Plant Soil (2015) 395:105–123
DOI 10.1007/s11104-014-2246-y

REGULAR ARTICLE

Phosphorus availability to beans via interactions between mycorrhizas and biochar

Steven J. Vanek · Johannes Lehmann

Received: 19 May 2014 / Accepted: 12 August 2014 / Published online: 28 August 2014

Abstract
Background and aims We sought to understand biochar's role in promoting plant phosphorus (P) access via arbuscular mycorrhizas (AM), focusing on whether P solubility and biochar-P proximity altered AM enhancement of P uptake in a mycorrhizal crop legume.
Methods A greenhouse study compared feedstock-derived P with 50 mg P pot^{-1} of sparingly soluble $FePO_4$ (Fe-P) or soluble NaH_2PO_4 (Na-P) at different proximities to biochar (co-pyrolyzed, mixed with biochar, mixed with soil) on *Phaseolus vulgaris* P uptake, specific root length (SRL), AM colonization, AM neutral lipids, and microbial biomass-P.
Results Biochar increased AM colonization by 6 % ($p<0.01$) and increased Fe-P uptake from 3.1 to 3.8 mg $plant^{-1}$, with AM-related Fe-P uptake increased by 12 % ($p<0.05$). Regardless of proximity, biochar applied with Fe-P was enriched (>2×) with AM hyphae. Biochar-P proximity did not alter P uptake, but shifted uptake towards AM for Fe-P and roots for Na-P. Soluble P located on biochar increased total plant+microbial P ($p<0.05$). Biochar reversed ($p<0.05$) reductions in SRL induced by AM.
Conclusions Biochar enhanced AM's access to sparingly soluble P, and root/microbial access to soluble P. Biochar augments sparingly soluble P uptake at scales larger than biochar particles, perhaps by reducing P sorption or facilitating root/hyphal exploration.

Keywords Biochar · Iron phosphate · Microbial biomass · Mycorrhizas · Phaseolus vulgaris · Phosphorus fixation · NLFA · Rhizosphere

Responsible Editor: N. Jim Barrow.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-014-2246-y) contains supplementary material, which is available to authorized users.

S. J. Vanek (✉)
Department of Geography, Penn State University,
State College, PA, USA
e-mail: sjv13@psu.edu

J. Lehmann
Department of Crop and Soil Sciences, Cornell University,
Ithaca, NY, USA

Introduction

Phosphorus (P) limits plant growth in both natural systems and low-input agriculture (Vance 2001). Root physiological adaptations as well as soil microbial symbioses expand plants' access to chemically recalcitrant P (Richardson et al. 2011; Balemi and Negisho 2012). In addition, soil organic matter (SOM) and organic additions to soil may also enhance P uptake by plants. Possible mechanisms include reductions in P chemisorption (Ahmad and Tan 1991; Guppy et al. 2005; Gerke 2010; Kudeyarova 2010; Cui et al. 2011;), improvements in soil physical and chemical properties that facilitate root nutrient uptake (Atkinson et al. 2010; Chakraborty et al. 2010; Silva et al. 2011), and nutrients and habitat provided by SOM to microbes that enhance soil biogeochemical cycling (Erich et al. 2002; Chacon et al. 2006; Alguacil et al. 2011; Gichangi et al. 2010).

Nevertheless, because organic additions generally contain labile P, the extent to which SOM functions per se to enhance P fertility has been challenged (Borggaard et al. 2005; Guppy et al. 2005).

To increase crop nutrient uptake and reverse SOM decline in agricultural soils, pyrolyzed biomass (biochar) has been proposed as an anthropogenic "boost" to SOM. It is proposed that biochar creates durable, positive effects on soil fertility (Kimetu et al. 2008; Woolf et al. 2010) via physico-chemical means as well as through impacts on soil biota (Atkinson et al. 2010; Lehmann et al. 2011), with biochar soil residence times that are one to two orders of magnitude longer than unpyrolyzed residues (Zimmerman 2010; Kuzyakov et al. 2014). Among these impacts, research has examined biochar's effect on arbuscular mycorrhizal (AM) symbioses that enhance plant P access to recalcitrant soil P (Warnock et al. 2007, 2010; Lehmann et al. 2011). Neutral to positive effects of biochar on AM root colonization were found by Quilliam et al. (2012a) and in studies reviewed by Warnock et al. (2007). Suggested mechanisms included changes in soil physical and chemical conditions, dilution of AM propagules and altered signaling between plants and AM, and sheltering hyphae from fungal grazing (Lehmann et al. 2011). However, Warnock et al. (2010) showed that AM root colonization and soil hyphal responses varied widely, including reduced colonization associated with high-P biochars, and possible toxicity to AM from low-temperature biochars. Elmer and Pignatello (2011) suggested that biochar increased AM colonization by sorbing allellopathic chemicals that depress colonization. LeCroy et al. (2013) also concluded that sorption of organic plant compounds that regulate colonization led to growth reduction by AM of young sorghum plants under high nitrogen conditions.

These findings suggest a number of nutrient and non-nutrient mechanisms for biochar impacts on AM but do not fully explain how the concentration and solubility of P in biochar affects the AM symbiosis. Additionally, hypothesized processes that promote P availability at biochar surfaces (Guppy et al. 2005; Joseph et al. 2010) suggest that biochar would promote root and AM access to nutrients in direct contact with biochar particles versus those nutrients mixed throughout the soil. In this study we tested the effects of P solubility and biochar-P spatial proximity on a mycorrhizal legume with high P demand in a low-P soil. We examined the effects of these factors on plant P nutrition, specific root length, soil microbial biomass P, and AM root colonization of *Phaseolus vulgaris*, a crop with global importance. Based on ecological theory defining AM symbioses in cost-benefit terms for plant and fungal symbionts (Johnson 2010; Lekberg et al. 2010) we hypothesized that:

1. The solubility of P accompanying biochar determines AM and biochar effects on P availability: for low or sparingly soluble P, application of both AM and biochar would increase plant P uptake, increasing shoot: root ratio and reducing specific root length (Atkinson et al. 2010). Positive AM/biochar effects would be absent when sufficient soluble P accompanies biochar.
2. Impacts on AM colonization are due to differences in P solubility: Because AM colonization is strongly linked to plant investment in acquiring a scarce resource, biochar added with low or sparingly soluble P would increase AM root colonization, while biochar with sufficient soluble P would depress or have no effect on AM colonization.
3. P sources located on or in biochar foster the greatest positive interactions of AM/biochar on P availability. Due to hypothesized mechanisms fostering P availability at biochar surfaces, P "co-located" or in close proximity to biochar particles would be more available to roots and AM hyphae than P mixed through a biochar-amended soil, and would create disproportionate abundance of AM mycelium foraging for P in these particles. Increased P availability near biochar particles and associated labile carbon on biochar would also increase microbial biomass-P with co-located P.

Materials and methods

Biochar, phosphorus, and mycorrhizal treatments

We tested these hypotheses in a greenhouse experiment with common bean (*Phaseolus vulgaris*, P-efficient variety BFS 10, CIAT, Colombia) in a factorial design with AM inoculation, biochar addition, P solubility, and co-location of P with biochar as experimental factors (Table 1). The sparingly soluble P source was $FePO_4$ powder (Fe-P; 100 % of particles passing a 54 μm sieve),

Table 1 Description of treatments in the biochar type/location and mycorrhizal inoculation factorial experiment. All biochar prepared from maple-hickory feedstock and adjusted to soil pH (4.2) and added to soil at 5 % v:v. after pyrolysis and any post-treatments

Treatment Description	Description of addition to soil	P content of biochar, g P kg^{-1}	Total P addition to pot, mg P pot^{-1}	Biochar pH before adjustment to pH4.2
	Phosphorus and biochar level and location			
Soil-only control	No addition to soil/sand pot medium	–	–	–
Unmodified biochar	Maple-hickory biochar (450°C, 1h slow pyrolysis)	0.3	2.5	7.2
Oxidized biochar	Unmodified biochar oxidized in 20 % H_2O_2 at 45 °C for 10 h, triple rinsed	0.1	0.4	3.6
Fe-P BEF	$FePO_4$ mixed in slurry with ground feedstock at 13.0 g kg^{-1} before pyrolysis	6.8[1]	50	6.7
Na-P BEF	$NaH_2PO_4 \cdot H_2O$ mixed in slurry with ground feedstock at 9.7 g kg^{-1} before pyrolysis.	7.1[1]	50	6.7
Fe-P AFT	$FePO_4$ mixed in slurry with unmodified biochar after pyrolysis at 31.1 g kg^{-1}, dried	6.4	50	7.2
Na-P AFT	$NaH_2PO_4 \cdot H_2O$ mixed in slurry with unmodified biochar after pyrolysis at 28.5 g kg^{-1}, dried	6.4	50	7.2
Fe-P soil+BC	231 mg pot^{-1} $FePO_4$ plus unmodified biochar	0.3	50	7.2
Na-P soil+BC	211 mg pot^{-1} $NaH_2PO_4 \cdot H_2O$ plus unmodified biochar	0.3	50	7.2
Fe-P soil	243 mg pot^{-1} $FePO_4$	–	50	–
Na-P soil	223 mg pot^{-1} $NaH_2PO_4 \cdot H_2O$	–	50	–
	AM inoculation			
+AM	*Glomus clarum*, INVAM strain WV235 (INVAM, West Virginia University), in 40 mL pot^{-1} attapulgite granular clay carrier (Oildri, Chicago, IL, USA) with spores and *Sorghum bicolor X sudanense* root fragments			
-AM	Spore-free attapulgite carrier with *Sorghum bicolor X sudanense* root fragments			

[1] Using these P concentrations, BEF biochars were diluted with unmodified biochar after analysis (see methods) to achieve the target P concentration of 6.4 mg kg^{-1}

thought to be unavailable to *P. vulgaris* (Yan et al. 1996), and soluble P was applied as NaH_2PO_4 (Na-P). P additions of 50 mg P pot^{-1} (50 kg P ha^{-1} equivalent) were intended to allow detection of effects on Fe-P availability. Treatments were also included with unmodified biochar (only P from feedstock), an oxidized and rinsed biochar with negligible P, and a soil-only control without P or biochar. Treatments were replicated three times in a randomized complete block design.

