A Thesis

entitled

Can we reduce phosphorus runoff into Lake Erie by stimulating soil biota?

by

Jessica R. Susser

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Biology

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An Abstract of

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A principle driver of water-polluting harmful algal blooms (HABs) in agricultural watersheds is fertilizer phosphorus (P) runoff from farm fields. Because P is essential to plant growth, eliminating P application is infeasible. However, much of the P that is added to soils as fertilizer binds tightly to soil particles and is relatively unavailable to plants. In natural systems, microbial and faunal decomposers can increase soil P availability to plants. In agricultural systems, stimulating these organisms may help maintain P availability with decreased P application rates, thereby increasing P application efficiency while reducing runoff potential.

We tested the hypothesis that stimulating soil fauna with sodium (Na^+) and microbes with carbon (C) would increase soil P availability to plants. We added corn stover and Na^+ solution to plots in conventionally-managed corn fields in Northwest Ohio. Stover treatments increased microbial biomass and activity and Na^+ and stover combined increased soil faunal activity. However, even in both control plots and plots with stimulation of soil microbes and fauna, soil biological activity was low, - and was not correlated with P availability. Therefore, in fields with low levels of decomposer

activity, organisms may play a limited role in soil P cycling. In these types of ecosystems, treatments to stimulate decomposers already in those systems may be ineffective in reducing P runoff potential, at least in the short term.

Acknowledgements

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List of Abbreviations

BG β-1,4-glucosidase

C .....Carbon

DOC .....Dissolved Organic Carbon

LAP .....Leucine Amino Peptidase

N.....Nitrogen
Na^+ .....Sodium
NAG.....β-1,4-N-acetyl-glucosaminidase

P .....Phosphorus
PO_4^{3-}.....Phosphate
PHOS .....Phosphatase

Chapter 1

Introduction

In temperate agricultural watersheds, excessive phosphorus (P, mostly in the form of PO_4^{3-}) fertilization of crop fields has contributed to harmful algal blooms and deterioration of freshwater resources. This includes the eutrophication of Lake Erie and the resulting water crisis of 2014 in Toledo, OH that led to the shutdown of municipal drinking water for over half a million people (Henry 2014). Between 1970 and 2010, PO_4^{3-} inputs into the Maumee watershed, the largest contributor to P inputs into Lake Erie, have been reduced by about one third (Powers et al. 2016). However, despite decreased PO_4^{3-} inputs, PO_4^{3-} export in the Maumee watershed has not decreased during this time period (Powers et al. 2016). This is partially because accumulated PO_4^{3-} from historical fertilizer applications continues to enter the watershed. One method with potential to maintain PO_4^{3-} availability to crops while decreasing PO_4^{3-} in these accumulated pools is to stimulate the activities of soil microbial and faunal decomposers that cycle soil PO_4^{3-}. These organisms' activities are often limited by the lack of micro (e.g. Na^+, Mg^{2+}) (Kaspari and Yanoviak 2009, Joern et al. 2012, Ott et al. 2014) or macro

(e.g. C, N, P) (Zak et al. 1994, Fierer et al. 2009, Kallenbach and Grandy 2011) nutrients. In particular, microbes in agricultural fields in Northwest Ohio are likely C limited due to depletion of soil organic matter and residue export (Fierer et al. 2009, Kallenbach and Grandy 2011). Soil fauna, on the other hand, may be attracted to Na in the soil because in inland ecosystems, soil Na concentrations do not meet faunal needs (Kaspari et al. 2010, Joern et al. 2012). We therefore hypothesize that alleviating C and Na^+ limitation in these fields may increase the potential for soil biota to cycle PO_4^{3-}.

1.1 Soil PO_4^{3-} Chemistry and Legacy P

Phosphate has the potential to bind to a variety of different sites in the soil. PO_4^{3-} availability to both biota and leaching in the soil depends on which binding sites the PO_4^{3-} attaches to, which, in turn, depends on the soil pH, geochemistry, biological activity, and PO_4^{3-} source (Cross and Schlesinger 1995, Nziguheba et al. 1998, Hinsinger 2001). Legacy P^-accumulations are caused by this PO_4^{3-} binding in soils. Typically, when PO_4^{3-} is added to agricultural fields, much of the PO_4^{3-} binds to sites in soil particles that have high affinity for PO_4^{3-} and PO_4^{3-} becomes relatively inaccessible to plants (Barrow and Shaw 1975, Plante 2007, Barrow 2015). As a result, some PO_4^{3-} on these sites remains bound to soil particles from season to season, and, if the soil is continuously fertilized, PO_4^{3-} builds up in the soil and becomes "legacy P" (Kleinman et al. 2011). After years of PO_4^{3-} fertilization, soils can become P-saturated and then cannot bind additional PO_4^{3-} (Barrow and Debnath 2014). Once soil is PO_4^{3-} saturated, less PO_4^{3-} fertilizer is necessary to maintain crop yields, and excess PO_4^{3-} is more likely to runoff, entering into and polluting waterways (Barrow 2015).

Liberating legacy P^- from inaccessible pools could reduce the need for additional fertilizer without reducing plant-available PO_4^{3-} supply. Depending on the soil type and PO_4^{3-} concentration, legacy P^- alone may be sufficient to sustain crop yields for 2-25 years (Rowe et al. 2015). Increasing the rate at which legacy P is released may extend the amount of time soils can maintain crop yields without additional fertilizer input. We hypothesized that the rate of legacy P^- release and the relative concentration of plant-available PO_4^{3-} in agricultural soils can be increased by stimulating decomposer activity.

In order to measure the impact of soil biota on the relative availability of soil PO_4^{3-} to plants, it is necessary to separately quantify inaccessible PO_4^{3-} pools vs. accessible PO_4^{3-} pools, which requires PO_4^{3-} fractionation (e.g. Hedley fractionation, Hedley et al. 1982). Only some of the PO_4^{3-} pools in a P fractionation are readily available to plants (Cross and Schlesinger 1995). We extracted some of the fractions that are indexes of plant availability and potential to leach from the soil as well as total soil PO_4^{3-} to test how stimulating biota would impact relative soil PO_4^{3-} availability (see Table 1.1 and methods).

Table 1.1: Descriptions of nutrient pools and words used for each pool.

Pool	Method	Description
Fraction(s) Relatively Available to Plants		
Cumulative Exchangeable	Resin strips (Saggar et al. 1990)	A relative metric of nutrients that are available for cation exchange over the course of deployment.
Water-soluble	Extracted with H_2O (Darrouzet-Nardi and Weintraub 2014)	Water extracts nutrients that are in soil pore water or loosely bound to soil particles, and this pool is correlated with PO_4^{3-} available in runoff (Pote et al. 1996). Much of this PO_4^{3-} should also be immediately available to soil organisms, although some will be spatially inaccessible (Darrouzet-Nardi and Weintraub 2014).
Salt-adsorbed	K_2SO_4 extractable - H_2O extractable nutrient (Darrouzet-Nardi and Weintraub 2014)	K_2SO_4 extracts nutrients that are adsorbed to the soil particle surfaces through ion exchange, including those that are spatially inaccessible. Most of the adsorbed pool will be available to organisms via cation exchange, although some will be spatially inaccessible
Olsen	Extracted with $NaHCO_3$ solution (Olsen-P) (Olsen et al. 1954, Cross and Schlesinger 1995)	Often considered to be "plant-available" PO_4^{3-}, Olsen-P is widely used and correlated with corn yields in the American Midwest (Mallarino 1997). It releases some PO_4^{3-} in Ca-P complexes and PO_4^{3-} on ion exchange sites. (Olsen et al. 1954). This test has been validated on soils with pH >5. (Olsen et al. 1954). Note that in this study, we assume that all PO_4^{3-} extracted by K_2SO_4 is also extracted using the Olsen protocol.
Alkaline-adsorbed	Olsen PO_4^{3-} - Salt-adsorbed PO_4^{3-} (Cross et al. 1995)	The proportion of Olsen PO_4^{3-} that is likely accessible to plants but not loosely adsorbed to soil particles or extractable by K_2SO_4

Pool	Method	Description
Fraction(s) Relatively Unavailable to Plants		
Bound	Total- PO_4^{3-} - Olsen PO_4^{3-}	P that is tightly bound to soil particles and likely relatively inaccessible to plants (Cross et al. 1995). Includes PO_4^{3-} sorbed by Al and Fe, bound in primary minerals, stably bound to Ca^{2+}, and occluded in soil aggregates, etc.
Microbial Fraction(s)		
Microbial Biomass	Chloroform fumigation and subsequent extraction in 0.5 M K_2SO_4 - K_2SO_4 extractable - PO_4^{3-}	Nutrients within microbial biomass.
Total		
Total	Soil is digested using strong acids while heated	Includes all nutrients, including those bound to soil particles, adsorbed pools and pools that are complexed and precipitated by secondary minerals (Al, Fe)

1.2 Effects of Soil Biota on P-cycling in Farm Fields

Heterotrophic soil organisms play an important role in PO_4^{3-} cycling by releasing PO_4^{3-} from organic matter or soil particles or immobilizing PO_4^{3-} from soil solution. Understanding how these organisms affect soil PO_4^{3-} cycling and plant availability can help us to manage agricultural PO_4^{3-}, especially legacy P, more efficiently (Richardson and Simpson 2011). One method to determine how soil biota affect soil P cycling is to increase their biomass and activities by alleviating C and Na^+limitations. We examined the effects that increasing the activities of two categories of soil organisms who exert

controls on PO_4^{3-} cycling: 1) microbes, which include bacteria and fungi, and 2) soil meso and macrofauna (hereafter fauna)—which typically include arthropods, earthworms, and other soil invertebrates has on the proportion of PO_4^{3-} in the soil that is plant available.

1.2.1 Microbial Effects on PO_4^{3-} Cycling

Microbes can affect soil PO_4^{3-} concentrations through several different mechanisms: they can mineralize PO_4^{3-} by decomposing organic matter (Oehl et al. 2004, Bünemann 2015), solubilize phosphate from minerals (Richardson and Simpson 2011), decrease PO_4^{3-} adsorption onto mineral particles by changing the particle surface charge (Hong et al. 2015), immobilize PO_4^{3-} in their biomass (Oberson and Joner 2005, Achat et al. 2010), and affect plant PO_4^{3-} uptake through symbiotic relationships and hormonal impacts on root architecture (Bonkowski 2004) (Figure 1-1). Although PO_4^{3-} that is released into the soil solution by microbes is the most readily available to plants in the short term, it also has the highest potential to leach from the soil. In contrast, PO_4^{3-} that is immobilized in microbial biomass may be unavailable to plants in the short term (days), but, in the long term (months-years), may be less likely to leach from soils or enter sparingly-available PO_4^{3-} pools (Olander and Vitousek 2004). The relative medium-term (days – weeks) availability of microbial P is supported isotopic tracer studies indicating that PO_4^{3-} held in microbial biomass turns over in 2-9 days (Oberson et al. 2001, Oehl et al. 2001, Achat et al. 2010) as every time microbial biomass turns over, plants have a chance to access the microbial P. Therefore, while microbial activities have

the potential to strongly influence soil PO_4^{3-} availability, the potential impact of these activities is not always straightforward.

Microbes exert a strong control on soil PO_4^{3-} cycling (Chen et al. 2008, Achat et al. 2012), but the precise effects of microbial activities on the distribution of PO_4^{3-} in different pools depends on the system and microbial community composition. Over a broad range of natural ecosystems, inorganic PO_4^{3-} availability is correlated with soil organic C content, which, in turn, is correlated with microbial activities and biomass (Achat et al. 2016). Although the mechanism for this increase in PO_4^{3-} availability with soil organic C content may, at least in part, be physio-chemical changes in the soil associated with increased C content, it is likely also driven by increasing biological activities (Achat et al. 2016). For example, in Orthieutric Albeluvisols and Albic Luvisols in a boreal forest ecosystem in Siberia dominated by *Populus tremula*, *Abies sibirica*, and *Aconitum septentrionale,* increased microbial activities were correlated with increased inorganic PO_4^{3-} availability (Achat et al. 2012). However, inorganic PO_4^{3-} does not always increase with increased biomass. When grassland systems were converted to pine plantations, decreased microbial biomass in the plantations are correlated with increases in inorganic PO_4^{3-} availability (Chen et al. 2008). The authors proposed that this is likely caused by a shift in the microbial community to include a higher fungi:bacteria ratio, as fungi tend to be more efficient at mineralizing PO_4^{3-} than bacteria. Therefore, in soil ecosystems, while microbes do exert a strong control on soil PO_4^{3-}, the direction and magnitude of this control is system and community dependent.

