure yields without taking into account the diversity of biological mechanisms that can access P across a gradient of accessibility in soil. By contrast, an ecological approach to soil P management would aim to reduce the size of soluble Pi pools while increasing crop access to Po and sparingly soluble P pools (Oberson et al. 1993, Drinkwater & Snapp 2023). A variety of techniques are available to pursue an ecological management strategy. For instance, increasing crop functional diversity by cover cropping, rotational diversification, and perennialization can influence P cycling and retention across different pools and soil types with a reduced need for external inputs. Increased plant functional diversity has been shown to increase P uptake and commensurately increase aboveground biomass in grasslands (Oelman et al. 2011) and in pot experiments (Karanika et al. 2007). In a comparison between barley and barley-forage rotation with inorganic or organic fertilizers over a 3 yr experiment, Zheng et al (2002) found that the $NaHCO_3$ & NaOH both were influenced by rotation and by input type, with significant differences based on fertilizer type across fractions and a drawdown of bicarb Po in monoculture plots. Two research projects conducted in sandy soils at the Kellogg Biological Station in Michigan found that tillage had variable impacts on P distributions depending on soil type (Daroub et al. 2000) and that pasture plots containing alfalfa "mined" residual P and increased organic NaOH P throughout the growing season and the pools supplying resin P are different in agricultural and natural (forest) systems (Daroub et al. 2001). Liao et al. (2021) found differences in which pools were depleted by monoculture faba bean, monoculture maize, and an intercrop of the two on a calcareous site, with simultaneous drawdown of some pools and accumulations in more recalcitrant pools depending on fertilization regime. Much research has looked at the capacity of the legume functional group to better access P than other functional groups by carboxylate production, root morphology, and PSM relationships as Dai et al. (2025) demonstrate, and

greater inclusion of legumes provides a system-level opportunity to reduce P consumption and improve food security (Stagnari et al. 2017, Meena & Lai 2018).

To reduce nutrient losses while maintaining agricultural productivity, there is a critical need to understand how soil type, current management practices, and management history interact to influence soil P availability. To address this knowledge gap, we collected soils from a wide range of management systems and soil types across working farms in Michigan's portion of the Western Lake Erie Basin. We then applied the Hedley sequential extraction protocol to explore how soil inorganic P is impacted by a set of diversification practices, including cover cropping, the introduction of harvested perennial plants, and crop diversity overall. The distribution of inorganic P is often mediated by geochemical factors, which we explored by sampling a wide range of soil types. However, short-term biological processes (which are influenced by diversification practices) can also influence P distribution because biological processes regulate the movement of labile P forms in soil. In addition to diversified crop rotations, we also assessed the effects of practices such as tillage and fertilization that have been more commonly studied in agricultural experiments. Additionally, we used paired forest plots adjacent to some of the farm sites to determine the impact of agriculture relative to a soil type-matched unmanaged soil.

Methods

2.1 Site Description

Soil P was evaluated across 50 agricultural field sites on working farms and 11 paired forest sites in Lenawee, Hillsdale, Monroe and Washtenaw counties in the Western Lake Erie Basin (WLEB). The study area has a mean annual temperature of 9.5C and mean annual total precipitation of 94 cm. Temperatures drop below freezing (0C) for 130 days on average, or over 4 months of the year. Soil types vary, with a range of textural types present including mostly sand, loam, and heavy clays (GLISA 2026).

2.2 Management Histories and Survey Data

Farmers were recruited for this project through a variety of sources in the summer of 2024 with the support of partners in MSU Extension and the local Conservation Districts. A gradient of agricultural management practices was sought and a total of 50 farm fields were enrolled in the study period. Of these 11 sites had paired deciduous forest plots which could be used to estimate background levels of C, N, and P. Management practices were fully characterized by interviewing farmer participants. Detailed 5-year management histories were collected for each field, including information on crop rotation (including any use of cover crops), crop yield, nutrient inputs, and other practices such as tillage.

2.3 Soil Sampling

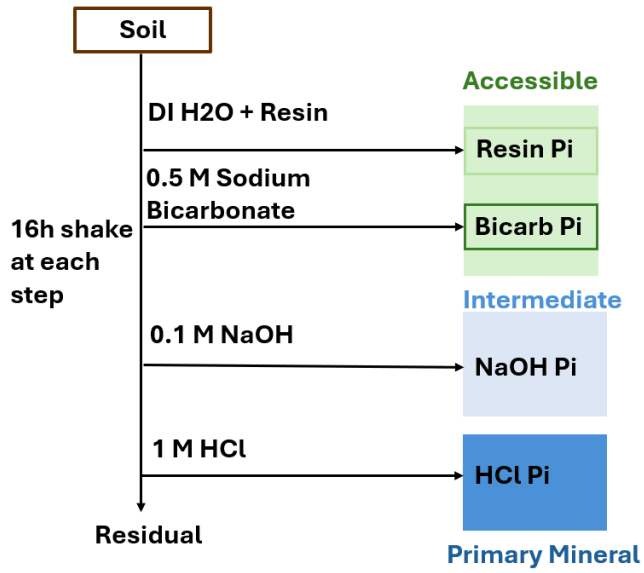
In fall of 2024, from mid-October through early December, soils were sampled to 20 cm depth after crop harvest. Due to the diversity in management strategies and farm types, fields varied widely in size (from 10-162 acres, with an average field size of 50 acres) . We collected 20 cores (XX diameter) from random locations within the dominant soil type in each field, and cores were homogenized while at site into one representative soil sample. Bulk density was assessed by taking two large-diameter cores to 20-cm from each field.

Soils were analyzed for a suite of chemical, physical and biological properties. Soil was sent to A&L Great Lakes Laboratories for analysis of soil macro- and micro-nutrients, pH, soil texture, and the conventional phosphorus tests Bray-1 and Mehlich-III. As part of a broader project, the research team also measured other biological and physical indicators of soil health including potentially mineralizable N and C, permanganate oxidizable C, particulate and mineral-associated organic matter fractions, microbial biomass C and N, wet aggregate stability and available water capacity.

Samples were air-dried and ball-ground to (2 mm size) prior to P extractions. Soil P fraction distribution was determined using the Tiessen & Moir (1993) modification of Hedley et al. (1982), following the first four extraction steps indicated in Tiessen & Moir to obtain the most biologically active fractions. Soil samples were processed in triplicate, with three separate samples from each field run through a complete extraction process. The extraction series begins with 0.5g of sample, which is transferred into a falcon tube. Two bicarbonate pre-charged anion-exchange membrane (AEM) resins (total surface area ~2000 cm2) were added to the tube along with 30 mL DI water. The tube was capped, turned on its side, and shaken vigorously

on a linear shaker for 16 hours. Resins were removed, rinsing sediment back into the original tube, and placed in a new tube along with 20 mL of 0.5 M HCl. The resin tube was shaken overnight to allow phosphorus to discharge from the resins before being decanted into a labelled scintillation vial which is immediately capped and frozen. The tube containing sediment was centrifuged at 0C and 2000 RPM for 15 minutes, then the DI water was filtered through a Whatman 40mm filter before being discarded. Any sediment on the filter was rinsed back into the tube using a pre-measured 30 mL volume of 0.5 M sodium bicarbonate which was added in its entirety to the falcon tube containing sediment. The falcon tube was then returned to the shaker for another 16 hour shake and centrifuged at 0C and 2000 RPM for 15 minutes. 0.5 M sodium bicarbonate was vacuum filtered through a Whatman 40mm filter into a sidearm flask, then decanted into a labelled scintillation vial which was immediately capped and frozen. The same steps were repeated for a 30 mL volume solution of 0.1 M sodium hydroxide, and a 30 mL volume of 1 M hydrochloric acid. All samples were frozen immediately at -20C and only thawed in advance of pH adjustment for colorimetry.