Biochar was made from mixed maple and hickory sawmill waste ground to 2 mm and pyrolyzed in a custom pyrolysis unit comprising a closed, mild steel drum (6-mm walls) with a central rotating paddle driven at 1 RPM. The unit was located in a programmable muffle furnace (Fisher Isotemp 126, Pittsburgh, PA, USA) programmed to maintain 450 °C for 1 h after ramping 3 °C·min^{-1}, with 1 L·min^{-1} argon sweep gas. To create biochar particles of similar size to soil aggregates, particles <250 μm were sieved from the biochar prior to analysis and use. Sparingly soluble and soluble P sources were co-located with biochar both before and after pyrolysis, as well as added separately to soil with and without biochar (Table 1). All biochar was pH-adjusted to the soil pH (4.2) with HCl and NaOH in a 4:1 v:v water:biochar mixture, until pH changed by <0.1 pH unit over 3 h, and then dried at 80 °C before addition to soil. Mycorrhizal treatments (+AM) incorporated a clay carrier with *Glomus clarum* (strain WV235, INVAM, West Virginia University) as spores and colonized root fragments of *Sorghum bicolor X sudanense*, while non mycorrhizal controls (−AM) used the same carrier with uninoculated roots (Table 1).

Soil and plant management

Soil/sand mix was prepared using subsoil of the Calhoun experimental forest in South Carolina, USA (BE and B22 horizons; Richter et al. 1994). This well characterized soil was used to represent weathered tropical soils with eroded surface horizons and challenging

conditions for crops and soil biota. Stones and SOM >2 mm were removed by sieving. For these horizons Richter et al. (1994) measured a clay content of 28.6 %, 4 mg kg^{-1} Mehlich-III extractable P, and DCB-extractable iron oxides of 252 mg kg^{-1}. We measured soil pH (4.2 in 0.01 mol L^{-1} $CaCl_2$) and an adsorption isotherm indicating substantial P sorption (Freundlich isotherm with n=2.36, K_d=93.0 for mg P kg^{-1} sorbed vs. mg P L^{-1} in water). Soil was mixed 3:1 v:v sand:soil with acid-washed pool filter sand (U.S. Silica, Frederick, MD, USA) to ameliorate poor structure from sieving and pot culture. Available P (Olsen) in the soil:sand mix was 0.17 μg P g^{-1}.

Beans were grown in 1.6-L pots (pot TP-49, Steuwe and Sons, Tangent, OR, USA) with a base layer of 200 mL acid-washed silica sand, followed by 800 mL soil:sand mix, followed by 100 mL additional washed sand and a perlite layer to conserve moisture. Biochar was incorporated at 5 % v:v of the 800 mL of soil:sand mix, equivalent to 7.8 Mg ha^{-1} to a depth of 0.15 m. In treatments without biochar, P sources were directly incorporated to soils. Pots were watered to field capacity and pre-incubated for 1 week to allow sorption of soluble P and avoid transient negative effects of biochar volatiles on plant growth (Deenik et al. 2011). Because the attapulgite mycorrhizal carrier contained P and had a pH higher than the soil, controls without the mycorrhizal carrier were also planted.

Two pre-germinated bean seeds per pot were planted and thinned after emergence to one plant for uniformity. We watered pots daily just to excess, with water recovered in catch dishes and returned to pots. We fertilized plants every 2 days with 100 mL nutrient solution containing 2 mmol L^{-1} $Ca(NO_3)_2$, 3 mmol L^{-1} K_2SO_4, 1 mmol L^{-1} $(NH_4)_2SO4$, 1 mmol L^{-1} $MgSO_4$, 50 μmol L^{-1} Fe-EDTA, 25 μmol L^{-1} H_3BO_3, 2 μmol L^{-1} $MnSO_4$, 2 μmol L^{-1} $ZnSO_4$, 0.5 μmol L^{-1} $CuSO_4$, 0.5 μmol L^{-1} $(NH_4)_6Mo_7O_{24}$ (modified from Snapp et al. 1995). We excised cotyledons at the first true leaf stage to accentuate plant P stress.

Plant growth and soil measurements

Plants were harvested in block order between 28 and 42 days after germination, when Fe-P and Na-P treatments were flowering. Soil pH in 0.01 mol L^{-1} $CaCl_2$ was measured to rule out liming effects of biochar, and fresh and dry (60 °C) shoot mass was determined. To test hyphal and root exploration of biochar, intact root segments were rinsed from soil: a 10-mm thick section of soil and roots was sliced from each pot, parallel to the taproot and 20 mm to one side, from top to bottom of the 800-mL soil-sand mix. This slice was gently immersed in 1 % sodium hexametaphosphate (HMP) dispersant followed by water with a gentle hand motion (1 Hz) that removed soil with minimal disturbance to hyphal and root hair connections to biochar particles. We spread rinsed roots and any attached biochar on a 0.21m by 0.29m flatbed scanner tray with a blue background (R,G,B≈1,70,170) to allow separation during image analysis of redder roots and black biochar from shadows on the background. Scanned roots were dried and weighed separately to avoid P contamination from HMP.

Taproots were rinsed and dried separately, and remaining soil from the soil/sand mix was sieved to 1.8 mm to recover fine roots. Roots from sieving were floated and rinsed in several changes of water. Three grab samples per pot of these roots were scanned to estimate root length, which was divided by the dry weight (60 °C) of the scanned roots to yield specific root length (SRL; m g^{-1}). A small composite grab sample of fine roots (~200–1,500 μm) was likewise placed in fixative (dilute acetic acid/ethanol) for AM root staining. Nodules from all fractions were picked from dried roots and weighed separately. Sieved soil was homogenized and ~100 mL frozen for analysis using ergosterol (see supplemental information) and signature lipid analysis (PLFA/NLFA). We refrigerated the remainder (2 °C) for microbial biomass P (P_{MB}) determination and subsequent drying.

Root colonization by arbuscular mycorrhizas

Roots were stained with trypan blue to visualize AM structures (Koske and Gemma 1989). Roots were cleared in 10 % w:v KOH at 90 °C for 10 min, acidified in 1 % HCl for 2–24 h, stained at 90 °C using 0.5 g L^{-1} trypan blue in acidic glycerol (50:45:5 v:v water:glycerol:1 % HCl), and destained 5d in acidic glycerol. Thirty root segments of ~15 mm length per microscope slide were mounted. One slide per treatment was then scanned twice manually at 400×, scoring colonization as the presence of AM hyphae (those with nearby arbuscules) in a root along the midline of the field of view. One hundred fields were scored to calculate the proportion of fields with colonized roots. To assess AM arbuscule density in roots (arbuscules cm^{-1}), arbuscules along the length of a root within ten 2.5-mm low-power (100×) fields were counted.

Microbial biomass phosphorus

Sieved, refrigerated soil (2 °C) was used for duplicate measurements of microbial biomass P (P_{MB}) by chloroform fumigation extraction within 10 days of harvest (modified from Gregorich et al. 1990). Ten grams of moist sand/soil mix were placed in 60-mL glass jars with Teflon-lined lids (Qorpak, Bridgeville, PA USA). Two jars per pot were fumigated with 0.5 mL washed chloroform (alcohol removed) pipetted directly onto the soil, followed by 40 mL Olsen extractant solution (0.5 mol L^{-1} $NaHCO_3$ adjusted to pH 8.5). Two additional jars were extracted but not fumigated. Uncorrected P_{MB} was then calculated as:

$$P_{MB-raw} = (P_{i-fum} V_{fum}/m_{s-fum}) - (P_{i-n} V_n/m_{s-n}),$$

with $P_{i\text{-}fum}$, V_{fum}, and $m_{s\text{-}fum}$ the Olsen P concentration, extract volume, and dry mass of soil of the fumigated extracts, respectively; and $P_{i\text{-}n}$, V_n, and $m_{s\text{-}n}$ those of the non-fumigated extracts, using gravimetric moisture (105 °C) to calculate m_s from moist soil weights. Moisture in soil at the time of extraction was incorporated into V_{fum} and V_n. To correct $P_{MB\text{-}raw}$ for soil sorption of fumigation-released P, a four-point sorption study was conducted using the Na-P BEF, Fe-P BEF, unmodified, and soil-only treatments. Fumigated and unfumigated extractant solutions were spiked with 0, 1, 4, and 12 mg P L^{-1} as K_2PO_4, and $P_{MB\text{-}raw}$ was corrected for P sorption using the sorption study and an extraction efficiency K_{ec} of 0.4 (Brookes et al. 1982):

$$P_{MB} = P_{MB-raw} \times 0.4/f_{recov}(P_{MB})$$

where f_{recov} is a function describing the recovery of added P per soil dry mass fitted to the four-point sorption study and evaluated at the uncorrected P_{MB} for each sample for the type of biochar addition (Na-P BEF, Fe-P BEF, unmodified, or soil without biochar).

