Canadian Journal of Soil Science
 Revue canadienne de la science du sol

Phosphorus uptake and availability and short-term seedling growth in three Ontario soils amended with ash and biochar

Journal:	<i>Canadian Journal of Soil Science</i>
Manuscript ID	CJSS-2017-0007.R2
Manuscript Type:	Article
Date Submitted by the Author:	06-Jul-2017
Complete List of Authors:	Noyce, Genevieve; University of Toronto, Department of Geography; University of Toronto at Scarborough, Physical and Environmental Sciences Jones, Trevor; Ontario Ministry of Natural Resources, Ontario Forest Research Institute Fulthorpe, Roberta; University of Toronto at Scarborough, Physical and Environmental Sciences Basiliko, Nathan ; Laurentian University, Biology; University of Toronto - Mississauga, Department of Geography
Keywords:	Forest soil, Phosphorus, Microbial biomass, Root growth

SCHOLARONE™
 Manuscripts

Phosphorus uptake and availability and short-term seedling growth in three Ontario soils
amended with ash and biochar

Genevieve L. Noyce^{1,2,*}, Trevor Jones³, Roberta Fulthorpe², and Nathan Basiliko^{4,5}

¹ Department of Geography, University of Toronto, Toronto, ON, Canada

² Department of Physical and Environmental Sciences, University of Toronto Scarborough,
Toronto, ON, Canada

³ Ontario Forest Research Institute, Ontario Ministry of Natural Resources, Sault Ste Marie,
ON, Canada

⁴ Department of Geography, University of Toronto Mississauga, Mississauga, ON, Canada

⁵ Department of Biology and the Vale Living with Lakes Centre, Laurentian University,
Sudbury, ON, Canada

* Corresponding author: G. Noyce; Smithsonian Environmental Research Center, 647 Contees
Wharf Rd, Edgewater MD 21037; noyceg@si.edu; 443.482.2351

ABSTRACT

Phosphorus (P) can be a limiting nutrient in terrestrial ecosystems and adding biochar or wood ash can increase plant-available P. We added wood ash and biochar to microcosms containing three acidic Ontario soils planted with red pine or sugar maple seedlings and observed seedling growth responses, as well as amendment-induced changes in soil P pools, microbial P, and enzyme activity. Neither ash nor biochar consistently increased seedling growth; instead sugar maple and red pine seedlings often had opposing responses to the same amendment/soil combination. Overall, these results indicate that it is important to carefully consider both the chemical and physical characteristics of the soil and the ash or biochar, as well as the nutrient requirements of the target tree species, in order to effectively use these amendments to reduce P-limitation.

Key words: forest soil, phosphorus, microbial biomass, root growth

INTRODUCTION

Phosphorus can be a limiting nutrient in terrestrial ecosystems with high levels of nitrogen (N) deposition, including Central Ontario (Watmough and Dillon 2003; Gradowski and Thomas 2006). Plants take up almost all of their P as monovalent or divalent orthophosphate anions (H_2PO_4^- or HPO_4^{2-}), but concentrations of these bioavailable phosphates are generally far below optimal concentrations for plant growth as occluded P can be protected chemically and physically (Hinsinger 2001; Whalen and Sampedro, 2010). When the orthophosphate concentration is low, the dominant P process is the adsorption of phosphate ions to organo-mineral soil constituents, such as iron (Fe) or aluminum (Al) oxides, Al silicates, calcium (Ca) carbonates, or the surface of clay minerals or organic matter (Hinsinger 2001; Richardson 2001; Plante 2007; Whalen and Sampedro 2010). These complexes are formed using weak electrostatic bonds, so the P is easily desorbed (Whalen and Sampedro 2010). Conversely, when the orthophosphate concentration is high, soluble P precipitates with metal cations such as Ca and magnesium (Mg) phosphates in alkaline soils or as Fe and Al phosphates in acidic soils, which are considerably more stable than the bonding of P to anion exchange sites (Hinsinger 2001; Richardson 2001; Plante 2007; Whalen and Sampedro 2010). These phosphate minerals make up a large percentage of soil P (Hinsinger 2001), but soils also typically contain many forms of organic P, such as phytate, nucleic acids, and phospholipids (Plante 2007), with organic P composing 30 to 50 % of total soil P (Richardson 2001; Plante 2007; Richardson et al. 2009). Additional P inputs from weathering are typically only a small portion of an ecosystem's P budget, so the recycling of these organic forms is quite important for overall soil P levels (Badaluco and Nannipieri 2007; Whalen and Sampedro 2010; Binkley and Fisher 2013).

In neutral and alkaline soils, pH plays a large role in the bioavailability of orthophosphates. Soil pH can affect both the presence of the metal cations with which orthophosphate precipitates as well as the ability of Fe and Al oxides to hold P ions (Hinsinger 2001; Plante 2007; Whalen and Sampedro 2010). A pH of 6.5 generally maximizes P availability, because there is minimal Al and Ca precipitation (Plante 2007; Whalen and Sampedro 2010). As the pH of the rhizosphere increases, phosphate salt solubilities increase, resulting in more free orthophosphates available for plant uptake (Richardson 2001; Badalucco and Nannipieri 2007; Whalen and Sampedro 2010). This is especially true for the dissolution of Ca-phosphates in the acidified rhizosphere (Hinsinger 2001; Whalen and Sampedro 2010). However, too much acidification reduces P bioavailability, because the ability of Fe and Al oxides to hold orthophosphate ions increases at a low pH (Hinsinger 2001).

Microorganisms can alter the soil pool of orthophosphates available to plants through four main pathways: increasing bioavailable P by solubilizing inorganic P, increasing bioavailable P by mineralizing organic P, producing phytohormones that increase root growth and thus enhance the plant's ability to take up P from the limited available pool, and decreasing P availability through immobilization of P in microbial biomass (Richardson 2001; Plante 2007; Richardson and Simpson 2011). While phosphatase activity is very important for transforming P into plant-available forms, insoluble inorganic phosphates need to be solubilized before phosphatases can work (Lapeyrie et al. 1991). Phosphorus-solubilization by the microbial community is consequently a major driver of P bioavailability and thus plant growth (Rodríguez and Fraga 1999; Richardson, 2001).

This highlights the importance of measuring plant-available P in soils and working to further understand what controls it. Furthermore, monitoring the nutrient concentration of

microbial biomass can indicate soil nutrient status trends; Powlson et al. (1987) proposed that a change in microbial biomass P may be an early indication of successfully remediating P-limited soils. Changes in plant-available P can be quantified in several ways, including by measuring an increase in extractable soil P (Biederman and Harpole, 2013). It is also possible to measure the quantity of P in plant tissue and microbial biomass, which can be particularly useful when comparing multiple soils.

Biochar and wood ash are soil amendments that have the potential to increase plant-available P, both by raising the pH of acidic soils and by directly supplying P in the biochar or ash particles themselves (Augusto et al. 2008; DeLuca et al. 2009; Saarsalmi et al. 2012; Biederman and Harpole 2013). In addition, biochar can adsorb cations such as Al^{3+} , Fe^{3+} , and Ca^{2+} that complex with P in the soil solution, increasing P availability (Xu et al. 2014). Biochar and ash can also affect the soil microbial community, which may indirectly increase soil P availability. Furthermore, microbial turnover of P is particularly important after ash addition, due to the slow dissolution of P from the ash granules (Clarholm 1994; Mahmood et al. 2002, 2003).

While biochar and ash typically increase soil P availability and consequently plant uptake of P (Augusto et al. 2008; DeLuca et al. 2009; Novak et al. 2009; Biederman and Harpole 2013), the mechanisms of this P provision are not thoroughly understood and could be either from chemical reactions or from microbial responses to additional P. Without a complete understanding of these mechanisms, biochar and ash amendments cannot be designed to maximize their utility in P-depleted soils. Biochar and ash effects on P availability can also vary widely depending on the soil texture and the dominant plant species. Acidic soils typically have stronger responses to biochar than alkaline soils (Cui et al. 2011; Biederman and Harpole 2013; Xu et al. 2014) and ash amendment effects on P availability are strongest in P-limited soils

(Wallander et al. 2005). However, despite this variability, few studies have systematically compared effects in different soils planted with different species under the same amendment and growth conditions. For example, Xu et al. (2014) compared P effects in three soils with varying rates of biochar addition, but did not incorporate a plant aspect and Erich and Ohno (1992) compared ash effects on P availability in eight different soils, but all were planted with corn seedlings. In addition, most previous biochar studies have occurred in agricultural soils; the nature of biochar in forest soils is relatively understudied, especially in terms of P provision. Ongoing field studies in Ontario forests have tested the effects of amending forest soils with 5 t ha⁻¹ of biochar (e.g. Sackett et al., 2014; Noyce et al. 2015) and 1 to 8 t ha⁻¹ of wood ash (e.g. Gorgolewski et al., 2016; Noyce et al. 2016) and found minimal effects, but there may be a higher threshold above which substantial changes in soil chemistry will occur.

