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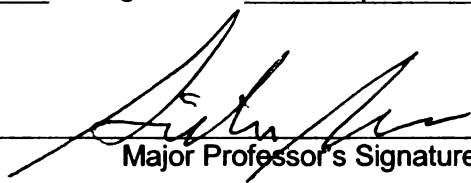
PHOSPHORUS AVAILABILITY IN ANNUAL AND PERENNIAL
CROPPING SYSTEMS

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COURTNEY MALOOF GALLAHER

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M.S. degree in Crop and Soil Sciences


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**PHOSPHORUS AVAILABILITY IN ANNUAL AND PERENNIAL CROPPING
SYSTEMS**

By

Courtney Maloof Gallaher

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

PHOSPHORUS AVAILABILITY IN ANNUAL AND PERENNIAL CROPPING SYSTEMS

By

Courtney Maloof Gallaher

Understanding mechanisms that promote efficient nutrient cycling is key to creating more sustainable agricultural landscapes. Many legumes have a unique ability to mobilize soil P. Phosphorus cycling was quantified at the Long Term Ecological Research (LTER) experiment at Kellogg Biological Station, in four systems initiated in 1989: 1) conventional corn-soybean-wheat, 2) organic corn-soybean-wheat with two years of red clover cover crops, 3) continuous alfalfa, and 4) a successional system, taken out of farmland in 1989. These treatments allow for comparisons between cropping systems with annual and perennial legumes and different intensities of legumes. In 2006, soybeans were planted as an assay of P bioavailability in the conventional, organic and alfalfa systems. Soil samples from the four systems, from 1992, 2001 and 2006, were analyzed for particulate organic matter phosphorus (POM-P), total organic P, total soil P, and soil extractable P (Bray P) to examine changes in soil P pools over time. Phosphorus bioavailability was greatest in the alfalfa system. Changes in soil pools occurred over time, with more P being stored in the organic and plant available soil pools in systems with a greater presence of legumes. Overall, these findings argue for an integrated approach to phosphorus nutrient management in low-input or organic agricultural systems, which utilize a variety of legumes to improve the bioavailability of P and build up P in soil pools with rapid turnover, such as POM-P.

To Shaun, with gratitude for your never ending love support on this journey, and to my
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Introduction

Phosphorus (P) is an essential nutrient for plants, needed for the production of ATP, DNA, and RNA and other cell constituents. It plays important roles in nearly all phases of the plant life cycle, including photosynthesis, flowering, seed production and maturation, and root growth. Phosphorus deficiency can cause severe stunting and significant yield losses (Haven et al., 1999). Despite being one of the most important plant nutrients, P is also one of the least mobile in soil and therefore highly unavailable to plants. Soluble forms of P are fixed by other soil minerals, making less than 0.01% of total soil P available to plants (Brady and Weil, 2000). Thus an understanding of soil P biogeochemistry is important for deciding how best to manage different agroecosystems.

Natural ecosystems have evolved to efficiently cycle soil nutrients, while modern agricultural systems are less efficient and rely heavily on external inputs, including inorganic fertilizers, to supplement the crop's nutrient demands. Depending on the agricultural system, farmers encounter a number of problems related to effective nutrient management. In conventional agricultural systems, excessive use of inorganic fertilizers often leads to non-point source pollution, which can have very detrimental impacts on aquatic ecosystems, leading to eutrophication (Carpenter et al., 1998).

In other agroecosystems, farmers must manage crops in nutrient deficient soils or with low nutrient inputs. Smallholder farmers in many areas of the developing world struggle to meet the nutrient demands of their crops; they may not have access to compost or manure, and supplemental inorganic fertilizers can be prohibitively expensive. In the U.S., many organic farmers use manure to provide nitrogen and phosphorus to their crops. However, organic farmers without access to manure would

also greatly benefit if their agroecosystems utilized crop rotations that better promote P cycling. Identifying combinations of plants, such as different cereals and legumes that can more efficiently cycle or increase the availability of soil nutrients, is extremely relevant in this context.

Legumes have long been recognized for their role in the nitrogen cycle, but it is also important to recognize that some legume species have unique mechanisms to access sparingly soluble P pools (Ae et al., 1990). Previous greenhouse and field trials have shown that legumes improve the bioavailability of phosphorus to subsequent crops (Cavigelli and Thien, 2003; El Dessougi et al., 2003; Kamh et al., 1999; Nuruzzaman et al., 2005). Preliminary studies have also suggested that phosphorus mobilized by legumes is moved from less available inorganic pools to more available soil organic pools (Maroko et al., 1999). However, the impact of different species and management practices of legumes on these soil pools is not well understood. Ultimately, improving our understanding of how legumes help to mobilize soil phosphorus and store it in different soil pools can help farmers design more efficient cropping systems.

A long-term agricultural experiment, located at Kellogg Biological Station (KBS), has maintained different cropping systems with varying legume inputs since 1989. Four of the treatments are based on corn-soybean-wheat rotations, with conventional, no-till, low-input and organic management, the last of which includes red clover as a leguminous winter cover crop. A continuous alfalfa treatment and a successional treatment, old farmland that is returning to prairie, have also been established. Focusing on four of these treatments (conventional, organic, continuous alfalfa, and successional) provides a contrast between the number of years legumes are present in a crop rotation as well as the

duration of legumes present (annual vs. perennial). These four treatments provide the context for answering questions about the impact of legumes on both availability of P to subsequent crops, and about the extent of the movement of P between different soil pools.

In order to answer questions about the ability of legumes to enhance soil phosphorus availability and cycling over time, we designed a study to look at 1) the bioavailability of phosphorus in the conventional, organic and alfalfa treatments to a subsequent soybean crop, and 2) to characterize different soil phosphorus pools from three time points in the conventional, organic, alfalfa and successional treatments of the LTER site.

Chapter 1. Literature Review

The Phosphorus Cycle

The global phosphorus cycle is the only major biogeochemical cycle in which the atmosphere does not play a significant role. Phosphorus primarily occurs in nature as a phosphate ion in rocks or ocean sediments. Chemical or biological weathering moves P into the soil solution, and makes it available for plant uptake. Decomposing plant or animal biomass returns P to the soil, increasing P in the organic pool (Fig. 1) (Newman, 1995).

In natural ecosystems, P is recycled efficiently between plants and soil, thus any P losses are primarily a result of runoff or erosion. In contrast, in agricultural systems additions and losses are directly impacted by different management strategies (Bundy and Sturgul, 2001). Fertilizer and livestock manure are the main sources of P inputs into agricultural systems, and P lost from these systems is primarily the result of runoff or crop removal. In a study of Wisconsin cropland, total fertilizer P additions have declined by 30% in recent decades, but crop removal of P has increased since 1970, largely because of increased crop yields, acreage and changes in crops grown, and was consistent with a national trend (Bundy and Sturgul, 2001). Phosphorus loss from an agricultural system can be minimized by practices such as decreasing fertilizer rates, point application of P, incorporating crop residues or managing fields to reduce runoff.

Although farmers may apply substantial amounts of P fertilizer to a field, this P is not readily available for plant uptake because soluble inorganic P is rapidly fixed by other inorganic soil components. In acid soils, P complexes with aluminum (Al) and iron (Fe)

and in alkaline soils P is fixed by calcium (Ca) (Brady and Weil, 2000). In acid soils, it is estimated that over 90% of P fertilizer added to a soil will be fixed by other soil minerals (Bundy and Sturgul, 2001). Some legume species are able to access these soil P stores and can improve overall P cycling in an agroecosystem.

Plant uptake of Phosphorus

Although the total amount of P in soil is large (300-5000 mg/kg), it is difficult for plants to access since available P concentrations in soil solution tend to be extremely low (Brady and Weil, 2000). Soil solution concentrations of inorganic P (Pi), the form of P most readily available to plants, are commonly 1μM and rarely exceed 10μM (Bielesky and Ferguson, 1983). The two most common available forms of inorganic P found in soil solution are H_2PO_4^- and HPO_4^{2-} , with the H_2PO_4^- most common at pH less than 7, and HPO_4^{2-} above pH 7 (Schachtman et al., 1998).

Mycorrhizal fungi aid in plant P uptake since P is relatively immobile in soil and the mycorrhizal mycelium markedly extend the surface area of the root systems for uptake. More than 90% of terrestrial plants form symbiotic relationships with mycorrhizal fungi (Bolan, 1991; Smith and Read, 1997). This symbiosis is based upon the mutualistic exchange of plant carbon for soil mineral nutrients scavenged by these fungal hyphae. Phosphorus uptake by plants infected by mycorrhizae can be 3 to 5 times higher than in non-infected plants. (Smith and Read, 1997).