Phosphorus content of biochar and plant biomass

Plant biomass (shoots, roots, nodules) was ground to 600 μm and analyzed for P using digestion with concentrated HNO_3 at 110 °C and H_2O_2 addition in the final step to oxidize organic carbon (Kalra 1998). Digested ash was dissolved in 20 mL 5 % HCl using sonication. Digests were analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, model ICAP 61E, Thermo Electron, Waltham, MA). Biochar was analyzed for P using ICP-AES after ashing for 5 h at 800 °C, followed by nitric acid digestion at 120 °C and dissolving of ash in 5 % HNO_3 (Enders and Lehmann 2012).

Lipid biomarker analysis in soil and biochar

To estimate fractions of AM biomass in soils and biochar, we used 16:1ω5 neutral lipid and phospholipid fatty acids (NLFA and PLFA; Van Aarle and Olsson 2003; Schnoor et al. 2011), with methods adapted from Bossio et al. (1998). For whole soils, 5 g freeze-dried (Kinetics dura-dry MP, Kinetics Systems, Fremont, CA, USA) and ground soil/biochar was extracted and centrifuged twice using a 2:1:0.8 mixture of methanol: $CHCl_3$: 0.1 mol L^{-1} phosphate buffer (pH 7). Decanted supernatants from centrifuging were allowed to separate overnight after adding additional buffer and methanol. Extracts from the $CHCl_3$ top phase of separation were dried under N_2 (28 °C) and lipids re-suspended in 1.5 mL $CHCl_3$ before separation using silica solid-phase extraction columns (Agilent, Foster City, CA, USA) of NLFAs and PLFAs using elution from columns with $CHCl_3$ and methanol. These neutral and polar lipid fractions were dried as before and their methyl esters formed using gentle heating (35 °C) in 1 mL 1:1 v:v methanol:toluene and 1 ml methanolic KOH. This solution was then neutralized (1 mol L^{-1} acetic acid) and methyl esters were washed into a non-polar hexane phase by shaking twice with 2 mL of 4:1 v:v hexane: chloroform. The resulting hexane with methyl esters was dried under N_2 (28 °C) in 4-mL vials, sealed with argon, and kept at −20 °C and dark until analysis. Methyl esters were re-suspended in 300 μL hexane-methyl tert-butyl ether with 9:0 and 19:0 fatty acid methyl ester external standards by the University of Wisconsin lipid analysis facility. Analysis was performed with an Agilent 6890 gas chromatograph (Agilent, Foster City, CA, USA) with split inlet and flame ionization detector, and a 25 m by 0.2 mm column with a 95 % methyl/5 % phenyl polysiloxane stationary phase. Peak identification was carried out using Sherlock-MIDI software (MIDI, Newark, Delaware, USA). The NLFA and PLFA 16:1ω5 were used as AM biomass indicators. We also analyzed soils and biochar for ergosterol as a biomarker of many non-AM fungi (methods and results in online supporting information).

To measure the proportion of 16:1ω5 NLFA and PLFA present in biochar, biochar was floated from freeze-dried soil samples: approximately 25 g soil was repeatedly rinsed and the supernatant decanted through a 53-μm sieve until all visible biochar particles were recovered. This sample was then rinsed through a 74-μm and 53-μm sieve to remove residual floated silt, with most biochar particles from soil recovered on the 74-μm sieve. The 53-μm fraction was retained for ignition (550 °C) and biochar mass was calculated by difference between oven-dried and ignited samples, compared to a similar decanted size fraction from soil-only treatments to control for SOM in fine sand. Biochar from the 74-μm sieve was washed into a 100-mL graduated cylinder, and hyphae in the supernatant (not associated with biochar) were decanted and discarded using agitation for 10 s with a hand-held blender followed by 2 min of biochar settling to the bottom. Rinsing/decanting was continued until floating hyphae visible using a stereoscope in the decanted water were absent. The washed biochar sample with attached and internal hyphae was then filtered on an ashed, weighed glass fiber filter, and the filter plus sample immediately extracted with solvent mixture for NLFA/PLFA as described above. The dry weight of biochar extracted was determined after oven drying of the biochar extraction tubes. We tested that lipid extraction did not change biochar mass. The results for NLFA/PLFA content from extracted biochar were adjusted to reflect the small additional amount of biochar measured through drying and ashing of the 53-μm filter fraction (above) assuming that unextracted 53-μm and extracted biochar had the same lipid concentrations. Signature lipid amounts were divided by the initial washed soil mass to determine NLFA/PLFA content associated with biochar. The ratio of biochar-associated to whole-soil NLFA/PLFA for each treatment was compared to 5 %, the expected ratio if signature lipids were evenly distributed between biochar and soil based on the 5 % v:v addition of biochar to soil.

Image processing of root flatbed scans

To calculate specific root length (SRL), 0.29 by 0.19 m flatbed scanned images of washed roots with known dry mass were thresholded using image-J software (Schneider et al. 2012), using conditionals on red, green, and blue (rgb) values of scan pixels: $55 \leq r \leq 200$, $80 \leq g \leq 200$, $90 \leq b \leq 200$, and $b\text{-}r > 80$. This yielded a monochromatic image with roots in white for analysis by WinRhizo (Regent instruments, Quebec, Canada) for root length and average diameter. Scan root length was divided by the dry mass of the scanned roots to determine SRL. The scans of HMP-rinsed roots with biochar attached were similarly thresholded (conditionals of $20 \leq r \leq 60$, $g \leq 70$, and $b \leq 80$ for biochar) and processed with WinRhizo to determine root length and biochar pixel area. A calibration factor of biochar mass per pixel area was developed by scanning small amounts of biochar of known dry mass. Biochar-root association was then expressed as mg biochar m^{-1} root length.

Statistical analyses

Hypotheses were evaluated using 21 preplanned orthogonal contrasts testing the impact of AM fungi, biochar, P type, and co-location of P on biochar (Table 2). Within P type, the effect of P-biochar co-pyrolysis (BEF) versus mixture after pyrolysis (AFT), and addition to soil before biochar (soil+BC; Table 1) was also tested. These contrasts also tested for interactive effects of P and biochar with AM inoculation. Data was transformed when needed to insure homoscedasticity. Linear regression models were used to test for correlation among plant and AM responses, with experimental factors as categorical predictors. For regressions on proportion root length colonized by AM, data was transformed using Lineweaver-Burk plots for saturating kinetics (Lineweaver and Burk 1934).

Results

Bean biomass, phosphorus uptake, shoot:root ratio, and nodule biomass

Total bean biomass (roots+shoots+nodules) increased under AM inoculation ($p<0.01$), and was also increased ($p<0.05$) by biochar from 3.1 to 3.6 g pot^{-1} for Fe-P treatments but not for low-P treatments (Table 2, preplanned contrasts 1, 5, 6). Soil liming from biochar did not contribute to P availability, since P uptake was highest at the lowest final soil pH (final soil pH range 4.2 to 4.8, regression $p < 0.0001$ for total P uptake against soil pH at harvest). Inoculation with *G. clarum* increased bean P uptake and tissue concentration under low P and Fe-P application (Fig. 1, Table 2). Contrasts

Table 2 Orthogonal contrasts describing main and interaction effects of mycorrhizae (AM) and biochar (BC) on nine response variables indicating plant biomass and P uptake, mycorrhization,, and microbial biomass P. Significance of contrasts denoted by: +$p<0.1$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