Fig 2.1 Visual Summary of Extraction Steps



Phosphorus samples were first treated by the colorimetry pH-adjustment steps described in Hedley et al (1982). In brief, for the alkaline extractions (NaOH and NaHCO3) organic matter is precipitated out of solution by acidification with dilute sulfuric acid and centrifugation. For the resulting clear extracts - and for the acid extractions directly - 4 para-nitrophenol is used as a color-changing indicator to titrate extracts to a pH of 3. Prior to pH adjustment, extracts are diluted using the extractant at consistent ratios (⅕ for HCl, ⅖ for NaOH, ⅗ for bicarbonate and resin) to bring resulting concentrations within range for the color developing agent.

Then malachite colorimetry was performed, following a protocol originally developed for river water (Motomizu et al. 1983) and later validated for soil extracts (Ohno & Zibilske 1991, D'Angelo & Vandiviere 2001). This protocol uses two sequential color developing agents; an

ammonium molybdate solution is added to extracts once they are in plate wells to form the spectrally active compound, then malachite carbinol is added after a 20 minute delay to stabilize the reaction before absorbance is read after another 20 minutes. This method has been compared with the molybdate blue method originally used in the Hedley protocol (Rahutomo et al. 2019). It is more accurate with low concentrations of phosphorus and as such samples were diluted to fall below 1000 ug/L before being run in a spectrophotometer at 630 nm on a 96-well plate. Each soil extract was dispensed into three wells to provide replication. Only Pi was produced as a result of this colorimetric determination process.

2.4 Data Cleaning

Reflectance readings from each cell from spectrophotometry were calibrated using an extract and pH matched standard curve, averaged across replicates, and then were corrected for pH adjustment additions to obtain concentration in ug/g soil for each extraction. Concentrations were averaged across replicate extractions, producing a mean assigned to a given field site. Where the coefficient of variation (CV) between replicate extractions was greater than 15, the median replicate concentration was assigned to that field site.

2.5 Statistical Analyses

Intratest correlations were assessed using cor() in base R (R Core Team, 2025), and visualized using the corrplot() R package (Simko 2024). The distribution of P concentrations for each of the measured fractions was not normally distributed, so a base 10 log-transform was applied to achieve normality prior to statistical analysis. Linear Mixed Models were analyzed using R (R Core Team, 2025). Linear mixed models were applied to the combined dataset using lmerTest() R package (Kunetsova et al. 2017). Models were generally structured as y ~ x + clay + (1|farm), where y was the transformed P fraction, x was the treatment of interest, clay percentage was a fixed effect and farm was a random effect. Models were run separately for each inorganic fraction and for Bray and Mehlich tests.

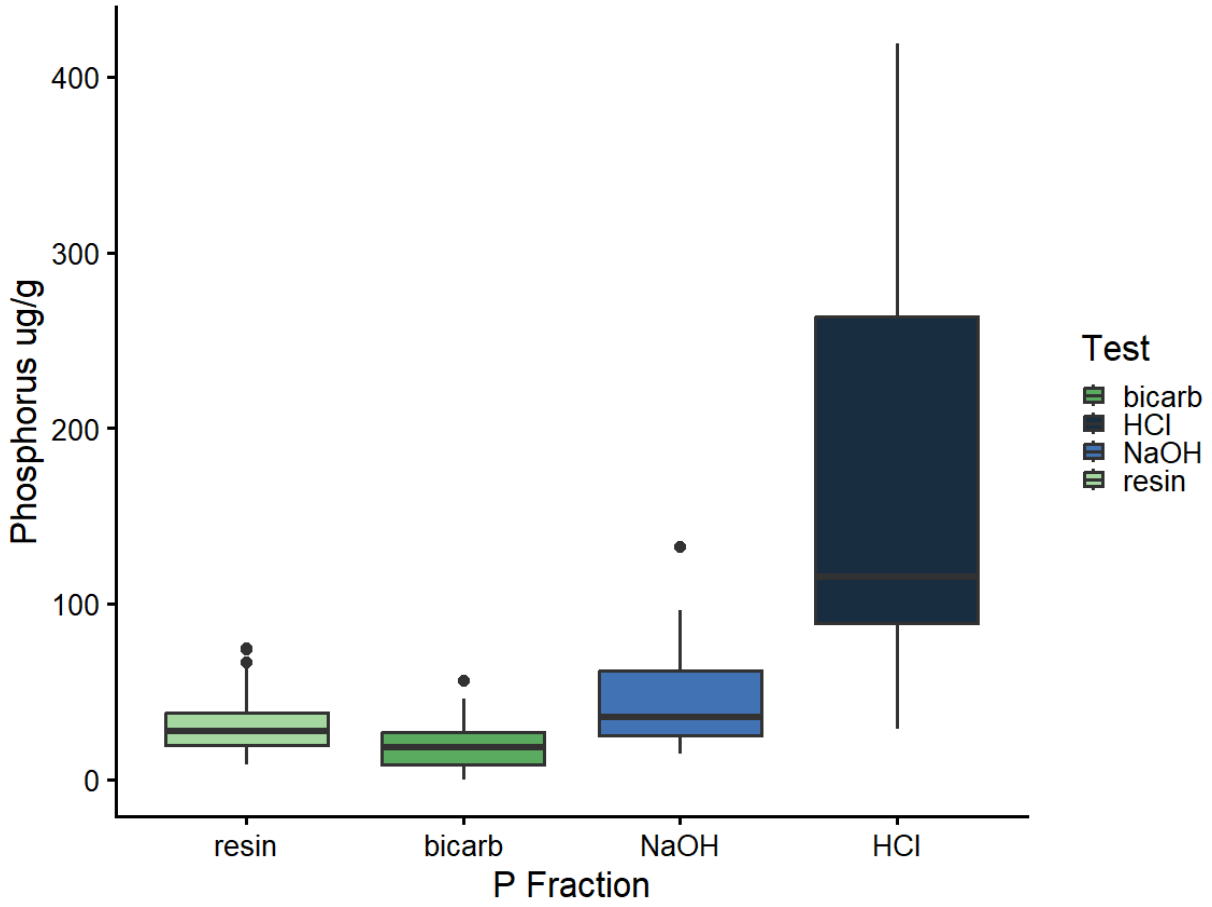
To assess tillage impact, a tillage rating variable incorporating tillage depth and frequency was used as the treatment. Three fertilizer treatments were used separately - P fertilizer lb/ac, P balance kg/ha, and N balance kg/ha - to examine the effect of inputs, p inputs relative to crop harvest, and N inputs relative to crop harvest. Crop diversity was considered by using a species count as treatment where each unique species grown was counted. For cover crop impacts, years of cover crop use (T_c) was used as the treatment, and the field sites were restricted to include only non-perennial growers using cover crops for 0 years or using cover crops for 3 or more years. Within cover crop users, functional group impacts of legumes were assessed by restricting the entire dataset to fields where cover crops were used and applying years a legume was grown as cover (sum_legume_cover) as treatment. Lastly, the impact of harvested perennial cultivation was assessed using the years a perennial was grown (T_p) as the treatment variable, again restricting to non-perennial growers (0 years) or persistent perennial growers (3+ years).

For forest soil comparisons, a two-sided t test (t.test() in R) was used to establish significance of difference at a threshold of $p < 0.05$.