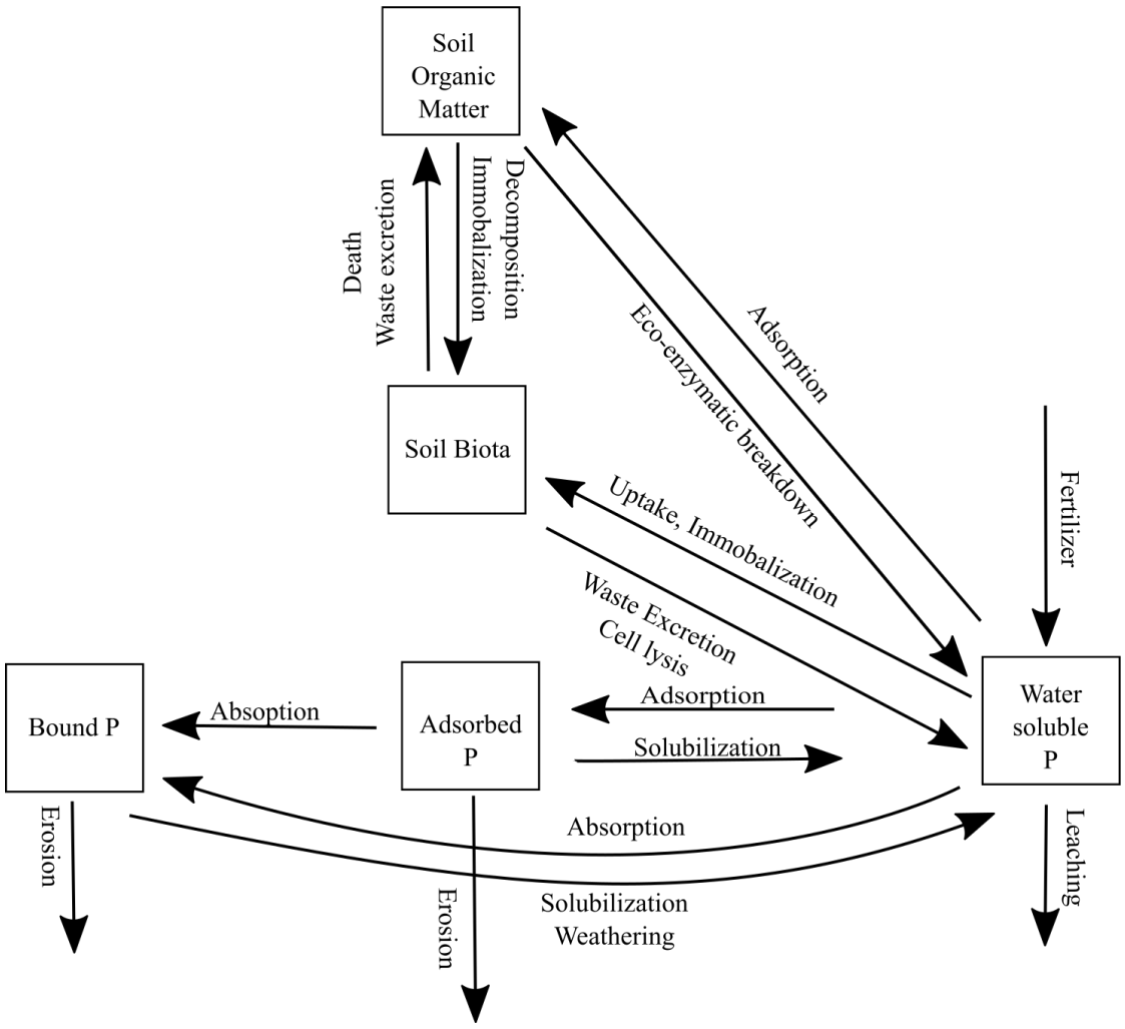

Figure 1-1: A conceptual box and flow diagram of how PO_4^{3-} moves between the PO_4^{3-} pools measured, with particular emphasis on how soil biota should affect PO_4^{3-} movement. Note that for simplicity, the absorbed PO_4^{3-} box includes both alkaline and salt-adsorbed PO_4^{3-} (see Table 1.1). The cumulative exchangeable PO_4^{3-} is theoretically all of the PO_4^{3-} that passes through the water-soluble pool at that location over the course of the season. See Table 1.1 for descriptions of the different pools.

1.2.2 Faunal Decomposers Effect on PO_4^{3-}-Cycling

Soil fauna mainly affect soil PO_4^{3-} concentration through direct and indirect effects on decomposition (Petersen and Luxton 1982, Seastedt 1984). Soil fauna, particularly detritivores, affect soil PO_4^{3-} cycling by changing the distribution of nutrients

via altered soil aggregate structure (e.g. earthworms creating casts) (Chapuis-Lardy et al. 2011), by changing nutrient distribution in soil through activities such as mound building (Beare et al. 1995, Chapuis-Lardy et al. 2011), shredding organic matter (Seastedt 1984), or consuming microbes and mineralizing nutrients held in microbial biomass through excretion (Petersen and Luxton 1982, Bonkowski 2004). Higher trophic levels, such as faunal predators, are generally C limited and will excrete mineralized N and PO_4^{3-} as they eat to obtain adequate C nutrition (Anderson et al. 1983). Through these mechanisms, soil fauna from a variety of functional groups can impact the spatial and chemical distribution of PO_4^{3-} in soils.

Most studies of soil fauna effect on nutrient cycling and decomposition focus on the effect of soil fauna exclusion on the rate of litter mass loss and, across a variety of ecosystems, litter mass loss rates during decomposition are 35% lower, on average, when soil macro fauna are excluded (Zhang et al. 2015). Although the magnitude of fauna effects on litter decomposition is mediated by climate and litter quality and this effect may be limited when faunal activities are limited (e.g. cold, drought), the presence of soil fauna increases decomposition rates under most conditions (Tian et al. 1992, Wall et al. 2008, García-Palacios et al. 2013). This trend holds true in agricultural systems. Studies comparing native grassland and conventional tillage and no-till agricultural fields have found that decomposition rates are correlated with soil fauna abundance (Reddy et al. 1994, Domínguez et al. 2010). For example, in a decomposition study of five different agricultural litters in an Oxic paleustalf soil in a tropical agricultural system in Nigeria,

litter bags that allowed the passage of macrofauna released N, PO_4^{3-}, Ca^{2+}, and Mg^{2+} at a higher rate than litterbags that did not allow the passage of these fauna (Tian et al. 1992).

These results provide evidence that fauna have the potential to affect PO_4^{3-} cycling in agricultural soils. However, this effect on mass loss may or may not translate to changes in soil nutrient concentrations. In fact, though soil fauna increase the rate of litter mass loss, they may not affect the rate of C mineralization, though relatively few studies have looked at this (Frouz et al. 2015). Whether this the same trend also holds true for PO_4^{3-} mineralization rates and how these organisms interact with soil microbes to control the availability of PO_4^{3-} to plants in crop fields is still unclear (Chapuis-Lardy et al. 2011, García-Palacios et al. 2013).

1.3 Effects of C and Na^+ Limitation on Soil Organisms

1.3.1 Microbial C Limitation

Microbial C limitation and the positive response of microbes to C amendments in agricultural systems have been well documented (reviewed in Zak et al. 1994, Fierer et al. 2009, Kallenbach and Grandy 2011). Conventionally managed agricultural fields are often C depleted due to intensive disturbance and export of plant materials. As a result, these soils tend to have low microbial biomass and the nutrient cycling ecosystem services provided by microbes is diminished (Diacono and Montemurro 2010, Kallenbach and Grandy 2011). As these systems are C limited, addition of C-rich substrate can increase microbial activities.

The stimulation of microbes with C has, indeed, been shown to stimulate microbial P-cycling in agricultural soils. In a study of the effects of C-substrate additions

on microbial P-uptake in maize fields with Kandiudalfic Eutrudox soils in Kenya, amendments of glucose or plant litter increased microbial P-uptake. In fact, microbial PO_4^{3-} concentration was more dependent on soil C concentrations than on soil PO_4^{3-} concentration (Bünemann et al. 2004a). Furthermore, fertilization of soils with C substrates can change the amount of PO_4^{3-} in different soil pools. In a laboratory incubation of Udic Haploboroll agricultural soils from Saskatchewan, Canada, cellulose and N additions decreased 0.5 M $NaHCO_3$ extractable inorganic-P while increasing 0.5 M $NaHCO_3$ and sonicated/0.1 M NaOH extractable organic-P (Hedley et al. 1982). This indicates that increasing soil C concentrations increases the amount of PO_4^{3-} that can be held in microbial biomass. In addition, stimulating microorganisms with C prevented the loss of extractable PO_4^{3-} in soils, most likely because PO_4^{3-} held in microbial biomass was released into soil gradually over time and was therefore less likely to enter pools that were tightly bound and therefore inaccessible to plants (Hedley et al. 1982). We therefore predict that stimulating soil microbes with C will decrease the proportion of P that is inaccessible to plants.

1.3.2 Sodium Limitation of Decomposer Fauna

In addition to macronutrient limitation, soil fauna are limited by micronutrients such as Ca^{2+}, Mg^{2+}, and Na^+ (Kaspari and Yanoviak 2009, Joern et al. 2012, Ott et al. 2014). Unlike other micronutrients, Na^+ is required by animals but not plants to maintain cellular osmotic gradients (Kaspari et al. 2009). Soil fauna, particularly detritivores, may be limited by Na^+ when levels in plant tissues do not meet demand (Kaspari et al. 2014). Therefore, detritivores and herbivores must obtain Na^+ from non-plant sources, such as

soils. Soil Na^+ concentrations tend to be higher near coastlines where Na^+ deposition is relatively high and lower on highly-weathered, inland soils where costal deposition is low (Kaspari et al. 2008). Therefore, Na^+ can limit decomposer fauna in inland ecosystems.

Several studies have found support for sodium limitation of soil fauna. Sodium fertilization in the Ecuadorian Amazon (Kaspari et al. 2014) and in Chinese sub-tropical forests (Jia et al. 2015) increased macroarthropod decomposer activity, increased decomposition rates, may have increased fungal hyphae density, and had mixed effects on microbial activities. Generally, herbivore (Joern et al. 2012) and decomposer (Ott et al. 2014) fauna can be stimulated by increased Na^+, but predator fauna are not (Ott et al. 2014). This suggests that organisms that feed primarily on plant matter may have a Na^+ deficient diet, but organisms with other food sources may not (Ott et al. 2014).

Although fewer studies have been done in temperate systems, ants in Western Massachusetts and herbivores in Eastern Nebraska are limited by Na^+ (Kaspari et al. 2010, Joern et al. 2012). According to coarse-scale Na^+ measurements, the soils of western Massachusetts and Eastern Nebraska should have broadly similar Na^+ concentrations to soils in NW Ohio (Smith et al. 2014). In additional, a preliminary experiment in Wood County, Ohio, in agricultural corn and soybean fields in Mesic Mollic Epiaqualf (Web Soil Survey) soils also suggest that Na^+ may attract soil fauna (Pelini 2015, unpublished data). In this experiment, litter bags of mixed oak and aspen leaves were soaked in either 1% NaCl solution or water and left in the field for 48 hours. The bags were then harvested and the fauna that had colonized the bags were extracted. More invertebrates colonized the NaCl soaked bags than the water-soaked bags (Pelini

2015, unpublished data), indicating that Na^+ may indeed be limiting to soil fauna in NW Ohio crop fields. We therefore predicted that Na^+ amendments would increase soil meso- and macro-fauna biomass and activities.

1.4 Goals and Objectives

Our goal was to determine whether increasing soil biological activities of soil organisms with Na^+ and/or plant litter additions affects the release of soil and detrital PO_4^{3-}. We attempted to increase microbial biomass by adding corn stover—a relatively C-rich substrate, and to increase faunal biomass by adding Na^+. We hypothesized that increasing microbial and faunal activities would correlate with an increase in the proportion of soil PO_4^{3-} available for plant uptake and a decrease in the concentration PO_4^{3-} in sparingly available pools, which include legacy P. Alternatively, soil organisms may immobilize PO_4^{3-}, decreasing the amount of soluble PO_4^{3-} immediately available for uptake (see conceptual diagram, Figure 1-1). However, PO_4^{3-} in soil organisms is likely relatively available to plants because of its 2-9 day turnover rate (Oberson et al. 2001, Oehl et al. 2001, Achat et al. 2010) as every time microbial biomass turns over, PO_4^{3-} is released into solution and can be taken up by plants. Therefore, though PO_4^{3-} in microbes is temporarily unavailable, it may be released into solution gradually over time and these PO_4^{3-} pools may be an important long-term (weeks-months) PO_4^{3-} source for plants. Thus, we predicted that the net result of alleviating C and/or Na^+ limitations would be increased PO_4^{3-}-liberating activities by soil decomposer organisms, thereby increasing relative soil PO_4^{3-} availability.

Chapter 2

Methods

2.1 Field Experiment:

2.1.1 Site Description

Our experiment was conducted in four crop fields in Wood County, Ohio (N 41° 27', W 83° 30') within the watershed of the Western Basin of Lake Erie. The clay-loam soil in the fields is in the Hoytville series, classified as a Mesic Mollic Epiaqualf (Web Soil Survey). The soil contains an average of 2.05% organic matter (preliminary measurement, analyzed by Spectrum Analytical Inc., Washington Court House, OH, USA) and an average pH of 6.7, although pH values varied between 5.1 and 8.3 (see below for description of measurement). Average annual precipitation in the region is 843 mm and average summer temperature is 22° C (Natural Resources Conservation Services Water and Climate Center 2002). The fields are conventionally managed by the same farmer, cycling between corn (*Zea mays*) and soybeans (*Glycine max*) on a two-year rotation. All fields were planted with corn for the duration of our field experiment. The fields were fertilized with 28% nitrogen (a 2:1:1 mixture of urea, ammonium, and nitrate)

at a rate of 349 liters per hectare during the week of June 6th, 2016. No phosphate fertilizer was added for the 2016 growing season. The liming history of these fields is unavailable.

2.1.2 Experimental Design

We applied four treatments: Na^+, corn stover, both stover and Na^+, and a control with no amendments. In each field, three 2 m x 2 m blocks were set up at least 10 m from any other block. Within each block, we established four 0.5 m x 0.5 m plots, each separated from the other plots by at least 1 m (Figure 2-1). Plots separated by corn rows had two rows of corn between them to maintain the 1 m minimum separation between plots. The plots were set up in-between corn rows to minimize microtopographical variation and to minimize interference with actively growing corn plants. We randomly applied one of the four treatments to each of the plots in a block, ensuring that each block received all four treatments. Each of the four fields had three blocks with three replicates of each treatment, resulting in a total of 48 plots with 12 replicates of each treatment. Treatments were deployed on July 8th and 9th, 2017 and the final harvest took place on September 30th, 2017, for a total experimental time of 12 weeks.