Orthogonal contrast number and definition	Total plant biomass ($g\ pot^{-1}$)	Shoot P uptake ($mg\ P\ pot^{-1}$)	Total P uptake ($mg\ P\ pot^{-1}$)	Shoot P concentration ($mg\ P\ g^{-1}$)	P Shoot: root ratio ($mg\ mg^{-1}$)	Nodule biomass ($mg\ P\ pot^{-1}$)	AM Root length colonized (%)	Arbuscule density (cm^{-1})	Microbial biomass P ($\mu g\ P\ g^{-1}\ soil$)
Main effects	*First contrast group mean* vs. *second contrast group mean*, *with contrast statistical significance*								
1. Main effect of *Glomus clarum* (+AM vs. -AM)	4.3 vs 4.1 **	5.1 vs.3.8 ***	8.3 vs.5.9 ***	1.6 vs 1.1 ***	ns	229 vs.181 ***	–	–	ns
Main effects of added P and biochar, combining +/− AM									
2. Na-P vs. Fe-P and low-P treatments	6.8 vs 2.7 ***	8.0 vs.2.5 ***	12.1 vs.4.1 ***	1.6 vs 1.2 ***	2.0 vs.1.4 ***	406 vs.90 ***	82 % vs.96 % ***	128 vs. 341 ***	ns
3. Fe-P vs. Low-P chars and soil-only control	3.5 vs 1.8 ***	3.6 vs.0.9 ***	6.0 vs.1.7 ***	1.5 vs 0.8 ***	1.5 vs.1.2 ***	146 vs.15***	ns	296 vs. 401 *	1.9 vs.0.5***
4. Na-P (BEF, AFT, soil+BC) vs. Na-P soil	ns	7.8 vs.8.6 *	11.6 vs.13.4 **	1.6 vs 1.7 +	ns	380 vs.482 **	ns	ns	ns
5. Fe-P (BEF, AFT, soil+BC) vs. Fe-P soil	3.6 vs 3.1 *	3.8 vs.3.1 **	6.2 vs.5.3 *	ns	1.6 vs.1.4 +	161 vs.103 **	ns	ns	1.6 vs.2.8*
6. Low-P biochars (unmodified, oxidized) vs. soil-only control	ns	ns	ns	ns	1.3 vs.1.1 *	ns	98 % vs.92 % **	ns	ns
7. [(Aft, soil+BC) vs. BEF], Na-P treatments	7.6 vs 4.8 ***	9.6 vs.4.1 ***	14.0 vs.6.9 ***	1.7 vs 1.3 ***	2.3 vs.1.5 ***	468.5 vs.203.8 ***	77 % vs.95 % **	80 vs. 223 ***	ns
8. [(Aft, soil+BC) vs. BEF], Fe-P treatments	ns	ns	ns	0.7 vs 0.4 ***	1.7 vs.1.4 *	ns	ns	ns	1.9 vs.1.0*
9. Na-P AFT vs. Na-P soil+BC	ns	ns	ns	ns	ns	ns	70 % vs.83 % **	55 vs. 105 *	2.7 vs.1.0 **
10. Fe-P AFT vs. Fe-P soil+BC	ns	ns	ns	ns	ns	ns	97 % vs.92 % *	436 vs. 252 *	ns
11. Unmodified vs. oxidized low-P biochars	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interaction effects	*Effect of AM (difference between + AM and –AM) in the first contrast group* vs. *effect of AM in the second contrast group, with contraststatistical significance*								
12. AM x [Na-P vs. Fe-P and low P treatments]	−1.1 vs 0.9 ***	−0.1 vs.2.2 ***	0.4 vs.3.6 ***	0.3 vs 0.7 ***	−0.2 vs.0.2 **	−56.1 vs.108.6 **	–	–	ns
13. AM x [Fe-P vs. low P treatments]	ns	3.3 vs.0.8 ***	5.2 vs.1.4 ***	1.0 vs 0.1 ***	ns	178.4 vs.15.6 ***	–	–	ns
14. AM x [BC vs. no BC], Na-P treatments	−1.2 vs −0.8 *	ns	ns	0.4 vs 0.1 ***	ns	ns	–	–	ns
15. AM x [BC vs. no BC], Fe-P treatments	ns	3.4 vs.3.0 ***	5.25 vs.5.15 ***	1.0 vs 1.0 ***	ns	193 vs.135 ***	–	–	ns
16. AM x [BC vs. no BC], low-P treatments	1.2 vs 0.9 *	0.9 vs.0.7 ***	1.42 vs.1.37*	ns	0.5 vs.-0.1 **	ns	–	–	ns
17. AM x [AFT and soil+BC vs. BEF], Na-P tmts.	ns	−0.8 vs.1.6 *	−0.9 vs.3.0 **	0.2 vs 0.8 *	ns	−122 vs.85 **	–	–	ns
18. AM x [AFT and soil+BC vs. BEF], Fe-P tmts	ns	3.5 vs.3.1 ***	5.4 vs.5.0 **	ns	ns	189 vs.201**	–	–	ns
19. AM x [AFT vs. soil+BC], Na-P treatments	ns	ns	ns	0.0 vs 0.3 +	ns	ns	–	–	ns
20. AM x [AFT vs. soil+BC], Fe-P treatments	ns	ns	ns	ns	ns	ns	–	–	ns
21. AM x [unmodified vs. oxidized BC]	ns	ns	ns	ns	ns	ns	–	–	ns

comparing Fe-P treatments with and without biochar showed an increase with biochar in shoot P uptake from 3.1 to 3.8 mg P pot^{-1} and total P uptake from 5.3 to 6.2 mg pot^{-1} (Table 2, contrast 5). This increase in P uptake included a modest but significant positive biochar-AM interaction, by which AM increased shoot P uptake 0.4 mg pot^{-1} (12 %) more with biochar than without, and total P uptake showed a similar positive interaction (Table 2, contrast 15). A positive biochar-AM interaction was also seen in comparing the unmodified and oxidized biochar treatments with the soil-only control (contrast 16). Biochar on average decreased Na-P uptake, due to dramatically lower P uptake when Na-P was added to feedstock before pyrolysis (Table 2, contrasts 2 and 7; Fig. 1), and statistically equivalent P uptake between Na-P AFT, Na-P soil+BC, and Na-P soil treatments (Fig. 1). Nodule biomass paralleled P uptake: a positive biochar main effect and AM by biochar interactions were observed for Fe-P treatments (Table 2).

Root colonization by AM and root-biochar connection by hyphae

Roots of uninoculated (−AM) controls were not colonized, while+AM beans had a higher proportion of root length colonized (%RLC) in the Fe-P and low-P treatments than the Na-P treatments. In Fe-P and low-P treatments, +AM plants took up two to three times as much P as –AM controls (Fig. 2b). In addition, within low P, Fe- P, and Na-P treatment groupings, particular treatments indicated shifts in the reliance on AM for P uptake. Low-P biochars increased %RLC compared to the soil-only control, while unmodified biochar had more root arbuscule density (arbuscules cm^{-1} root) compared to the oxidized and soil-only control (Table 2, contrast 6; Fig. 2b). In low-P biochar and soil-only treatments shoot P uptake was also positively correlated to %RLC (R^2=0.90, p=0.003), although not root arbuscule density. In Na-P and Fe-P treatments, biochar per se did not induce differences in %RLC (Table 2, contrasts 4 and 5). However, Fe-P placed on biochar after charring (AFT) increased %RLC by 5 % absolute and almost doubled AM arbuscule density compared to Fe-P mixed with soil before biochar addition (Table 2, contrast 10). Na-P placed on biochar after charring reduced %RLC by 13 % absolute, and roughly halved AM arbuscule density, compared to the Na-P soil+BC treatment (contrast 9). In these two AFT treatments co-located P on biochar altered the levels of AM colonization, although their total P uptake was not significantly different from the same P added to the adjacent soil (Fig. 2). This suggested differences in the relative allocation to AM and root P uptake that we confirmed using post-hoc statistical contrasts showing that Fe-P AFT with the higher AM colonization had lower fine root biomass, and a higher shoot:root ratio than other+AM Fe-P biochar treatments (p=0.03 and 0.02 for the contrast [Fe-P AFT] vs. [Fe-P BEF, Fe-P soil+BC] applied to fine root biomass and shoot:root ratio, respectively; see also Fig. 2).

Flatbed scans of rinsed roots with biochar showed that root-biochar adhesion was associated with AM colonization. The mean linear density of biochar on roots in+AM treatments was 16±2.1 (S.E.) mg m^{-1} root. For -AM treatments biochar density on roots was over 100 times lower (0.08±0.02 mg m^{-1}). Among+AM treatments, biochar adhering to roots correlated strongly to %RLC, so that Na-P AFT and Na-P soil+BC mycorrhizal plants had the lowest density of biochar adhered to roots (Fig. 3a).

Lipid biomarkers for AM in soils and biochar

The 16:1ω5 NLFA and PLFA biomarkers for AM fungi in whole soils were correlated (R=0.51, p=0.02) with NLFA approximately 50 times more abundant than PLFA, similar to other AM fungi (Larsen et al. 1998). Yield of PLFA in the small amounts of biochar recovered by washing were difficult to distinguish from noise, so that the 16:1ω5 NLFA fraction is used here as an indication of AM biomass in soils and biochars (see online supplementary information for other PLFA/NLFA results). The 16:1ω5 NLFA biomarker was strongly correlated to AM colonization (R=0.91, p<0.0001; Fig. 3b) and root arbuscule density (R=0.70, p=0.0001). Total 16:1ω5 NLFA was higher in Low-P, Fe-P AFT and Fe-P soil treatments than in those with soluble P added (p<0.05, Fig. 4a). Also, when Fe-P was added without biochar or co-located with biochar (Fe-P AFT) levels of 16:1ω5 NLFA were higher than when Fe-P was mixed with soil before biochar (Fig. 4a, post-hoc contrast, p=0.015).

The proportion of 16:1ω5 NLFA between biochar and whole soils was highest for treatments with both Fe-P and biochar, as well as the treatments Na-P AFT (co-located soluble P) and unmodified biochar (Fig. 4b). For all biochar additions except for the oxidized, low-P biochar, the proportion of 16:1ω5 NLFA in biochar