Results

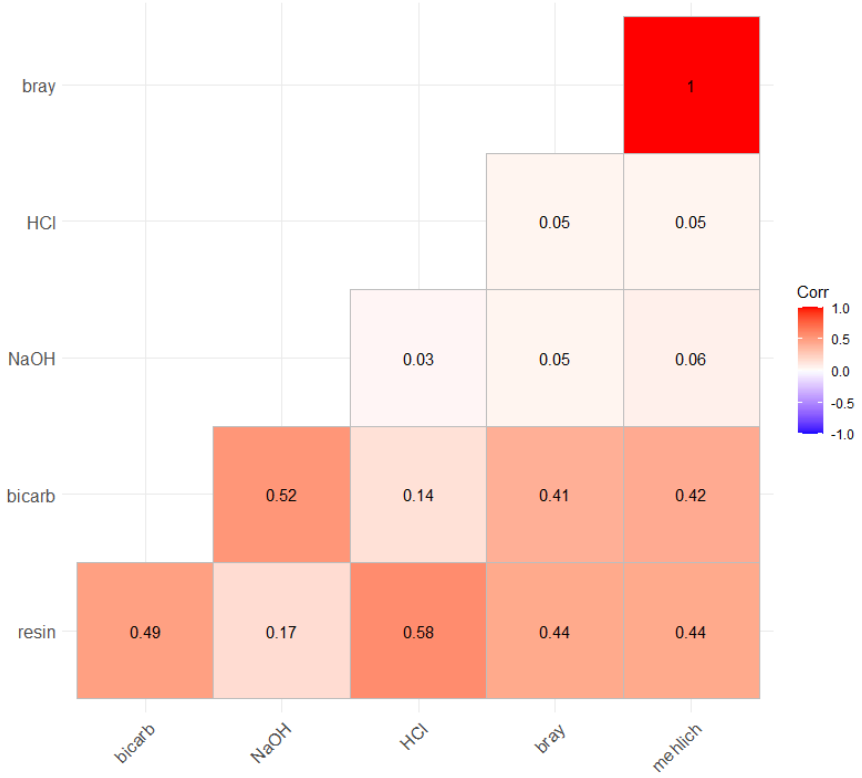
Among soils in this study, the bicarbonate extractable P had the smallest mean concentration at 18.9 ug/g, followed by resin exchangeable P at 31.9 ug/g, NaOH (45.9 ug/g) and HCl (170.9 ug/g). The HCl had the largest range of all tests with a range of 390.4 ug/g among all soils. The inorganic bicarbonate extractable P was the only fraction with undetectable P concentration among all sites and all fractions. (See appendix for tabulated aggregate distribution and summary data.)

Fig. 3.1 Mean Pi concentration (y-axis) for sequential soil fraction (x-axis) from most labile (resin) to least (HCl).



For this dataset, Bray-1 and Mehlich-III tests were linearly correlated with a coefficient of correlation of 1. This was the strongest correlation between test types. The non-alkaline extractions (resin and HCl) were also strongly correlated (r=0.58), as were the two base extractions (bicarbonate and NaOH extracts, r=0.52). The most plant-available (or most labile) extracts followed at a coefficient of 0.49, and the correlation between the most plant-available sequential extractions and the conventional soil tests followed with coefficients of 0.44 for conventional tests and resin and 0.42 for conventional tests and bicarbonate.

Fig. 3.2 A correlation matrix comparison between Hedley sequential extractions and conventional soil P tests (Bray-1 and Mehlich-III).



Following initial data exploration Pi concentrations (ug Pi/g soil) were transformed using log-10 to meet assumptions for normality and variance distribution prior to application of linear mixed models. Using linear mixed models to explain P concentration from sequential Hedley extraction series, the model using the tillage rating did not show a significant effect.

The effects of inorganic P fertilizer inputs in pounds per acre was marginally significant, and P inputs in manure were not significant. Phosphorus balance - or the sum of inputs minus exports via harvest - had a small but statistically significant negative effect on HCl-Pi. The estimate of impact of inorganic P fertilizer and P balance was the same. Nitrogen balance produced no significant effects. Crop diversity - or the number of unique species in the previous 5-year crop rotation, including harvested crops and cover crop species - was associated with a statistically significant increase in the NaOH-Pi fraction. There was no significant effect of cover cropping on the Pi fractions, even when comparing longer term cover crop use (3+ years) to no cover crops. However, when analyzing the subset of fields with cover crops (1+ years of cover crop use in the previous 5 years), the inclusion of legume cover crops had a statistically significant and positive effect on the resin-Pi and NaOH-Pi fractions. Fields planted to harvested perennials (i.e., primarily alfalfa) were correlated with a marginally significant decrease in the most labile forms of inorganic P - both resin and bicarbonate.

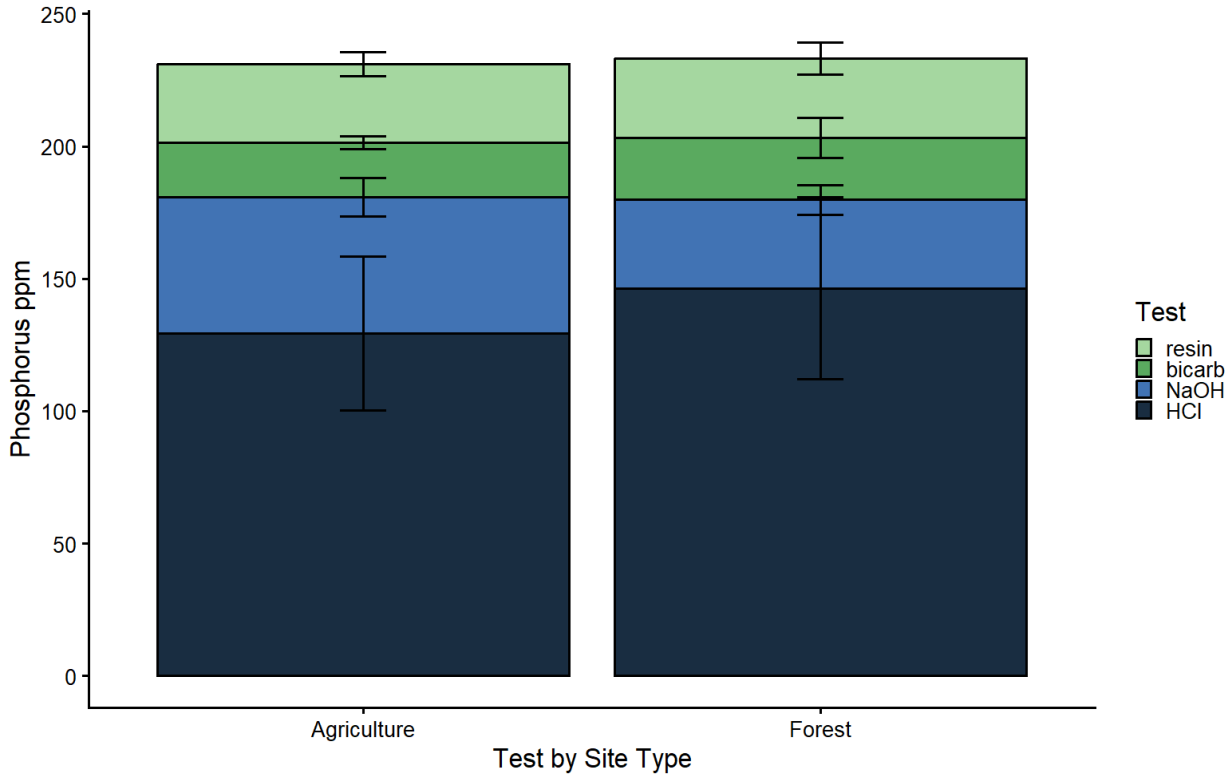
Table 3.1. Linear Mixed Model effects using fertilizer inputs contrasted with P balance, analyzing annual crop fields (excluding harvested perennials), effects of crop diversity, analyzing annual crop fields (excluding harvested perennials), effects of legume cover crops, analyzing only fields with cover crops grown at least once during the management history, and effects of harvested perennials, comparing fields with harvested perennials grown for 3+ years to fields with no perennials. Significance codes: p < 0 '***', < 0.001 '**', < 0.01 '*', < 0.05 '.'