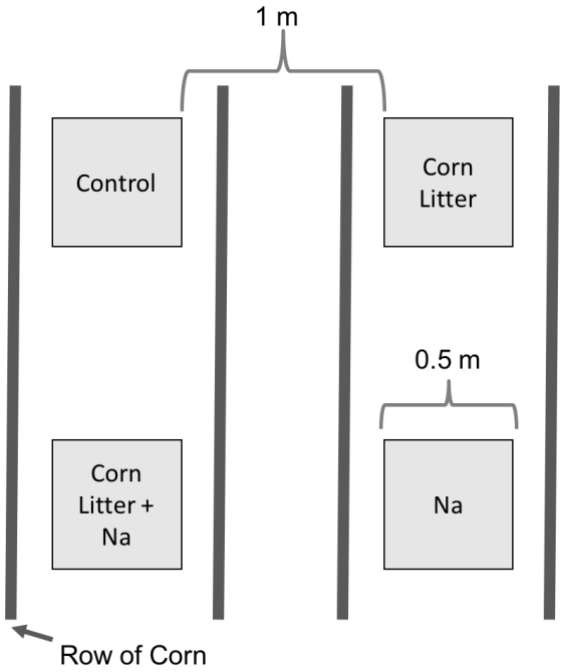

Figure 2-1: The layout of the plots within one block. Each plot was 0.5 m x 0.5 m and each block was 2 m x 2 m. Plots were arranged in a square and separated by 1 m. One of four treatments were randomly applied by blindly picking a pin flag that was labeled with one of the four treatments. Each block received all four treatments.

One liter of 0.72 M NaCl was added to each Na^+ amendment plot using a hand pump garden sprayer (Chapin 20000 1-gallon lawn sprayer, Chapin International Inc. Batavia, NY). Assuming the solution infiltrated the top 5 cm of soil and an estimated bulk density 1.32 g per cm^3, each gram of soil received 1 mg of Na^+. Plots without Na+ addition were watered with one liter of water to ensure that all plots received an equal amount of water.

Corn stover was obtained from a farm in Defiance County in NW Ohio. The stover was harvested in the fall of 2015 and stored indoors overwinter. All corn litter was

the SQC10715 variety, obtained from Shininger's Quality Crop Seed in Fulton, OH. We ground the stover with a meat grinder, a garden mulcher, and/or a coffee mill, then sieved it through a 5 mm sieve. Each plot received 169 g of corn stover raked into the top 5 cm to simulate disking in, so that the top 5 cm of each plot received an average of 1 g of litter for every 100 g of soil, based on our bulk density estimate of 1.32 g per cm^3. Plots without stover addition were raked in the same manner as plots with stover addition to ensure that all plots were disturbed by the experiment the same amount.

2.1.3 Soil Sampling

We sampled soils at three time points: 5-6 days, 9 weeks, and 12 weeks after treatment application. In each plot, we collected two samples of the top 5 cm of soil using a 5 cm diameter bulb planter. We focused on the top 5 cm of soil because PO_4^{3-} tends to be concentrated in the top 5 cm in fields such as this one (Johnson 2013). After collection, all soils were hand homogenized for 5 minutes and a 10 g subsample was immediately weighed out for moisture content determination, with the remainder stored at 20° C until analysis (within 48 h). To measure pH, we mixed 1 g of air dried soil with 10 mL of nanopure water and then allowed it to equilibrate for 10 minutes before measurement.

2.1.3.1 Cumulative Phosphate Availability

Ion exchange resins are used to measure cumulative exchangeable nutrient concentrations *in situ* (Binkley and Matson 1983, Saggar et al. 1990). At a random point in each plot, we installed one 2 cm wide x 5 cm long anion and one cation exchange resin strip (GE Power and Water ion exchange membranes; Maltz Sales, Foxborough, MA,

anion # AR204SZRA-MKIII and cation # CR67-MKIII) perpendicular to the soil and with the top flush with the soil. Resin strips were installed during plot set-up (July 8th and 9th 2016) and harvested 30 days later. One day after the first strips were harvested, new strips were installed and were harvested 71 days later during the final plot harvest. We left resin strips in the ground for a shorter time period at the beginning of the season because the soil had been recently fertilized and had higher nutrient concentrations. Except for the one day between the first harvest and the second installation, resin strips were in the plots for the duration of the experiment. After the strips were removed from the soil, they were rinsed with deionized water, shaken on an orbital shaker at approximately 120 rpm in 35 mL of 2 M KCl for one hour, then vacuum filtered through Whatman #1 filter paper. Extracts were stored frozen at -20° C until analysis for nutrients as described below.

2.2 Laboratory Analyses

2.2.1 Soil Extractions

To measure nutrients that are bound at varying strengths to soil particles, we extracted each sample in three different solutions: water (extracts nutrients not adsorbed to ion exchange sites), 0.5 M potassium sulfate (K_2SO_4) (extracts nutrients adsorbed to cation exchange sites), and 0.5 M bicarbonate ($NaHCO_3$) adjusted to a pH of 8.5 (Olsen P, a more comprehensive metric of available soil P than water or K_2SO_4 extraction; see Table 1.1). The PO_4^{3-} that is extracted with H2O is water soluble and correlated with PO_4^{3-} concentration in runoff (Pote et al. 1996). K2SO4 should extract a fraction of P that is adsorbed to soil particles. While water and K_2SO_4 exactions are sufficient to

extract soil nitrogen, they are not adequate for extracting phosphorus because PO_4^{3-} binds more tightly to soils than most bio-available forms of N or C. We chose to use Olsen bicarbonate extractions as they are widely used as an indicator of plant-available PO_4^{3-} in the Midwestern US and should provide a relative index of the amount of PO_4^{3-} available to plants. Water and K_2SO_4 extractions were conducted following a modified version of the method of Weintraub et al. (2007). In brief, we shook 5 g of soil on an orbital shaker at approximately 120 rpm for one hour with 25 mL of solution (either nanopure water or 0.5 M K_2SO_4 solution) for 1 hour and then vacuum filtered the mixture through Whatman #1 filter paper. For the $NaHCO_3$ extraction, we shook 1 g of soil with 20 mL solution for 30 minutes (Olsen et al. 1954) on an orbital shaker and then vacuum filtered through Whatman #1 filter paper. Extracts were frozen at -20°C for subsequent nutrient analyses. See Table 1.1 for descriptions of the nutrient pools captured by these different extraction methods.

2.2.2 Chemical Analyses

We analyzed all soil and resin strip extracts for PO_4^{-3}-P, dissolved organic C (DOC), and total dissolved N (TDN). Phosphate-P was measured using a malachite green colorimetric microplate assay (D'Angelo et al. 2001). We then analyzed the microplates for absorbance at 630 nm using a BioTek Synergy HT microplate reader (BioTek Instruments Inc., Winooski VT, USA). PO_4^{3-} concentration is reported in µg PO_4^{3-}-P g^{-1} dry soil. To analyze for DOC and TDN, we extracted 5 g subsamples of soil from each plot in 0.5 M K_2SO_4 as described above, diluted the extracts at a rate of 10:1, and analyzed the diluted samples using a Shimadzu TOC-Vcpn total organic carbon analyzer

with a total N module (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). DOC and TDN concentrations are reported as µg DOC or TDN per gram dry soil.

Total soil P and Na^+ were analyzed on dried soil samples by Ward Laboratories Inc. (Kearney, NE, USA). Total Na^+ and P are reported in µg g^{-1} dry soil. Total soil C and N were measured by the United States Department of Greenhouse Production Research Group (USDA-GPRS) in Toledo, OH using an Elementar Vario Micro Cube Select (Elementar, Langenselbold, Germany).

2.2.3 Microbial Analyses

2.2.3.1 Microbial Biomass C, N, and P

Samples used to measure microbial biomass C, N, and PO_4^{3-} content were prepared using a modified version (Scott-Denton et al. 2006) of the chloroform fumigation-extraction method (Brookes et al. 1985). 5 g subsamples of soil were each separately fumigated with 2 mL chloroform in sealed 250-ml Erlenmeyer flasks for 24 hours. These fumigated samples were then extracted with 0.5 M K_2SO_4 and the extracts were analyzed for PO_4^{3-}, DOC, and TDN as described above. The difference in PO_4^{3-}, DOC, and TDN content between the fumigated and non-fumigated samples is assumed to be the concentration of PO_4^{3-}, DOC, and TDN released from biomass under chloroform fumigation. Due to the high signal: noise ratio of this method, several samples had microbial biomass C, N, or PO_4^{3-} below detection. These samples were excluded from further analysis. There is not a known extraction efficiency coefficient (K_{EC}, K_{EN}, K_{EP}) for these soils, so none was applied to these measurements. Therefore, microbial biomass C, N, and PO_4^{3-} should be considered relative, rather than absolute, measurements.

2.2.3.2 Microbial Respiration

Within 24 hours of soil harvest, we placed 20 g of soil into 236 mL mason jars (Ball half pint wide mouth canning jars, Jarden Corp., Rye, NY, USA) that had lids with septa installed, adjusted the soils to 55% of water holding capacity (determined during preliminary experiments and based on estimation of moisture content of a subsample of field moist soils by repeatedly drying soils in a microwave on low power for short intervals until they reached a constant mass), capped the jars loosely, and incubated the jars at 20 °C overnight.

Prior to respiration measurements, the samples were removed from the incubator and vented using a small handheld fan to ensure that the CO_2 concentration in the jars was equilibrated with ambient air. We then sealed the jars and incubated them at 20 °C for approximately four hours. We measured microbial respiration by sampling the carbon dioxide (CO_2) in 2 mL head-space samples collected through the septa in the jar lids using a Li-820 Infrared Gas Analyzer (LI-COR Biosciences, Lincoln NE, USA). The Li-820 was calibrated with two CO_2 standards: 2500 and 5000 ppm.

2.2.3.3 Enzyme Assays

We quantified the activities of ecoenzymes to determine relative activities and nutritional needs of soil microorganisms. To this end, we measured the activities of four ecoenzymes: β-1,4-glucosidase (BG), β-1,4-N-acetyl-glucosaminidase (NAG), leucine amino peptidase (LAP), and phosphatase (PHOS). BG, NAG, LAP, and PHOS each catalyzes a terminal step in C, N, or PO_4^{3-} acquisition. BG breaks terminal non-reducing 1,4 linked β-D-glycosidic bonds in the glucose dimer cellobiose (the terminal step in

cellulose breakdown) and releases glucose. NAG catalyzes the terminal step in chitin and chitodextrin breakdown and releases N-acetyl glucosamine. LAP catalyzes the hydrolysis of peptide bonds on the N-terminus of peptides and releases amino acids, particularly leucine. PHOS hydrolyzes phosphate monoesters, releasing ortho-phosphate from organic molecules. We chose these ecoenzymes because they are considered to be indicative of the relative C (BG), N (NAG and LAP), and phosphate (PHOS) requirements of the microbial community (Sinsabaugh and Follstad Shah 2012).

To measure ecoenzyme activities, we used a modification of the fluorometric microplate method outlined by Saiya-Cork et al. (2002), using a modified universal buffer adjusted a pH of 7.68 (based on preliminary soil pH measurements) instead of acetate buffer with a pH of 5. Fluorescently labeled substrate for each ecoenzyme was separately added to soil slurries (created by mixing 1 g of soil with 125 mL modified universal buffer with a tissue-homogenizer (BioSpec Tissue Tearer, BiosSpec Products, Bartlesville, OK) and incubated at 20°C for four hours. The substrates are labeled with methylumbelliferone (MUF) or 7-amino-4-methylcoumarin (MC) and the substrates used to measure BG, NAG, LAP, and PHOS are: 4-MUB-β-D-glucoside, 4-MUF-N-acetyl-β-D-glucosaminide, 7-amido-4-MC (hydrochloride), and 4-MUF phosphate, respectively. When these labeled substrates are acted upon by the appropriate ecoenzymes, the fluorophore is released and then fluoresces, and this fluorescence is then quantified by a microplate reader (BioTek Instruments Inc., Winooski VT, USA). To correct for the soil particles' interference on fluorescence (quench), we mixed sample slurry with either MUF or MC reference standard, depending on the assay, and that was compared to the

fluorescence of MUF or MC and buffer to calculate a correction factor (quench coefficient). We also measured the autofluorescence of each sample, substrate, and buffer, which was used to correct the final fluorescence value. BG samples from July 7 2016 were excluded from analysis due to substrate contamination. LAP and NAG activities were summed to create an estimate of N-acquiring ecoenzyme activities.

2.3 Invertebrate Analyses

To measure relative rates of faunal activities, we deployed pitfall traps on July 20th, July 27th, August 3rd, and September 21st. Each trap was constructed using a specimen cup (118 mL, 6 cm diameter; Dynarex, Orangeburg, NY; product # 4256) and buried in the center of each plot so that the rim of the cup was flush with the surrounding soil. Each cup was filled with 45 mL of 70% ethanol and left out for 48 hours on rain-free days. After 48 hours, the cups were collected, filtered, and stored in ethanol for further analysis. Invertebrates were identified to taxonomic family and classified as either herbivores, scavengers (hereafter called detritivores), or predators by the Pelini lab (Bowling Green State University). Due to low faunal capture rates, cumulative plot activity was calculated by summing the activities for all dates for each plot. This cumulative activity measure is used for subsequent analyses unless otherwise noted.

2.4 Statistical Analysis

All statistical analyses were done using the software package R (R Core Team 2017). We managed data using the reshape2 (Wickham 2007) package. Relationships between treatment, biological activities, and PO_4^{3-} pools were evaluated using mixed effects models (see appendix for in-depth descriptions of all statistical tests). We

constructed plots using the ggplot2 (Wickham 2009) and ggpubr (Kassambara 2017) packages for R. Each harvest took place over three days, and t-test results indicated that plots did not differ significantly over the three-day span of the harvest. Therefore, data from all three days during each harvest were pooled.

Chapter 3

Results

3.1 Phosphorus Pools

Overall, approximately 0.7% ± 0.5% of the total PO_4^{3-} (reported mean ± standard deviation; standard deviation is used to illustrate the data's variability) was Olsen extractable, 0.09% ± 0.01%$^{-}$ was held in microbial biomass, and the remaining 99.3% ± 0.5% was bound tightly to soil particles (Figure 3-1). Of the easily accessible PO_4^{3-} fractions, about 16.1% ± 15.8% was water soluble, 18% ± 15.2% was held in microbial biomass, 65.0% ± 24.8% was alkaline-adsorbed, and no significant proportion was in the salt-adsorbed pool (Figure 3-1).