This study focused on the effects of three amendment rates of biochar and wood ash on growth and biomass allocation of two common Ontario tree species (sugar maple and red pine), as well as P uptake and bioavailability in three acidic forest soils during a 12-week growth chamber incubation experiment. These species represent two major forest conditions found in south central Ontario (red pine plantations and natural hardwood forests dominated by sugar maple), have varying nutrient requirements, and are both economically important in the region. Specific aims of this study included: (1) Do biochar and ash enhance sugar maple and red pine growth in P-limited, acidic forest soils? (2) Do these amendments increase bioavailable P in soils and are effects consistent across different combinations of soil texture and tree species? (3) If bioavailable P does increase, is it partially mediated by the soil microbial community or solely by chemical reactions?

Increases in bioavailable P were assessed as changes in P in the soil, plant tissue, and microbial biomass. An increase in microbial biomass P is typically an early indicator of P-limitation remediation, because once the microbial biomass turns over, the microbial P will be released for plant uptake (Powlson et al. 1987; Clarholm 1998). To determine if increases in bioavailable P were microbially-mediated, we assessed experimental effects on both microbial biomass P and phosphatase activity.

MATERIALS AND METHODS

Experimental design and sampling

All three soils used for this experiment were collected from Great Lakes–St. Lawrence forests overlying the granitic Precambrian Shield; a coarse acidic sand (pH 5.6) was collected from a white pine plantation, an acidic sandy-loam (pH 4.7) was collected from a tolerant hardwood forest dominated by sugar maple, and an acidic fine silty-sand (pH 4.7) was collected from a red pine plantation. All three soils were classified as Dystric Brunisols (Soil Classification Working Group 1998), based on personal observations of soil pits dug at each site. Hereafter these three soils are referred to as sand, sandy-loam, and silty-sand, respectively, however it is acknowledged that other factors also varied across the three soils, such as vegetation, climate, and nutrient availability. At all sites, the forest floor organic layer was removed and mineral soil was collected down to 40 cm. Soil was thoroughly homogenized prior to use.

Sugar maple (*Acer saccharum*) and red pine (*Pinus resinosa*) seeds from the Ontario Tree Seed Facility (Angus, ON) were germinated in 125-mL cone-shaped pots with a 4-cm diameter opening (Ray Leach SC10 Super) filled with each soil type. After establishment, seedlings were

randomly assigned to one of seven treatments, with seven replicates of each soil/species/treatment combination. The treatments consisted of controls with no ash or biochar added or pots with 0.63 g biochar, 2.51 g biochar, 6.29 g biochar, 0.50 g ash, 2.01 g ash, or 5.03 g ash added directly to the soil surface. These addition rates were calculated based on the surface area of the pots and were equivalent to adding 5, 20, or 50 t biochar ha⁻¹ or 4, 16, or 40 t ash ha⁻¹, to match addition rates in ongoing field-studies. Biochar was batch-processed from sugar maple sawdust and pyrolyzed at a maximum temperature of 450 °C, which should mean minimal phosphorus was lost during the pyrolysis process (Bridle and Pritchard 2004; Atkinson 2010), as P volatilizes between 700 and 800 °C (DeLuca et al. 2006). Ash was mixed fly and bottom ash from a biomass boiler burning spruce, pine, and fir bark. Biochar and ash properties are provided in Table 1. Throughout the experiment, seedlings were kept in a growth chamber with a 14 h photoperiod (photosynthetic photon flux density 265 µmol m⁻² s⁻¹), 50 % relative humidity, and temperatures of 24 °C day/18 °C night and watered as needed based on visual assessment of the soil.

Seedlings were destructively harvested after 12 weeks, before they started to senesce. Individual root, shoot, and leaf/needle biomass were obtained and leaf and needle area were measured using WinFOLIA and WinSEEDLE (Regent Instruments). Soil in each pot was thoroughly homogenized and a subsample from each was immediately frozen for microbial analyses; the remainder was air-dried and sieved to 2 mm for chemical analysis. Due to time and resource limitations, chemical and microbial analyses were conducted only on controls and the medium amendment rate treatments (2.51 g biochar and 2.01 g ash, or the equivalent of 20 t biochar ha⁻¹ and 16 t ash ha⁻¹).

Soil and plant tissue chemistry

Soil and plant chemical analysis was conducted in the OFRI Geochemical Laboratory (Sault Ste. Marie, Ontario, Canada) following the methods described by Kalra and Maynard (1991). Soils were air-dried and analyzed for Bray-1 extractable P. Ca, K, Mg, Mn, and Na were determined by an ammonium acetate digest with selenium dioxide as a catalyst followed by analysis on an Genesis ICP-OES (Spectro Analytical Instruments, Germany) and total C and N were determined by combustion analysis using a VarioMax CN analyzer (Elementar Analysensystem, Germany). Soil pH was measured in extracts of 10 g soil to 40 mL 0.5 M K_2SO_4 . Soil moisture was determined gravimetrically. To provide sufficient mass for analysis, plant tissue was rinsed, dried, ground, and pooled per treatment. The ground samples were then digested in a Se solution and analyzed for P, Ca, K, and Mg on an ICP. Total C and N were determined as for the soil samples.

Microbial biomass

Microbial biomass was assessed through chloroform fumigation followed by extraction with 0.5 M K_2SO_4 (Vance et al. 1987). Extracted samples were filtered through 0.45 μm glass fiber filters and frozen until analysis. Microbial biomass was calculated as the difference in C, N, or P between fumigated and unfumigated samples. An extraction efficiency coefficient of 0.4 was used for microbial biomass P, based on the assumption that only 40 % of the P in the microbial biomass is extractable following chloroform fumigation (Brookes et al., 1982). Microbial biomass C (MB-C) and N (MB-N) were measured on a Shimadzu TOC/TN analyzer. Microbial biomass P (MB-P) was determined using a microplate malachite green colorimetric procedure (D'Angelo et al., 2001) as described by Jeannotte et al. (2004). Each sample was analyzed in duplicate.

Extracellular phosphatase activity

Potential phosphatase activities were measured in soil suspensions using colorimetry, following a modification of the procedure described by Popova and Deng (2010). A 3.00 g subsample of soil was mixed vigorously with 240 mL deionized water for 30 min. While continuously mixing, 16 150- μ L aliquots were pipetted into a 96-well plate. A 50- μ L aliquot of 60 mM *p*-nitrophenol substrate was added to the 8 sample wells and a 50- μ L aliquot of modified universal buffer (Tabatabai 1994) was added to the 8 control wells. Substrates were made using modified universal buffer adjusted to either pH 6 or pH 11, to assess acid and alkaline phosphatase activity, respectively, and four analytical replicates were conducted for each sample/substrate combination. Microplates were incubated at 37 °C while continuously shaking, after which all reactions were terminated with 50 μ L 0.50 M NaOH. Absorbance of each well was measured at 405 nm using a microplate spectrophotometer.

Statistical analyses

Statistical analyses were performed in R, version 3.1.2. Grubbs' test was used to identify and remove outliers (<1 % of dataset). ANOVA was used to assess overall effects of amendment addition on seedling biomass, microbial biomass, soil pH, and phosphatase activity, with Tukey's HSD for post hoc tests. Pearson's correlation tests were used to investigate relationships between seedling characteristics and ash and biochar addition rates, as well as between other components of the dataset. Non-normal data (according to Shapiro-Wilk test) were log- or square-root-transformed prior to calculating correlations.

RESULTS

Soil physicochemical properties

Physicochemical characteristics of the silty-sand-, sand-, and sandy-loam-textured soils differed, with the silty-sand and sandy-loam having lower pH than the sand (Figure 1a). Ash addition of 16 t ha^{-1} increased soil pH by at least 0.4 and 0.6 pH units in all microcosms (Figure 1a), though effects were strongest in the sand. In contrast, biochar addition of 20 t ha^{-1} had variable pH effects, depending on the soil and seedling species (Figure 1a). Biochar effects were always less pronounced than ash effects (Figure 1a). Soil moisture was much higher in the finer-textured soils (Figure 1b).