Although mycorrhizal associations remain an important factor in legume uptake of soil P, many legumes have additional adaptations which allow them to chemically

modify the rhizosphere in order to access sparingly soluble sources of P. Legume roots may increase P solubility of the dominant P fraction either through acidification (Ca-P) or alkalization (Al-P, Fe-P) of the rhizosphere via root exudates (Gahoonia and Nielsen, 1992; Riley and Barber, 1971). Under P deficient conditions, the non-mycorrhizal white lupin, develops proteoid roots that excrete citrate in order to solubilize calcium-bound soil phosphate (Dinkelaker et al., 1989; Gardner et al., 1983). Although pigeon pea (*Cajanus cajan* L.) is mycorrhizal, it has been shown that additional soil P taken up by this plant is due to its ability to utilize iron-bound phosphate (Fe-P) by excreting piscidic acid, which chelates Fe (Ae et al., 1990). Additional studies have shown that pigeon pea is able to access aluminum bound phosphate (Al-P) using a similar mechanism. Groundnut (*Arachis hypogaea* L.) has also been shown to access sparingly soluble soil phosphorus pools by excreting organic acids, including malonic, oxalic and piscidic acid (Otani et al., 1996). In P limiting conditions, alfalfa (*Medicago sativa*) acidifies the rhizosphere by excreting malic, citric and succinic acids which solubilize iron-bound P (Lipton et al., 1987; Masaoka et al., 1993). Red clover (*Trifolium pretense*), a common leguminous cover crop, has also been shown to excrete carboxylic acids which mobilize sparingly soluble soil P by ligand exchange and occupation of P sorption sites (Gerke, 1995).

Some legumes are less efficient at mobilizing sparingly soluble soil P. For example, soybean develops fibrous root systems which exude only trace levels of organic ions in response to P limited conditions (Ohwaki and Hirata, 1992). However, these root systems appear to be better adapted to acquire more readily available soil P than other legumes (Braun and Helmke, 1995).

Crop rotations to manage soil phosphorus fertility

In view of the potential of different legumes to improve mobilization of phosphorus, it is important to consider their overall role in maintaining soil fertility as part of a crop rotation. Soil fertility is a fundamental factor in determining the overall productivity of a farming system. Conventional and organic farming systems approach the management of soil fertility in different ways, with conventional agriculture using solutions based on input substitution, such as inorganic fertilizer to improve soil fertility, and organic systems using longer term, preventative solutions such as crop rotation (Stockdale et al., 2001). Since one of the greatest challenges faced by organic farming systems without access to manure is the management of nutrient supplies, within these systems, there is generally an emphasis on alternative strategies to build soil fertility, such as through the use of green manures and grain legumes (Watson et al., 2002). These legumes are traditionally used to manage nitrogen fertility, but given the potential of legumes to mobilize soil phosphorus, enhanced phosphorus fertility could be an additional benefit. However, this potential benefit has not been much investigated.

The high crop yields achieved in most developed countries today are highly dependent on phosphorus fertilizer, primarily from fertilizers derived from rock phosphate (Newman, 1997). Organic systems that operate without the use of phosphate inputs often run a P deficit, as calculated by system budgets (Berry et al., 2003), yet a few authors have found that annual P deficits have caused no decline in the content of extractable P over 10 to 20 years (Kaffka and Koepf, 1989; Oehl et al., 2002). Building simple nutrient budgets based on crop rotations can be a useful tool to understand

potential long-term deficits and to develop long-term strategies for phosphorus nutrient management. However, it is important to recognize potential problems with the oversimplification of nutrient budgets, which reflect assumptions about transfers from available to unavailable pools that do not necessarily accurately predict crop available P.

Positive rotational effect of legumes on P cycling

The unique ability of some legume species to acquire sparingly soluble soil P has the potential to improve the P use efficiency of crops grown subsequently in a rotation. Decomposition of organic P in legume tissues provides a relatively labile form of P to the next crop, thus supplementing the small soluble inorganic P pool with a larger pool of mineralizable soil organic P in systems under organic or low fertilizer management (Tiessen et al., 1994).

Crops grown following legumes are often higher yielding with more vigorous growth because of these organic P pools. A greenhouse study by Cavigelli and Thien (2003) found that sorghum yields were significantly higher following the incorporation of legume residues in an experiment involving alfalfa, red clover, yellow sweet clover (*Melilotus officinalis*), white lupin (*Lupinus albus* L.), winter pea (*Pisum sativum* L. ssp. *arvense*) and hairy vetch (*Vicia villosa*), as well as winter wheat used as a control. Yield increases were highest following alfalfa, which had the highest tissue P content prior to incorporation, consistent with alfalfa having best facilitated movement of P into the soil organic pool.

In another greenhouse study, the authors (Nuruzzaman et al., 2005) investigated the effect that grain legumes have on P uptake of a subsequently grown wheat crop. In

this study, the legumes white lupine, field pea (*Pisum sativum*) and faba bean (*Vicia faba*) all significantly improved the growth of a subsequent wheat crop as compared to the growth of wheat after wheat. Tissue concentrations of P in wheat were 6%, 13% and 19% higher in wheat following field pea, white lupine and faba bean respectively. In addition, carboxylic acids were present in significantly higher concentrations in the rhizosphere soils of all the legume treatments. Because the system was not nitrogen limited the authors concluded that the improved yields were likely due to mobilization of sparingly soluble soil P.

Kamh et al. (1999) carried out a greenhouse study in P-limited soils from northern Nigeria to examine the effect of nine legume species on a subsequent maize crop in an effort to characterize legume P efficiency in comparison to maize. Legume species studied included *Mucuna pruriens* (velvet bean), *Lablab purpureus* (hyacinth bean), *Glycine max* (soybean), *Phaseolus vulgaris* (common bean), *Cajanus cajan* (pigeon pea), *Chamaecrista rotundifolia* (round-leafed cassia), *Clitoria ternatea* (butterfly pea), and *Centrosema pubescens* (centro). Of these species, velvet bean, common bean and pigeon pea were the most efficient at providing soil P to a subsequent maize crop, and were nearly as effective as the application of P fertilizer, at rates of 15 to 150 kg/ha P. Because adequate mineral N was supplied to all the treatments, the authors also concluded the improved maize yields following legumes were a result of mobilization of sparingly soluble soil P.

In a subsequent greenhouse study, the same authors (Kamh et al., 2002) compared the effect of the same legume species on maize growth using two P-limited, high P-fixing soils from Nigeria and Guinea. Their results were similar to their previous study, but

further indicated that on high-P fixing soils growth of leguminous plants cannot alone provide sufficient P nutrition to a subsequent maize crop. These results were confirmed in a field trial with the same soils (Horst et al., 2001). However Horst et al. (2001) suggested that growing leguminous crops can still contribute to the overall sustainability of a system by reducing the need for external inputs and shifting P cycling towards more plant available P pools.

Intercropping P-efficient legumes with cereal crops may allow for efficient cycling of P and increased yields in a similar manner to legume rotations. El Dessougi et al. (2003) examined P cycling in a greenhouse study where maize was intercropped with white lupine, sugar beet, oilseed rape or groundnut. Dry matter yield of maize from the maize and groundnut intercrop was shown to be three times higher than maize grown alone, where neither system received P fertilizer. In addition, soil fractionation indicated that intercropped maize had higher available P fractions. Because all treatments had sufficient available N, improved maize yields were most likely due to improved P availability solubilized by groundnut.

Partitioning Soil Phosphorus Pools

Phosphorus is stored in three different soil pools designated primary inorganic, secondary inorganic, and organic (Walker and Syers, 1976). These soil pools all release biologically available P to soil solution but at very different rates, depending on soil conditions such as soil texture, pH, moisture content, plant and microbial activities. (Sanyal and De Datta, 1991). The primary inorganic pool includes primary soil minerals,

such as apatite, which is a source of soluble P early during ecosystem development. Phosphorus released from the weathering of primary minerals is incorporated into plant biomass and cycled back into organic P or secondary inorganic P pools. The secondary inorganic P pool consists of secondary minerals that form as a result of chemical and biological weathering. Over time, as primary minerals are depleted of P and most of the P is found in organic and secondary inorganic P pools, the productivity of an ecosystem is dependent on its ability to cycle P between organic matter and living organisms (Miller et al., 2001). Soil P pools are difficult to quantify in a biologically relevant way, and thus different laboratory methods, such as extractable inorganic phosphorus, particulate organic matter phosphorus (POM-P), and total soil phosphorus, have been developed to partition soil P pools and relate them to plant available P.

Extractable inorganic phosphorus

Three methods are commonly used today to measure extractable inorganic phosphorus, which is a measure of plant available soil phosphorus. These methods are dependent upon four basic reactions between P and the extracting solution: 1) dissolving action of acids, 2) anion replacement to enhance desorption, 3) complexing of cations binding P, and 4) hydrolysis of cations binding P (Elrashidi, 2005). In acidic soils, the primary sources of P are Al- and Fe- phosphates, while calcium phosphates are the dominant P mineral in alkaline and calcareous soils. Bray-1 and Melich 3 are most appropriate for acidic to neutral soils while the Olsen method is an effective predictor of plant available P in alkaline or calcareous soils.