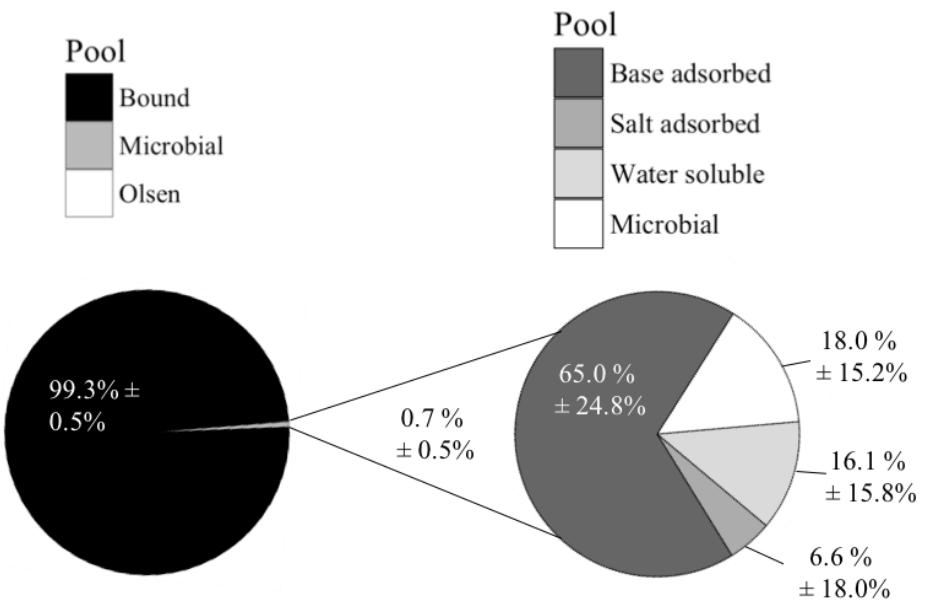

Figure 3-1: The distribution of PO_4^{3-} in different pools (mean ± standard deviation). The left side of the figure shows PO_4^{3-} in all soil pools. The right side of the figure shows an enlarged version of the easily accessible PO_4^{3-} pools (< 2% of total PO_4^{3-}). Note that standard deviation, rather than standard error, is used to illustrate the dispersion of the data.

All phosphorus pools varied spatially and some varied over time (Table B.1; Table B.2). The Olsen and alkaline-adsorbed pools decreased during the experiment by 27% and 82%, respectively, but these decreases were not significant ($p = 0.61$, $p = 0.57$, respectively). Bound PO_4^{3-} and total PO_4^{3-} pools did not change significantly over time ($p = 0.7$, $p = 0.95$). The PO_4^{3-} proportion that was water soluble was significantly correlated with soil moisture — a 1% elevation of soil moisture was correlated with a 20% elevation in water soluble PO_4^{3-} ($p = 0.0009$; Figure 3-2). Soil pH was not significantly correlated with the instantaneous proportion of PO_4^{3-} in any given pool. However, a one-unit higher average pH in a plot was significantly correlated with 2.57 ± 1.23 µg per g soil (mean ±

standard error) more PO_4^{3-} depletion (as soil pH goes from 7 to 8, the amount of PO_4^{3-} depleted from this pool is elevated by an average of 48%) over the experiment from the Olsen pool ($p = 0.05$; Figure 3-3; Table B.2). In addition, pH was insignificantly correlated with the amount of PO_4^{3-} that was depleted from all pools (Table B.2).

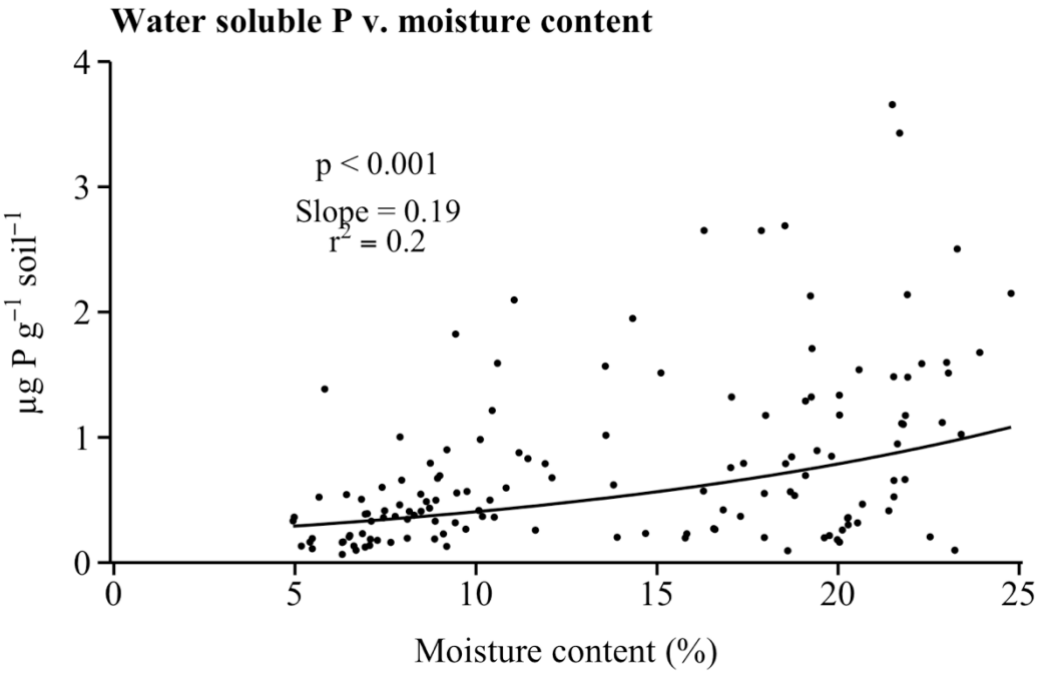

Figure 3-2: Water soluble PO_4^{3-} vs. soil moisture content. Mixed effects models with field as a random effect indicate that the water soluble PO_4^{3-}concentration is significantly correlated with moisture content ($p < 0.01$). The absolute water-soluble PO_4^{3-} concentration is shown here rather than the proportion of water-soluble PO_4^{3-} because we only have total PO_4^{3-} for two-thirds of the samples (and thus, can only calculate the proportion for two-thirds of the samples). The same trend is true for the proportion of water-soluble PO_4^{3-}, with a 1% change in moisture content correlated with a 20% change in the proportion of water soluble PO_4^{3-}. P-values and slope were calculated using mixed effects models, r^2 values were calculated by linearly correlating the log transformed variables.

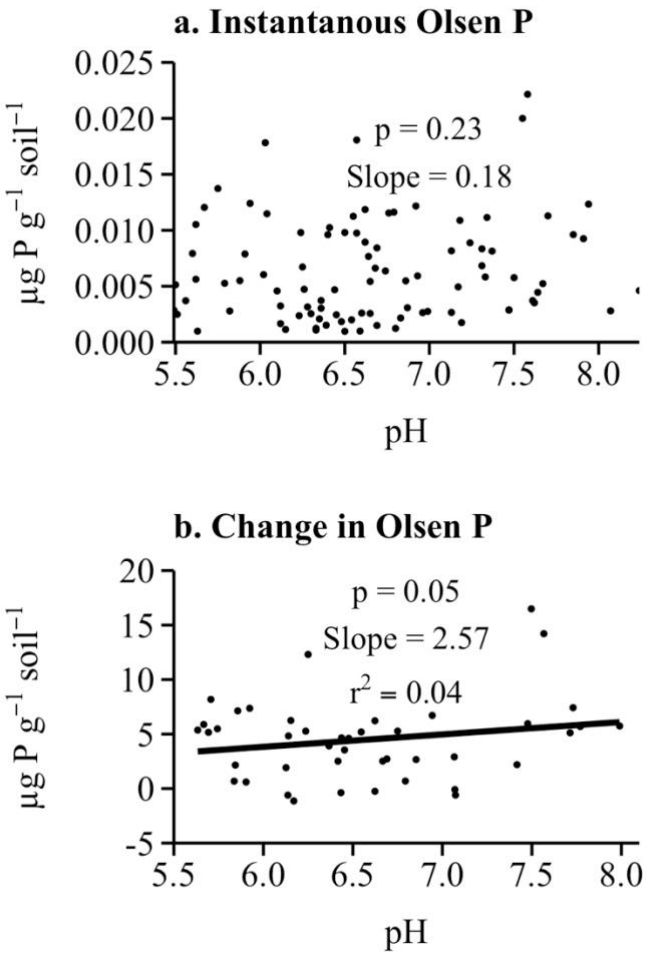

Figure 3-3: **a.** The proportion of PO_4^{3-} in the Olsen P pool vs. pH. **b.** the change in Olsen P v. pH. pH had no effect on the instantaneous Olsen P pool but it was slightly, and significantly, correlated with more Olsen P depletion over the course of the season. P-values and slope were calculated using mixed effects models, r^2 values were calculated by linearly correlating the variables.

Few samples had a salt-adsorbed PO_4^{3-} pool. The best predictor for the presence or absence of a salt-absorbed pool was total P. Salt-adsorbed PO_4^{3-} had a threshold relationship with total P — below approximately 875 µg total P per g dry soil there was

usually no salt-adsorbed pool, above this threshold the size of the salt-adsorbed PO_4^{3-} pool was positively correlated with total PO_4^{3-} (Figure 3-4).

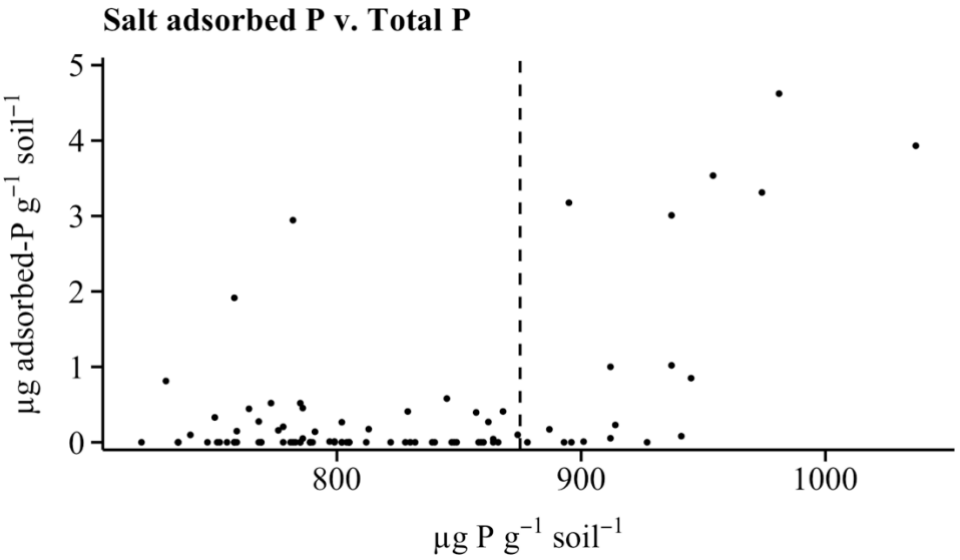

Figure 3-4: Salt-adsorbed PO_4^{3-} vs. total P. The dashed line indicates the approximate threshold at 875 µg total PO_4^{3-} per g dry soil. Below this threshold, most samples do not have a significant salt-adsorbed PO_4^{3-} pool. Above this threshold salt-adsorbed PO_4^{3} positively correlated with total PO_4^{3-}.

3.2 Treatment Effects

Sodium concentration was significantly higher in soils receiving Na^+ throughout the experiment (Figure B-1). The largest effect was during week one, when Na^+ addition elevated average soil Na^+ concentration from 17 ± 1.4 µg per g dry soil to 926 ±45.4 µg per g dry soil (from here on, all values are reported as mean ± standard error). At the end of the experiment, Na^+ remained significantly elevated in Na^+ addition plots, with a concentration of 268.6 ±14.0 µg per g dry compared to 11.0 ± 1.0 µg per g dry in plots

without added Na^+. Na^+ concentration decreased significantly throughout the season in both Na^+ treated and control plots ($p < 0.05$; Figure B-1).

Corn stover addition approximately increased DOC on the first sampling date ($p < 0.01$; Figure B-1). On the last sampling date, DOC remained elevated in the stover treatments, but was also increased in the Na treatment ($p = 0.02$). Stover addition was also associated with elevated microbial biomass and respiration (Figure 3-5; Table B-3). On the first sampling date, respiration was seven times higher in stover treatment plots than in controls ($p < 0.00001$), and on the last sampling date respiration was four times higher in the stover treatment, although there was no significant interaction between date and treatment ($p = 0.52$). Sodium treatment alone had no significant effect, but when Na^+ was added in combination with stover, respiration was suppressed insignificantly by about 50% compared with the stover-only treatment ($p = 0.34$). Microbial biomass C was 65% higher in stover addition soils than unamended soils and was not discernibly affected by the Na^+ treatment alone. Microbial biomass in the stover and Na^+ combined plots was 40% higher than in the control plots ($p= 0.0029$). While this was a slight reduction in biomass compared with stover-only plots, microbial biomass did not differ significantly between stover and stover & Na^+ treatments. Overall, microbial biomass decreased over time but there was no significant interaction between treatment and time for microbial biomass C. Microbial biomass N and PO_4^{3-} were 54% and 46% higher (respectively) in the stover addition plots than control plots, but these increases were not significant ($p = 0.10$, $p = 0.079$ respectively).