Amendment effects on extractable soil P varied between treatments. Extractable P concentrations in the sand were three times higher than in the other two soils, for both species (Table 2). In the sugar maple microcosms, biochar addition had no effect, but ash addition increased extractable P in the sandy-loam and silty-sand by 18 % and 30 %, respectively (Table 2). In the pine microcosms, however, ash addition increased extractable P by 70 % in the sand and both biochar and ash had significant positive effects in the sandy-loam.

Other macronutrients (Ca, K, and Mg), as well as some micronutrients (Na and Mn) also varied between soils, with the sand microcosms typically having the lowest nutrient availability (Table 2). The magnitude of amendment effects was variable, but ash addition significantly increased Ca, K, and Mg concentrations in all microcosms and biochar addition significantly increased K in all microcosms (Table 2). Ash effects on nutrient availability were generally substantially larger than biochar effects for the same soil/seedling combination (Table 2).

Seedling growth and nutrient content

Without amendments, sugar maple root biomass was lowest in the silty-sand microcosms, but there was no soil (and interrelated texture class) effect on aboveground biomass (Table 4). Red pine root biomass was not affected by soil type, but pines grown in the silty-sand had

significantly more aboveground biomass than those in the sand or sandy-loam and consequently a lower root-to-shoot ratio (Table 4; Fig. 2). Average leaf and needle surface area was similar for seedlings grown in sandy-loam and silty-sand, but pines grown in sand had less needle area and maples grown in sand had more leaf area, compared to the other soil types. Red pine seedlings grown in the sand also had a higher concentration of all macronutrients than pines grown in the other two soils, but the lowest total N concentration (Table 3). Sugar maple seedlings showed less consistent trends, though maples in sand still had the highest P and lowest total N of the three soils (Table 3).

In general, ash addition reduced root biomass of both maple and pine, though not always significantly, whereas biochar effects varied by species (Table 4, Supplementary Table 2). Seedling responses to biochar and ash addition were also strongly dependent on soil type/textural class (Table 4, Supplementary Table 2). The largest reductions occurred in the sandy-loam with 5 t ha⁻¹ of added biochar and 40 t ha⁻¹ of added ash (Table 4). Red pine root responses to both biochar and ash were more variable, but 5 t ha⁻¹ biochar and 40 t ha⁻¹ ash in sandy-loam also significantly reduced pine root biomass (Table 4). For sugar maple, root biomass was typically negatively correlated with increasing addition rates of ash, but not biochar (Table 4, Supplementary Table 2). Red pine root biomass was positively correlated with the biochar addition rate in the silty-sand-textured soil, with low addition rates decreasing root biomass but high addition rates increasing root biomass; this was the only positive response to treatments for either species (Table 4, Supplementary Table 2).

The response of aboveground biomass to the amendments also varied by species and soil texture (Table 4, Supplementary Table 2). Maple stem biomass was only significantly altered in the silty-sand, where stem biomass was negatively correlated with the amendment rates of both

biochar and ash (Table 4, Supplementary Table 2). Maple leaf biomass had no significant correlations (Table 4, Supplementary Table 2). Pine aboveground biomass also had minimal responses to biochar addition, but pine stem biomass also decreased with ash addition in the silty-sand (Table 4, Supplementary Table 2). The only positive correlation between biomass and increased rates of amendments occurred for pine seedlings grown in sand-textured soil with added ash, but biochar added to pine seedlings in sand produced a significantly negative relationship (Table 4, Supplementary Table 2). Leaf and needle surface area was never significantly correlated with addition rates of either amendment (Supplementary Table 2).

In contrast, root-to-shoot ratios of both seedling species had strong responses to amendments (Fig. 2, Supplementary Table 2). In the sandy-loam and sand microcosms, both biochar and ash addition reduced the root-to-shoot ratio for sugar maples and ash addition reduced the root-to-shoot ratio for red pines (Fig. 2). In the sand, pine root-to-shoot ratio increased with biochar addition (Fig. 2). Responses in silty-sand microcosms were more varied (Fig. 2).

Pine seedlings had higher tissue concentrations of P than sugar maple seedlings, but exhibited smaller responses to biochar and ash addition (Table 3). The P content of the sugar maples grown in sandy-loam and silty-sand-textured soils was only negligibly altered by amendments (Table 3), but biochar and ash both increased seedling P for maples grown in sand (Table 3). In contrast, red pines in sand showed no response in P content to amendments and effects in the other soils were varied (Table 3).

Microbial biomass

Microbial C and P were 2.5 to 5.5 times higher in the sandy-loam and silty-sand compared to the sand, before biochar or ash addition (Table 5). Amendment effects on microbial

C varied widely, but biochar addition did significantly reduce microbial C in microcosms planted with red pine (Table 5). Amendment effects on microbial P were strongly soil-dependent (Table 5). In the sandy-loam and silty-sand, both biochar and ash addition reduced microbial P, whereas the amendments generally increased microbial P in sand, though not always significantly (Table 5).

In the red pine microcosms only, microbial biomass C and P were both positively correlated with root biomass and microbial biomass P was negatively correlated with extractable P (Supplementary Table 1). Microbial biomass C in both pine and maple microcosms was positively correlated with soil moisture and negatively correlated with extractable P (Supplementary Table 1).

Phosphatase activity

Across all treatments, acid phosphatase activity was higher than alkaline phosphatase activity. Acid phosphatase activity was significantly lower in treatments with sugar maple compared to red pine, but there was no difference between control soils (Table 5). Biochar and ash addition decreased acid phosphatase activity in silty-sand, but not in sandy-loam or sand (Table 5).

Alkaline phosphatase activity was significantly lower in sand than in either sandy-loam or silty-sand, but was not affected by seedling species in the control soils (Table 5). Biochar reduced alkaline phosphatase activity in all sugar maple microcosms, whereas ash only had an effect in the sand microcosms (Table 5). In red pine microcosms, ash had negligible effects, but biochar significantly decreased alkaline phosphatase in the sand and significantly increased alkaline phosphatase activity in the silty-sand (Table 5). Acid phosphatase was not correlated with either microbial biomass or root biomass, but alkaline phosphatase was positively correlated

with microbial C and soil moisture (Supplementary Table 1). Alkaline phosphatase was also negatively correlated with extractable P (Supplementary Table 1).

DISCUSSION

Physicochemical, microbial, and seedling responses to both biochar and ash addition varied widely between the three soil textures, but neither amendment consistently increased seedling growth nor bioavailable P in all microcosms. In addition, sugar maple and red pine seedlings had some opposing responses to the same amendments. Overall, effects were more dependent on the seedling species and soil textural class, than on the amendment type, which was unexpected, given the inherent differences between biochar and wood ash. These results highlight the extreme importance of tailoring biochar and ash amendments to both the native soil conditions and the target tree species, in order to effectively use these amendments to increase tree nutrition.

Seedling growth responses to soil amendments

In the sandy-loam and sand, sugar maples responded more positively to biochar and ash addition than red pines did. These responses manifested as a decline in root biomass, compared to the controls, without an accompanying decline in aboveground biomass, potentially implying that the seedlings could meet their nutrient requirements while allocating fewer resources to root growth, as a decline in root-to-shoot ratio is typically evidence of increased soil fertility (Chapin 1980). Aboveground biomass was only significantly altered in one amended microcosm (red pine in silty-sand with 40 t ash ha⁻¹), indicating that while biochar and ash did not increase seedling growth during this experiment, the amendments also generally did not have negative effects. Varying responses between tree species are typical and studies have also often observed

larger biochar effects on angiosperms than gymnosperms (Pluchon et al. 2014; Thomas and Gale 2015). In this study, the different responses of the two tree species are most likely due to the variability in their nutrient requirements; the P demand of the sugar maple seedlings may have been met with the tested amendment rates, while the red pine seedlings were still P-limited. However, these results do contrast with a recent meta-analysis, in which biochars were shown to have consistent, strong, growth-promoting effects, particularly in short-term seedling studies (Thomas and Gale 2015). These positive biochar effects are typically attributed to fertilization and nutrient retention, as well as adsorption of allelopathic compounds (Pluchon et al. 2014; Thomas and Gale 2015). The null effects observed here may have been an artifact of the seedling microcosms. For example, the biochar used in this experiment was produced in a batch reactor, which can result in condensation of toxic volatile contaminants such as benzene and toluene (Spokas et al. 2011). These compounds have been shown to negatively affect plant growth, especially in a small soil volume (Deenik et al. 2010; Marks et al. 2014), which could explain the reduction of root biomass in some biochar treatments.