The Bray-1 method, developed by Bray and Kurtz (1945), uses a combination of HCl and NH_4F as extractants to remove easily acid soluble forms of P, such as Al- and Fe-phosphates. In 1953, A. Mehlich developed an extraction method (Mehlich-1) that used a combination of HCl and H_2SO_4 acids to extract P from soils in the north-central region of the U.S. (Mehlich, 1953). Due to the sulfate ions in solution, Mehlich-1 dissolves Al- and Fe-phosphates as well as P adsorbed to colloidal surfaces in soils. In the 1980's, Mehlich further refined his initial methods to develop an extractant appropriate for multi-elemental analysis (Mehlich, 1984). The Mehlich 3 extractant is a combination of acids and salts, including dilute acetic acid (HOAc), nitric acid (HNO_3), ammonium fluoride (NH_4F) and ammonium nitrate (NH_4NO_3) as well as the chelating agent ethylenediaminetetraacetic acid (EDTA). Although versatile, the Mehlich soil tests are only suitable for use in acid and neutral soils. In 1954, Olsen developed a soil P test to extract inorganic P from calcareous, alkaline or neutral soils that uses a 0.5 M sodium bicarbonate (NaHCO_3) solution at a pH of 8.5. The sodium carbonate in solution causes calcium carbonate to precipitate, thus increasing the dissolution of Ca-phosphates. In addition, the sodium bicarbonate extractant also removes dissolved and adsorbed P from calcium carbonate and Fe-oxide surfaces (Olsen et al., 1954).

Total soil phosphorus

Total soil phosphorus can be partitioned into either inorganic or organic forms. There are several commonly accepted methods for determination of total soil P (P_t), including the sodium carbonate (Na_2CO_3) fusion method and the perchloric acid (HClO_4)

digestion method (Olsen and Sommers, 1982). The sodium carbonate fusion method is most reliable, but it is very time consuming and requires the use of platinum crucibles which are expensive and require special handling. The perchloric acid soil digestion is more suitable for determination of phosphorus in large numbers of samples, although samples must be digested under a perchloric acid fume hood (Olsen and Sommers, 1982). A more recent study has suggested that determination of total soil P using the standard Kjeldahl digestion, currently used for total soil N analysis, can also yield reliable results (Taylor, 2000).

Particulate organic matter

Understanding soil organic matter (SOM) cycling is relevant to understanding phosphorus cycling because productivity may be reduced in well fertilized soils where there has been a loss of SOM (Aref and Wander, 1997; Johnston, 1991). In low-input or organic farming systems, SOM is an essential source of important plant nutrients. Soil organic matter levels can be improved using processes that increase carbon inputs and reduce carbon losses, including a variety of management practices such as pasture (Sbih et al., 2003), amending with compost (Stone et al., 2001), cover crops (Stevenson et al., 1998), and no-till systems (Frey et al., 1999).

Analysis of SOM fractions has been undertaken as a means to detect changes in processes of carbon assimilation in relationship to management practices. Historically, SOM characterization focused on extraction methods that separated organic matter from the soil mineral matrix, allowing for HPLC, GC, or elemental analyses (Wander, 2004).

These methods were judged based on their ability to isolate pure, reproducible and homogenous components for analysis (Stevenson, 1994). More recent efforts at characterizing SOM have focused on fractionating SOM into labile, slow and recalcitrant pools, relating carbon retention to function. The labile, or active, organic matter pool represents material recently incorporated into the soil and is thought to be of high nutrient supply potential. Thus, this labile pool has the potential to greatly contribute to nutrient cycling (Wander, 2004).

Particulate organic matter (POM) is commonly used to assess labile SOM pools. POM has been shown to be responsive to changes in management practices, and some research indicates it is better than other fractions at representing labile SOM, such as carbon mineralization potential (Alvarez and Alvarez, 2000; Conteh et al., 1998; Franzluebbers et al., 2000). POM can vary significantly with plant inputs, seasonality, soil depth, and patterns of sampling so care must be taken to standardize sampling regimes (Wander and Traina, 1996; Wander et al., 1998). Nutrient analysis of POM provides an indicator of labile nutrient pools. Particulate organic matter phosphorus (POM-P), a measure of total P in the organic matter collected, could be a useful an indicator of labile soil phosphorus.

The two basic methods used to characterize soil POM separate organic matter from the soil mineral matrix either by size or density. The POM or coarse fraction (CF) typically refers to organic matter that is sand-sized or larger. Size-based methods separate organic matter from soil using different sizes of sieves. These methods generally focus on large residues, varying in size from 100-2000 μm , that can be clearly identified as plant residues (Wander, 2004). Density separations separate sand from

organic matter by dispersing a soil sample in a liquid ranging in density from 1.6- 2.6 g/cm³. Lower densities tend to favor recovery of larger POM particles whereas use of denser liquids results in the recovery of a wider range of particle sizes (Ladd and Amato, 1980). Liquids commonly used for density separations include sodium or potassium iodide, sodium polytungstate (NaPT), and silica gels (Wander, 2004). Some methods of POM measurement combine size and density separations.

Chapter 2. The influence of legumes on the bioavailability of phosphorus and the partitioning of soil phosphorus pools

Introduction

Effective nutrient management presents a variety of challenges for farmers in different agricultural systems. Conventional agricultural systems, which rely heavily on inorganic fertilizers, have developed as the dominant agricultural system in the U.S. only in the last one hundred years. Nutrient management in these systems is based on inorganic fertilizers to supplement nutrient deficiencies, while organic or many smallholder systems are managed through the use of long-term crop rotations (Stockdale et al., 2001). Management of phosphorus presents unique challenges, because although the total amount of P in soil is large (300-5000 mg/kg), it is difficult for plants to access since available P concentrations in soil solution tend to be extremely low (Brady and Weil, 2000). Farmers may apply substantial amounts of P fertilizer to a field, but this P is not readily available for plant uptake because soluble inorganic P is rapidly fixed by other inorganic soil components. Phosphorus complexes with aluminum (Al) and iron (Fe) in acidic soils, and with calcium in alkaline soils (Brady and Weil, 2000). In acid soils, it is estimated that over 90% of fertilizer added to a soil will be fixed by other soil minerals (Bundy and Sturgul, 2001). Thus an understanding of soil P biogeochemistry is important when deciding how best to manage different agroecosystems, especially in P limited conditions.

Plant species mediation of nutrient cycling mechanisms requires in-depth study, as a key regulator of nutrient cycling in intensively managed systems. Highly reactive

nitrogen and excess phosphorus application will continue to be relied upon to support cropping system productivity as long as inefficient cycling remains the norm. Natural ecosystems have evolved to efficiently cycle soil nutrients, while modern agricultural systems are less efficient and rely heavily on external inputs, including soluble inorganic fertilizers, to supplement the crop's nutrient demands. Some legume species are able to access these soil P stores, although the impact on overall P cycling in an agroecosystem has not been studied in detail. Identifying combinations of plants, such as different cereals and legumes that can more efficiently cycle or increase the availability of soil nutrients, may be extremely relevant in this context.

Mycorrhizal associations are one factor in legume uptake of soil P, but many legumes have other adaptations which allow them to chemically modify the rhizosphere in order to access sparingly soluble sources of P. Legume roots increase P solubility of the dominant P fraction through mechanisms such as acidification (Ca-P) or alkalization (Al-P, Fe-P) of the rhizosphere via root exudates (Gahoonia and Nielsen, 1992; Riley and Barber, 1971). Genotype influences the mechanisms of plant-soil P interaction. For example, under P deficient conditions, the non-mycorrhizal white lupine develops proteoid roots that excrete citrate in order to solubilize calcium-bound soil phosphate (Dinkelaker et al., 1989; Gardner et al., 1983). In P-limiting environments, alfalfa (*Medicago sativa*) acidifies the rhizosphere by excreting malic, citric and succinic acids that solubilize iron-bound P (Lipton et al., 1987; Masaoka et al., 2004). Red clover (*Trifolium pretense*), a common leguminous cover crop, has also been shown to excrete carboxylic acids which mobilize sparingly soluble soil P by ligand exchange and occupation of P sorption sites (Gerke, 1995). Other legumes appear to be less efficient at

mobilizing sparingly soluble soil P. For example, soybean develops fibrous root systems which exude only trace levels of organic ions in response to P limited conditions (Ohwaki and Hirata, 1992). However, these root systems appear to be better adapted to acquire more readily available soil P than other legumes (Braum and Helmke, 1995). Species effects on mechanisms of accessing sparingly soluble P have only begun to be elucidated.

The impact of different species and management practices of legumes on these soil pools is currently not well understood. In numerous greenhouse and field trials, legumes have been shown to improve the bioavailability of phosphorus to subsequent crops (Cavigelli and Thien, 2003; El Dessougi et al., 2003; Kamh et al., 1999; Nuruzzaman et al., 2005). In additional studies, phosphorus mobilized by legumes was moved from less available inorganic pools to soil organic pools, or to readily available plant available phosphorus pools (Maroko et al., 1999). For example, with a nutrient budget conducted on farm in Malawi the potential of the legume pigeonpea residues to improve P nutrition in intercropped corn was demonstrated (Snapp et al., 1998). Improving our understanding of how legumes help to mobilize soil phosphorus and store it in different soil pools can help farmers design more efficient cropping systems.