	resin		bicarb		NaOH		HCl	
Intercept	1.395	***	1.112		1.625	***	1.790	***
clay	0.004		0.005		-0.001		0.016	***
Pi Fertilizer	0.000		-0.003		0.000		-0.002	.
Intercept	1.353	***	0.878		1.605	***	1.597	***
clay	0.004		0.006		0.000		0.017	***
P Balance	0.000		0.000		0.000		-0.002	**
Intercept	1.332	***	0.891		1.717	***	1.624	***
clay	0.002		0.001		-0.008	.	0.015	*
Legume Cover	0.174	*	0.362		0.211	**	0.083	
Intercept	1.338	***	0.856		1.547	***	1.678	***
clay	0.003		0.003		-0.003		0.017	***
Crop Diversity	0.013		0.024		0.023	*	-0.004	
Intercept	1.342	***	0.963		1.606	***	1.657	***
clay	0.005		0.004		-0.001		0.016	***
Harvested Perennial	-0.052	.	-0.160	.	0.008		0.010	

The above models were all run on Mehlich-III and Bray-1 tests as well. There was no statistically significant effect on either test for P or N fertilizers or balances, crop diversity, RCI, or harvested perennials. The incorporation of legumes versus only non-legumes for cover cropping had a marginally significant effect for Mehlich only. The tillage rating factor was significant for both tests with a small positive effect. Consistent cover cropping (3+ years versus 0) produced a significant, small negative effect on both tests.

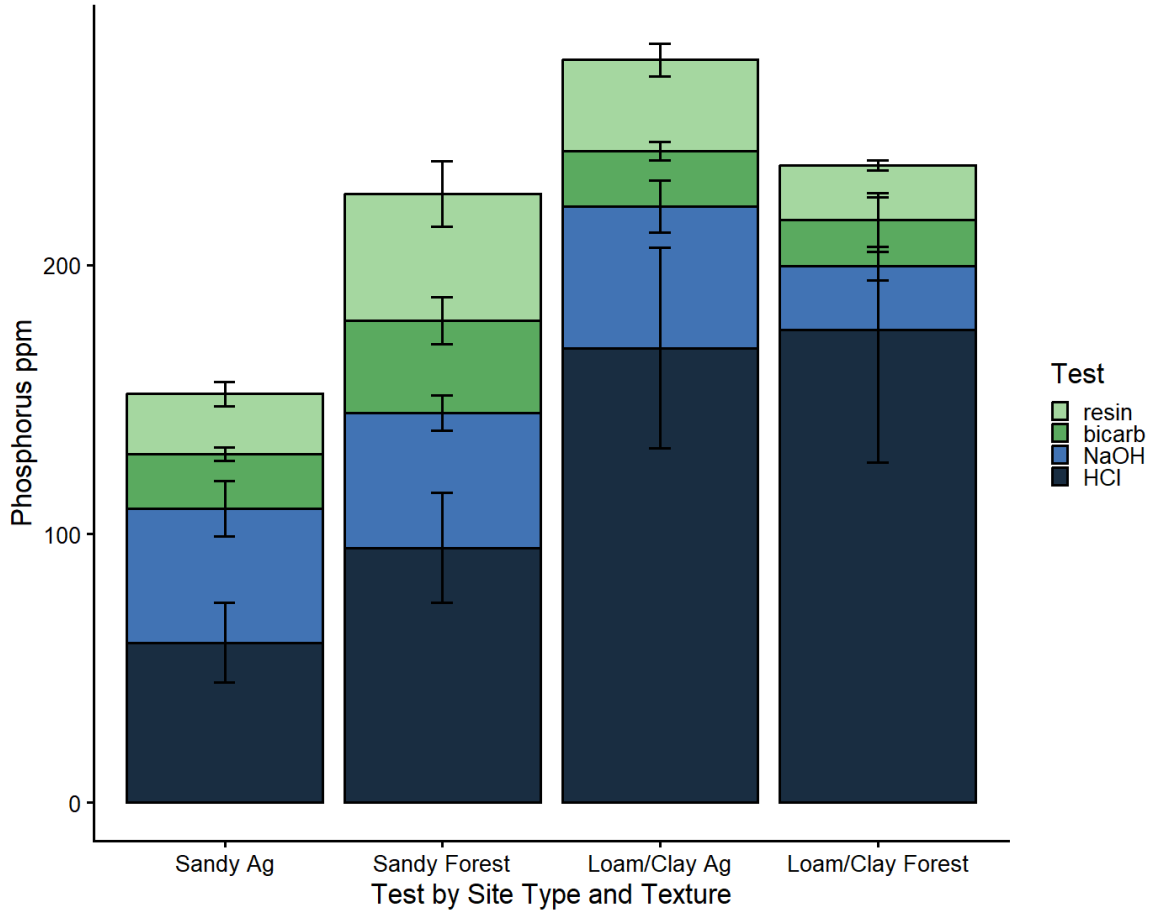
Across the paired subset, there was substantial heterogeneity in the relationships between forest plots and their agricultural pairs. The NaOH-Pi fraction was significantly larger (18.3 ug/g larger, on average, p = 0.046) for agricultural sites than forest sites. When analyzed as a proportion of total Pi (i.e., to account for differences in the total quantity of Pi between sites), there was no statistically significant difference in any Pi fraction between agricultural and forest sites.

Fig. 3.3 A comparison of average sequential extraction distributions between forest sites and their paired agricultural sites. 22 total sites are included, 11 of each type.