Biochar and ash effects on P uptake

Overall, the only microcosms that exhibited signs of increased P uptake after amendment were the maple-sand pots. Despite the significant reductions in root biomass with 20 t ha⁻¹ of biochar and 16 t ha⁻¹ of ash, P content per unit biomass of the seedlings increased by 30 to 50 % relative to the controls after both biochar and ash amendment, and total P uptake was also considerably higher after ash addition. Previous studies have also found soil type and species effects on plant nutrient responses to biochar and ash. For example, Yeboah et al. (2009) discovered that biochar increased maize nutrient uptake in a sandy-loam, but not in a silty-sandy-loam soil; Jin et al. (2016) observed stronger P response to biochar addition in silty-sand loam

compared to clay loam; Jacobson et al. (2004) noticed differing effects of ash in pine-dominated forests compared to mixed-coniferous forests; and Erich and Ohno (1992) found that P uptake by corn after ash addition was higher in loamy soils than sand-textured ones. However, all of these prior studies have only tested biochar or ash addition, not both.

The variable responses observed in this study may be partly due to changes in soil acidity. Seedling P effects were largest in the sand-ash microcosms, perhaps because the ash-amended soils were closest to the ideal 6.5 pH for P availability (Plante 2007; Whalen and Sampedro 2010). The sandy-loam and silty-sand were initially almost 1 pH unit more acidic than the sand, so we expected those soils to have strong pH responses to the amendments (Xu et al. 2014). In contrast, adding 20 t ha⁻¹ of biochar to the sandy-loam and silty-sand in this study did not alter pH, while adding biochar to the sand increased soil pH by an average of 0.24 pH units. Phosphorus compounds are more likely to be adsorbed and removed from the soil solution when pH is below 7 (Xu et al. 2014), which may explain why the soils that were around 4.7 after biochar addition had less P available for plant uptake. On the other hand, Erich and Ohno (1992) compared eight different soils and found that the soils with the highest pH buffering capacity had the highest rates of plant P-uptake, presumably because the elevated levels of H⁺ led to more dissolution of wood ash and thus higher amounts of P solubilization. As the sandy-loam and silty-sand clearly had higher buffering capacity than the sand, more P may have been released from the ash amendment in those soils, which fits with ash addition significantly increasing extractable soil P in the maple sandy-loam and silty-sand treatments.

An alternative explanation is that responses were affected by initial nutrient concentrations or other aspects of soil chemistry in the three soils. For example, N-limited forest sites typically do not exhibit growth responses or increased foliar nutrient concentrations after

ash addition because ash does not add N back to the soil (Jacobson et al. 2004; Wallander et al. 2005). However, the sandy-loam soil, in particular, is known to have excess N and to be P-limited (Gradowski and Thomas 2006, 2008), so it should thus be a strong candidate for positive responses to ash amendments, though that was not seen in this study. High concentrations of Al^{3+} or Fe^{3+} can also limit the PO_4^- present in the soil solution (Giesler et al. 2002), so it is possible that the sandy-loam and silty-sand contained more Al and Fe than the sand, which could contribute to the lack of increased plant tissue P in those microcosms. Limited effects on soil P availability can also occur when the Ca in the added ash or biochar forms insoluble Ca phosphates (Jacobson et al. 2004; Xu et al. 2013). Because the same ash and biochar was used for all three soils, any differences in Ca:P ratio would be resultant of the initial soil properties. For example, since the silty-sand soils were more Ca-rich, initially, more of the added ash-P might be complexed. On the other hand, many microbes can solubilize Ca-P complexes, whereas very few microbes can solubilize Fe-P or Al-P complexes (Banik and Dey 1983; Kucey et al. 1989). Heavy metals, such as those often found in ash, can also inhibit phosphomonoesterases, which could reduce P availability (Tabatabai 1994). However, because ash did not reduce phosphatase activity more than biochar did, it is unlikely that heavy metal concentrations were high enough to have an effect.

Variability in amendment effects could also be due to secondary-compound formation from the humic material incorporated into these microcosms (Jacobson et al. 2004) or from differences in soil surface area. Biochar particles can act as a P sink when applied at a high enough rate (Parvage et al. 2013), but the same application rate was used in all three soils, so this is less likely to be a factor in these results. However, as the sand had the lowest initial surface area, biochar addition likely substantially increased relative surface area, which could lead to

more nutrient retention. Jacobson et al. (2004) also suggested that variability could be due to differences in plant nutrient-uptake ability. Variability in P-uptake between the two species could explain the differences in soil P between sugar maple and red pine microcosms.

There were no significant increases in microbial biomass P in the maple-sand pots, which likely indicates the soil microbial community may only have had a minimal role in the observed increase in plant and soil P in these microcosms, especially given the significant negative correlation between microbial biomass P and extractable soil P in the pine microcosms. However, it is possible that sorption by the biochar particles resulted in underestimation of microbial biomass in the biochar treatments (Thies et al. 2015). Similarly, both biochar and ash addition in these microcosms significantly reduced the activity of alkaline phosphatase, which was also negatively correlated with extractable and total soil P. Acid phosphatases derive from plants, fungi, and bacteria, so changes in potential activity cannot be attributed solely to microbial responses (Nannipieri et al. 2011), but alkaline phosphatases are produced only by bacteria and fungi, not plants (Tabatabai 1994). Consequently, the decline in alkaline phosphatase activity observed in this study, especially combined with the significant correlation between alkaline phosphatase activity and microbial carbon biomass, may indicate that soil microbes are playing a lesser role in P mobilization after biochar and ash addition.

However, there are limitations to using spectrophotometry to assess phosphatase activity that could impact our interpretation of these results. The source of the phosphatase enzymes is unknown, since the stabilized extracellular enzymes, i.e. those complexed with organic matter, cannot be separated from activity associated with active microbial cells (Nannipieri et al. 2011). In addition, biochar particles can sorb enzymes, affecting colorimetric assays (Thies et al. 2015). Nonetheless, because microbial biomass C and alkaline phosphatase activity were significantly

correlated, this may indicate that the enzymes are more likely to be produced by the extant microbial community (Taylor et al. 2002). These methods also measure potential enzyme activity, assuming the concentration and diffusion of substrate are non-limiting, which most likely overestimates in situ activity (Wallenstein and Weintraub 2008). Increased enzyme activity with nutrient addition indicates more enzyme production, but it is also necessary to account for enzyme degradation, enzyme stabilization, and microbial community changes (Waldrop et al. 2000) that could be affected by the presence of biochar and ash. Conclusively separating acid phosphatase activity from alkaline phosphatase activity can be challenging, as well, since alkaline phosphatase can still be active at pH 6.5 (Nannipieri et al. 2011). Similarly, alkaline phosphatase activity is strongly correlated with soil moisture, so any drying of the soil that occurred during analysis may have decreased the measured activity (Tabatabai 1994).

In addition, this study only determined the potential activity of phosphomonoesterases, which hydrolyze organic phosphate compounds with a single ester bond, whereas a substantial portion of the P in plant-derived biochar is pyrophosphate (Qian et al. 2013; Uchimiya and Hiradate 2014). Pyrophosphate has two ester bonds, and is thus broken down by inorganic pyrophosphatase (pyrophosphate phosphohydrolase), which is produced by bacteria and plants (Tabatabai 1994). Even though multiple biochars have been shown to contain pyrophosphate, there have been no studies of the relationship between biochar and inorganic pyrophosphatase activity. Consequently, it is possible that microbial mineralization of pyrophosphates contributed to the increased P availability in the biochar treatments, so this needs to be addressed in future work.

Most likely, physicochemical reactions in the soil are mainly responsible for the isolated increases in bioavailable P. This corresponds to previous studies, in which biochar applied to a

sandy soil increased P availability by directly releasing P from the biochar particles, in addition to indirectly by raising soil pH (Xu et al. 2013). Ohno (1992) observed the same effects with wood ash applied to 10 acidic soils; soil P increased through a combination of direct release and ash-induced pH effects. Omil et al. (2013) also found that adding wood ash to a sandy soil increased tree growth and P nutrition due to both direct ash effects and stimulation of the soil microbial community, though that study focused on pine growth, not maple. However, to fully characterize any microbial effects on P availability in these soils, a complete P budget needs to be created, along with a detailed analysis of the functional genes present in the microbial community. In addition, while this experiment only assessed the microbial community after 12 weeks, closer examinations of the dynamics of plant-soil-microbial interactions through time are likely warranted.