Phosphorus in the soil is stored in three different pools, designated primary inorganic, secondary inorganic, and organic (Walker and Syers, 1976). The rate at which these pools release biologically available P to the soil solution varies depending on soil conditions such as soil texture, pH, moisture content, plant and microbial activities. (Sanyal and De Datta, 1991). Because soil P pools are difficult to quantify in a biologically relevant way, different laboratory methods, such as extractable inorganic phosphorus, particulate organic matter phosphorus (POM-P), and total soil phosphorus,

have been developed to partition soil P pools and characterize relationships to plant-available P. Extractable inorganic P, a measure of plant-available P which describes easily exchangeable iron and aluminum phosphates, is quantified in acidic to neutral soils using the Bray-1 or Melich-3 extraction methods. Particulate organic matter (POM) is a measure of soil organic matter that is commonly used to assess labile SOM pools, which responds to changes in management practices more rapidly than other labile SOM fractions (Alvarez and Alvarez, 2000; Conteh et al., 1998; Franzluebbers et al., 2000). Nutrient analysis of POM provides an indicator of labile nutrient pools. Although few studies have been published relating total phosphorus in POM to P nutrient cycling, particulate organic matter phosphorus (POM-P) could be a useful indicator of labile soil phosphorus (Salas et al., 2003).

In this study, we explored the ability of legumes to enhance soil P cycling and availability over time, by examining how legumes in three long-term crop rotations enhanced bioavailability of phosphorus to a subsequent crop. In addition we characterized different soil phosphorus pools from three points in time within four treatments of a long-term agricultural trial to look at the dynamics of soil phosphorus over time.

In the Long Term Ecological Research (LTER) row-crop trial, located at Michigan State University's Kellogg Biological Station (KBS) in SW Michigan, USA, different cropping systems have been maintained with varying legume inputs since 1989. For this study, we collected soils from a conventionally managed corn-soybean-wheat rotation, an organic corn-soybean-wheat rotation with two years of winter red clover as a cover crop, a continuous alfalfa treatment, and a successional system (old farmland

allowed to return to prairie). In addition, soybeans were planted as a bioassay into microplots within the conventional, organic and alfalfa systems. These treatments were chosen because they contain different numbers of annual and perennial legumes ranging from few legumes (conventional) to intermediate legumes (organic) to continuous legumes (perennial alfalfa). The overall objectives of this study were to 1) examine the bioavailability of phosphorus in the conventional, organic, and alfalfa treatments to a subsequent soybean crop, and 2) to characterize movement of phosphorus between different soil pools in conventional, organic, alfalfa and successional treatments of the LTER.

Methods

Site description and agronomy

This study was conducted at the Long Term Ecological Research (LTER) experiment at the W.K. Kellogg Biological Station (KBS) in southwest Michigan, (42° 24' N, 85° 24' W, elevation 288 m). Soils at the LTER site developed on glacial outwash deposited 12,000 years ago, and are primarily Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) series (Crum and Collins, 1996). Average annual rainfall at KBS is around 890 mm y⁻¹ with about half falling as snow, and the mean annual temperature is 9.7 °C. (2006).

Established in 1989, the LTER is designed to test hypotheses using a randomized complete block design testing treatments that vary in management intensity. Four treatments were selected that ranged in legume intensity from annuals to perennials: 1) a conventionally managed corn-soybean-wheat rotation that receives standard levels of chemical inputs, 2) a certified organic corn-soybean-wheat rotation, with red clover cover crop planted following wheat, 3) perennial alfalfa, and 4) an early successional treatment, where old farmland has been allowed to return to prairie.

Treatments in the LTER are replicated six times with a plot size of 1 ha, but we chose to use four of the replicates (reps 1, 2, 4 and 5) based on the uniformity of soil characteristics among the replicates (Kravchenko et al., 2006).

Soybean bioassay

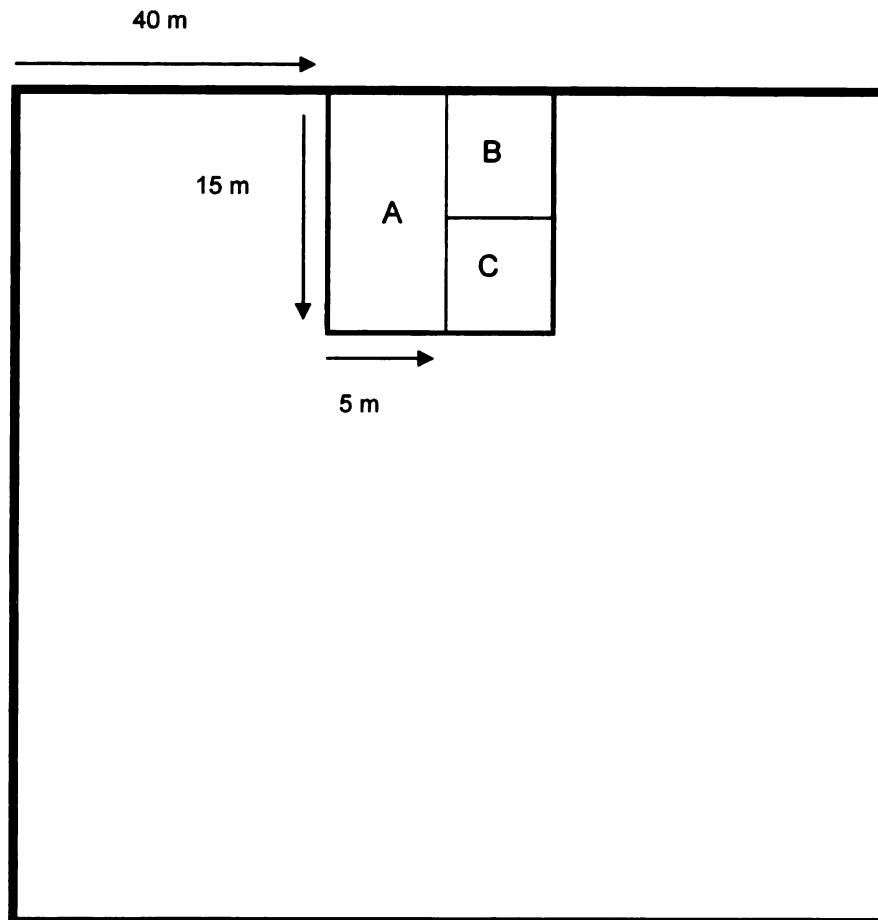
Soybeans were used as an assay of P bioavailability following the conventional, organic and alfalfa cropping systems, in expectation that a legume crop could access sparingly soluble P pools enhanced through historical legume residue inputs. Soybeans were planted June 19 and 20, 2006 into microplots (10 x 15 m) at the north end of each 1 ha replicate (reps 1, 2, 4 and 5) in the conventional, organic and alfalfa treatments. Each microplot was divided into three sub-plots (see fig. 1) in order to test the effects of long term cropping system history, short term recent legume effects and the addition of inorganic P fertilizer. In subplot A soybeans were planted in both 2005 and 2006. In subplots B and C, soybeans were planted in 2006 only, but either B or C was randomly selected to receive 30 kg per hectare phosphorus applied as triple super phosphate (TSP) fertilizer in June 2005. Alfalfa was killed using glyphosate and incorporated three weeks prior to planting soybeans.

Precipitation in 2006 was 54.9 cm, distributed as follows over the months of the growing season; 15.7 cm in May, 4.3 cm in June, 10.7 cm in July, 15.7 cm in Aug., and 8.9cm in September. Total rainfall over the 2006 growing season was within the range of the 10 year average 50.3 +/- 13 cm (mean +/- std. dev.).

Soybean biomass was harvested September 8, 2006 from three 0.5 m row-lengths in each sub-plot. Each row-length contained 5-8 plants, and row-lengths were composited and dried as one sample. Soybean biomass was dried at 60°C for a minimum of 72 hours, weighed, ground to pass through a 1mm sieve and thoroughly mixed, and analyzed, using a cold digestion, for total tissue P content by A&L Great Lakes Laboratories in Fort Wayne, Indiana. A small amount of sample (~0.2 grams) was

weighed into a digestion vessel, and the samples were digested in a microwave with hydrogen peroxide and concentrated HCl. The digests were diluted to 20 mls (1:100 digestion) and then transferred to an ICP for mineral analysis.

Figure 2.1 Description of microplot areas within treatments of the LTER



Microplot area within the conventional, organic, alfalfa and successional treatments of the LTER. Soybeans were grown in subplot A in 2005 and 2006, and were grown in subplots B and C in 2006 only. In addition, either B or C was randomly selected in 2005 to receive 30 kg/ha triple superphosphate fertilizer.

Soil Sampling

Composite soil samples were collected June 5 and 6, 2006 from the microplot areas at depths of 0-20 cm and 20-50 cm. Samples were air dried and ground to pass through a 2mm sieve. Additional soil samples from 1992 and 2001 were obtained from the LTER archives. The unground archived samples were collected at a depth of 0-20 cm. The years 1992 and 2001 were chosen to represent the same phase of the rotation of the rotation as the 2006 samples, all of which were sampled following corn.