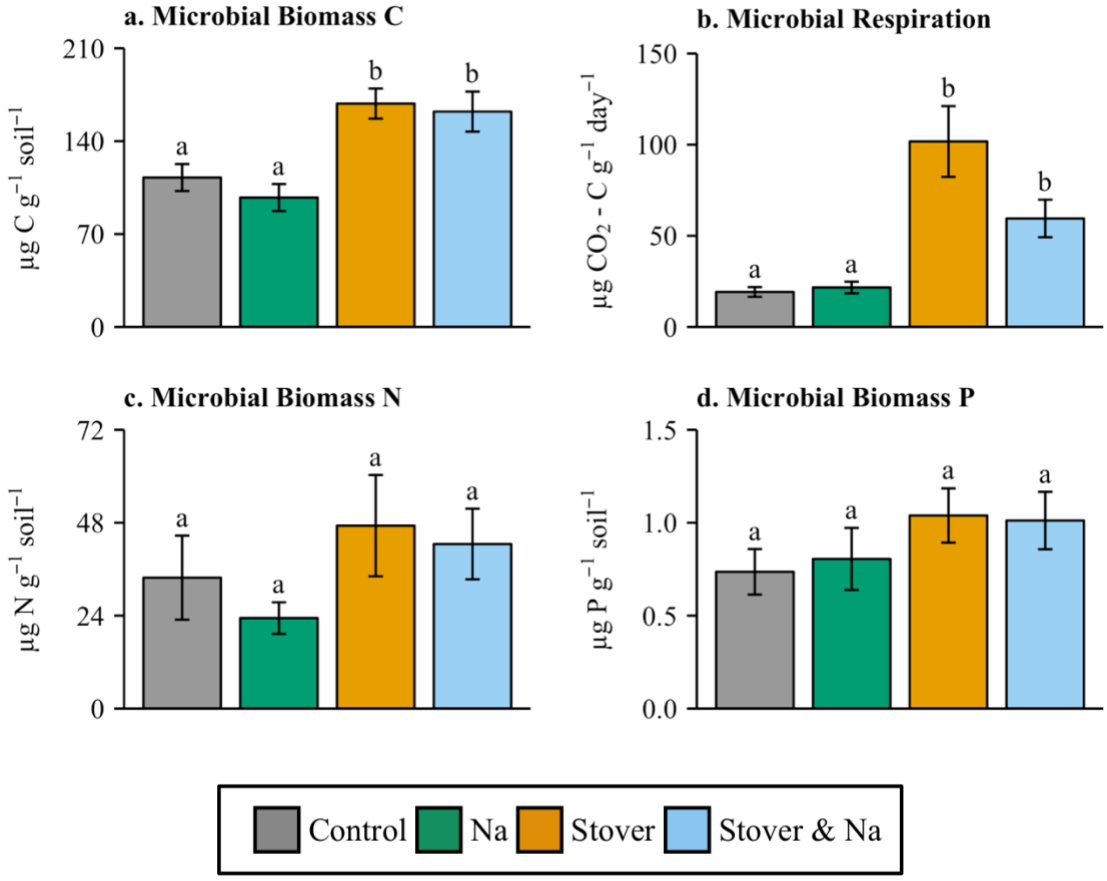

Figure 3-5: Mean **a.** microbial biomass C, **b.** microbial respiration, **c.** microbial biomass N, and **d.** microbial biomass PO_4^{3-} vs. treatment. Stover treatment significantly elevated microbial biomass C and total respiration, ($p < 0.0001$). Na^+ treatment alone had no effect. Error bars show standard error, and bars with different lower-case letters are significantly different from one another. Statistics were calculated using mixed-effects models with treatment and sampling event as fixed effects and field as a random effect.

Potential BG, NAG, and LAP ecoenzyme activities were elevated by 25-172% (depending on the ecoenzyme) in stover amended soils, but these higher activities were not significantly different from the controls on any given day (Table B.3). Stover addition significantly elevated PHOS activities by 75% - 148% (depending on the date) (p =

0.0001). Na^+ treatment had no significant effect on ecoenzyme activities. Ecoenzyme activities were all significantly affected by date, and were typically higher on the later sampling dates, particularly in the stover treatments.

Overall fauna and detritivore activities were higher in stover and Na^+ amended soils than in the controls (Figure 3-6; Table B.4). In control plots, average cumulative faunal activity over the four sampling dates was 16 ± 1.1 individuals. Activities were insignificantly higher by 15% ± 10% ($p = 0.13$) and 8% ± 10% ($p = 0.46$) in stover plots and Na^+ plots, respectively. However, when the two were added in combination, activities were 22% ± 10% higher than the control ($p = 0.039$), but were not significantly different from either the stover or Na^+ treatment alone. Similarly, detritivore activities, with a cumulative activity level of 3.3 individuals in the control plots, was elevated in the stover plots by 33% ± 22% ($p = 0.15$), by 16% ± 21% ($p = 0.43$) in the Na^+ plots, and by 64% ± 22% ($p = 0.01$) in plots with stover and Na^+. Sodium and stover addition had no significant interaction effect ($p > 0.90$) on faunal activities. Neither herbivore nor predator functional group abundances were significantly affected by either stover or Na^+ treatment.

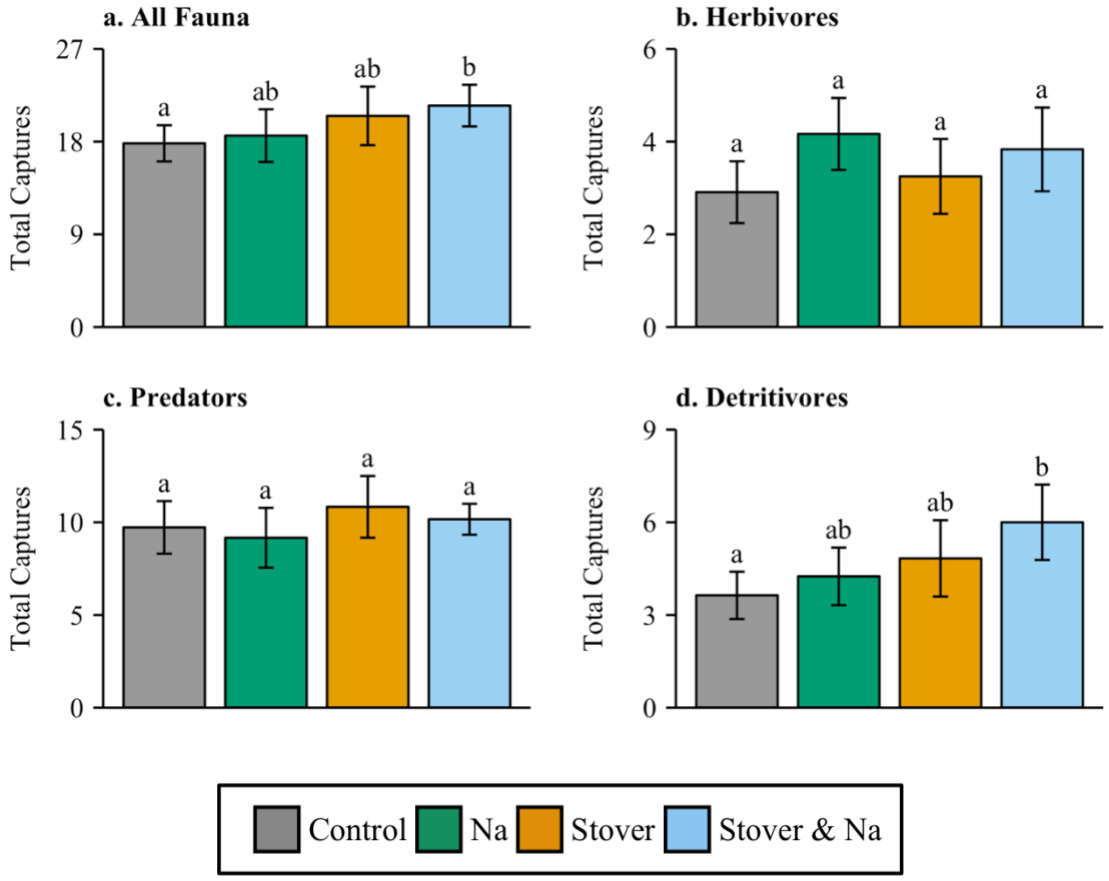

Figure 3-6: Cumulative invertebrate activity vs. treatment for: **a.** All soil fauna, **b.** Herbivores, **c.** Predators, and **d.** Detritivores. Stover and Na^+ addition alone insignificantly elevated detritivore activities by 29% ± 20% (p = 0.15) and 16% ± 21% (p = 0.44), respectively. Adding stover and Na^+ in combination significantly elevated detritivore activities by 51% ± 20% (p = 0.01). Treatment did not significantly affect herbivore (p > 0.23) or predator (p > 0.35) activities. Statistics were calculated using mixed-effects models with treatment and sampling event as fixed effects and field as a random effect.

Treatment had no significant effect on the proportion of PO_4^{3-} available in each pool (Figure 3-7, Table B.1). However, over the course of the season, the salt-adsorbed pool increased more over time in the Na^+ treatment plots than the control plots (by 3.05 ± 0.87 µg P per g soil, p =0.03). It should be noted, however, that this is based on just nine

out of 48 plots that had an average total P level > 875 (and, thus, the potential for a significant absorbed PO_4^{3-} pool). In the Na^+ treatments (both Na^+ alone and Na^+ and stover combined), total soil PO_4^{3-} was 2-3% higher than in control plots ($p = 0.01$, $p = 0.04$, respectively; Table B.1).

3.3 Phosphorus Pool Relationships to Biological Activities

Apart from cumulative exchangeable PO_4^{3-}, the instantaneous or overall proportion of PO_4^{3-} in each pool was not correlated with microbial activities ($p > 0.05$; Figure 3-8, Table B.5). Cumulative exchangeable PO_4^{3-}, however, was significantly correlated with average microbial respiration rates and phosphatase activities ($p < 0.01$, Figure 3-9, Table B.5), but not with microbial biomass C. Similarly, only one measured PO_4^{3-} pool was significantly correlated with a metric of faunal activity — a 1% elevation in cumulative herbivore activities was correlated with a 0.6% elevation in salt-adsorbed PO_4^{3-} ($p = 0.02$), although this significant correlation was based on just seven sample points because most soil samples lacked a detectable salt-adsorbed PO_4^{3-} pool.

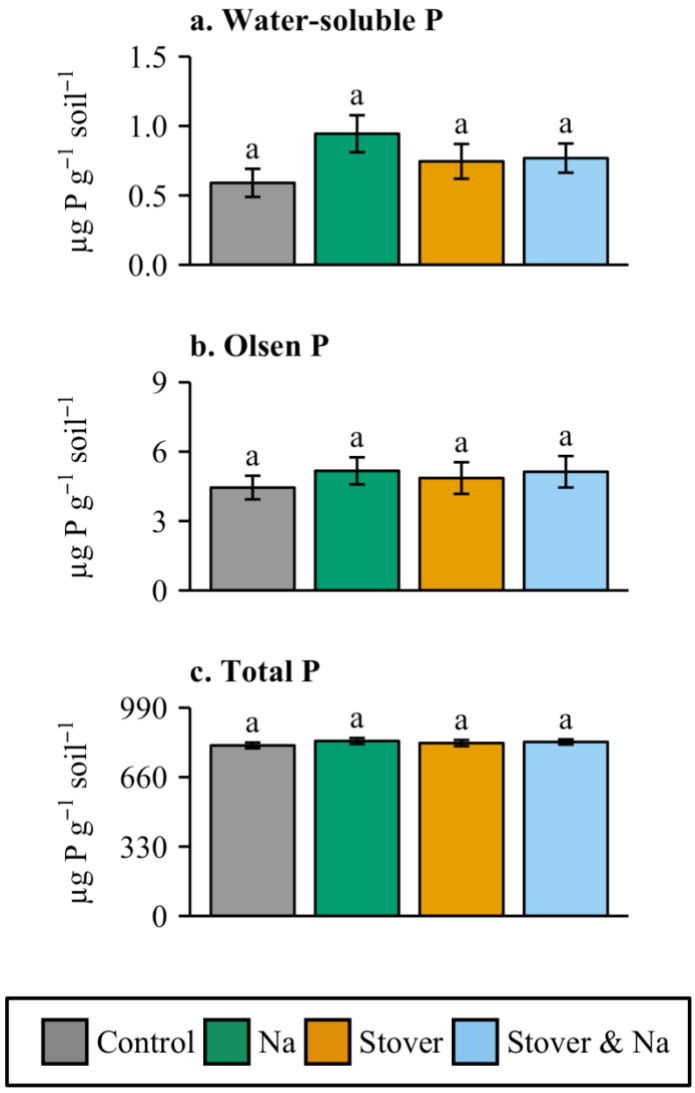

Figure 3-7: **a.** Water-soluble, **b.** Olsen, and **c.** total PO_4^{3-} pools vs. treatment. Treatment had no significant effect on the amount of PO_4^{3-} in each pool ($p > 0.05$), Na^+ treatment alone had an insignificantly higher proportion of PO_4^{3-} in the water-soluble pool ($p = 0.06$). Other PO_4^{3-} pools (not shown) were not significantly affected by treatment. Error bars represent standard errors. Statistics were calculated using mixed effects models with treatment, pH, and moisture content as fixed effects and field as a random effect.