Conclusions

Neither ash nor biochar consistently increased seedling growth and instead sugar maple and red pine seedlings often had opposing responses to the same amendment/soil combination. Sugar maples grown in sand were the only set of microcosms to show signs of increased bioavailable P and they responded similarly to both biochar and ash. This increase in plant-available P after amendment addition was likely mainly due to chemical reactions, rather than microbially-mediated processes. Overall, these results indicate that biochar and ash addition may be able to alleviate soil P deficiencies and increase sugar maple P nutrition in some acidic forest soils, but may have minimal effects in other soil types and on red pine growth. Because these effects can range from neutral to negative, it is necessary to account for soil type, tree species, and application rate when planning an amendment scheme.

It is also important to interpret these results in the context of this being a microcosm experiment; incubation experiments are not always an accurate reflection of how soil amendments will influence soil and microbial properties under field conditions and short-term experiments may not portray long-term biochar and ash effects. For example, biochar can increase soil P by reducing P leaching (Laird et al. 2010), but this is less important in incubations than in the field. It is most likely that field conditions will only exacerbate the variability observed in the microcosms, making it especially important to properly tailor amendment protocols. Augusto et al. (2008) also caution that when ash causes a short-term (1 to 5 years) increase in foliar P, that effect can disappear by years 6 to 10, further suggesting that short-term incubation results should be interpreted with caution. In addition, the added ash and biochar were concentrated near the surface of each microcosm, rather than evenly distributed throughout the entire volume of soil. While this technique mimics how amendments would be applied in a field-scale context, where mixing the ash or biochar into the forest soil is not logistically feasible, it likely contributes to the high variability observed in these results. For example, the nutrients in the added particles need to move from the amendment into the soil and only then into the seedlings. There may also be a soil texture interaction, where the ash and biochar particles move more easily through the coarse sand, compared to the other soils.

REFERENCES

- Atkinson, C. 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil* **337**: 1–18.
- Augusto, L., Bakker, M.R., and Meredieu, C. 2008. Wood ash applications to temperate forest ecosystems—potential benefits and drawbacks. *Plant Soil* **306**: 181–198.
doi:10.1007/s11104-008-9570-z
- Banik, S. and Dey, B.K. 1983. Phosphate-solubilizing potentiality of the microorganisms capable of utilizing aluminium phosphate as a sole phosphate source. *Zentralblatt Für Mikrobiol.* **138**: 17–23. doi:10.1016/S0232-4393(83)80060-2
- Biederman, L.A. and Harpole, W.S. 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *GCB Bioenergy* **5**: 202–214. doi:10.1111/gcbb.12037
- Bridle, T.R. and Pritchard, D. 2004. Energy and nutrient recovery from sewage sludge via pyrolysis. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* **50**: 169–175.
- Brookes, P.C., D.S. Powlson, D.S. Jenkinson. 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* **14**(4): 319–329. doi:10.1016/0038-0717(82)90001-3
- Chapin, F.S. 1980. The mineral nutrition of wild plants. *Annu. Rev. Ecol. Syst.* **11**: 233–260.
doi:10.1146/annurev.es.11.110180.001313
- Clarholm, M. 1998. Wood ash to counteract potential phosphorus and potassium limitations in a Norway spruce forest subjected to air pollution. *Scand. J. For. Res.* **2**: 127–136.
- Clarholm, M. 1994. Granulated wood ash and a “N-free” fertilizer to a forest soil—effects on P availability. *For. Ecol. Manag.* **66**: 127–136. doi:10.1016/0378-1127(94)90152-X
- Cui, H.-J., Wang, M.K., Fu, M.-L., and Ci, E. 2011. Enhancing phosphorus availability in phosphorus-fertilized zones by reducing phosphate adsorbed on ferrihydrite using rice straw-derived biochar. *J. Soils Sediments* **11**: 1135–1141. doi:10.1007/s11368-011-0405-9

- D'Angelo, E., Crutchfield, J., and Vandiviere, M. 2001. Rapid, sensitive, microscale determination of phosphate in water and soil. *J. Environ. Qual.* **30**: 2206–2209.
- Deenik, J.L., McClellan, T., Uehara, G., Antal, M.J., and Campbell, S. 2010. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. *Soil Sci. Soc. Am. J.* **74**: 1259–1270.
- DeLuca, T.H., MacKenzie, M.D., and Gundale, M.J. 2009. Chapter 14: Biochar effects on soil nutrient transformations. Pages 251-270 in J. Lehmann and S. Joseph, eds. *Biochar for Environmental Management*. Earthscan, Washington, DC, USA.
- DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., and Holben, W.E. 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. *Soil Sci. Soc. Am. J.* **70**: 448–453. doi:10.2136/sssaj2005.0096
- Erich, M.S. and Ohno, T. 1992. Phosphorus availability to corn from wood ash-amended soils. *Water, Air, Soil Pollut.* **64**: 475–485. doi:10.1007/BF00483357
- Giesler, R., Petersson, T., and Högberg, P. 2002. Phosphorus limitation in boreal forests: effects of aluminum and iron accumulation in the humus layer. *Ecosystems* **5**: 300–314. doi:10.1007/s10021-001-0073-5
- Gorgolewski, A., J. Caspersen, P. Hazlett, T. Jones, H. Tran, and N. Basiliko. 2016. Responses of Eastern Red-backed Salamander (*Plethodon cinereus*) abundance 1 year after application of wood ash in a northern hardwood forest. *Can. J. For. Res.* **46**(3): 402-409. doi:10.1139/cjfr-2015-0230.
- Gradowski, T. and Thomas, S.C., 2008. Responses of *Acer saccharum* canopy trees and saplings to P, K and lime additions under high N deposition. *Tree Physiol.* **28**: 173–185. doi:10.1093/treephys/28.2.173

- Gradowski, T. and Thomas, S.C., 2006. Phosphorus limitation of sugar maple growth in central Ontario. *For. Ecol. Manag.* **226**: 104–109. doi:10.1016/j.foreco.2005.12.062
- Jacobson, S., Högbom, L., Ring, E., and Nohrstedt, H.-Ö. 2004. Effects of wood ash dose and formulation on soil chemistry at two coniferous forest sites. *Water. Air. Soil Pollut.* **158**: 113–125. doi:10.1023/B:WATE.0000044834.18338.a0
- Jeannotte, R., Sommerville, D.W., Hamel, C., and Whalen, J.K. 2004. A microplate assay to measure soil microbial biomass phosphorus. *Biol. Fertil. Soils* **40**: 201–205. doi:10.1007/s00374-004-0760-4
- Jin, Y., Liang, X., He, M., Liu, Y., Tian, G., and Shi, J. 2016. Manure biochar influence upon soil properties, phosphorus distribution and phosphatase activities: A microcosm incubation study. *Chemosphere* **142**: 128–135. doi:10.1016/j.chemosphere.2015.07.015
- Kalra, Y.P. and Maynard, D.G. 1991. Methods manual for forest soil and plant analysis. For. Can., Northwest Reg., North. For. Cent., Edmonton, AB. Inf. Rep. NOR-X-319.
- Kucey, R.M.N., Janzen, H.H., and Leggett, M.E. 1989. Microbially mediated increases in plant-available phosphorus. *Adv. Agron.* **42**: 199–228.
- Laird, D.A., Fleming, P., Davis, D.D., Horton, R., Wang, B., and Karlen, D.L. 2010. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma* **158**: 443–449. doi:10.1016/j.geoderma.2010.05.013
- Mahmood, S., Finlay, R.D., Fransson, A.-M., and Wallander, H. 2003. Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. *FEMS Microbiol. Ecol.* **43**: 121–131. doi:10.1111/j.1574-6941.2003.tb01051.x