Total and extractable inorganic soil phosphorus

Soil samples from 1992, 2001 and 2006 were measured for total P to allow investigation of potential net losses between the treatments. Soils were air dried and ground to pass through a 2mm sieve. Samples were analyzed for total soil phosphorus, using the Kjeldahl total P method, by the Central Analytical Laboratory at Oregon State University (Taylor, 2000).

Soil samples were also analyzed for extractable inorganic soil phosphorus as a measure of plant available phosphorus. Soil samples were ground to pass through a 2mm sieve and analyzed for Bray-P 1 by A&L Great Lakes laboratories in Fort Wayne, IN (NCR-13 and North Dakota Agric. Exp. Stn. 1, 1980).

Phosphorus budget

A phosphorus budget was assembled for the years 2000 to 2005 in the conventional, organic, alfalfa and successional treatments of the LTER. Phosphorus inputs, in the form of P fertilizer, were calculated based on fertilizer application records in the LTER field log (2007). Exports from the system included phosphorus removed in harvested plant biomass. Calculations for the P removed from crops in the conventional and organic systems were based on grain yield data for the years 2000 to 2005, available on the LTER data archives (LTER data catalog, 2007). The amount of phosphorus removed in alfalfa cuttings was calculated based on biomass data available in the LTER archived data catalogs. Phosphorus tissue and grain concentrations for alfalfa and soybean were based on analysis of total tissue P in eight composite samples from the LTER in 2005 and 2006, respectively. Grain phosphorus concentrations for corn and wheat were based on average literature values (USDA, SR19).

Particulate organic matter

Particulate organic matter (POM) was analyzed from un-ground soil samples (0-20 cm) from 1992, 2001 and 2006. POM was collected in two steps; first, sand and organic matter were separated from the soil based on a size separation, and then a density separation was used to isolate the particulate organic matter.

Size separation: Twenty five (25) grams of soil were shaken with 30 mL of 0.05 M NaCl in 50 mL centrifuge tubes for 2 hours. Samples were passed through a 53 μ m sieve and

rinsed with distilled water until a clear solution was obtained. Materials retained on the sieve were a mixture of sand and POM. This mixture was washed, using distilled water, into a 500 mL glass canning jar and placed in an oven to dry overnight at 60°C.

Density separation:

Dried sand and POM mixtures were carefully scraped from the glass jars into 50-mL conical centrifuge tubes containing 35 mL of sodium polytungstate of density 1.85 Mg/m³. Suspended material was mixed by slow reciprocal shaking by hand (10 strokes), and the tube was placed in a vertical position and centrifuged at 2500 x *g* for 30 minutes. POM, floating at the top of the tube, was carefully poured onto a 20 µm mesh nylon filter using a vacuum filtration system, and rinsed thoroughly with water to remove excess sodium polytungstate. Materials retained on the 20 µm filter were washed, using distilled water, into a pre-weighed aluminum weighing pan and dried for 24h at 60°C. Once dry, pans were weighed and then POM was ground by hand using a porcelain mortar and pestle. Sodium polytungstate was recycled using the methods described by (Six et al., 1999).

Ground POM was analyzed for total tissue P content by A&L Great Lakes laboratory in Fort Wayne, IN using the method described above for soybean tissue samples.

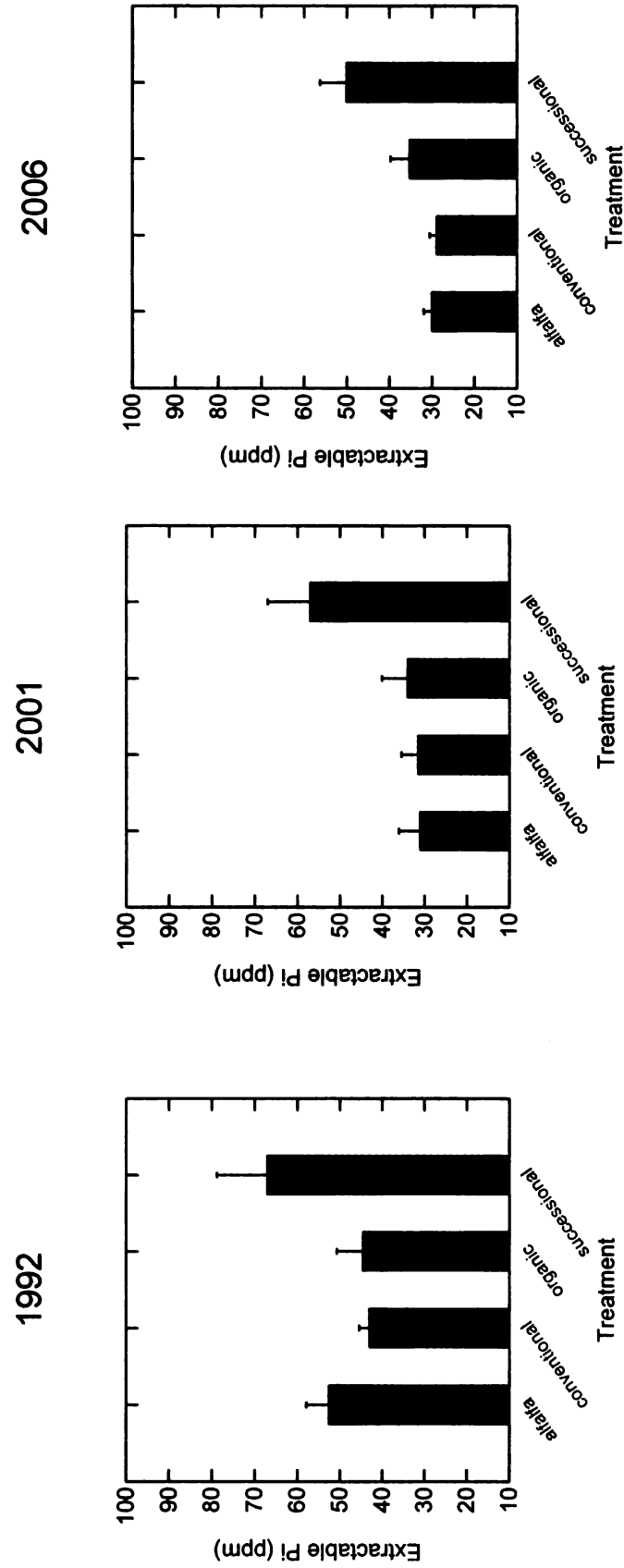
Results

Total and extractable inorganic soil phosphorus

Total soil phosphorus ranged from 210 to 564 ppm P, with an average value of 348 ppm across treatments. There were no significant differences in the total P content of soil by cropping system (p -value = 0.684), nor did the application of P fertilizer in 2005 impact total soil P concentrations in 2006 (p -value = 0.989). In addition, the total amount of P in soil stayed relatively stable over time (Tables 2.1 and 2.2).

Significant changes in extractable inorganic phosphorus (Bray-P) were seen over time. The high levels of inorganic P measured in the 1992 soil samples reflect the fact that the LTER experiment began on soils that had anthropogenically elevated levels of soil P due to extensive application of P fertilizer when the farmland was in a corn-soybean rotation (Daroub et al., 2001). Extractable inorganic phosphorus changed significantly over time in soils sampled at 0-20 cm (Figure 2.2), with decreases in plant available phosphorus occurring in all treatments between 1992 and 2001 (p -value = 0.0309) (Tables 2.1 and 2.3). If the year 2006 is considered alone, significant differences can be seen in the amount of plant available phosphorus between treatments, at depths of 0-20 cm and 20-50cm (p -value = 0.006), with more plant available phosphorus in the organic and successional treatments than in the conventional system (Figure 2.3). The application of phosphorus fertilizer to subplots in 2005 had no significant impact on plant available phosphorus in 2006.

Figure 2.2 Extractable inorganic P in 1992, 2001, and 2006



Extractable inorganic phosphorus (Bray P) in soil samples (0-20cm) from four cropping systems from 1992 to 2006. Plant available phosphorus fell initially in all treatments, then leveled off. Inorganic phosphorus was least available in the conventional corn-soybean-wheat rotation, and highest in the successional treatment. The alfalfa and conventional treatments received phosphorus fertilizer while the organic and successional treatments did not.

Figure 2.3 Extractable inorganic P in 2006

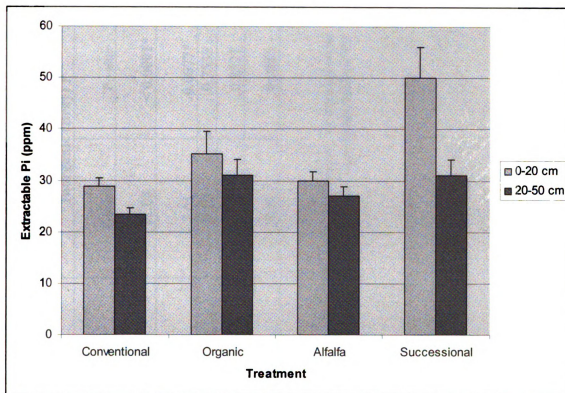


Fig. 2.3 Averages plus standard errors are presented for extractable inorganic P (Bray P) from 2006 soil samples at two depths from four cropping systems in a 17 year experiment at KBS, Hickory Corners, MI. The four systems include two management systems (conventional and organic) for three year sequences of corn-soybean-wheat, a perennial alfalfa system, and a successional system, of old farmland allowed to return to prairie.