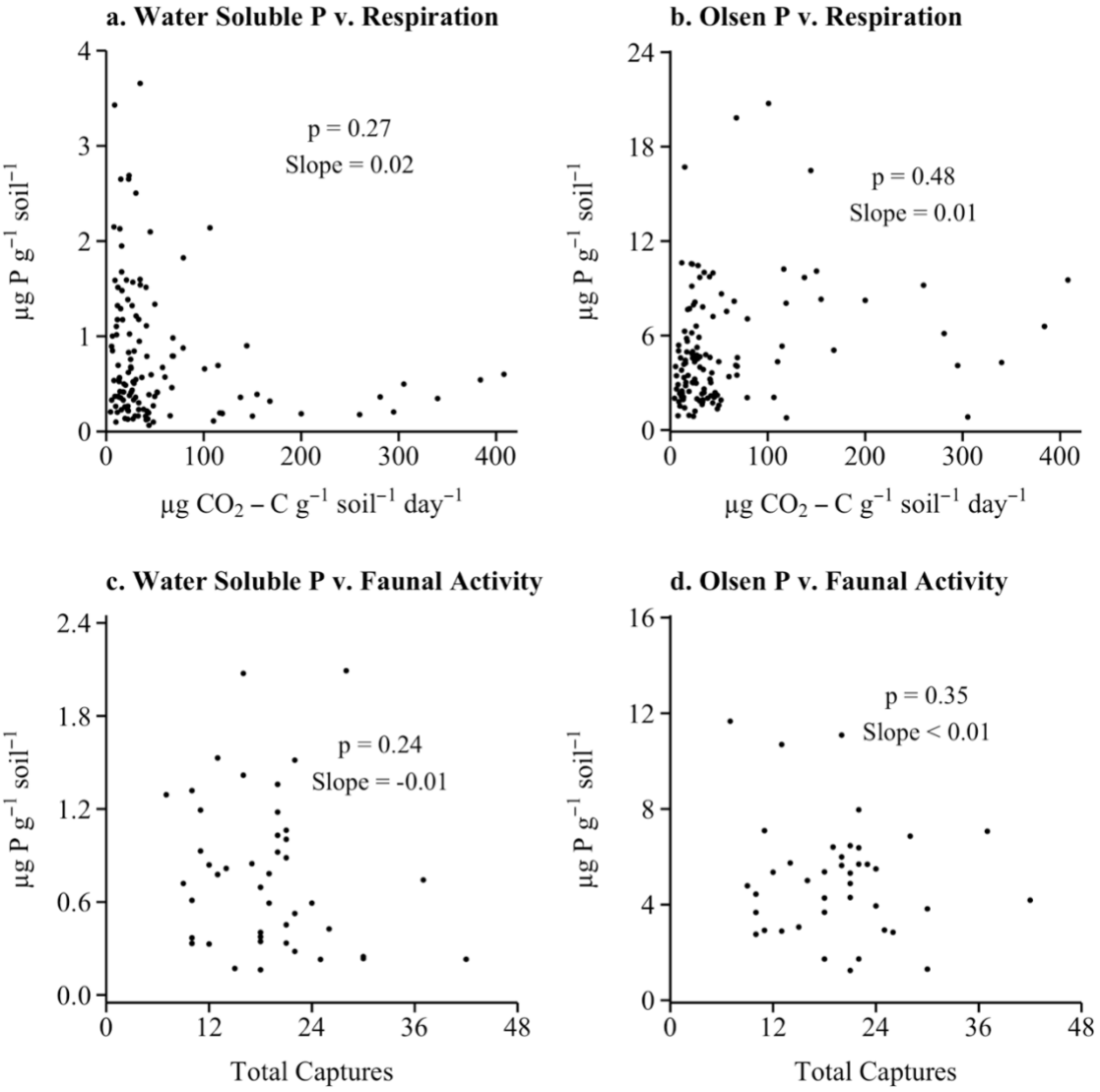

Figure 3-8: **a.** Water soluble PO_4^{3-} vs. microbial respiration, **b.** Olsen PO_4^{3-} vs. microbial respiration, **c.** mean water soluble PO_4^{3-} vs. cumulative faunal activities, and **d.** mean Olsen PO_4^{3-} v. cumulative faunal activities. P values and slope were calculated using multi-level models. Overall, PO_4^{3-} pools were not significantly correlated with microbial or faunal activities.

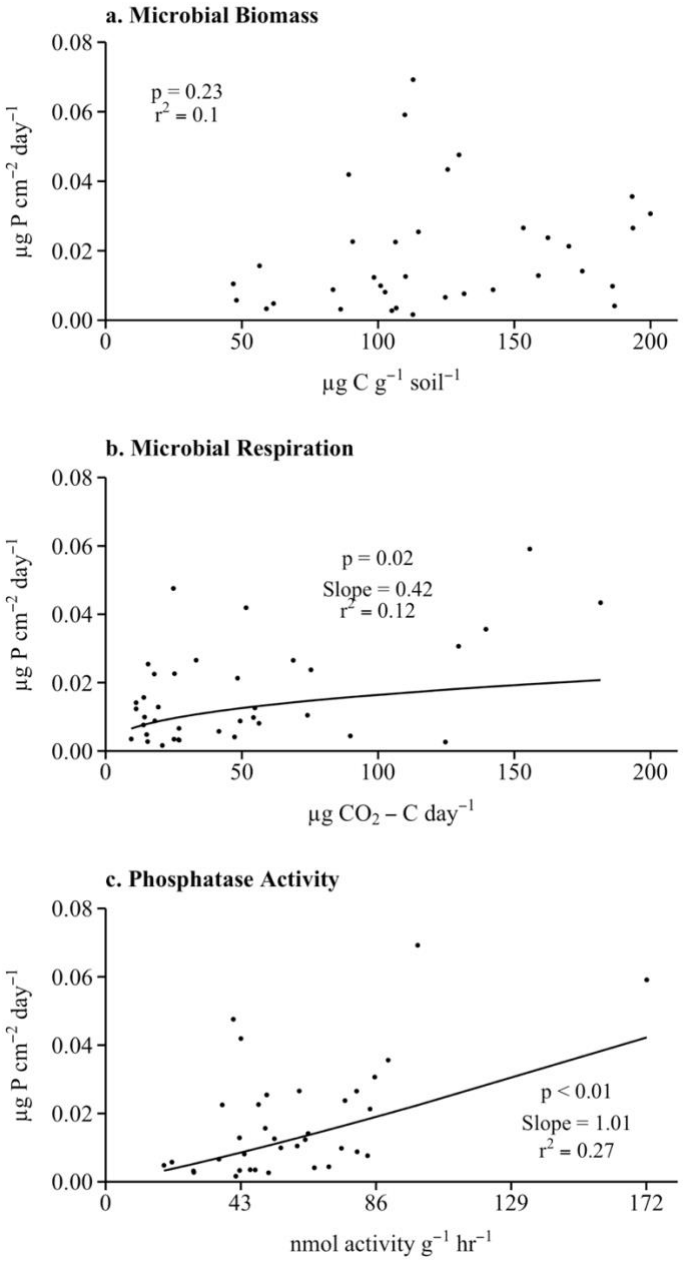

Figure 3-9: Cumulative exchangeable PO_4^{3-} vs. plot mean for **a.** microbial biomass C, **b.** microbial respiration, and **c.** potential phosphatase activities. Microbial respiration and phosphatase activities were significantly correlated with cumulative exchangeable PO_4^{3-} whereas microbial biomass was not. P-values and slope were calculated using mixed effects models, r^2 values were calculated by linearly correlating the log transformed variables.

Chapter 4

Discussion

4.1 Biological Responses to Corn Stover and Sodium Additions

As expected, microbial activities were higher in corn stover than control plots. The strong positive response of microbial biomass, respiration, and ecoenzyme production to C addition is consistent with other studies that found strong microbial C limitation in conventionally managed fields (Zak et al. 1994, Fierer et al. 2009, Kallenbach and Grandy 2011). In contrast, Na^+ had limited effects on microbes: Na^+ had no significant effect on biomass and suppressed respiration only in the presence of corn stover. Perhaps when C strongly limits microbes they do not respond to other stressors, and/or baseline activities are so low that any changes are undetectable.

Unlike microbes, fauna, particularly detritivores, responded positively and significantly to a combination of Na^+ and stover. Although stover or Na^+ alone appeared to attract fauna, these elevations in activities were statistically insignificant. The low faunal response to either stover or Na^+ addition alone may be because activities were minimal in these fields (an average of 3-4 individuals per plot per 48-hour sampling

period). This low level of faunal activity may have been due to, at least in part, the relatively dry soil conditions of 2016 caused by hotter (23.8 C compared to a 10 year average of 22.1 C) and drier (209 mm rainfall compared to a 10-yer average of 230 mm rainfall) weather conditions than average. In corn fields, drought can limit soil faunal response to treatment (Pavuk et al. 1997). Given the dry conditions of 2016, water and/or other factors (e.g. a lack of habitat, such as litter, in these fields) may have limited fauna, making the community too depauperate to respond strongly to Na^+ or stover, at least over the course of 12 weeks.

4.1.1 Is There Evidence for Sodium Limitation?

Overall, our results provide limited support for the hypothesis that Na^+ limits soil fauna who primarily feed on plant matter. In this experiment, detritivore, but not herbivore or predator fauna, were stimulated by Na^+. The response of detritivores, but not predators, is consistent with other studies in temperate systems indicating that Na^+ limits fauna that scavenge or feed on plant material (Ott et al. 2014, Kaspari et al. 2017), but prey density limits predators (Ott et al. 2014). However, herbivores, based on their diet, should respond like detritivores, and, in fact, in other temperate systems herbivore fauna are positively correlated with litter Na^+ concentration (Joern et al. 2012, Ott et al. 2014). The lack of herbivore response to our treatments may be driven by the Na^+ application directly to the soil, and not living plants— herbivores' primary food source, rather than a lack of Na^+ limitation of herbivores, or by the general lack of fauna in our study sites. Overall, we can conclude that while faunal detritivores in this temperate agricultural system may be limited by Na^+, predators are most likely not limited by Na^+, and

herbivores do not respond to Na^+ applied directly to the soil that is not assimilated into plant biomass.

Soil microbes were not stimulated, and indeed may have been suppressed by Na^+ addition. This differs slightly from tropical systems where low-levels of Na^+ addition may stimulate microbes (Kaspari et al. 2014, Jia et al. 2015). It is possible that Na^+ may have caused some community shifts, but if so, it did not result in changes in soil nutrient availability or microbial biomass. Overall, these results suggest that soil microbes are not limited by Na^+ in these systems.

4.2 PO_4^{3-} Pools

4.2.1 PO_4^{3-} Pools Response to Treatment

Treatment had no effect on the distribution of most soil PO_4^{3-} across most pools. However, total PO_4^{3-} was slightly (~2%), but significantly ($p < 0.04$) elevated in the Na^+ only treatments. In addition, the rate at which the salt-adsorbed pool increased over the season was higher in Na^+ only treatment plots ($p = 0.03$). Although total PO_4^{3-} was elevated under Na^+ treatment, the effect size was small and likely of little practical significance. The greater gain in adsorbed PO_4^{3-} over time in the Na^+ treatments, however, is consistent with observations that PO_4^{3-} solubility is positively correlated Na^+ concentration, possibly because Na^+ reacts with $PO_4^{3-}$to form NaH_2PO_4, a soluble compound or because Na^+ blocks the access of PO_4^{3-} to the surface of the soil mineral particle (Curtin et al. 1992, Buckingham et al. 2010, Mahmood et al. 2013). Over the course of the experiment, Na^+ concentrations in the Na^+ treated soils decreased and the salt-adsorbed PO_4^{3-} pool simultaneously decreased. The statistically insignificant trends

of higher (40% ± 21%; p = 0.06) water soluble PO_4^{3-} in the Na^+ only treatment and the decrease water soluble PO_4^{3-} with a decrease Na^+ concentration over the course of the experiment are also consistent with this interpretation. The lack of response of the combined Na^+ and stover may be because soil organic matter, perhaps by providing more ion exchange sites for both Na^+ and PO_4^{3-} to bind to, reduces the competition for binding sites and the potential for Na^+ and PO_4^{3-} to interact and form soluble NaH_2PO_4. In fact, in saline soils, organic matter can counteract the effects of NaCl on PO_4^{3-} in soil (Mahmood et al. 2013).

4.2.2 Correlation of Biological Activities and PO_4^{3-} Pools

Most PO_4^{3-} pools did not significantly correlate with biological activities. There was a negative correlation between herbivore activities and salt-adsorbed P, but it was small (a reduction of ~15% per herbivore captured) and likely of little practical significance. Cumulative exchangeable PO_4^{3-} was, however, positively correlated with average microbial respiration and phosphatase activities. This suggests that while instantaneous microbial activities may be poor indicators of PO_4^{3-} availability, cumulative microbial activities may be correlated with the PO_4^{3-} availability over the season. While we cannot conclusively determine whether higher microbial activities were the cause or result of elevated PO_4^{3-} availability, we speculate that, while stimulating soil fauna on a small-scale did not detectibly affect soil instantaneous PO_4^{3-} pools, some microbial activities may be positively correlated with cumulative PO_4^{3-} availability over the course of the season.

Given the strong controls that organisms exert on PO_4^{3-} cycling in natural ecosystems, the limited response of PO_4^{3-} to stimulated biological activities in the stover addition plots was surprising. This may be because, in natural systems, PO_4^{3-} availability is correlated with organic matter concentration and the potential for soil organisms to mineralize PO_4^{3-} (Harrison 2008, Achat et al. 2016). However, this relationship breaks down in agricultural systems (Achat et al. 2016). Agricultural soils, including those we studied, have low soil organic matter content when compared with most natural systems (Kallenbach and Grandy 2011, Xu et al. 2013), which limits the potential for organic PO_4^{3-} mineralization. In addition, large amounts of PO_4^{3-} inputs from fertilizer may obscure any changes in P availability caused by mineralization. Although soil microbes can liberate PO_4^{3-} by other means than mineralizing PO_4^{3}, such as solubilizing PO_4^{3-} on mineral surfaces^{-} (Richardson and Simpson 2011), stimulating microbial activities with corn stover did not affect the concentrations or proportions of PO_4^{3-} in soil pools.

In some agricultural systems, microbial activities do increase the amount of plant-available PO_4^{3-} (e.g., Hedley et al. 1982, Oehl et al. 2001). However, those findings are from agricultural fields that were managed for more soil organic matter than those in this study. In fact, in fields with more soil organic matter, microbes mineralize PO_4^{3-} at a greater rate and those fields have more available PO_4^{3-}(Oehl et al. 2004). In addition to the effect of organic matter, PO_4^{3-} cycling by microbes may also depend on soil amendment composition; for example, in one study, corn stover did not affect soil solution PO_4^{3-}, but added glucose lowered soil solution PO_4^{3-} in comparison to control plots (Bünemann et al. 2004b). Both stover and glucose treatments, however, increased

microbial PO_4^{3-} concentration. The authors suggest that in the stover treatment, microbial PO_4^{3-} increased without a reduction in soil solution PO_4^{3-} because increased microbial demand for PO_4^{3-} was balanced by the release of PO_4^{3-} from corn stover. Therefore, amendment quality will alter effects on PO_4^{3-} availability and should be considered when managing soil PO_4^{3-}. It is likely that the lack of response of soil PO_4^{3-} pools to stover addition in this study was caused by all of the above factors: low soil organic matter content, low microbial activities, and amendment composition.