- Mahmood, S., Finlay, R.D., Wallander, H., and Erland, S. 2002. Ectomycorrhizal colonisation of roots and ash granules in a spruce forest treated with granulated wood ash. *For. Ecol. Manag.* **160**: 65–74. doi:10.1016/S0378-1127(01)00462-5
- Marks, E.A.N., Alcañiz, J.M., and Domene, X. 2014. Unintended effects of biochars on short-term plant growth in a calcareous soil. *Plant Soil* **385**: 87–105. doi:10.1007/s11104-014-2198-2
- Nannipieri, P., Giagnoni, L., Landi, L., and Renella, G. 2011. Role of phosphatase enzymes in soil. Pages 215-243 in E. Bütemann, A. Oberson, and E. Frossard, eds. *Phosphorus in Action, Soil Biology*. Springer Berlin Heidelberg, Berlin, Germany.
- Novak, J.M., Busscher, W.J., Laird, D.L., Ahmedna, M., Watts, D.W., and Niandou, M.A.S. 2009. Impact of biochar amendment on fertility of a southeastern Coastal Plain soil: *Soil Sci.* **174**: 105–112. doi:10.1097/SS.0b013e3181981d9a
- Noyce, G.L., N. Basilko, R. Fulthorpe, T.E. Sackett, and S.C. Thomas. 2015. Soil microbial responses over 2 years following biochar addition to a north temperate forest. *Biol. Fert. Soils.* **51**(6): 649-659. doi: 10.1007/s00374-015-1010-7.
- Noyce, G.L., R. Fulthorpe, A. Gorgolewski, P. Hazlett, H. Tran, and N. Basiliko. 2016. Soil microbial responses to wood ash addition and forest fire in managed Ontario forests. *Appl. Soil. Ecol.* **107**: 368-380. doi: 10.1016/j.apsoil.2016.07.006
- Ohno, T. 1992. Neutralization of soil acidity and release of phosphorus and potassium by wood ash. *J. Environ. Qual.* **21**: 433. doi:10.2134/jeq1992.00472425002100030022x
- Omil, B., Piñeiro, V., and Merino, A. 2013. Soil and tree responses to the application of wood ash containing charcoal in two soils with contrasting properties. *For. Ecol. Manag.* **295**: 199–212. doi:10.1016/j.foreco.2013.01.024

- Parvage, M.M., Ulén, B., Eriksson, J., Stroock, J., and Kirchmann, H. 2013. Phosphorus availability in soils amended with wheat residue char. *Biol. Fertil. Soils* **49**: 245–250. doi:10.1007/s00374-012-0746-6
- Plante, A.F., 2007. Chapter 15: Soil biogeochemical cycling of inorganic nutrients and metals. Pages 283-302 in E.A. Paul, ed. *Soil Microbiology, Ecology, and Biochemistry*. Academic Press, Oxford, U.K.
- Pluchon, N., Gundale, M.J., Nilsson, M.-C., Kardol, P., and Wardle, D.A. 2014. Stimulation of boreal tree seedling growth by wood-derived charcoal: effects of charcoal properties, seedling species and soil fertility. *Funct. Ecol.* **28**: 766–775. doi:10.1111/1365-2435.12221
- Popova, I.E. and Deng, S. 2010. A high-throughput microplate assay for simultaneous colorimetric quantification of multiple enzyme activities in soil. *Appl. Soil Ecol.* **45**: 315–318. doi:10.1016/j.apsoil.2010.04.004
- Powlson, D.S., Prookes, P.C., and Christensen, B.T. 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biol. Biochem.* **19**: 159–164. doi:10.1016/0038-0717(87)90076-9
- Qian, T., Zhang, X., Hu, J., and Jiang, H. 2013. Effects of environmental conditions on the release of phosphorus from biochar. *Chemosphere* **93**: 2069–2075. doi:10.1016/j.chemosphere.2013.07.041
- Richardson, A.E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Funct. Plant Biol.* **28**: 897–906.
- Richardson, A.E. and Simpson, R.J. 2011. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* **156**: 989–996. doi:10.1104/pp.111.175448

- Saarsalmi, A., Smolander, A., Kukkola, M., Moilanen, M. and Saramäki, J. 2012. 30-Year effects of wood ash and nitrogen fertilization on soil chemical properties, soil microbial processes and stand growth in a Scots pine stand. *For. Ecol. Manag.* **278**: 63–70. doi:10.1016/j.foreco.2012.05.006
- Sackett, T.E., N. Basiliko, G.L. Noyce, C. Winsborough, J. Schurman, C. Ikeda, and S.C. Thomas. 2014. Soil and greenhouse gas responses to biochar addition in a temperate hardwood forest. *GCB Bioenergy* **7**(5): 1062-1074. doi:10.1111/gcbb.12211
- Soil Classification Working Group. 1998. The Canadian System of Soil Classification, 3rd ed. - Agriculture and Agri-Food Canada Publication 1646, 187 pp.
- Spokas, K.A., Novak, J.M., Stewart, C.E., Cantrell, K.B., Uchimiya, M., DuSaire, M.G., and Ro, K.S. 2011. Qualitative analysis of volatile organic compounds on biochar. *Chemosphere* **85**: 869–882. doi:10.1016/j.chemosphere.2011.06.108
- Tabatabai, M.A., 1994. Chapter 37: Soil enzymes. Pages 801-834 in R.W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, and A Wollum, eds. *Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, Wisconsin, USA.
- Taylor, J.P., Wilson, B., Mills, M.S. and Burns, R.G. 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil Biol. Biochem.* **34**: 387–401. doi:10.1016/S0038-0717(01)00199-7
- Thies, J.E., M.C. Rillig, and E.R. Graber. 2015. Chapter 13: Biochar effects on the abundance, activity, and diversity of the soil biota. Pages 327-389 in J. Lehmann and S. Josepd, eds. *Biochar for Environmental Management: Science, Technology, and Implementation*. Routledge, London, U.K.

- Thomas, S.C. and Gale, N. 2015. Biochar and forest restoration: a review and meta-analysis of tree growth responses. *New For.* **46**: 931–946. doi:10.1007/s11056-015-9491-7
- Uchimiya, M. and Hiradate, S. 2014. Pyrolysis temperature-dependent changes in dissolved phosphorus speciation of plant and manure biochars. *J. Agric. Food Chem.* **62**: 1802-1809. doi:10.1021/jf4053385
- Vance, E.D., Brookes, P.C. and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **19**: 703–707. doi:10.1016/0038-0717(87)90052-6
- Vitousek, P.M., Porder, S., Houlton, B.Z. and Chadwick, O.A. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen—phosphorus interactions. *Ecol. Appl.* **20**: 5–15.
- Waldrop, M.P., Balser, T.C., and Firestone, M.K. 2000. Linking microbial community composition to function in a tropical soil. *Soil Biol. Biochem.* **32**: 1837–1846. doi:10.1016/S0038-0717(00)00157-7
- Wallander, H., Fossum, A., Rosengren, U. and Jones, H. 2005. Ectomycorrhizal fungal biomass in roots and uptake of P from apatite by *Pinus sylvestris* seedlings growing in forest soil with and without wood ash amendment. *Mycorrhiza* **15**: 143–148. doi:10.1007/s00572-004-0312-7
- Wallenstein, M.D. and Weintraub, M.N. 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. *Soil Biol. Biochem.* **40**: 2098–2106. doi:10.1016/j.soilbio.2008.01.024
- Whalen, J.K. and Sampedro, L. 2010. *Soil Ecology and Management*. CABI, Oxford, U.K.

- Xu, G., Sun, J., Shao, H., and Chang, S.X. 2014. Biochar had effects on phosphorus sorption and desorption in three soils with differing acidity. *Ecol. Eng.* **62**: 54–60.
doi:10.1016/j.ecoleng.2013.10.027
- Xu, G., Wei, L.L., Sun, J.N., Shao, H.B. and Chang, S.X., 2013. What is more important for enhancing nutrient bioavailability with biochar application into a sandy soil: Direct or indirect mechanism? *Ecol. Eng.* **52**: 119–124. doi:10.1016/j.ecoleng.2012.12.091
- Yeboah, E., Ofori, P., Quansah, G.W., Dugan, E. and Sohi, S.P. 2009. Improving soil productivity through biochar amendments to soils. *Afr. J. Environ. Sci. Technol.* **3**: 34–41.