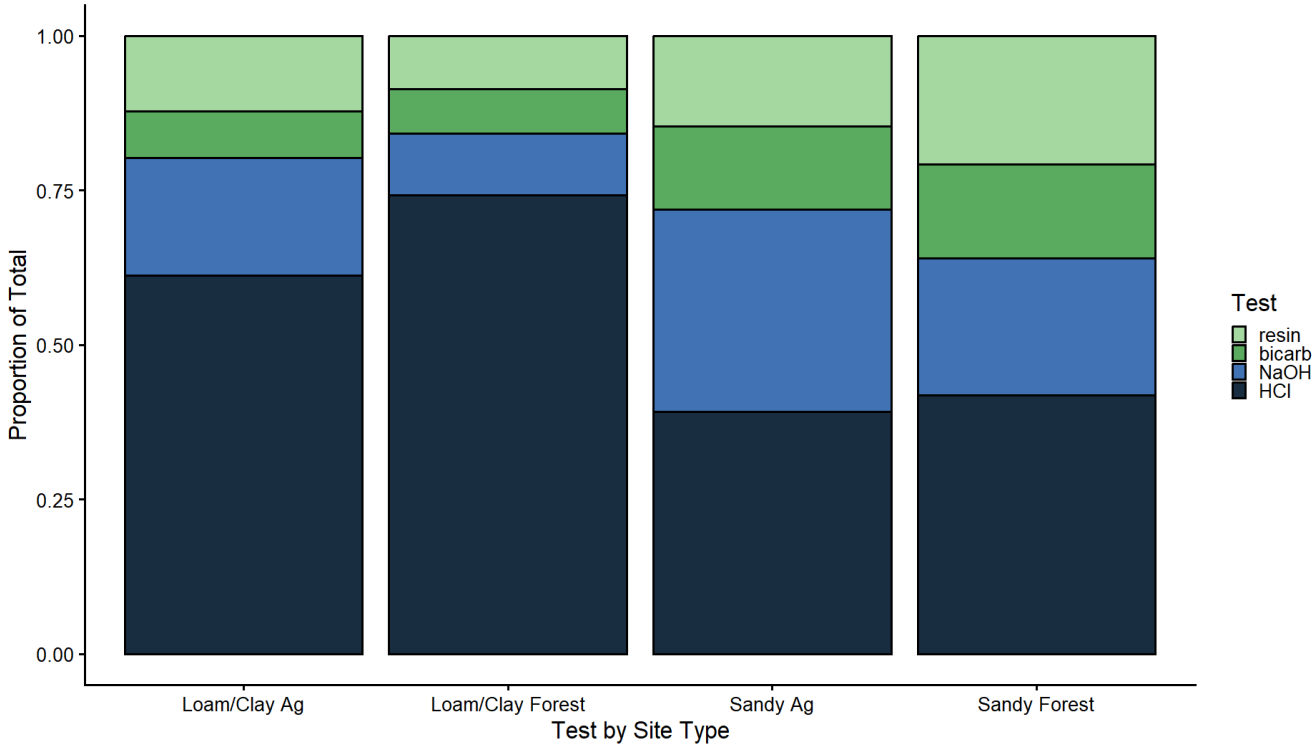
A sub-comparison of forest sites was performed between those with sandy soils and clay or loam soils. Soils were considered sandy if both soils had sand percentages above the aggregate median of 49.5%, a criteria which 8 sites (4/11 pairs) met. On sandy soils, no statistically significant differences existed between Pi fractions in forest and agricultural sites. On clay/loam soils, resin-Pi and NaOH-Pi fractions were significantly greater on agricultural sites than forest sites (13.5 ppm average difference, $p = 0.042$ for resin and 28.9 ppm average difference, $p = 0.029$ for NaOH).

Fig. 3.4 A comparison of average sequential extraction distributions between forest sites and their paired agricultural sites broken into categories by soil texture.



Proportional distribution was also broken out by texture. No statistically significant difference in proportion analyzed between agriculture and forest sites existed for either texture set.

Fig. 3.5 A proportional comparison of average sequential extraction distributions between forest sites and their paired agricultural sites broken into categories by soil texture.



Discussion

The overall Pi distribution across the working farm sites in the WLEB was comparable to the distributions found in global datasets of P, such as the one referenced in Nakayama et al. (2021). In comparisons between tests, Bray and Mehlich - the regionally common commercial tests for orthophosphates - were found to be directly correlated and also generally smaller than the sum of the most labile Pi from the sequential extraction (resin + bicarb). This is typical of the literature, especially on high-P soils (Ketterings & Flock 2005, Culman et al 2020, Gutierrez Boem et al 2011). Of the Hedley sequential extraction fractions, the Bray and Mehlich soil tests were most closely correlated with the inorganic P in resin and bicarbonate fractions, indicating that these traditional tests are related to the most plant-accessible P.The lack of a strong correlation between the Hedley fractions and traditional P tests may be due to missing P from organic pools, especially in the bicarbonate pool, which was not characterized for this analysis. The low correlation between these tests and the NaOH fraction makes sense because these tests do not fully capture microbially accessible P or P bound to the surfaces of sesquioxides. Given the role of P-solubilizing microbes in plant P acquisition (Tian et al 2021), especially on farms with greater levels of crop rotation complexity, and the large influence of legacy P in nutrient pollution to waterways (Kleinman et al 2011), it is likely that these tests are inadequate for informing reduction of P surpluses on farms. As expected, the impact of texture and parent material was high and especially evident in the HCl-Pi fraction of this sample. Clay was the strongest correlated of the textural classes, probably due to the ability of charged clay complexes to retain orthophosphate directly and increased organic matter retention which also provides phosphate binding sites.

While statistically significant, the model estimate of soluble P fertilizer inputs and P balance (i.e., the difference between P inputs and P removed in harvested crops) on Pi in any fractions was very small. This was counter to what has been observed in the other work (Zhang & Mackenzie 1997, Yu et al. 2024) where P inputs were the primary management predictor of total P and P allocation. One possible reason for this discrepancy is that, on average, farmers in our sample had slightly negative 5-year P balances, in contrast to much of the previous work on fertilization where P inputs were higher. There is general awareness of the high legacy P levels in agricultural soils in the WLEB, and it is likely that farmers are scaling their inputs accordingly. This would align with the review results from Negassa & Leinweber (2009), where P inputs 5 times greater than agronomic recommendations were needed to produce a significant effect on P distribution in soil. Manure in particular is correlated with changes to the bicarbonate/NaOH Po pools, which were not assessed in this research, and it is possible that more significant effects of P fertilization or manure addition would be apparent in the organic P pools. In a meta-analysis that analyzed N and P inputs in temperate systems for studies lasting 5-10 years, N inputs led to a small increase in NaOH-P in the top 10cm of soil, and decreases in labile P and Ca-bound P (Yu et al 2024). The effects of N in this study were only significant at high levels of N input (>200 kg N/ha/yr), which is higher than our average at 76.2kg N/ha/yr, so it is possible that the application rate in our sample was insufficient to cause the effect. This meta-analysis did not disaggregate organic and inorganic P pools, so it is also possible that N inputs impact primarily the organic pool by increasing primary production, which would not be

captured by our analysis. In research on low-P soils, N fertilization was found to increase the uptake of soil P in grass-pasture systems primarily increasing plant enzyme and biomass production (Touhami et al. 2021), which in a P-sufficient soil would probably present as accumulations to Po.

By mixing deeper soil layers with surface layers, tillage could bring more primary P in contact with dense rooting zones and increase primary P weathering rates. It also breaks apart fungal hyphae, which could decrease biological P-solubilization rates. In this analysis tillage had no impact on any Pi fractions in our model, which aligns with the mixed results in prior studies. For instance, Daroub et al (2000) did not find any impact of tillage on Pi or Po fractionation, and neither did O'Halloran (1993), in contrast to earlier research finding higher available P in surface layers with tillage (Karlen et al. 1991). Many of the earlier studies testing tillage effects on soil P, such as Tracy et al (1990) and Weil et al (1988), analyzed only the top 2.5cm of the soil profile, while our analysis went to 20-cm depth. Our sample could have been too deep to capture possible shifts in location of soil P identified in this earlier research. Crop plants tend to root more deeply than the top 2.5cm, with Fan et al (2016) estimating an average mean of root depth distribution for 14 different crops to 15cm, which limits the relevance of those prior assessments to plant growth and the influence of crops on internal P cycling and distribution in soil.