Table 2.1 ANOVA table for total and extractable inorganic soil P (0-20")

	Total Phosphorus		Inorganic (Bray) P	
	F-value	P-value	F-value	P-value
Treatment	0.50	0.684	11.08	< 0.0001*
Year	0.83	0.367	7.61	0.0077*
Fertilizer	0.00	0.989	0.10	0.7533
Recent crop history	0.00	0.980	0.18	0.8321
Treatment*year	0.06	0.981	0.32	0.8085

There were no significant differences in total soil phosphorus due to treatment, application of fertilizer, or management history. In addition, there were no significant changes over time. Both treatment and year were significant factors for inorganic (bray) P.

Table 2.2 Total soil phosphorus (means +/- SEM)

	1992	2001	2006
Conventional	321.6 +/- 10.8	291.1 +/- 11.7	347.1 +/- 21.7
Organic	354.7 +/- 21.1	344.8 +/- 20.6	380.1 +/- 33.5
Alfalfa	350.35 +/- 8.1	333.6 +/- 20.5	320.7 +/- 21.2
Successional	382 +/- 32.3	345.6 +/- 22.7	372.4 +/- 39.2

Total soil phosphorus concentrations and standard deviations (n = 4) for 0-20" soil samples from the conventional, organic, alfalfa and successional systems.

Table 2.3 Extractable inorganic soil P (Bray P) (means +/- SEM)

	1992 <i>a</i>	2001 <i>b</i>	2006 <i>b</i>
Conventional <i>a</i>	43.0 +/- 2.0	31.5 +/- 3.3	28.0 +/- 1.6
Organic <i>a</i>	44.0 +/- 5.2	34 +/- 5.1	35.2 +/- 4.3
Alfalfa <i>a</i>	52.5 +/- 4.5	31.0 +/- 4.3	30.0 +/- 1.8
Successional <i>b</i>	67.0 +/- 9.9	57.0 +/- 7.6	50.1 +/- 5.9

Extractable inorganic soil P and standard deviations (n = 4) for soils (0-20") from the conventional, organic, alfalfa and successional systems. Different letters indicate significant differences ($P < 0.05$) between treatments and years.

Phosphorus Budget

Average annual P budgets were negative for each single rotation period in the conventional, organic and alfalfa cropping systems, as well as over a nine year period from 1997 to 2005. Over the nine year period, the net deficits were 12.0, 64.2, and 28.0 kg/ha P for the conventional, organic and alfalfa cropping systems, respectively (Table 2.4), which is a net negative per year of 1.3, 7.1, and 3.1 kg/ha P for the conventional, organic, and alfalfa systems. This indicates that in all three systems, P removal in harvested biomass exceeded the amount of P added in the form of inorganic fertilizer. This net negative balance was not reflected in the measured extractable inorganic soil P, or in the phosphorus uptake of crops from these systems.

Table 2.4 Phosphorus budget for the conventional, organic and alfalfa treatments of the LTER from 1997 to 2005

Treatment	Year	Crop	Biomass		Losses		Gains	Net P	Net P
			Avg (kg/ha)	Std Dev (kg/ha)	Avg P removed (kg)	Std Dev P removed (kg)	P fertilizer added	per year	5 years
T1	1997	soybean	1653.6	542.6	7.2	2.4	22.4	15.2	
T1	1998	wheat	2599.1	620.7	5.7	2.7	0.0	-5.7	
T1	1999	corn	4475.8	1707.0	12.9	7.5	0.0	-12.9	
T1	2000	soybean	2656.7	254.6	11.6	1.1	0.0	-11.6	
T1	2001	wheat	4203.0	336.0	12.1	1.0	0.0	-12.1	
T1	2002	corn	5398.3	1430.5	11.3	3.0	18.3	7.0	
T1	2003	soybean	1620.8	192.4	7.1	0.8	19.3	12.2	
T1	2004	wheat	4075.0	127.3	11.7	0.4	0.0	-11.7	
T1	2005	corn	6460.0	1128.9	13.6	2.4	21.3	7.7	-12.0
T4	1997	soybean	1634.2	517.5	7.2	2.3	0.0	-7.2	
T4	1998	wheat	949.4	314.9	0.9	0.9	0.0	-0.9	
T4	1999	corn	4911.9	1847.4	3.9	3.9	0.0	-3.9	
T4	2000	soybean	2899.5	185.5	12.7	0.8	0.0	-12.7	
T4	2001	wheat	2715.2	230.0	7.8	0.7	0.0	-7.8	
T4	2002	corn	5453.3	1518.8	11.5	3.2	0.0	-11.5	
T4	2003	soybean	1009.5	192.7	4.4	0.8	0.0	-4.4	
T4	2004	wheat	2299.2	268.5	6.6	0.8	0.0	-6.6	
T4	2005	corn	4413.3	661.6	9.3	1.4	0.0	-9.3	-64.2
T6	1997	alfalfa	7922.1	1271.1	19.8	3.2	0.0	-19.8	
T6	1998	alfalfa	8611.2	1089.5	21.5	2.7	0.0	-21.5	
T6	1999	alfalfa	1906.2	459.9	4.8	1.1	0.0	-4.8	
T6	2000	alfalfa	281.8	91.9	0.7	0.2	0.0	-0.7	
T6	2001	alfalfa	171.7	98.0	0.4	0.2	0.0	-0.4	
T6	2002	alfalfa	12009.3	904.1	30.0	2.3	0.0	-30.0	
T6	2003	alfalfa	10717.1	698.5	26.8	1.7	48.2	21.4	
T6	2004	alfalfa	10282.2	1122.3	25.7	2.8	0.0	-25.7	
T6	2005	alfalfa	9195.2	1407.5	23.0	3.5	76.5	53.5	-28.0

Particulate organic matter

The amount of particulate organic matter in soil, a measure of the active organic matter pool, increased significantly over time in the organic, alfalfa and successional systems of the LTER, but remained relatively constant in the conventional treatment. Treatment differences in the percentage of POM in soil were noticeable as early as 1992, just three years after the start of the LTER experiment and these trends continue through 2006 (Figure 2.4). In addition to long-term cropping system effects, recent crop history also significantly influenced the amount of POM present in soil (p -value = 0.01).

Phosphorus concentrations of POM (POM-P) ranged from 0.039 to 0.088 percent, with a mean concentration of 0.053 across all treatments. Treatment differences in the phosphorus concentration of POM were significant (p -value = 0.0024) as were changes in the P concentration of POM over time (p -value = 0.0036). The P concentration of POM decreased in the conventional, alfalfa and successional treatments from 1992 to 2006, while it remained fairly constant in the organic treatment (Fig. 2.5). The application of fertilizer in 2005 did not significantly impact the phosphorus concentration of POM in 2006 (p -value = 0.1403). There was an unclear correlation between treatment and POM-P concentration (Figure 2.6). Because there has been a significant increase in the quantity of POM over time, more soil phosphorus is being stored in the POM pool for the organic and alfalfa cropping systems.

Figure 2.4 Particulate organic matter (as a percentage by weight of soil)

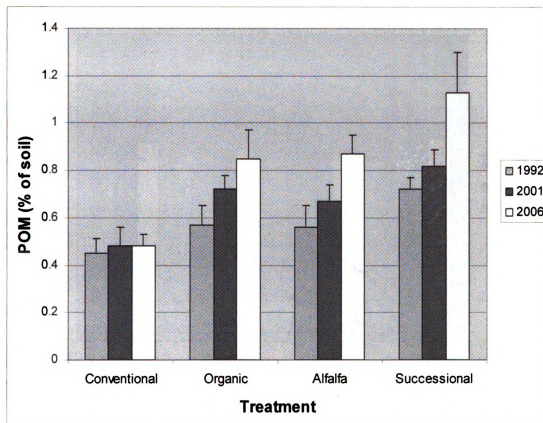


Figure 2.4 Averages plus standard errors of particulate organic matter, as a percentage of soil by weight, in four cropping systems from 1992 to 2006.

Figure 2.5 Phosphorus concentration of Particulate Organic Matter

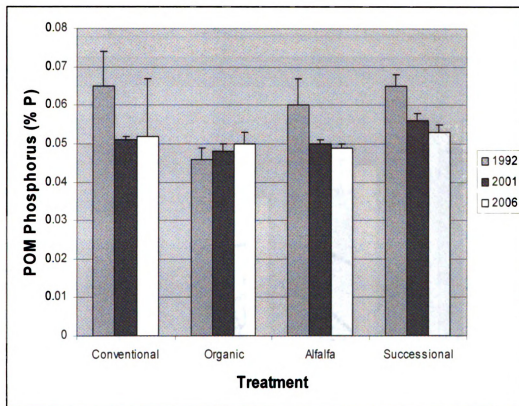


Figure 2.5 Averages plus standard errors of phosphorus content as a percentage of particulate organic matter (POM) in soil in four cropping systems of the LTER from 1992 to 2006.