TABLES

Table 1. Properties of biochar (derived from maple sawdust) and wood ash (derived from conifer bark) used in this experiment

	Biochar	Ash
pH (H ₂ O)	7.22	10.5
pH (CaCl ₂)	6.90	10.1
Total N (%)	0.32	0.19
Total C (%)	80.0	18.9
LOI (%)	97.8	21.6
P (%)	0.02	0.43
K (%)	0.32	3.99
Ca (%)	0.50	7.86
Mg (%)	0.06	0.95
Fe (ppm)	49.7	17900
Cu (ppm)	5.01	60.5
Mn (ppm)	368	6,790
Zn (ppm)	23.9	1720
Al (ppm)	--	3200
Na (ppm)	52.9	11300

Table 2. Summary of soil nutrient availability for each treatment.

	SUGAR MAPLE								
	SAND			SANDY-LOAM			SILTY-SAND		
	Control ^a	Biochar effect (%) ^b	Ash effect (%) ^b	Control	Biochar effect (%)	Ash effect (%)	Control	Biochar effect (%)	Ash effect (%)
Extractable P (mg P L ⁻¹)	52.8 (.19) <i>a</i>	- 1.3 (.74)	- 0.80 (.59)	15.7 (.31) <i>b</i>	- 0.96 (1.9)	+ 18 (1.9)	11.1 (1.1) <i>c</i>	- 0.36 (3.7)	+ 30 (4.9)
Total C (%)	1.29 (.03) <i>a</i>	+ 52 (5.7)	+3.2 (3.1)	4.4 (.09) <i>b</i>	+ 28 (6.2)	+ 3.2 (4.0)	n/a ^c	n/a ^c	n/a ^c
Ca (mg L ⁻¹)	221 (5.3) <i>a</i>	-1.2 (3.0)	+ 96 (4.5)	243 (12) <i>b</i>	-3.2 (3.2)	+ 111 (10)	412 <i>d</i>	+ 5.0 (6.0)	+ 109 (9.2)
K (mg L ⁻¹)	16.8 (1.0) <i>a</i>	+ 102 (16)	+ 428 (25)	30.3 (1.9) <i>b</i>	+ 92 (7.1)	+ 312 (11)	22.0 <i>d</i>	+ 183 (34)	+ 566 (56)
Mg (mg L ⁻¹)	24.3 (.73) <i>a</i>	- 3.4 (2.7)	+ 64 (4.5)	31.2 (2.0) <i>b</i>	- 9.7 (5.7)	+ 60 (7.7)	37.7 <i>d</i>	+ 3.6 (6.6)	+ 88 (8.5)
Mn (mg L ⁻¹)	0.8 (.04) <i>a</i>	+ 8.2 (2.8)	+ 309 (27)	2.5 (.11) <i>b</i>	- 1.6 (6.1)	+ 182 (19)	4.2 <i>d</i>	+ 22 (10)	+ 151 (33)
Na (mg L ⁻¹)	79.9 (8.0) <i>a</i>	- 14 (6.0)	+ 34.5 (12)	141 (20) <i>b</i>	- 20 (7.7)	+ 8.3 (9.4)	119 <i>d</i>	+ 21 (5.0)	+ 30 (13)

	RED PINE								
	SAND			SANDY-LOAM			SILTY-SAND		
	Control	Biochar effect (%)	Ash effect (%)	Control	Biochar effect (%)	Ash effect (%)	Control	Biochar effect (%)	Ash effect (%)
Extractable P (mg L ⁻¹)	52.4 (.32) <i>a</i>	- 0.63 (.27)	+ 72 (9.9)	16.3 (.34) <i>b</i>	+ 5.6 (1.3)	+ 15 (2.5)	10.7 (.29) <i>c</i>	+ 0.28 (3.1)	+ 12 (27)
Total C (%)	1.25 (.02) <i>a</i>	+ 77 (13)	+ 8.3 (3.2)	4.39 (.23) <i>b</i>	+ 20 (2.9)	+ 0.8 (1.7)	n/a ^c	n/a ^c	n/a ^c
Ca (mg L ⁻¹)	255 (5.5) <i>a</i>	- 4.8 (2.1)	+ 104 (7.5)	263 (17) <i>a</i>	- 11 (2.4)	+ 99 (6.8)	439 (5.4) <i>b</i>	+ 1.1 (2.4)	+ 62 (14)
K (mg L ⁻¹)	28.5 (.69) <i>a</i>	+ 83 (3.4)	+ 301 (14)	67.0 (11) <i>b</i>	+ 57 (22)	+ 174 (27)	45.6 (2.1) <i>c</i>	+ 55 (17)	+ 339 (52)
Mg (mg L ⁻¹)	29.9 (1.1) <i>a</i>	- 4.7 (2.0)	+ 37 (3.8)	33.1 (2.3) <i>a</i>	- 12 (3.1)	+ 55 (7.3)	41.7 (.64) <i>b</i>	- 3.6 (2.3)	+ 42 (13)
Mn (mg L ⁻¹)	0.7 (.05) <i>a</i>	+ 26 (6.0)	+ 512 (34)	4.8 (.37) <i>b</i>	-1.8 (4.7)	+ 136 (17)	6.8 (.67) <i>b</i>	+ 17 (16)	+ 54 (28)
Na (mg L ⁻¹)	91.7 (12) <i>a</i>	- 25 (3.7)	+ 6.2 (17)	135 (10) <i>b</i>	- 22 (3.2)	+ 35 (10)	211 (12) <i>c</i>	- 7.6 (5.1)	- 0.84 (16)

Note: Values represent means (SE).

^aControl columns are absolute values; letters indicate significant differences between soils for each seedling species.

^bBiochar and ash effect columns are the percent change relative to their respective control soil after addition of either 20 t ha⁻¹ biochar or 16 t ha⁻¹ ash; bold font indicates a significant amendment effect.

^cBelow detection limit.

^dOnly one replicate with detectable values.

For Review Only

Table 3. Chemical analysis of sugar maple and red pine seedlings (pooled roots, stems, and leaves) from each soil/amendment combination.

SUGAR MAPLE									
	SAND			SANDY-LOAM			SILTY-SAND		
	Control	Biochar	Ash	Control	Biochar	Ash	Control	Biochar	Ash
P (mg Kg ⁻¹)	824	1055	1218	668	626	619	645	669	708
Ca (mg Kg ⁻¹)	8194	8344	7860	8013	7267	7395	8828	9551	7031
K (mg Kg ⁻¹)	4261	6262	9505	5131	6486	7038	3644	5355	6903
Mg (mg Kg ⁻¹)	2079	2229	1948	1319	1359	1298	1351	1596	1074
TN (%)	0.66	0.67	0.64	1.32	1.29	1.42	0.94	0.92	1.06
TC (%)	45.2	44.6	44.7	44.6	44.7	44.7	45.2	45.2	44.5
TS (%)	0.07	0.09	0.10	0.11	0.09	0.11	0.10	0.09	0.10
RED PINE									
	SAND			SANDY-LOAM			SILTY-SAND		
	Control	Biochar	Ash	Control	Biochar	Ash	Control	Biochar	Ash
P (mg Kg ⁻¹)	1959	1973	1963	1053	1220	1104	1283	1346	1124
Ca (mg Kg ⁻¹)	3757	4007	3693	3108	2944	3438	2890	3046	3478
K (mg Kg ⁻¹)	7577	6836	10323	6200	6783	9047	5989	8531	8839
Mg (mg Kg ⁻¹)	1704	1674	1728	973	911	1063	988	973	1055
TN (%)	0.83	1.06	0.89	1.68	1.38	1.84	1.49	1.70	1.83
TC (%)	46.4	46.1	45.6	46.1	45.9	45.6	46.2	46.3	45.8
TS (%)	0.10	10.11	0.11	0.10	0.10	0.11	0.11	0.12	0.15

Table 4. Seeding biomass data.