As with microbes, soil fauna activities and soil PO_4^{3-} availability were also not significantly correlated, likely because the C content of our soil is low (~2.5%), limiting faunal abundance (mean of < 5 individuals captured per 48-hour sampling period) and effects on decomposition and PO_4^{3-} cycling. In these conventionally managed fields with low organic matter and soil faunal activities, fauna likely have little, if any, detectable impact on soil PO_4^{3-} cycling during the growing season.

4.2.3 Correlations Between Abiotic Factors and PO_4^{3-} Pools

While pH poorly predicted the concentration of PO_4^{3-} pools on individual dates, pH was correlated with a decrease in Olsen PO_4^{3-} and insignificantly correlated with a decrease in total PO_4^{3-} over the course of the season. The lack of a pH effect on soil PO_4^{3-} availability is surprising given the well documented relationship between pH and PO_4^{3-} availability (Brady and Weil 2008) and the large pH range in our soils (5.1 - 8.3). We speculate that high PO_4^{3-} levels in all of our soils minimized pH based differences between plots and treatments. However, Olsen PO_4^{3-} was depleted during the growing season at a higher rate in soils with higher pH, perhaps because of slightly higher PO_4^{3-}

solubility, but not high enough to be detected with instantaneous measurements. Therefore, while other factors more strongly influence instantaneous PO_4^{3-} concentrations in these fields, pH may influence the medium or long-term PO_4^{3-} availability and retention.

Although the proportion of water-soluble PO_4^{3-} was unaffected by pH, it was positively correlated with soil moisture. This may be due to soil drying and rewetting, which causes microbes to release PO_4^{3-} into soil solution during cell lysis (Turner and Haygarth 2001, Gordon et al. 2008). Although this finding is correlative rather than causal, the relationship between soil moisture and PO_4^{3-} solubility in the soils in the area that was formerly the Great Black Swamp (Forsyth 1970) should be investigated further.

While some of the variation in the PO_4^{3-} proportions in different pools can be explained by soil moisture or pH, most remains unexplained by the variables measured. Additional factors such as land-use history, climate, PO_4^{3-} application rate, liming history, native soil PO_4^{3-} concentration, and mineralogy can all impact soil PO_4^{3-} distribution (Negassa and Leinweber 2009, MacDonald et al. 2012). The fact that soil PO_4^{3-} pools varied even within fields suggests that fine spatial-scale factors, such as uneven PO_4^{3-} fertilizer application, micro-topography, and climate variation, or community composition in a plot may also affect in PO_4^{3-} availability.

4.2.3.1 The Salt-adsorbed PO_4^{3-} Pool Depends on Total Soil P

Only soils with high levels of total P (> ~ 875 µg P per g soil) had a detectable salt-adsorbed PO_4^{3-} pool, although these results should be interpreted with some caution, as only 11 of our 96 samples were in this group, and all but one of these samples were

taken from the same field. The presence of a salt-adsorbed PO_4^{3-} pool at only high levels of PO_4^{3-} may be due to a shift in the chemical binding of PO_4^{3-} anions to the soil particle surfaces at high soil PO_4^{3-} levels. Phosphate sorbs onto soil colloids in two-steps: initially, PO_4^{3-} ions react with binding sites on the soil particle surfaces; then, over time, PO_4^{3-} diffuses into the particle itself via solid state diffusion (Barrow 1983). However, once PO_4^{3-} concentrations in a soil particle are high enough to inhibit diffusion, PO_4^{3-} ions may remain bound to sites on the soil surface (Barrow and Debnath 2014). We hypothesize that a portion of this PO_4^{3-} is what we observed in our salt-adsorbed fraction (K_2SO_4 extractable - H_2O extractable).

Chapter 5

Conclusion

Our results indicate that soil microbes and fauna play a limited role in PO_4^{3-} release in the C limited soils of conventionally managed temperate corn fields, where biological activities are limited. Neither PO_4^{3-} concentrations in different soil pools nor total PO_4^{3-} were correlated with decomposer activities. This suggests that under conventional corn management regimes, which may include residue export (Ladd et al. 1994, Blanco-Canqui 2013), tile drainage (Bardgett et al. 1999), and/or inorganic fertilizer application (Fließbach et al. 2007), soil decomposer activities and the P-cycling ecosystem services that they provide are minimal.

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Appendix A

Description of Mixed Effects Statistical Models

To evaluate treatment effects on microbial biomass and activities, we used constructed variable-intercept mixed-effects models using the package nlme (Pinheiro et al. 2017) with field and block as random effects (with block as a variable within field). Sampling date and treatment were included in the models as fixed-effects. We evaluated whether harvest date and treatment interacted, interaction was only included in the models if it was significant at the 0.05 confidence level. Microbial respiration, biomass C, P, and N, and enzyme activities were log-transformed to fit the assumption of linearity. The assumptions of the heteroskedacity and normality of the residuals were checked graphically.

To assess the effect of treatment on faunal activities overall and on the activities of each functional group (predator, herbivore, and detritivore), we fit generalized linear mixed models with Poisson distributions using the glmer function in the lme4 package (Bates et al. 2015). Because capture rates were low (average capture rate of > 5 individuals per plot per 48 hour sampling period), data from all days were added together for each plot to create a cumulative activity variable. In the models of activity, treatment was treated as a fixed effect and field and block were treated as random effects (with block as a variable within field). The assumptions of heteroscedasticity and normality of residuals for all models were checked graphically.

We assessed the effect of treatment on the relative amount of P in each of the pools (P-pool ÷ total P) measured by constructing a mixed effects model in the nlme package (Pinheiro et al. 2017) with block nested in field as a random intercept. Because pH, moisture content, and date are likely to influence P solubility, they were included in the models as fixed effects. Models were simplified using the methods outlined by Gelman and Hill 2007, that is, models were constructed using all of the independent (x) variables, and then variables were eliminated from the model based on whether they were significant or sensible. That is, variables that had coefficients that were statistically insignificant and a did not make sense were excluded (Gelman and Hill 2007). The p-pool values were natural-log transformed to fit the assumption of linearity. The salt adsorbed P pool was not significantly different from zero unless total P was > 875 µg g soil^{-1} (see figure 3-4). Therefore, we only included soils in our model for the salt adsorbed P-pool that had a total P concentration > 875 µg g soil^{-1} . Similarly, we assessed the effect of the change in the amount of P in each pool over the course of the study by constructing mixed effects models with the same criteria used above, except only pH and treatment were included in the models. The change in p-pools was not transformed as such transformations were not required to meet the assumptions of a linear model. The assumptions of heteroscedasticity and normality of residuals for all models were checked graphically.

To assess the correlation of microbial activities and biomass on the proportion of P in each pool, each of the P pools were correlated separately against microbial biomass C, microbial respiration rate, and phosphatase activity. We used a mixed effects model using the nlme package (Pinheiro et al. 2017) with random effects of field and block(with block as a variable within field). The fit of this model was poor for the resin pool, so block was excluded for this model only. We also controlled for soil moisture and pH, and model fit was evaluated and adjusted using the criteria outlined above.

We assessed the correlation between cumulative invertebrate activities and the p in each pool by constructing mixed effects models using the lme function nlme package (Pinheiro et al. 2017) with average plot pH and cumulative activities as fixed effects and plot nested in field as the random effect. Cumulative activities were correlated with average P pools because of low invertebrate activities and asynchrony between soil P and invertebrate sampling. Similarly, to study the effect of biological activities on the change in each p pool over the course of the season, created separate models for each p pool against mean microbial respiration, biomass, phosphatase activity, and cumulative faunal activities. The random-intercept mixed-effects models were constructed using the lme function in the nlme package with the biological activity and average plot pH as fixed effects and block nested in field as random effects. The assumptions of heteroscedasticity and normality of residuals for all models were checked graphically.

Appendix B

Supplemental Figures and Tables

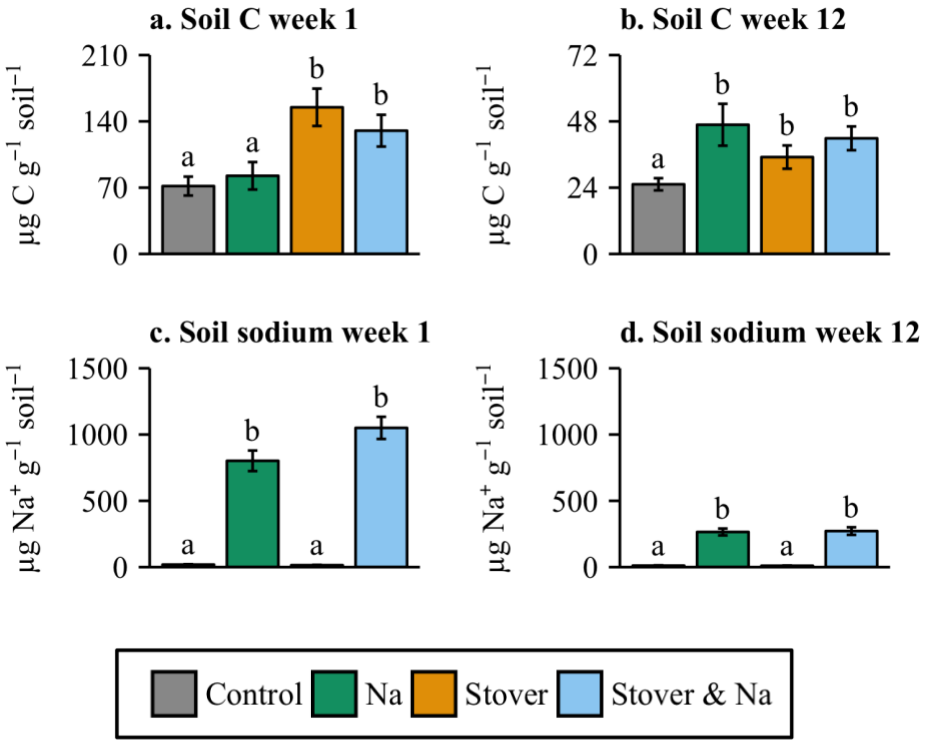

Figure B-1: a. Mean DOC for each treatment in week 1 b. Mean DOC for each treatment in week 12 c. Mean Na^+ concentration for each treatment in week 1 d. Mean Na^+ concentration for each treatment in week 12. Lower case letters indicate statistically different groups. Statistics were calculated using linear models with treatment and date as predictor variables. There was significant interaction between treatment and date. Error bars represent standard error

Table B.1: Output of mixed effects models for each P pool. Pools were log transformed to meet the assumption of normality. Reported as mean ± standard error.

P pool	**Fixed Effect**	**trend (Beta)**	**t-value**	**p (≤)**
Olsen P df = 74 $NaHCO_3$ extraction	Control	-6.05 ± 0.96	-6.33	0.00
	Na	0.27 ± 0.14	1.89	0.06
	Stover	0.15 ± 0.15	1.01	0.32
	Na & Stover	0.25 ± 0.14	1.71	0.09
	WC (%)	-0.04 ± 4.43	-0.81	0.42
	pH	0.18 ± 0.15	1.20	0.23
	date 9/30	-0.31 ± 0.62	-0.51	0.61
Alkaline adsorbed df = 71 Olsen P - K_2SO_4 P	Control	-3.54 ± 3.1	-1.14	0.26
	Na	-0.48 ± 0.8	-0.61	0.55
	Stover	-0.57 ± 0.83	-0.68	0.50
	Na & Stover	-0.51 ± 0.8	-0.64	0.52
	WC (%)	-4.97 ± 21.62	-0.23	0.82
	pH	-0.1 ± 0.5	-0.20	0.84
	date 9/30	-1.72 ± 2.98	-0.58	0.57
Bound df = 70 Total - labile	Control	-0.005 ± 0.006	-0.85	0.40
	Na	-0.001 ± 0.001	-1.09	0.28
	Stover	-0.001 ± 0.001	-1.26	0.21
	Na & Stover	-0.002 ± 0.001	-1.92	0.06
	WC (%)	0 ± 0.03	0.70	0.49
	pH	-0.001 ± 0.001	-0.77	0.44
	date 9/30	0.002 ± 0.004	0.39	0.70
Total df = 77	Control	6.72 ± 0.09	76.38	0.00
	Na	0.03 ± 0.01	2.51	0.01
	Stover	0.01 ± 0.01	1.20	0.23
	Na & Stover	0.02 ± 0.01	2.05	0.04
	WC (%)	-0.19 ± 0.33	-0.55	0.58
	pH	0 ± 0.01	-0.10	0.92
	date 9/30	0.03 ± 0.05	0.56	0.58

Table B.2: Statistical output of mixed effects models for the change in each P pool over the course of the experiment (July 13th or 15th – September 30th). Trend values are reported as mean ± standard error.