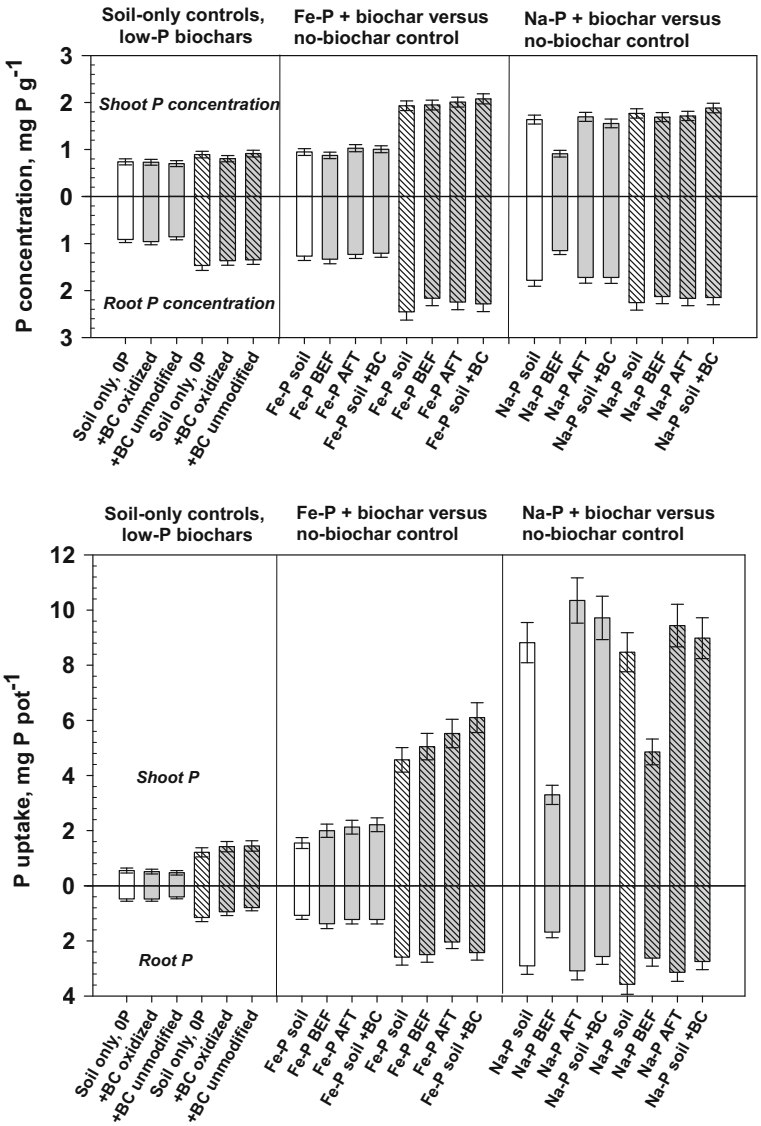

Fig. 1 Shoot and root P concentration (top) and P uptake (bottom) for uninoculated (−AM, solid bars) and *Glomus clarum*- inoculated (+AM, hatched bars) bean plants. Low-P biochars and BEF, AFT, and P in soil+BC locations of P for Fe-P and Na-P are compared to the 0P soil control and the same amount and type of P supplied without BC. Error bars show±one standard error; see Table 2 for statistical analysis

versus the total significantly exceeded 5 %, the expected value if NLFA were evenly distributed across biochar and soil volumes (Fig. 4b).

Microbial biomass phosphorus and bioavailable soil phosphorus

Microbial biomass P (P_{MB}) did not change with AM inoculation and interactions between AM and P type or co-location on biochar were absent (Table 2). However, P_{MB} in pots with addition of Na-P and Fe-P, with or without biochar, was greater than P_{MB} with low-P biochars and 0P controls (Table 2, Fig. 5a). In contrast to the higher plant uptake of Fe-P with biochar, P_{MB} was half in Fe-P treatments with biochar than those without (contrast 5 in Table 2). This was the reverse of the biochar effect on Fe-P uptake, so that P uptake (P_{tot}) in Fe-P treatments was negatively associated with the estimated stock (P_{MBtot}) of P_{MB} in pots (P_{MBtot} based on 800 mL of soil in pots, P_{tot} (mg P pot^{-1})=−0.80× P_{MBtot}(mg P pot^{-1})+7.9, $p<0.05$, $R^2=0.13$). Total bioavailable P per pot, the sum of total bean P uptake and

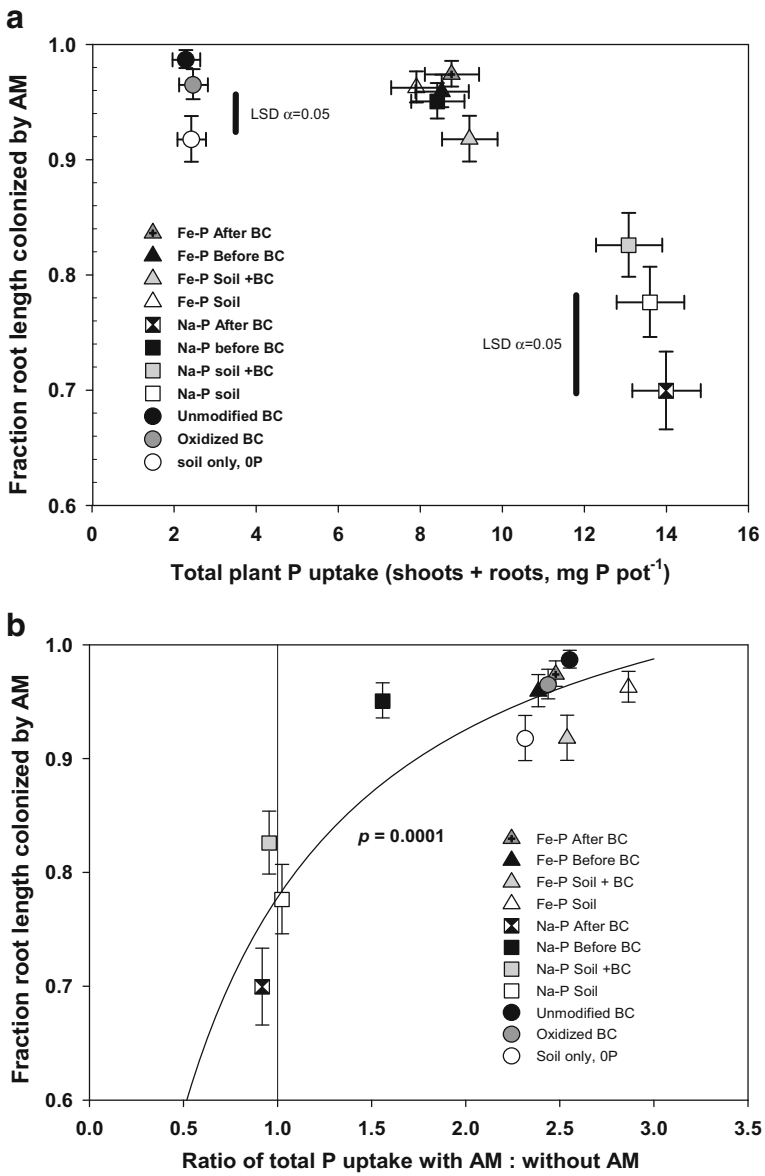

Fig. 2 **a.** Biplot of fraction root length colonized against total plant P uptake, showing reduced AM root colonization in soluble P treatments. LSD bar shows the least significant difference between treatments at high and low P uptake; **b**. Regression plot of fraction root length colonized against the benefit of AM inoculation (ratio of P uptake between+AM and –AM treatments). Data is fitted to Lineweaver-Burk saturation plot. Error bars show±one standard error

this microbial biomass P stock, was relatively even across Fe-P treatments, confirming this trend (Fig. 5b); however Fe-P availability was lower for co-pyrolyzed Fe-P than other Fe-P treatments (Fig. 5b; post-hoc contrast, $p=0.003$). In addition, Na-P co-located with biochar after pyrolysis had higher total microbial+plant P than either the Na-P soil or the Na-P soil+BC treatments because both plant and microbial biomass P were high in the Na-P AFT pots (Fig. 5b; contrasts averaged across+AM and –AM, [Na-P AFT vs. Na-P soil+BC], $p=0.005$ and [Na-P AFT vs. Na-P soil], $p<0.05$).

Specific root length

The effects of P and biochar addition on SRL depended on AM inoculation (Fig. 6). Without AM neither P plant availability or co-location position changed the mean SRL of approximately 200, although biochar decreased SRL for Fe-P in -AM treatments (Fig. 6a). Meanwhile, in+AM treatments SRL significantly increased with plant P uptake, refuting the hypothesis that SRL would decrease with more available P. Specific root length was also lower in+AM, Fe-P and Na-P treatments without biochar than those with biochar (Fig. 6b). Because

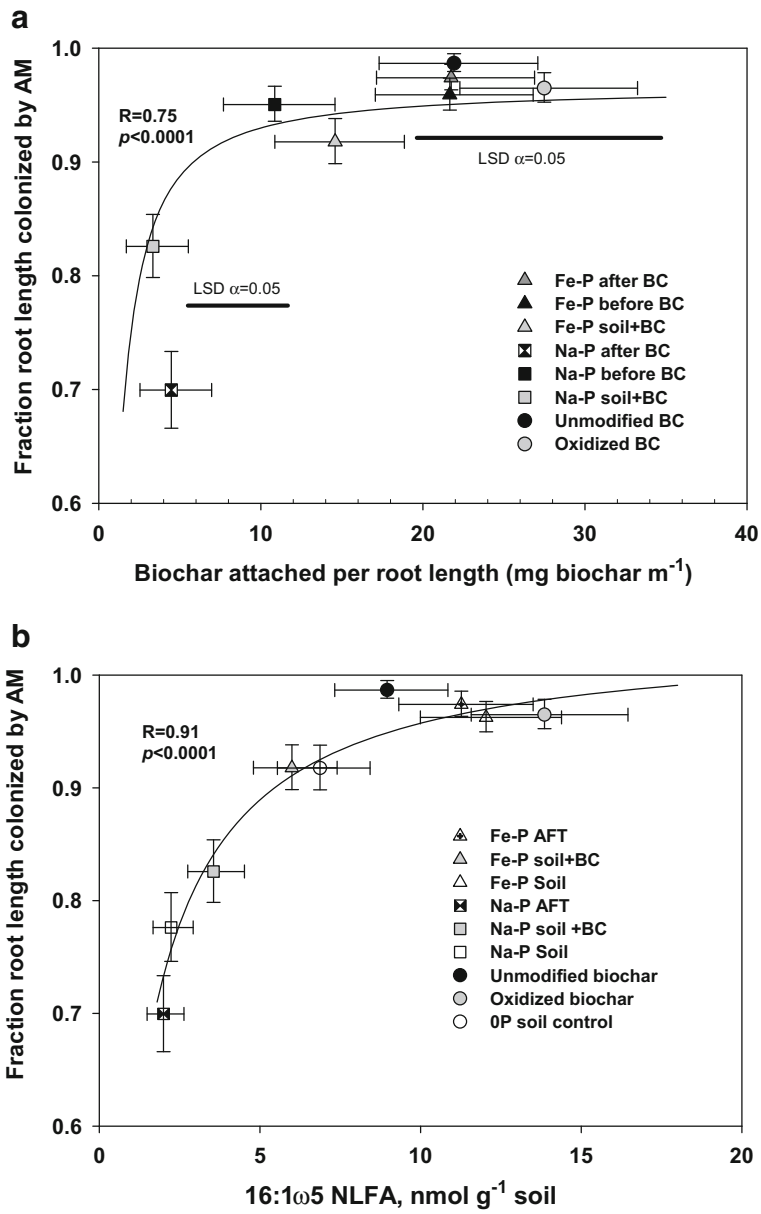

Fig. 3 **a**. Correlation between fraction root length colonized and biochar adhered to roots (mg·m^{-1} root length) Error bars show±one standard error and LSD bars show the least significant difference between two treatments; **b**. Relation between fraction root length colonized to the 16:1ω5 NLFA AM biomarker in soil. Colonization data in both graphs are fitted to Lineweaver-Burk saturation plot