Figure 2.6 Total P in the POM pool

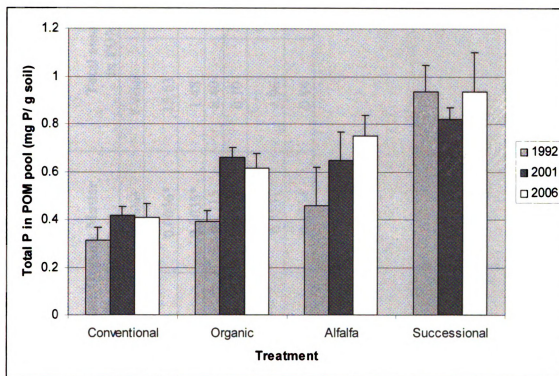


Figure 2.6 Averages plus standard errors of the total about of P found in the particulate organic matter (POM) pool in soils from the conventional, organic, alfalfa and successional cropping systems at the LTER from 1992 to 2006.

Table 2.5 ANOVA results for particulate organic matter

	Particulate Organic Matter (% by weight in soil)		Particulate Organic Matter Phosphorus		Total amount of P in POM pool	
	F-value	P-value	F-value	P-value	F-value	P-value
Treatment	9.99	< 0.0001*	4.80	0.0046*	15.63	< 0.0001*
Year	3.37	0.0714	9.26	0.0035*	1.48	0.2284
Recent crop history	4.30	0.0425*	1.23	0.2715	8.40	0.0053*
P fertilizer	1.03	0.3150	2.25	0.1391	0.19	0.6652
Treatment*Recent crop history	2.58	0.0623	1.16	0.3331	4.96	0.0039*
Treatment*year	0.39	0.7590	1.81	0.1554	0.84	0.4796

Table 2.6 POM as a percentage of soil, by weight (means +/- SEM)

Treatment	1992 <i>a</i>	2001 <i>a</i>	2006 <i>b</i>
Conventional <i>a</i>	0.45 +/- 0.06	0.48 +/- 0.08	0.48 +/- 0.05
Organic <i>ab</i>	0.57 +/- 0.08	0.72 +/- 0.06	0.85 +/- 0.12
Alfalfa <i>b</i>	0.56 +/- 0.09	0.67 +/- 0.07	0.87 +/- 0.08
Successional <i>c</i>	0.72 +/- 0.05	0.82 +/- 0.07	1.13 +/- 0.17

POM as a percentage of soil by weight, and standard deviations (n = 4) for soils (0-20") from the conventional, organic, alfalfa and successional systems. Different letters indicate significant differences ($P < 0.05$) between treatments and years.

Table 2.7 Tissue P concentration of POM (means +/- SEM)

Treatment	1992 <i>a</i>	2001 <i>b</i>	2006 <i>b</i>
Conventional <i>a</i>	0.065 +/- 0.009	0.051 +/- 0.001	0.052 +/- 0.012
Organic <i>a</i>	0.046 +/- 0.003	0.048 +/- 0.002	0.050 +/- 0.003
Alfalfa <i>ab</i>	0.060 +/- 0.007	0.050 +/- 0.001	0.049 +/- 0.001
Successional <i>b</i>	0.065 +/- 0.003	0.056 +/- 0.002	0.053 +/- 0.002

Phosphorus concentration of POM, with standard deviations (n = 4) for soils (0-20") from the conventional, organic, alfalfa and successional systems. Different letters indicate significant differences ($P < 0.05$) between treatments and years.

Table 2.8 Total amount of P in POM pool (mg/kg) (means +/- SEM)

Treatment	1992	2001	2006	Average across years
Conventional	0.313 +/- 0.055	0.415 +/- 0.037	0.409 +/- 0.057	0.391 +/- 0.037 <i>a</i>
Organic	0.392 +/- 0.044	0.660 +/- 0.040	0.615 +/- 0.062	0.0571 +/- 0.042 <i>b</i>
Alfalfa	0.457 +/- 0.160	0.648 +/- 0.121	0.749 +/- 0.087	0.680 +/- 0.658 <i>b</i>
Successional	0.936 +/- 0.111	0.820 +/- 0.051	0.937 +/- 0.166	0.914 +/- 0.101 <i>c</i>

Total amount of P in the POM soil pool, with standard deviations (n = 4) for soils (0-20") from the conventional, organic, alfalfa and successional systems. Different letters indicate significant differences ($P < 0.05$) between treatments, averaged across years.

Soybean bioassay

Average biomass of soybeans harvested from microplots ranged from 4.0-4.5 Mg/ha, but was not statistically different by cropping system (p -value = 0.31) or due to the application of phosphorus fertilizer (p -value= 0.3224) (Figure 2.7). The long term cropping system history did, however, influence tissue phosphorus concentrations of soybeans. Tissue phosphorus concentrations of the soybean pods ranged from 0.41 to 0.48 percent P, and the P concentration of the stems and leaves ranged from 0.12 to 0.2 percent P. This range is consistent with literature values (USDA, SR19). There were significant differences in the tissue phosphorus concentrations of the soybeans between the conventional, organic, and alfalfa treatments (p -value= <0.001), with the concentration of P following alfalfa being the highest (see Figure 2.8).

We also examined the different soybean plant tissue types to look at allocation of P within the plant. As expected, phosphorus concentrations of the soybean pods were much higher than in the stems and leaves (p -value = <0.001), but the P concentrations of both tissue types followed the same overall trend in terms of treatment differences.

Short term history did not influence soybean biomass or P tissue concentrations. This is reflected in soybeans planted microplots where soybeans were grown for 2 years, vs. 1 year following the general crop rotation of the LTER (p -value= 0.535) (Fig. 1).

Figure 2.7 Biomass of soybeans collected from microplot areas

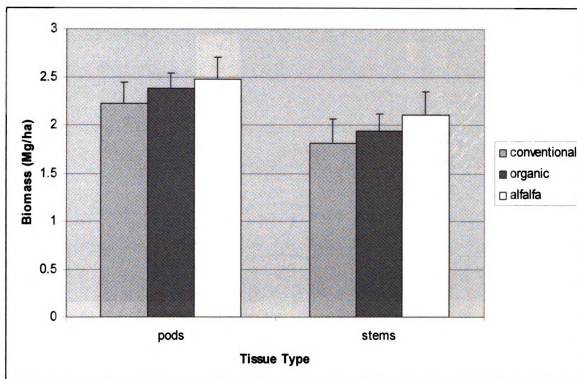


Figure 2.7 Averages plus standard errors of biomass of soybean pods and stems and leaves collected from soybean microplots in three cropping systems of the LTER in 2006.

100

100

100

Figure 2.8 Tissue P concentration of soybeans

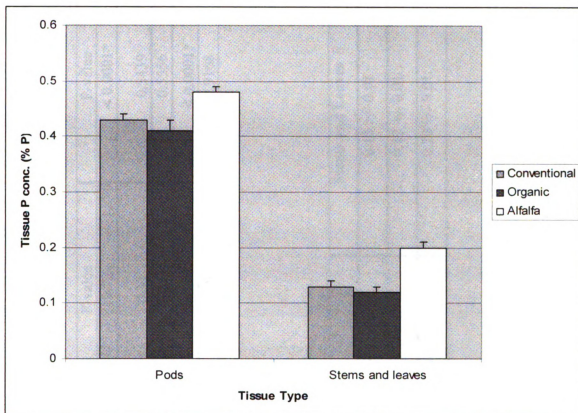


Figure 2.8 Averages plus standard errors of tissue phosphorus concentrations of soybeans grown as bioassays of phosphorus availability in conventional, organic, and alfalfa cropping systems of the LTER in 2006.

Table 2.9 ANOVA for soybean tissue P concentration

	DF	F-value	P-value
Treatment	2	19.72	< 0.0001*
Recent crop history	1	0.37	0.5439
Fertilizer	1	0.35	0.5556
Tissue Type	1	788.48	< 0.0001*
Treatment* Tissue type	2	0.09	0.9168

Table 2.10 Soybean tissue P concentration (mg/kg) (means +/- SEM)

Treatment	Pods <i>a</i>	Stems and Leaves <i>b</i>
Conventional <i>a</i>	0.43 +/- 0.01	0.13 +/- 0.01
Organic <i>a</i>	0.41 +/- 0.02	0.12 +/- 0.01
Alfalfa <i>b</i>	0.48 +/- 0.01	0.20 +/- 0.01

Tissue P concentration of soybeans, with standard deviations (n = 4) from the conventional, organic and alfalfa systems. Different letters indicate significant differences ($P < 0.05$) between treatments.

Discussion

Total and extractable inorganic soil P

Total soil phosphorus was measured to investigate differences among treatments and over time, as this is important to understand when comparing movement of phosphorus between soil pools. Unlike other studies of long-term cropping systems, (Oehl et al., 2002; Richards, 1995) which showed declines in Pt over time, the total amount of phosphorus remained relatively constant between all the treatments from 1992 to 2006 (Table 2.1), despite modest additions of phosphorus fertilizer in 2002 in the conventional treatment, and 2003 and 2005 in the conventional and alfalfa treatments (Fig. 2.8). Short term management, growing soybean for one versus two years, or adding P fertilizer in 2005, did not alter the total soil P in 2001 and 2006 (Table 2.1).