By increasing a diversity of root architecture and microbial partners, increases in plant diversity would be expected to increase total root scavenging and P-solubulizing microbe activity, which would decrease resin, bicarb, and NaOH Pi. Practices which leave biomass on the farm (like cover cropping) would be expected to increase Po without changing Pi in any forms, although it would be anticipated to increase P cycling in general. Simultaneously, increases in root presence throughout the season (i.e. including during the winter and edges of the growing season) would be expected to reduce the availability of Pi in resin and possibly bicarb as easily-scavenged Pi is incorporated quickly into plants. Cover cropping (i.e., the use of non-harvested crops in rotation with cash crops) did not significantly impact any of the inorganic P pools in this analysis. Other studies testing the effects of cover crops on soil P report conflicting results, and the lack of significant results in our sample is in line with more closely geographically tied results. Daroub et al. 2001 in southwest Michigan found similar trends where a winter cover crop compared with winter fallow did not significantly impact any P fractions, inorganic or organic, although they found a trend towards increased Po at some of the cover cropped plots. Takeda et al. 2009 examined *Brassica Napus* and rye cover crops and didn't find a significant difference in fractions after 3 years of cover cropping; however cover cropped fields started with lower resin and NaOH Pi, so in practice the cover cropping did correspond with a slight accumulation of Pi in those fractions. In that dataset results were also masked by larger changes induced by compost addition, so it is possible that historical fertility or background soil effects also reduced the statistical significance of changes. Kuo et al. 2004 used a modified extraction protocol that included bicarbonate Pi and NaOH Pi fractions. They found increases in Pi in those pools, specifically NaOH Pi with a rye cover crop and bicarbonate Pi with annual ryegrass and cereal rye as well as hairy vetch. Cereal rye was the most common cover crop in the fields we sampled, so it is noteworthy that this crop can differentially influence Pi in different contexts.

Our index of crop diversity, which was simply species richness over the 5-year management history, was positively correlated with NaOH Pi. This pool is thought to be an intermediately available source of P, and is the pool which has been documented in other research as being drawn down under continuous maize growth in particular (Li et al. 2023). In addition to decreasing under maize monoculture, Liao et al.(2021) found increases to the NaOH Pi pool when faba bean was grown either as an intercrop or monocrop. This could represent a difference in functional group response because many legume species can increase NaOH Pi. This would align with results from pot experiments conducted by Sugihara et al. (2015), where cow pea and pigeon pea both increased allocation to NaOH Pi in surrounding bulk soil in clay and sandy soils, and from work in Brazil by Pavinato et al (2008) that identified radish and soybean as the two crops (among a subset of primarily grain crops) to increase P availability in more labile fractions.

At our field sites, all growers incorporated a legume cash crop in rotation - soy for annual fields or alfalfa for harvested perennial fields - so it was not possible to isolate the effect of having legume crops in rotation in this study. However, we could assess the inclusion of legume cover crops, which were present in 14 of 31 cover cropping fields in our sample. Since they form rhizobial partnerships for N acquisition and are therefore less N limited than other plants, the legume functional group has a wide range of adaptations to reduce their P limitation. Legumes produce unique combinations of organic acids, notably high citric and malic acids which increase P desorption from sesquioxides (Dai et al 2025, Hinsinger 2001). In this analysis, functional diversity did impact differences in Pi allocation. We found a large and significant increase in resin Pi and in NaOH Pi for the fields where legume species were included in the cover crop. The increase in resin Pi could be due to increased direct mobilization from other soil pools, either more recalcitrant inorganic pools or organic pools. Elevated NaOH Pi appears to be a consistent trait of increased legume presence in a rotation, and probably a more general result of elevated inorganic available P - increased soil solution Pi is bound into sesquioxides of Fe and Al, and legume production of organic acids would increase soil solution Pi.

Another strategy to increase cropping system diversity is to incorporate perennial plants. Perennials, could decrease the available Pi fractions (resin and bicarb) by maintaining a living root presence year-round, and when crops like alfalfa are included in rotation could mine intermediately available P to decrease legacy pools of P. In our dataset, perennials were primarily harvested perennials, with most fields containing alfalfa for at least a portion of the rotation. Many of these fields are harvested as forage for animal feed, and most of the aboveground biomass is harvested at least once per year. In our linear mixed model assessing the impacts of harvested perennials, we found that harvested perennials were associated with smaller resin and bicarbonate Pi fractions. This suggests a drawdown in plant-available Pi and contrasts with the results from Daroub et al. (2001), in which 7 years of continuous alfalfa cropping decreased the intermediately available NaOH Po but did not impact the Pi extractions included here. The sum of Pi in our perennial field sites is very similar on average (212 ppm) to Daroub et al. (221ppm), but since they do not report the P distribution it also possible that there was less secondary P to be scavenged in our field sites, as the fields in Daroub et al. were

transitioned from a relatively high input conventional farming system at the beginning of the trials which could have induced an accumulation to P pools prior to their experiment. pH could also play a role, as our perennial soils had an average pH of 6.2 which was 1-0.5 points more alkaline than the Daroub et al. sites. In this analysis, perennialization (harvested or unharvested) does correlate with a drawdown in resin and bicarb Pi, leaving less available for loss to runoff and leaching.

In the subset of 11 sites, we were able to directly compare agricultural fields to a paired forest plot on the same soil type. We found that a history of agricultural management was associated with a larger NaOH Pi pool than our forest pairs on average. However, the average results across sites mask a very high degree of variation in relationships between paired soils in both textural categories. A number of soils had significantly more total P in the agricultural field across some or all of the fractions, while a number had the opposite directionality - higher total P in the forest site. The separate texture analyses demonstrate that soil texture may make a difference in which pools may accumulate P or which fields may accumulate legacy P. The similarities in pools with higher P as a result of legume inclusion in the cover cropping with pools with higher P as a net result of agricultural practice provide a second indication that legume inclusion may be impacting soil P availability similarly to fertilizer applications. Further research is needed to understand what agricultural management characteristics impact P distribution, principally in its deviation from natural ecosystems and ideally with longer time frames of management records to better understand the mechanisms behind these differences.

Conclusions

This research used the Hedley sequential extraction protocol to assess the influence of a set of agricultural parameters (tillage, P inputs, N&P balance) and cropping practices (cover cropping, harvested perennials, legume inclusion, and species diversity) on inorganic phosphorus on working farms in an ecologically sensitive watershed. Soil texture was found to significantly correlate with Pi, especially HCl Pi. Conventional tests, while associated with the directly plant-available pools, do not adequately quantify Pi in microbially accessible pools to inform ecological nutrient management. Some practices, like tillage, N balance, and cover cropping, were not found to have a significant impact on any fraction although this does not exclude the possibility of impacts on Po. P fertilizer inputs and balance were correlated with HCl Pi, but less correlated with plant available Pi than expected. This could be due to the fact that most farmers in our sample had a negative P balance in their recent management history. Harvesting perennial plants (principally alfalfa with some mixed grasses) was found to draw down plant-available resin and bicarbonate Pi but did not mine NaOH Pi in this set. Increased species diversity and the inclusion of legumes in cover cropping practice increased NaOH Pi, and legume inclusion increased resin Pi as well. Analysis on paired forest-agriculture plots found that a large degree of heterogeneity existed in all Pi pools and in the size differences and directions between pairs. In general, agricultural practice increased the size of the NaOH Pi fraction. These findings demonstrate that in working farms in the basin, crop diversity, functional group selection, and P inputs all impact Pi fractions, presenting an opportunity for the

substitution of P fertilizers with increased functional diversity to maintain yields in the basin while drawing down legacy P.

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Appendices

Table 1

Summary of data for all field sites from all tests for phosphorus in ug/g soil. Resin, bicarb, NaOH, and HCl represent the sequential extraction results determined from laboratory extractions, and Hedley sum is the summed total Pi extracted from soils. Bray-1 and Mehlich-III results are from air-dried soil samples submitted to Great Lakes A & L Laboratories.