higher P availability decreased AM colonization, SRL was also negatively related to AM arbuscule density in roots (Fig. 6c).

Discussion

Biochar effects on AM-facilitated phosphorus uptake

The experiment confirmed our first hypothesis that biochar's impact on P availability would be determined by the amount and solubility of P accompanying the biochar. Positive biochar-mycorrhizal interactive effects on bean P uptake were fostered by sparingly soluble Fe-P and absent when soluble P was combined with biochar. This result is consistent with plant functional equilibrium: the mutualistic benefit of P availability from AM declines when roots can take up soluble P directly (Johnson 2010). Biochar may thus not provide P nutrition benefits via AM in environments where soluble P availability approximates that in our Na-P treatments (~50 kg ha^{-1}). This agrees

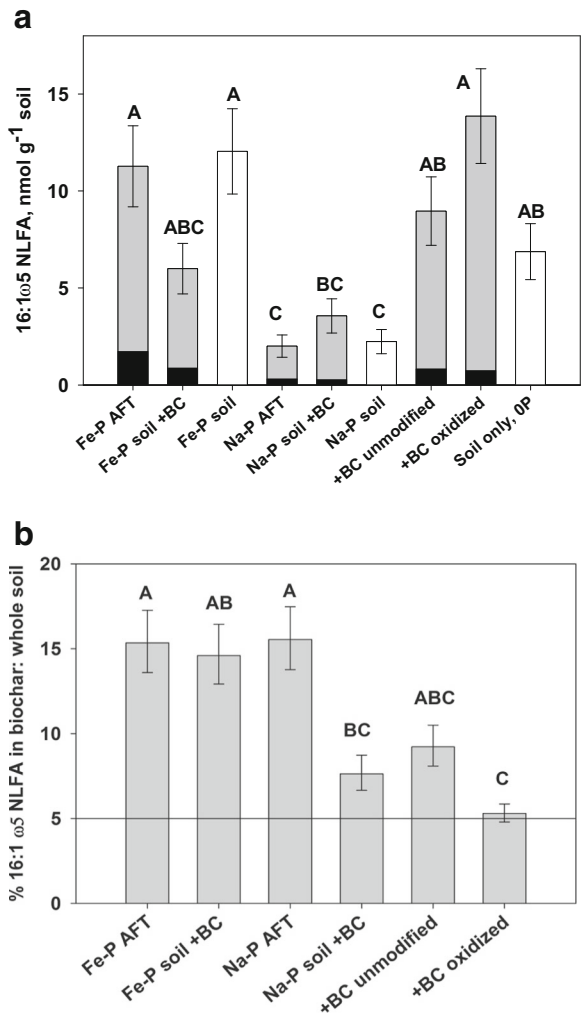

Fig. 4 **a**. Concentration of 16:1ω5 AM biomarker in whole soil+ biochar (upper portion of bars) and amount found in biochar separated by flotation (black portion of bar). Greyed bars are treatments with biochar. Error bars show±one standard error and letters show means differentiation by Tukey's test at α=0.05. **b**. Proportion of 16:1ω5 NLFA AM biomarker found within biochar for data shown in Fig. 4a. Error bars show±one standard error and reference line at 5 % is the expected value for 16:1ω5 AM NLFA if this were homogeneously distributed between soil and biochar in every biochar treatment

with Nzanza et al. (2012) who found no positive biochar/AM interaction in a soil with greater available P than used here (7 vs 0.17 μg P g^{-1}), and where 37 kg P ha^{-1} as fertilizer was applied to tomatoes. However, the positive interaction of biochar and AM on Fe-P availability for this legume/AM combination in a low-P soil is novel, and suggests that biochar per se can improve plant P nutrition from sparingly soluble sources such as those found in weathered tropical soils, via interactions with AM symbioses and not due merely to soil liming, improved water availability or biochar P additions. Enhancing the availability of sparingly soluble soil P has been proposed for soil organic additions more generally (Lobartini et al. 1998; Haynes and Mokolobate 2001; Guppy et al. 2005), and our results support this hypothesis regarding biochar. Meanwhile, increases in P availability from low-P biochar were more difficult to ascribe to biochar per se versus their P content. These unmodified and oxidized biochars contained a small fraction (4 %, <1 % respectively) of the P in biochars with Fe-P or Na-P but still increased plant shoot P vs. root P. They also fostered a positive biochar-AM interactive effect on shoot P. Interpreting this interaction is complicated by the lack of controls adding similar small P amounts without biochar: AM allowed plants to exploit either the scarce P in the biochar or additional soil P in the presence of biochar.

Biochar and phosphorus co-location effects on AM colonization

Our experiment also verified our second hypothesis that differences in P solubility drove the largest impacts on AM colonization. In agreement with previous research on AM (Johnson 2010; Ahmed et al. 2011; Gollner et al. 2011), colonization declined most dramatically in our experiment with addition of soluble Na-P, with or without biochar. However, biochar superimposed smaller but significant variations on these large solubility impacts. Unmodified biochar increased root colonization over the 0P control, which is the most typical comparison made in previous tests of biochar's effects on AM colonization (Warnock et al. 2010; e.g. Quilliam et al. 2012b). Given the P stress induced in the control, this may have resulted from small amounts of P in unmodified biochar that would have created a patchy P distribution in soils, which is known to favor AM root colonization (Cui and Caldwell 1996). The geometry of biochar particles of 1–2 mm mean size at 5 % v:v in soil leads to inter-particle distances between 2.5 and 5 mm, which may be patchy enough to favor mycorrhizal versus root exploration to access patches, resulting in greater AM benefit to plants and increased colonization. The chemical form of P in biochar may have also been more amenable to uptake by AM, since precipitated calcium phosphates likely predominate in biochar (Amonette and Joseph 2009) including our hardwood

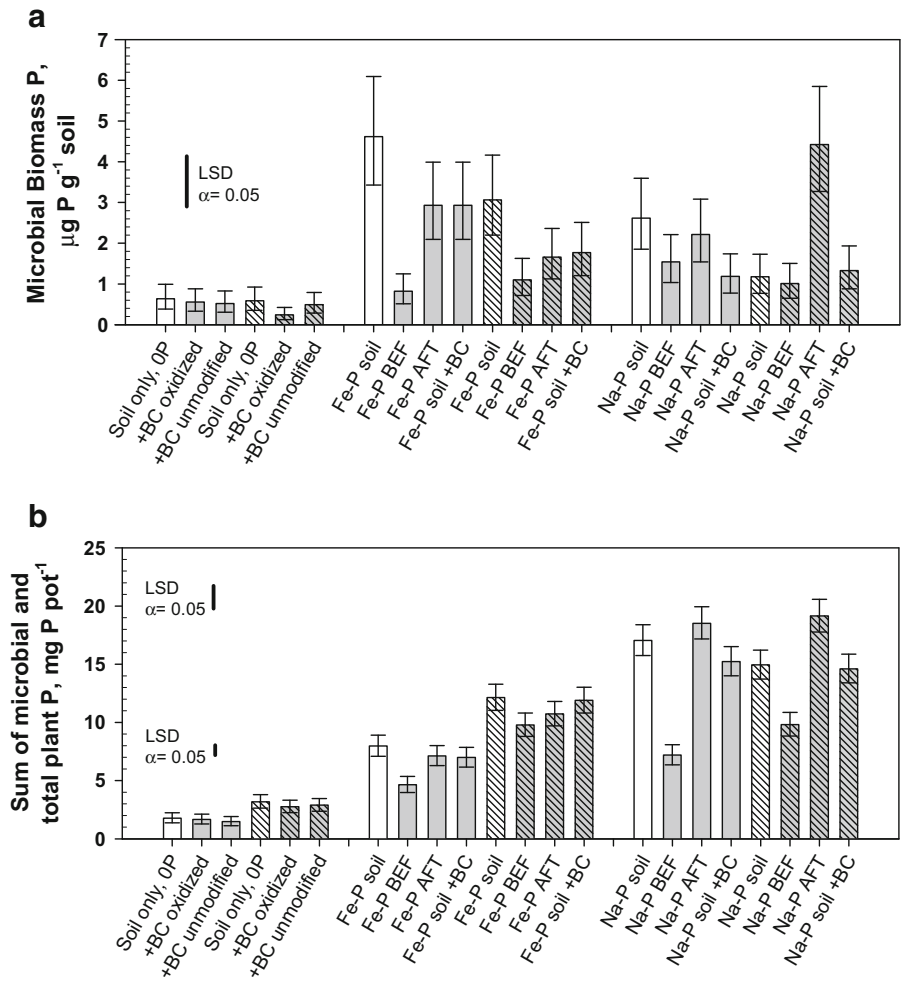

Fig. 5 **a**. Microbial biomass P (P_{MB}) for uninoculated (−AM, solid bars) and *Glomus clarum*- inoculated (+AM, hatched bars) pots. Error bars show±one standard error. **b**. Total P summed across plant biomass and microbial biomass. Microbial biomass calculated based on 800 mL soil with measured bulk density of 1.38 g mL^{-1}

biochar which had a Ca:P ratio of approximately 18:1 that would have favored such precipitation. Calcium phosphates could be solubilized by organic acids from AM hyphae, which would also release added Fe-P (Shi et al. 2011; Seguel et al. 2013).