Extractable inorganic phosphorus (Bray P) declined in all treatments from the 1992 levels (Figure 2.2), which were anthropogenically high due to the large amounts of fertilizer applied to farmland prior to the start of the LTER experiment in 1989. Over time, some of this phosphorus appears to have moved into other soil pools, whereas no detectable changes were found in the total amount of soil phosphorus from 2001 to 2006. Differences in allocation of P to different soil pools were noted as early as 1995, in an earlier study of this experiment, with a greater magnitude of soil P in the organic fraction of the alfalfa and successional treatments than in the conventional and organic (Daroub et al., 2001).

Extractable inorganic P from 2006 soil samples did not reflect the same pattern observed with the bioavailability of phosphorus, as measured through the soybean

bioassay (Figures 2.3 and 2.8). Discrepancies between extractable Pi and measured bioavailability have been found in other studies as well (Cavigelli and Thien, 2003; Nuruzzaman et al., 2005). Bray P levels in 2006 were more available in the organic corn rotation than the alfalfa system, while based on the bioassay, soybean tissue P concentrations were highest following the alfalfa system. This indicates the value of multiple measures of plant available pools as well as soil extractions represent plant-nutrient availability to variable degrees.

Phosphorus inputs to the conventional, organic and alfalfa treatments have varied over the years, with only the conventional and alfalfa treatments receiving inorganic P fertilizer. Despite this, extractable inorganic phosphorus was higher in the organic treatment than in the conventional or alfalfa treatments (Figure 2.3). This is consistent with the idea that long term management is an important determinant of nutrient availability. In cases such as this site, with coarse soil and moderately high soil P, P fertilizer inputs may have limited effects on P availability.

Phosphorus budget

Based on a nine year phosphorus budget for the LTER (Table 2.4), there was a net deficit for phosphorus in the conventional, organic and alfalfa cropping systems. This deficit was reflected in the extractable inorganic phosphorus (Bray P) tests, which declined over time. Decisions about P fertilizer applications in the conventional and alfalfa systems are based on P soil test levels, and as the soils started out at an anthropogenically high level, fertilizer applications to these systems have been small,

resulting in a negative net balance. However, it is important to note that plant available P was highest in the organic system in 2006, which showed the greatest net loss of P over the nine year period. This illustrates some of the problems inherent in creating nutrient budgets, which often do not accurately reflect P transfers into organic P or sparingly soluble P pools. Watson et al. (2002) argue that studying trends in soil organic matter and nutrient analyses, particularly in organic systems, is far more useful than using chemical analyses that provide snapshots in time, because of the long-term approaches to nutrient management in most organic systems. Results from our study demonstrate changes in soil organic matter pools and P_i over time in the alfalfa and organic systems, which are not adequately explained by a simple nutrient budget.

Particulate organic matter

In agreement with previous authors (Alvarez and Alvarez, 2000; Carter, 2000), we found that particulate organic matter (POM) in our study was highly responsive to changes in management practices. In 1992, just three years after the start of the LTER experiment, the organic corn-soybean-wheat, alfalfa and successional treatments had elevated quantities of POM, as a percentage of soil by weight as compared to the conventional corn-soybean-wheat rotation. This trend grew more pronounced over time (Figure 2.4). In addition, POM samples collected from the subplots with either one or two years of soybean grown in the microplot experiment in 2006 demonstrated that short term management strategies impact POM. Quantities of POM differed significantly between microplots with soybeans grown just one year following the main rotation vs. those with two years of soybean (Table 2.5).

In the organic treatment, red clover is established as a winter cover crop in the wheat, once in three years. The years chosen for analysis of POM followed the corn phase of the rotation, and thus came a full year after red clover was incorporated. Any residual red clover biomass was likely small in comparison to corn stalk biomass entering the conventional and organic rotations. Smaller quantities of POM in the conventional treatment may be due to increased mineralization with the addition of inorganic nitrogen fertilizers. Differences between these two treatments also appear to illustrate that both quantity and quality of the residue is important when considering ways to build soil organic matter. Consistent with Drinkwater et al. (1998), leguminous cover crops appear to be an important addition to a cropping system in terms of building soil organic matter. The greater quantity of POM in the alfalfa and successional treatments is likely due to the diversity and quality of residue inputs in these systems, as well as the presence of continuous living plant cover over the winter compared to the conventional system (one in three years of winter cover) and organic (two in three years of winter cover).

Particulate organic matter phosphorus (POM-P) was not a useful predictor of the bioavailability of phosphorus, as there was little correlation with the soybean tissue P concentrations (Figures 2.5 and 2.8). The phosphorus concentration of POM was much higher in 1992 than in 2001 and 2006 for the conventional, alfalfa and successional treatments. This correlates well with the elevated quantity of extractable inorganic phosphorus in these years, which dropped significantly in subsequent years (Figure 2.2). Most likely this is because POM is highly influenced by recent vegetation, and thus plants which take up more available soil P will contribute to P-enriched POM. The concentration of P in POM in the organic treatment remained relatively constant over

time, despite a significant decline in the amount of plant available phosphorus from 1992 to 2001. This is not readily explainable.

Although in general the concentration of P in POM declined over time, the total amount of P stored in the POM pool increased over time, most notably in the organic and alfalfa treatments, as a result of increases in the total quantity of POM in soil. Thus more phosphorus is stored in an organic pool with rapid turnover, which should impact plant availability of P. These results agree with the bioavailability results from the soybean bioassay as well as the data on plant available phosphorus (Bray P) (Figure 2.6). Thus it appears that it is possible to enrich the P content of POM with the application of P fertilizer, but in terms of moving more soil P into the POM pool it is necessary to consider longer-term management and rotation practices that enhance the quantity of POM in soil.

Soybean Bioassay

The ability of legumes to enhance bioavailability of phosphorus to a subsequent crop has been demonstrated in previous greenhouse studies (Cavigelli and Thien, 2003; Kamh et al., 2002; Nuruzzaman et al., 2005), but little research has been performed relating this back to field studies. In their paper, Cavigelli and Thein (2003) argue in favor of long-term field studies that examine the effects of green manures on bioavailability of phosphorus, since the capacity of native and agricultural systems to supply P to plants from organic P mineralization develops over time and may not be detected in shorter field studies.

In our experiment, P was more bioavailable to soybeans following perennial alfalfa than following the conventional or organic corn-soybean-wheat rotations. There was no significant difference in the amount of soybean biomass collected from the conventional, organic and alfalfa treatments, but the tissue concentrations of P were much higher in soybeans from the alfalfa plot. This suggests that the presence of a perennial legume, as well as long term management history positively impacted the availability of phosphorus. Notably, the management and rotational history appears to have been more important than application of phosphorus fertilizer in determining the availability of phosphorus for plant uptake. Both conventional and alfalfa treatments had phosphorus fertilizer applications in the last five years, but only in the alfalfa systems was P highly available, despite consistent P removal in alfalfa hay biomass. There were no significant differences in P concentration between soybean subplots that did or did not receive P fertilizer. Short term management history (one vs. two years of soybeans) also did not affect the bioavailability of P.

Our findings are consistent with previous research by Cavigelli and Thien (2003) who demonstrated phosphorus was more bioavailable to sorghum following incorporation of legume residues such as alfalfa and red clover. In their study, alfalfa had the highest P concentration prior to incorporation, and yields of sorghum were highest following alfalfa suggesting that it was best able to facilitate movement of P into the soil organic pool. Previous research conducted in the alfalfa treatment of the LTER (Daroub et al., 2001) documented an increase in the organic phosphorus fraction of the soil over time, which they attributed to the turnover of the extensive alfalfa root system (Daroub et al., 2001).

Conclusion

Two lines of evidence are consistent with a legume role in improving P bioavailability to a subsequent crop in the alfalfa and organic cropping systems. Phosphorus was most bioavailable to soybeans following alfalfa, and equally bioavailable to soybeans following the conventional and organic rotations. Unlike the conventional system, the organic rotation never received inorganic phosphorus inputs and thus it is plausible that increased P availability was aided by the red clover cover crop in the rotation. Extractable inorganic P (Bray P) was most related to long-term crop rotation, while in contrast, the phosphorus concentration of POM seemed to be related to fertilizer applications. However, the total amount of P located in the POM soil pool was closely related to the long-term management of the cropping system, with increasing quantities of P moving into the POM pool over time in the organic and alfalfa treatments. This is due to the increase in the total quantity of POM in the organic and successional treatments. Apparently, the quality not quantity of residue input was an important factor in increasing the percentage of soil POM.

Overall, these findings argue for an integrated approach to phosphorus nutrient management in low-input or organic agricultural systems, which uses a variety of legumes to improve the bioavailability of P and build up P in soil pools with rapid turnover, such as POM-P. Our results also demonstrate that organic systems which integrate legumes can be sustainable over a long time period with regard to soil P.

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