	SUGAR MAPLE							RED PINE						
	Biochar			Ash (t ha ⁻¹)				Biochar (t ha ⁻¹)				Ash (t ha ⁻¹)		
	Control ^a	5 t ha ⁻¹	20 t ha ⁻¹	50 t ha ⁻¹	4 t ha ⁻¹	16 t ha ⁻¹	40 t ha ⁻¹	Control	5 t ha ⁻¹	20 t ha ⁻¹	50 t ha ⁻¹	4 t ha ⁻¹	16 t ha ⁻¹	40 t ha ⁻¹
SAND														
Root biomass (g)	0.65 (.05) <i>ab</i>	0.55 (.06)	0.45 (.07)	0.54 (.05)	0.55 (.06)	0.48 (.06)	.053 (.08)	0.08 (.01) <i>a</i>	0.09 (.00)	0.09 (.01)	0.08 (.01)	0.07 (.00)	0.07 (.01)	0.06 (.01)
Stem biomass (g)	0.13 (.02) <i>a</i>	0.11 (.02)	0.10 (.01)	0.13 (.01)	0.12 (.01)	0.11 (.01)	0.11 (.02)	0.01 (.00) <i>a</i>	0.02 (.00)	0.02 (.00)	0.01 (.00)	0.02 (.00)	0.02 (.00)	0.02 (.00)
Leaf biomass (g)	0.21 (.02) <i>a</i>	.019 (.02)	.019 (.02)	.021 (.02)	0.21 (.01)	0.21 (.02)	0.20 (.03)	0.06 (.01) <i>a</i>	0.08 (.01)	0.06 (.01)	0.05 (.00)	0.06 (.00)	0.08 (.01)	0.08 (.01)
Total biomass (g)	0.99 (.08) <i>a</i>	.84 (.08)	0.74 (.10)	0.88 (.07)	0.89 (.07)	0.79 (.08)	0.84 (.12)	0.15 (.02) <i>a</i>	0.18 (.01)	0.17 (.02)	.015 (.01)	0.15 (.01)	0.17 (.01)	0.17 (.02)
SANDY-LOAM														
Root biomass (g)	0.62 (.03) <i>a</i>	0.31 (.06)	0.46 (.05)	0.49 (.08)	0.46 (.05)	0.56 (.08)	0.40 (.04)	0.09 (.01) <i>a</i>	0.07 (.00)	0.09 (.01)	0.09 (.02)	0.08 (.01)	0.07 (.01)	0.05 (.01)
Stem biomass (g)	0.08 (.01) <i>b</i>	0.08 (.01)	0.10 (.01)	0.10 (.01)	0.09 (.01)	0.11 (.01)	0.09 (.00)	0.02 (.00) <i>b</i>	0.02 (.00)	0.02 (.00)	0.02 (.00)	0.02 (.00)	0.02 (.00)	0.02 (.00)
Leaf biomass (g)	0.30 (.03) <i>a</i>	0.28 (.05)	0.32 (.03)	0.33 (.06)	0.27 (.04)	0.36 (.04)	0.29 (.02)	0.10 (.02) <i>ab</i>	0.10 (.01)	0.11 (.02)	0.09 (.01)	0.14 (.02)	0.09 (.01)	0.10 (.02)
Total biomass (g)	1.00 (.05) <i>a</i>	0.67 (.12)	0.88 (.06)	0.93 (.14)	0.81 (.09)	1.03 (.11)	0.77 (.06)	0.21 (.03) <i>ab</i>	0.18 (.02)	0.22 (.03)	0.19 (.02)	0.25 (.04)	0.18 (.02)	0.17 (.02)
SILTY-SAND														
Root biomass (g)	0.52 (.03) <i>b</i>	0.56 (.07)	0.47 (.07)	0.51 (.10)	0.59 (.04)	0.49 (.04)	0.43 (.05)	0.09 (.01) <i>a</i>	0.08 (.00)	0.12 (.01)	0.14 (.01)	0.10 (.01)	0.13 (.01)	0.07 (.01)
Stem biomass (g)	0.12 (.01) <i>a</i>	0.12 (.02)	0.10 (.01)	0.09 (.01)	0.14 (.01)	0.11 (.01)	0.10 (.01)	0.03 (.00) <i>b</i>	0.03 (.00)	0.03 (.00)	0.03 (.00)	0.02 (.00)	0.03 (.00)	0.02 (.00)
Leaf biomass (g)	0.23 (.03) <i>ab</i>	0.23 (.03)	0.26 (.04)	0.21 (.02)	0.30 (.04)	0.21 (.02)	0.23 (.03)	0.12 (.02) <i>b</i>	0.14 (.02)	0.15 (.02)	0.14 (.01)	0.10 (.01)	0.13 (.01)	0.08 (.01)
Total biomass (g)	0.87 (.06) <i>a</i>	0.92 (.10)	0.84 (.10)	0.81 (.12)	1.03 (.07)	0.81 (.07)	0.76 (.08)	0.25 (.03) <i>b</i>	0.25 (.03)	0.30 (.03)	0.31 (.03)	0.21 (.03)	0.28 (.02)	0.18 (.01)

Note: Values represent means (SE).
^aLetters indicate significant differences between soils for each seedling species.
^bBold font indicates a significant amendment effect.

Table 5. Summary of microbial biomass and phosphatase activity in each treatment.

	SUGAR MAPLE								
	SAND			SANDY-LOAM			SILTY-SAND		
	Control ^a	Biochar effect (%) ^b	Ash effect (%) ^b	Control	Biochar effect (%)	Ash effect (%)	Control	Biochar effect (%)	Ash effect (%)
Microbial biomass C ($\mu\text{g C g}^{-1}$ soil)	14.8 (4.8) ^a	+ 17.0 (38)	+38.7 (31)	71.3 (13) ^b	-0.92 (29)	+ 9.75 (19)	82.3 (11) ^b	- 10.9 (8.2)	+ 2.88 (11)
Microbial biomass P ($\mu\text{g P g}^{-1}$ soil)	0.66 (.21) ^a	+ 106 (77)	+ 125 (63)	1.71 (.29) ^b	- 12.1 (21)	- 12.2 (26)	2.25 (.31) ^b	- 46.3 (16)	- 54.2 (14)
Acid phosphatase activity ($\mu\text{g pNP g}^{-1}$ soil)	0.29 (.04) ^a	+ 33.9 (21)	- 6.32 (14)	0.35 (.04) ^a	- 7.97 (12)	+ 0.78 (11)	0.37 (.05) ^a	- 26.4 (6.0)	- 31.1 (9.9)
Alkaline phosphatase activity ($\mu\text{g pNP g}^{-1}$ soil)	0.13 (.07) ^a	- 56.1 (2.9)	- 71.4 (5.2)	0.15 (.05) ^a	- 41.2 (26)	+ 23.4 (42)	0.17 (.05) ^a	- 41.8 (19)	+ 6.96 (18)
	RED PINE								
	SAND			SANDY-LOAM			SILTY-SAND		
	Control	Biochar effect (%)	Ash effect (%)	Control	Biochar effect (%)	Ash effect (%)	Control	Biochar effect (%)	Ash effect (%)
Microbial biomass C ($\mu\text{g C g}^{-1}$ soil)	28.7 (5.4) ^a	- 66.0 (7.1)	- 38.9 (23)	82.7 (20) ^b	- 29.1 (12)	+ 103 (58)	112 (23) ^b	- 16.9 (6.5)	- 10.3 (11)
Microbial biomass P ($\mu\text{g P g}^{-1}$ soil)	0.91 (.23) ^a	+ 31.6 (60)	+ 19.2 (45)	2.49 (.79) ^{ab}	- 52.1 (20)	- 43.2 (19)	2.07 (.44) ^b	- 4.88 (25)	- 20.2 (22)
Acid phosphatase activity ($\mu\text{g pNP g}^{-1}$ soil)	0.40 (.04) ^a	+ 4.30 (13)	+ 4.51 (19)	0.42 (.05) ^a	+ 3.64 (17)	- 13.2 (14)	0.49 (.06) ^a	- 24.8 (9.2)	- 5.27 (13)
Alkaline phosphatase activity ($\mu\text{g pNP g}^{-1}$ soil)	0.05 (.02) ^a	- 48.1 (12)	+ 32.9 (33)	0.24 (.04) ^b	- 7.22 (28)	- 15.0 (22)	0.09 (.04) ^c	+ 100 (48)	+ 60.8 (38)

Note: Values represent means (SE).

^aControl columns are absolute values; letters indicate significant differences between soils for each seedling species.

^bBiochar and ash effect columns are the percent change relative to their respective control soil after addition of either 20 t ha⁻¹ biochar or 16 t ha⁻¹ ash; bold font indicates a significant amendment effect.

FIGURE LEGENDS

Figure 1. Soil pH (a) and soil moisture (b). Data are treatment means $\pm$ SE for control soils and soils with 20 t ha⁻¹ biochar or 16 t ha⁻¹ ash for each species/soil combination. Colors indicate amendment type.

Figure 2. Mean root-to-shoot ratios for all red pine (top) and sugar maple (bottom) seedlings. Error bars represent SE. Colors indicate amendment type.

For Review Only

239x325mm (300 x 300 DPI)

157x128mm (300 x 300 DPI)