Increased colonization due to unmodified biochar in soil may also represent interference in root-AM signaling that compromises roots' ability to modulate colonization as reported by LeCroy et al. (2013). However, in our experiment increase in colonization occurred without the growth costs from AM infection they observed in young sorghum plants. In fact, the positive interactive effects we observed on P shoot uptake and the shoot:root P ratio of low-P biochar treatments indicated decreased P stress with higher AM colonization. Also, when unmodified biochar was combined with soil-applied sparingly soluble P, AM colonization and 16:1ω5 NLFA was lower rather than higher in comparison to sparingly soluble P in soil without biochar (Fig. 2a). These two opposite effects of unmodified biochar are both consistent with nutrient impacts of biochar: creating patchy distributions that increase colonization when applied alone, while increasing the plant and AM availability of homogeneously distributed P to reduce colonization when applied with Fe-P in soil. Co-located soluble Na-P also had strong effects on colonization: co-pyrolyzed Na-P increased AM colonization compared to other Na-P treatments, likely due to precipitation of P in biochar during pyrolysis that favored AM uptake. Meanwhile soluble P mixed with biochar

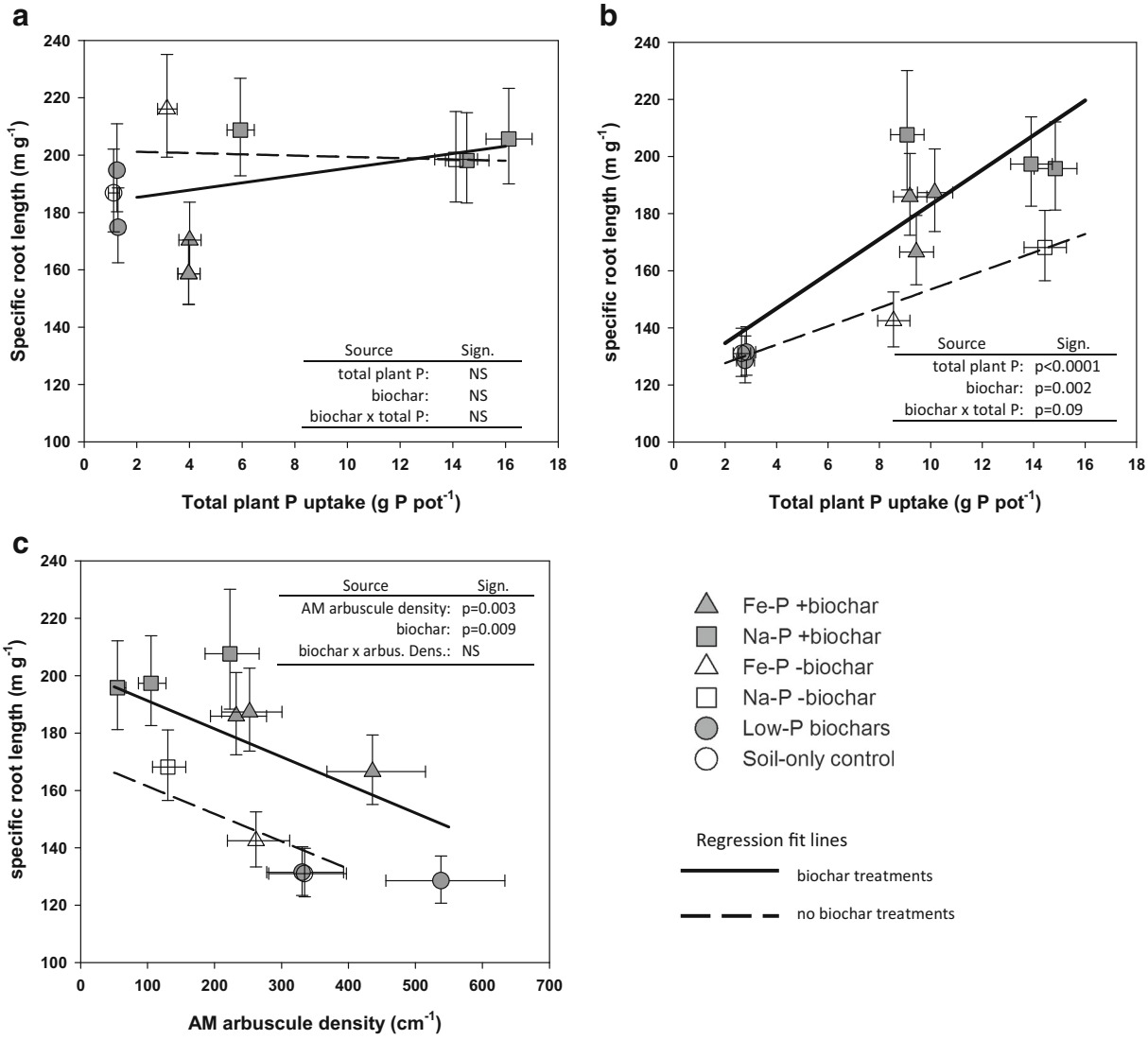

Fig. 6 **a**,**b**: Specific root length (SRL) regressed against total plant P uptake for -AM (**a**) and+AM treatments (**b**), with biochar addition to soil as a categorical predictor; **c**: SRL regressed against AM colonization intensity with biochar addition as a categorical predictor. Error bars show±one std. error. Table inset in each graph shows statistical significance of predictors and interaction with biochar factor

after pyrolysis reduced colonization rates, either by favoring root uptake or by dramatically increasing the effectiveness of AM uptake so the fewer hyphae and arbuscules were needed. Nutrient impacts of co-location that enhance mycorrhizal vs. plant root uptake may be thus be as important as effects on AM-root signaling in determining how biochar changes AM colonization. More experimentation should confirm and elucidate how biochar and AM affect root development, nutrient flows, and arbuscule formation at a cellular and root tissue level.

Co-location effects on phosphorus uptake and allocation to root and AM uptake pathways

In contrast to co-location impacts on AM colonization, our experiment refuted the hypothesis that co-location of P sources with biochar would increase bean P uptake. Plant reliance on root and AM uptake pathways to drive productivity (in beans, C and N fixation) can be visualized in a plot that shows shoot plus nodule P uptake on a vertical axis, versus normalized measures of 16:1ω5 NLFA on the x-axis and fine root biomass on the y-

axis (Fig. 7). Greater distance from the origin within the x-y plane can be read as greater total investment to acquire P. Among the Fe-P treatments assessed with NLFA, biochar plus Fe-P in soil had the lowest total root/AM investment and lower AM root colonization than biochar with co-located Fe-P. One explanation consistent with these results is that an overall facilitation effect of biochar on root and mycorrhizal uptake of Fe-P (considered below) acts in parallel with a nutrient patch effect on AM uptake that increases plant reliance on AM uptake, or makes hyphal exploration for Fe-P less efficient per unit hyphal biomass, when Fe-P is located on biochar particles than when it is distributed homogeneously in soil between biochar particles. Nevertheless, P uptake into shoots and nodules was not significantly increased by the lower investment costs for soil-applied Fe-P with biochar that Fig. 7 suggests. Further research should target this patch hypothesis by varying biochar particle size, inter-particle distance, and P solubility.

Effects on microbial biomass phosphorus and total phosphorus availability

Microbial biomass (MB) P increased with P addition, as expected, but did not increase uniformly with biochar addition, consistent with mixed biochar impacts on MB-C and MB-N in other short-term studies (Belyaeva and Haynes 2012; Case et al. 2012; Dempster et al. 2012; Schomberg et al. 2012). Interestingly, for Fe-P there was lower MB P under biochar addition, while the total of MB+plant P was conserved among treatments with and without biochar; this was not seen with all our biochar additions, ruling out a biochar toxicity effect in reducing MB. The inverse relationship of plant to MB P for sparingly soluble P suggests that biochar denied Fe-P to microbes by shifting uptake to hyphae and roots in a zero-sum way, rather than altering the total P available to microbes, hyphae, and plant roots. By contrast, the increased MB+plant P for soluble P placed on biochar after pyrolysis was the only case where we observed increased P availability due to co-location. Soluble P combined with mineralizable carbon on fresh biochar (Ameloot et al. 2013) may explain why MB P was especially high for soluble P placed close to biochar particles. Given the importance of MB for retaining soil P in bioavailable forms, these findings should be further tested by examining impacts of biochar and nutrients on MB C:N:P stoichiometry in a wider range of soils, as well as the durability of these impacts and effects on microbial functional groups beyond AM, using biomarker or molecular probing. Co-located soluble P and biochar could be useful in increasing soil microbial biomass P in the short term, even if plant uptake is not increased by co-location of soluble or sparingly soluble P.