P pool	**Fixed Effect**	**Trend (Beta)**	**t-value**	**p(≤)**
Water soluble df = 28 H_2O extraction	Control	-1.39 ± 1.81	-0.76	0.45
	Na	-0.68 ± 0.34	-2.00	0.06
	Stover	-0.2 ± 0.36	-0.57	0.57
	Na & Stover	-0.09 ± 0.34	-0.26	0.80
	pH	0.15 ± 0.27	0.56	0.58
Salt Adsorbed df = 4 K_2SO_4 - H_2O extraction	Control	0.27 ± 1.13	0.24	0.82
	Na	-3.05 ± 0.87	-3.50	0.03
	Stover	0.34 ± 0.99	0.34	0.75
	Na & Stover	-0.58 ± 0.87	-0.66	0.54
Olsen P df = 28	Control	-12.68 ± 8.16	-1.55	0.13
	Na	-1.92 ± 1.44	-1.33	0.19
	Stover	1.17 ± 1.48	0.79	0.44
	Na & Stover	0.85 ± 1.44	0.59	0.56
	pH	2.57 ± 1.23	2.10	0.05
Alkaline adsorbed df = 25 Olsen P - K_2SO_4 extraction	Control	-9.44 ± 8.45	-1.12	0.27
	Na	-1.02 ± 1.35	-0.76	0.46
	Stover	1.23 ± 1.45	0.84	0.41
	Na & Stover	1.03 ± 1.3	0.79	0.44
	pH	2.18 ± 1.27	1.71	0.10
Bound df = 24 Total - labile	Control	-102.01 ± 89.41	-1.14	0.27
	Na	-1.32 ± 17.22	-0.08	0.94
	Stover	19.09 ± 17.92	1.07	0.30
	Na & Stover	-1.88 ± 16.17	-0.12	0.91
	pH	14.09 ± 13.45	1.05	0.31

P pool	**Fixed Effect**	**Trend (Beta)**	**t-value**	**p(≤)**
Total df = 32	Control	-156.53 ± 86.94	-1.80	0.08
	Na	5.29 ± 15.09	0.35	0.73
	Stover	16.7 ± 15.1	1.11	0.28
	Na & Stover	-2.33 ± 15.1	-0.15	0.88
	pH	22.96 ± 13.03	1.76	0.09

Table B.3: Statistical output of mixed effects models for microbial activities v. treatment. Microbial activities were log transformed prior to analysis to fit the assumption of normality. Interaction effect are included when they are statistically significant ($p < 0.05$). A ":" is used to indicate an interaction. Trend values are reported as mean ± standard error.

Microbial Activity	**Fixed Effect**	**Trend (β)**	**t-value**	**p (≤)**
Microbial respiration df = 109	Control	3.4 ± 0.26	13.30	0.00
	Na	-0.12 ± 0.36	-0.33	0.74
	Stover	1.98 ± 0.37	5.42	0.00
	Na & Stover	1.28 ± 0.36	3.56	0.00
	sampling 9/10/16	-0.77 ± 0.36	-2.16	0.03
	sampling 9/30/16	-1.82 ± 0.42	-4.36	0.00
	Na:9/10/16	-0.19 ± 0.51	-0.37	0.71
	Stover: 9/10/16	-0.93 ± 0.51	-1.82	0.07
	Stover & Na: 9/10/16	-0.37 ± 0.51	-0.72	0.47
	Na: 9/30/16	1.44 ± 0.57	2.53	0.01
	Stover: 9/30/16	-0.35 ± 0.56	-0.63	0.53
	Stover & Na: 9/30/16	0.17 ± 0.57	0.30	0.76

Microbial Activity	**Fixed Effect**	**Trend (β)**	**t-value**	**p (≤)**
Microbial Biomass C df = 114	Control	4.96 ± 0.14	35.15	0.00
	Na	-0.15 ± 0.11	-1.30	0.20
	Stover	0.5 ± 0.11	4.42	0.00
	Na & Stover	0.34 ± 0.11	3.04	0.00
	sampling 9/10/16	-0.41 ± 0.1	-4.24	0.00
	sampling 9/30/16	-0.6 ± 0.1	-5.94	0.00
Microbial Biomass N df = 97	Control	3.48 ± 0.25	14.06	0.00
	Na	-0.04 ± 0.26	-0.15	0.88
	Stover	0.43 ± 0.26	1.68	0.10
	Na & Stover	0.32 ± 0.25	1.29	0.20
	sampling 9/10/16	-0.28 ± 0.22	-1.26	0.21
	sampling 9/30/16	-0.94 ± 0.23	-4.20	0.00
Microbial Biomass P df = 109	Control	-1.17 ± 0.3	-3.83	0.00
	Na	0.06 ± 0.22	0.28	0.78
	Stover	0.39 ± 0.22	1.77	0.08
	Na & Stover	0.33 ± 0.22	1.51	0.13
	sampling 9/10/16	0.91 ± 0.19	4.91	0.00
	sampling 9/30/16	0.72 ± 0.19	3.73	0.00
Total Enzyme Activities df = 97	Control	5.27 ± 0.14	36.77	0.00
	Na	-0.13 ± 0.19	-0.67	0.50
	Stover	0.31 ± 0.2	1.58	0.12
	Na & Stover	0.04 ± 0.2	0.23	0.82
	sampling 9/10/16	-0.37 ± 0.17	-2.19	0.03
	sampling 9/30/16	0.46 ± 0.17	2.71	0.01
	Na:9/10/16	0.27 ± 0.24	1.16	0.25
	Stover: 9/10/16	0.56 ± 0.24	2.29	0.02
	Stover & Na: 9/10/16	0.76 ± 0.24	3.11	0.00

Microbial Activity	**Fixed Effect**	**Trend (β)**	**t-value**	**p (≤)**
Total Enzyme Actifities (cont.)	Na: 9/30/16	0.31 ± 0.24	1.31	0.19
	Stover: 9/30/16	0.21 ± 0.25	0.87	0.39
	Stover & Na: 9/30/16	0.44 ± 0.24	1.82	0.07
BG Activities df = 24	Control	3.91 ± 0.26	15.28	0.00
	Na	-0.14 ± 0.28	-0.50	0.62
	Stover	0.24 ± 0.29	0.82	0.42
	Na & Stover	0.14 ± 0.28	0.52	0.61
	sampling 9/10/16	-0.66 ± 0.25	-2.70	0.01
	sampling 9/30/16	-0.38 ± 0.25	-1.54	0.13
	Na:9/10/16	0.29 ± 0.34	0.84	0.40
	Stover: 9/10/16	0.77 ± 0.35	2.18	0.03
	Stover & Na: 9/10/16	0.9 ± 0.34	2.62	0.01
	Na: 9/30/16	0.31 ± 0.34	0.89	0.37
	Stover: 9/30/16	0.28 ± 0.36	0.78	0.44
	Stover & Na: 9/30/16	0.45 ± 0.34	1.32	0.19
N Acquiring Enzyme Activities df = 118	Control	4.08 ± 0.12	34.16	0.00
	Na	-0.12 ± 0.14	-0.87	0.39
	Stover	0.28 ± 0.14	1.97	0.05
	Na & Stover	0.1 ± 0.14	0.69	0.49
	sampling 9/10/16	0.18 ± 0.14	1.27	0.21
	sampling 9/30/16	1.33 ± 0.14	9.51	0.00
	Na:9/10/16	0.27 ± 0.2	1.35	0.18
	Stover: 9/10/16	0.5 ± 0.2	2.51	0.01
	Stover & Na: 9/10/16	0.58 ± 0.2	2.93	0.00
	Na: 9/30/16	0.31 ± 0.2	1.53	0.13
	Stover: 9/30/16	0.22 ± 0.2	1.08	0.28
	Stover & Na: 9/30/16	0.38 ± 0.2	1.92	0.06

Microbial Activity	**Fixed Effect**	**Trend (β)**	**t-value**	**p (≤)**
PHOS Activities df = 118	Control	3.70 ± 0.16	23.81	0.00
	Na	-0.03 ± 0.17	-0.18	0.86
	Stover	0.68 ± 0.17	3.93	0.00
	Na & Stover	0.32 ± 0.17	1.90	0.06
	sampling 9/10/16	-0.19 ± 0.17	-1.13	0.26
	sampling 9/30/16	0.09 ± 0.17	0.55	0.59
	Na:9/10/16	0.19 ± 0.24	0.81	0.42
	Stover: 9/10/16	0.24 ± 0.24	1.01	0.32
	Stover & Na: 9/10/16	0.55 ± 0.24	2.29	0.02
	Na: 9/30/16	0.28 ± 0.24	1.15	0.25
	Stover: 9/30/16	-0.11 ± 0.24	-0.46	0.64
	Stover & Na: 9/30/16	0.12 ± 0.24	0.48	0.63

Table B.4: Statistical output of mixed effects models for the activity of all fauna and each functional group vs treatment. Trend values are reported as mean ± standard error.

Fauna group	**Fixed Effect**	**Trend (β)**	**z-value**	**p(≤)**
All fauna df = 47	Control	2.77 ± 0.13	22.05	<2e-16
	Na	0.08 ± 0.1	0.74	0.46
	Stover	0.15 ± 0.1	1.48	0.14
	Na & Stover	0.2 ± 0.1	2.06	0.04
Herbivore df = 47	Control	1.05 ± 0.25	4.26	0.00
	Na	0.27 ± 0.23	1.21	0.23
	Stover	0.03 ± 0.24	0.11	0.92
	Na & Stover	0.19 ± 0.23	0.83	0.41
Predator df = 47	Control	2.22 ± 0.16	13.48	<2e-16
	Na	-0.04 ± 0.14	-0.32	0.75
	Stover	0.12 ± 0.13	0.94	0.35
	Na & Stover	0.06 ± 0.13	0.45	0.65
Detritivore df = 47	Control	1.2 ± 0.25	4.77	0.00
	Na	0.16 ± 0.21	0.78	0.44
	Stover	0.29 ± 0.2	1.43	0.15
	Na & Stover	0.51 ± 0.2	2.59	0.01

Table B.5: Correlation of microbial activities and the proportion of P in each pool. Output was calculated using mixed effects models with field as a random effect and the microbial activity as a fixed effect. Soil moisture and pH were also controlled for as fixed effects. Note that cumulative exchangeable P is an absolute value rather than a proportion as a proportion would not be an appropriate calculation for a cumulative pool. All correlations of microbial activity were take simultaneously to the measurements of the P pool except for cumulative exchangeable P as it is a measure of all of the cumulative exchangeable P over the course of the experiment. Mean microbial activity (averaged in each plot) was correlated against cumulative exchangeable P. Trend values are reported as mean ± standard error.

P Pool	**Microbial activity**	**Trend (β)**	**t-value**	**p (≤)**
Water Soluble	Microbial respiration (df = 39)	0.02 ± 0.02	1.11	0.27
	Microbial biomass C (df = 37)	0.002 ± 0.02	-0.14	0.89
	Phosphatase (df = 41)	0.12 ± 0.16	0.70	0.49
Salt adsorbed	Microbial respiration (df = 2)	-0.14 ± 0.27	-0.52	0.66
	Microbial biomass C (df = 2)	-0.40 ± 0.35	-1.12	0.38
	Phosphatase (n = 1)	1.08 ± 4.05	0.27	0.83
Olsen	Microbial Respiration (df = 39)	0.01 ± 0.01	0.71	0.48
	Microbial biomass C (df = 36)	0.00 ± 0.01	0.13	0.89
	Phosphatase (df = 38)	0.14 ± 0.12	1.17	0.25
Alkaline adsorbed	Microbial respiration (df = 36)	-0.07 ± 0.07	-1.06	0.29
	Microbial biomass C (df = 35)	0.08 ± 0.07	1.19	0.24
	Phosphatase (df = 35)	0.22 ± 0.6	0.37	0.71
Bound	Microbial respiration (df = 36)	0 ± 0	-1.61	0.12
	Microbial biomass C (df = 36)	0 ± 0	-0.81	0.42
	Phosphatase (df = 34)	0 ± 0	-1.82	0.08
Cumulative exchangeable	Microbial respiration (df = 31)	0.42 ± 0.17	2.43	0.02
	Microbial biomass C (df = 29)	0.55 ± 0.44	1.24	0.23
	Phosphatase (df = 30)	1.01 ± 0.29	3.43	0.00

Table B.6: Correlation of plot mean soil fauna activity and the mean proportion of P in each pool. Statistical output was calculated using mixed effects models with field as a random effect and the faunal activity as a fixed effect. Mean soil pH was also controlled for as fixed effects. Note that cumulative exchangeable P is an absolute value rather than a proportion as a proportion would not be an appropriate calculation for a cumulative pool. Trend values are reported as mean ± standard error.

P pool	**Fixed Effect**	**trend (Beta)**	**t-value**	**p (≤)**
Water soluble	Abundance (df = 30)	-0.01 ± 0.01	-1.20	0.24
	Herbivore (df = 30)	0.01 ± 0.03	0.24	0.81
	Predator (df = 30)	-0.01 ± 0.02	-0.73	0.47
	Detritivore (df = 30)	-0.03 ± 0.02	-1.63	0.11
Salt adsorbed	Abundance (df = 5)	0 ± 0	-2.52	0.05
	Herbivore (df = 6)	0 ± 0	-3.37	0.02
	Predator (df = 6)	0 ± 0	-2.33	0.06
	Detritivore (df = 6)	0 ± 0	-1.67	0.15
Olsen	Abundance (df = 30)	0 ± 0	-1.00	0.33
	Herbivore (df = 29)	0 ± 0	-0.95	0.35
	Predator (df = 29)	0 ± 0	-0.14	0.89
	Detritivore (df = 29)	0 ± 0	-1.40	0.17
Alkaline adsorbed	Abundance (df = 27)	0 ± 0	-0.95	0.35
	Herbivore (df = 26)	0 ± 0	-0.30	0.77
	Predator (df = 26)	0 ± 0	-0.71	0.49
	Detritivore (df = 26)	0 ± 0	-0.98	0.34
Bound	Abundance (df = 26)	0 ± 0	1.07	0.30
	Herbivore (df = 25)	0 ± 0	0.76	0.45
	Predator (df = 25)	0 ± 0	0.79	0.44
	Detritivore (df = 25)	0 ± 0	1.21	0.24
Total	Abundance (df = 34)	-0.41 ± 0.55	-0.75	0.46
	Herbivore (df = 33)	-0.02 ± 1.8	-0.01	0.99
	Predator (df = 33)	-0.39 ± 1.06	-0.37	0.72
	Detritivore (df = 33)	-1.15 ± 1.17	-0.98	0.33

P pool	**Fixed Effect**	**trend (Beta)**	**t-value**	**p (≤)**
Cumulative exchangeable	Abundance (df = 25)	-0.02 ± 0.02	-1.15	0.26
	Herbivore (df = 24)	0.04 ± 0.06	0.66	0.51
	Predator (df = 24)	-0.04 ± 0.03	-1.34	0.19
	Detritivore (df = 24)	-0.04 ± 0.04	-1.06	0.30