Extraction	Min	Median	Mean	Max
Resin	8.3	27.6	31.9	74.6
Bicarb	0.0	17.9	18.9	56.1
NaOH	14.7	35.4	45.9	132.5
HCl	28.7	115.3	170.9	419.1
Hedley Sum	72.0	230.3	267.6	597.4
Bray-1	4.0	22.5	26.4	88.0
Mehlich-3	7.0	30.5	36.1	114.0

Table 2

Distribution of soil properties measured across agricultural sites

Soil Properties - 50 sites (Agricultural Only)					
Variable	**min**	**med**	**mean**	**max**	**unit**
pH	5.4	6.5	6.4	6.9	
CEC	3.0	9.8	10.4	17.7	cmol (+)/kg
K	33.0	93.5	97.0	170.0	ppm
Mg	50.0	220.0	228.1	370.0	ppm
Ca	250.0	11250.0	1391.0	2750.0	ppm
K_sat	1.1	2.3	2.5	5.7	%
Mg_sat	11.6	18.3	18.6	25.7	%
Ca_sat	42.4	65.5	65.0	79.0	%
PWP	0.032	0.103	0.107	0.210	$g\ g\ soil^{-1}$
AWC	0.033	0.079	0.075	0.109	$g\ g\ soil^{-1}$
C	0.991	1.763	1.914	5.169	%
N	0.082	0.149	0.171	0.428	%
POXC	224.1	481.6	501.5	1120.9	$mg\ kg\ soil^{-1}$
PMN	1.720	5.940	6.283	22.060	$mg\ kg\ soil^{-1}\ week^{-1}$
MAON	657.6	1218.0	1374.1	3358.6	$mg\ kg\ soil^{-1}$
sand	18.0	45.0	47.0	89.0	%
silt	5.0	27.0	24.7	36.0	%
clay	4.0	28.5	28.3	50.0	%

Table 3

Distribution of key management factors for agricultural sites.

Management Properties - 50 sites (Agricultural Only)							
Variable	**min**	**med**	**mean**	**max**	**Y**	**N**	**Variable Description**
Any_CC					31	19	binary presence of cover crops
Any_Cash_perennials					42	8	binary presence of harvested legumes
sum_legume_cover	0.0	0.0	0.3	2.0			numeric sum of years a leguminous cover crop was grown
Any_cashcrop_legumes					50	0	binary presence of cashcrop legumes
sum_legume_cash	1.0	2.0	2.6	5.0			numeric sum of years a leguminous cash crop was grown
L	1.0	2.4	2.4	4.0			numeric length of years of crop rotation
Crop_diversity	1.0	3.0	5.4	19.0			numeric sum of different species grown in the past 5 years
T_c	0.0	1.0	1.9	5.0			numeric years a cover crop was grown
T_p	0.0	0.0	0.5	5.0			numeric years a harvested perennial was grown
RCI	1.4	3.3	3.7	8.9			Sqrt ((Number of SpeciesxRotation Length) + Years Cover Crop + Years Perennial)
Tillage_rating	0.0	5.0	4.6	13.0			Tillage rating according to Sprunger Lab (MSU) Tillage rubric and methods
sum_till_passes	0.0	3.0	3.9	15.0			numeric tillage passes in the past 5 years
Tillage_score_year	0.0	4.2	5.9	28.0			Tillage score according to Sprunger Lab (MSU) rubric divided by management history length in years
Till_YN					32	18	binary presence of tillage
All Fertilizer P lb/ac	0.0	57.7	61.4	156.8			numeric inorganic phosphorus applied to the field per acre over the past 5 years
P_balance_kg/ha	-144.7	-17.4	-24.0	123.9			phosphorus balance (inputs-exports) in kg per ha for the past 5 years
All Fertilizer N lb/ac	0.0	396.4	381.2	788.0			numeric inorganic nitrogen applied to the field per acre over the past 5 years
N_balance_kg/ha	-326.7	99.8	108.9	482.6			nitrogen balance (inputs-exports) in kg per ha for the past 5 years

Table 4

Linear Mixed Model - Tillage rating, cover crop, and legume cover crop results for Bray and Mehlich.

	Bray-1		Mehlich-III	
Intercept	1.621	***	1.762	***
clay	-0.013	**	-0.012	**
Tillage Rating	0.021	.	0.019	.
Intercept	1.932	***	2.038	***
clay	-0.016	***	-0.015	***
Cover Cropping	-0.051	.	-0.046	.
Intercept	1.665	***	1.804	***
clay	-0.017	*	-0.016	**
Legume Cover	0.194		0.178	.

Figure 1
Paired Site Forest Comparisons - Stacked

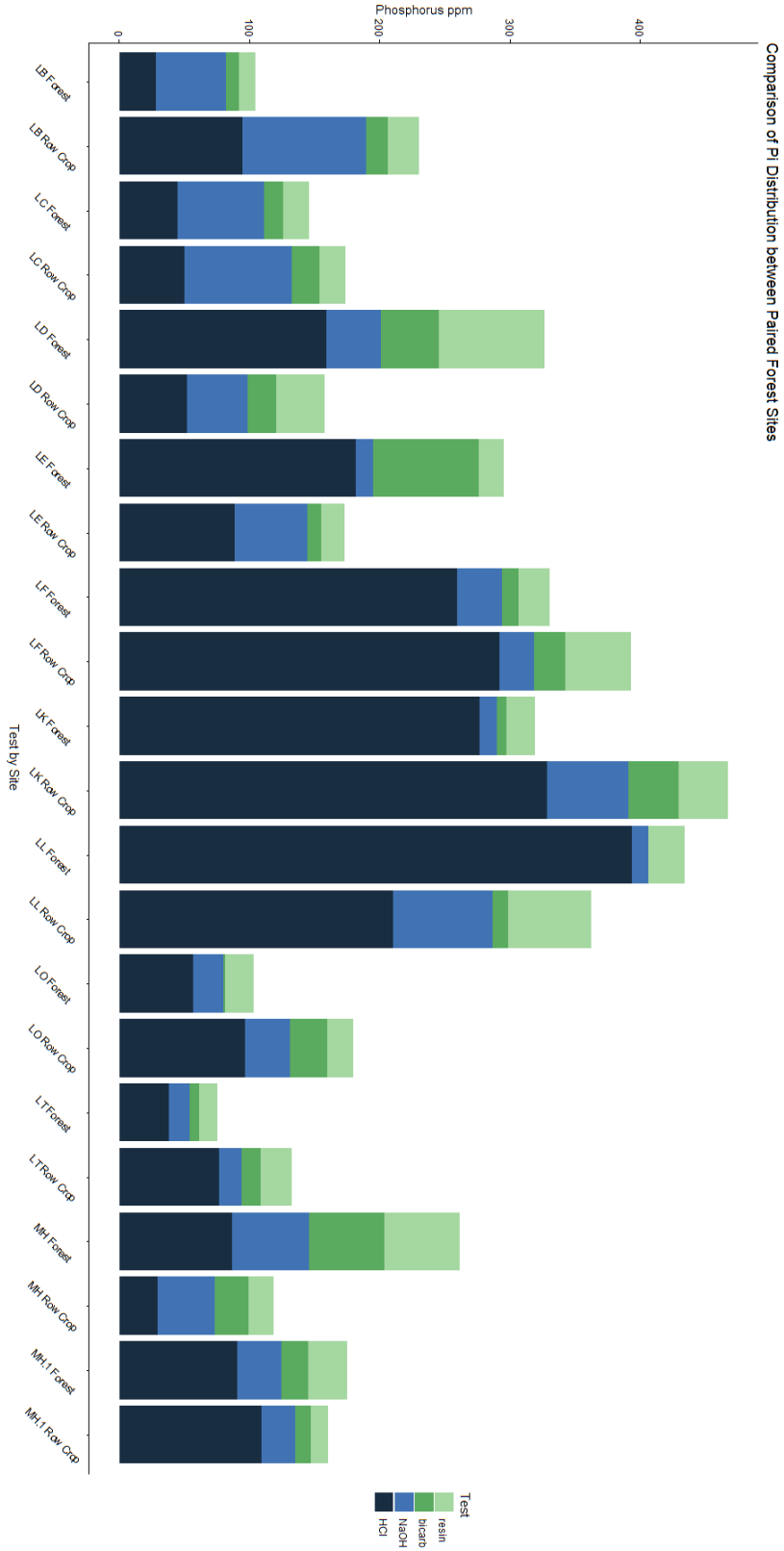


Figure 2

Paired Site Forest Comparisons - Proportional

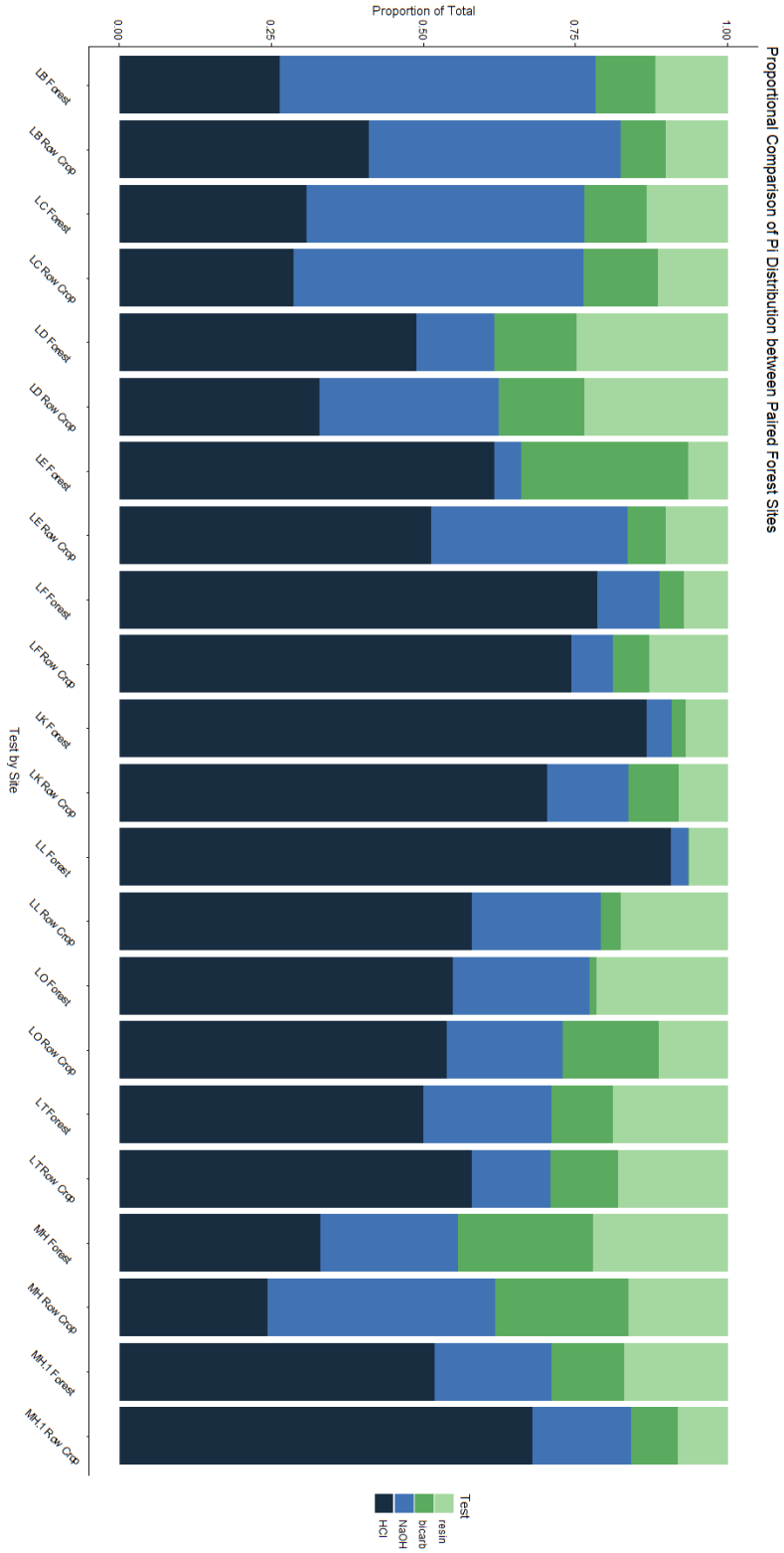


Table 5

Distribution of Pi sequential extraction results for Forest Paired Site Comparisons

All Pairs	Forests			Agricultural Sites		
Extraction	**min**	**mean**	**max**	**min**	**mean**	**max**
Resin	12.4	30.0	81.0	13.1	29.6	63.5
Bicarb	0.0	23.4	80.8	10.7	20.6	38.7
NaOH	12.6	33.3	66.2	17.1	51.6	95.0
HCl	27.4	146.4	393.0	28.7	129.3	328.3

Sandy	Forests			Agricultural Sites		
Extraction	**min**	**mean**	**max**	**min**	**mean**	**max**
Resin	19.5	47.0	81.0	13.1	22.3	37.0
Bicarb	14.8	34.5	58.0	12.4	20.4	25.8
NaOH	33.6	50.1	66.2	26.0	49.8	82.6
HCl	44.5	94.9	158.8	28.7	59.5	108.4

Clay	Forests			Agricultural Sites		
Extraction	**min**	**mean**	**max**	**min**	**mean**	**max**
Resin	12.4	20.3	27.9	17.6	33.8	63.5
Bicarb	0.0	17.1	80.8	10.7	20.7	38.7
NaOH	12.6	23.7	53.9	17.1	52.6	95.0
HCl	27.4	175.9	393.0	76.4	169.2	328.3

Table 6

Significance for Forest Comparison Paired t-tests, comparing Forests to Row Crops, including all site and texture-separated, and proportions. P-values less than a 0.05 significance threshold are bolded.

Dataset	**Test**	**t**	**p**
All-site	Resin	0.051	0.960
All-site	Bicarb	0.323	0.754
All-site	NaOH	-2.282	**0.046**
All-site	HCl	0.701	0.499
All-site	Hedley Sum	0.063	0.951
All-site	Resin Proportion	0.451	0.662
All-site	Bicarb Proportion	-0.048	0.962
All-site	NaOH Proportion	-1.463	0.174
All-site	HCl Proportion	1.104	0.296
Sand	Resin	2.415	0.095
Sand	Bicarb	1.678	0.192
Sand	NaOH	0.041	0.970
Sand	HCl	1.216	0.311
Sand	Hedley Sum	1.216	0.311
Sand	Resin Proportion	2.526	0.086
Sand	Bicarb Proportion	0.383	0.727
Sand	NaOH Proportion	-1.590	0.210
Sand	HCl Proportion	0.391	0.722
Clay/Loam	Resin	-2.584	**0.042**
Clay/Loam	Bicarb	-0.278	0.790
Clay/Loam	NaOH	-2.847	**0.029**
Clay/Loam	HCl	0.188	0.857
Clay/Loam	Hedley Sum	-1.040	0.338
Clay/Loam	Resin Proportion	-0.484	0.645
Clay/Loam	Bicarb Proportion	-0.117	0.911
Clay/Loam	NaOH Proportion	-0.784	0.463
Clay/Loam	HCl Proportion	1.010	0.352