Particle-independent effects of biochar on availability of sparingly soluble P to AM and plants

In contrast to these positive effects on total Na-P bioavailability and neutral effects on Fe-P bioavailability,

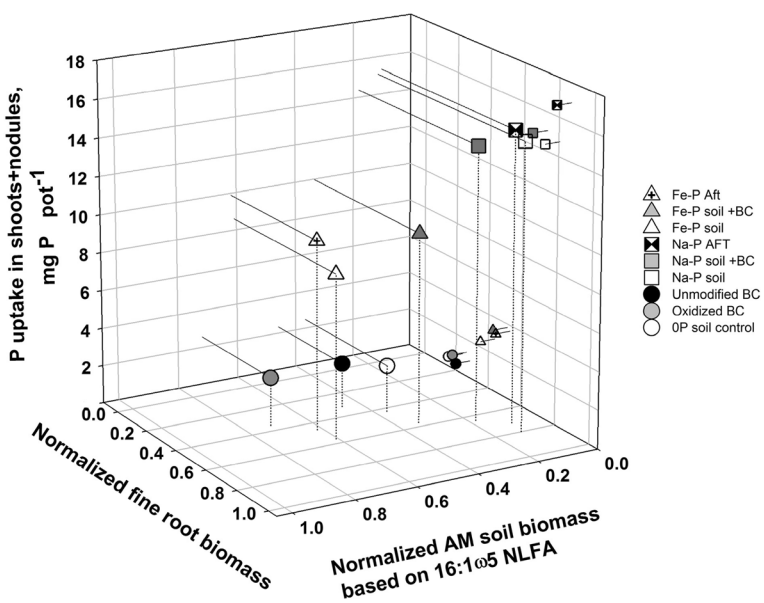

Fig. 7 P uptake into bean plant structures related to production (shoots+nodules) versus normalized measures of root biomass and AM soil hyphal biomass (16:1ω5 NLFA). Data points along the right-hand backplane are uninoculated treatments with negligible 16:1ω5 NLFA soil content. Normalized measures were generated by dividing each pot's root biomass and 16:1ω5 NLFA soil content by the maximum of the data in the experiment

our experiment demonstrated that biochar facilitated plant and AM uptake of sparingly soluble P at scales larger than biochar particles. One plausible explanation for this is that biochar facilitated root and hyphal exploration via "islands" of favorable habitat or porosity within less favorable soil environments such as the subsoil used in this experiment, or by lowering the mechanical impedance of soil as suggested by Atkinson et al. (2010). Biochar applied with Fe-P was preferentially enriched in AM regardless of whether or not the Fe-P was co-located with the biochar, which is consistent with this explanation: distributing Fe-P homogeneously in soil was insufficient to cause AM hyphae to "abandon" the biochar, even when colonization was reduced and the total AM (soil plus biochar) was lower for homogeneous Fe-P. This was corroborated by the high degree of biochar attachment to roots by hyphae in the Fe-P treatments (Fig. 3; see online supplement for images). Small amounts of feedstock-derived P in the unmodified biochar may have been important in initiating this AM exploration of biochar, as indicated by the lower proportion of AM biomarker in rinsed, oxidized biochar.

Given that both bean roots and AM were capable in our experiment of solubilizing Fe-P when compared to 0P controls, another possibility is that at a whole-soil level, biochar addition either facilitated solubilization from P-fixing oxides or prevented the re-sorption of solubilized P in this soil high in iron oxides. Previous evidence of biochar blocking sorption has usually been ascribed to the P content of the biochar (e.g. Morales et al. 2013) and liming effects of high-pH biochars (Cui et al. 2011; Xu et al. 2013), which we controlled for. Absent these nutrient and pH effects, dissolved organic carbon (DOC) in the form of low-molecular weight acids and other substances could play a role in blocking sorption sites (Hue 1991; Joseph et al. 2010). In a leaching experiment using a P-fixing ultisol, oak biochar released appreciable amounts of DOC (Mukherjee and Zimmerman 2013), and DOC from biochars has been shown to include low molecular weight organic acids (Joseph et al. 2010). Nevertheless given the concern that DOC levels in soils are rarely high enough to appreciably alter P-sesquioxide interactions (Guppy et al. 2005), this hypothesis should be carefully tested in future experimentation incorporating both plant-free conditions and AM/root uptake.

Biochar and AM interactive effects on root morphology

Specific root length (SRL) showed markedly different patterns depending on inoculation with AM. In inoculated treatments SRL increased with P availability, refuting the hypothesis that plants produce thinner roots under P stress to increase the ratio of root length (absorption benefit) to construction cost (Ostonen et al. 2007). We note that this interpretation of SRL and P stress has been disputed (Zobel et al. 2006; Useche and Shipley 2010). Our findings also contradict studies showing increases in SRL with AM inoculation (Miyauchi et al. 2008; Pang et al. 2010). More generally, declines in SRL have been shown to also result from salt, drought, aluminum stress, and compaction in soils (Zaifnejad et al. 1997; Sheng et al. 2009; Alameda and Villar 2012). Our experiment nevertheless conforms to Hetrick (1991) and Hetrick et al. (1992) who showed that plants dependent on AM symbioses have thicker roots under low P with high AM colonization. Hyphae of AM essentially act as ultra-thin roots and replace the high-SRL plant physiological response seen without AM, as we observed. Biochar also increased SRL when AM were present, and decreased SRL for Fe-P treatments without AM, outcomes that are more difficult to explain without invoking superposition of different effects on SRL. One plausible if partial explanation is that biochar alleviated high mechanical strength or aeration of soil thus increasing SRL with AM additions (Simojoki 2001; Alameda and Villar 2012). Alternatively, in+AM treatments biochar may have damped AM-root signaling in mycorrhizal development and reversed root thickening normally brought on by AM colonization (Lynch and Brown 1997; Guinel and Geil 2002; Spokas et al. 2010; Elmer and Pignatello 2011; LeCroy et al. 2013). Without AM, increased plant access to sparingly soluble P fostered by biochar may have lessened the need for thinner roots typically seen under P stress without AM; however this decrease was not similarly seen in -AM soluble P treatments where P stress was also alleviated, suggesting chemical or other effects linked to the interaction of iron phosphate, roots, and biochar. Additional mechanistic research on the rhizosphere is needed to further understand questions regarding biochar-AM interactive effects on nutrient uptake and root morphology, including field as well as greenhouse studies.

Acknowledgments The authors appreciate financial support from the NSF-Basic Research for Enabling Agricultural Development program (BREAD grant number IOS-0965336), and the Fondation des Fondateurs. Any opinions, findings and conclusions or recommendations expressed in this material are those of

the authors and do not necessarily reflect the views of the donors. We thank Daniel Richter for soil, Steve Beebe and CIAT for bean seed, Joseph Morton for AM inoculant and advice on AM in pot experiments, Harry Read for PLFA GC analysis and consultation, and Sara Nason, Kelly Hanley, Akio Enders, and Nick Vail for help in conducting the experiment. Electron microscopy in the online supplement made use of the Cornell Center for Materials Research Shared Facilities which are supported through the NSF MRSEC program (DMR-1120296). We also thank several anonymous referees for their valuable comments.

References

Ahmad F, Tan KH (1991) Availability of fixed phosphate to corn (zea mays l.) seedlings as affected by humic acids. Indones J Trop Agric 2:66–72

Ahmed FRS, Alexander IJ, Mwinyihija M, Killham K (2011) Effect of superphosphate and arbuscular mycorrhizal fungus *Glomus mosseae* on phosphorus and arsenic uptake in lentil (*Lens culinaris* L.). Water Air Soil Pollut 221:169–182

Alameda D, Villar R (2012) Linking root traits to plant physiology and growth in *Fraxinus angustifolia* Vahl. seedlings under soil compaction conditions. Environ Exp Bot 79:49–57

Alguacil MD, Roldan A, Salinas-Garcia JR, Querejeta JI (2011) No tillage affects the phosphorus status, isotopic composition and crop yield of *Phaseolus vulgaris* in a rain-fed farming system. J Sci Food Agr 91:268–272

Ameloot N, De Neve S, Jegajeevagan K, Yildiz G, Buchan D, Funkuin YN, Prins W, Bouckaert L, Sleutel S (2013) Short-term CO_2 and N_2O emissions and microbial properties of biochar amended sandy loam soils. Soil Biol Biochem 57: 401–410

Amonette JE, Joseph SD (2009) Characteristics of biochar: microchemical properties. In: Lehmann J, Joseph SD (eds) Biochar for environmental management: science and technology. Earthscan, London, pp 35–52

Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant Soil 337:1–18

Balemi T, Negisho K (2012) Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: A review. J Soil Sci Plant Nutr 12: 547–561

Belyaeva ON, Haynes RJ (2012) Comparison of the effects of conventional organic amendments and biochar on the chemical, physical and microbial properties of coal fly ash as a plant growth medium. Env Earth Sci 66:1987–1997

Borggaard OK, Raben-Lange B, Gimsing AL, Strobel BW (2005) Influence of humic substances on phosphate adsorption by aluminium and iron oxides. Geoderma 127:270–279

Bossio D, Scow K, Gunapala N, Graham K (1998) Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb Ecol 36:1–12

Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. Soil Biol Biochem 14: 319–329

Case SDC, McNamara NP, Reay DS, Whitaker J (2012) The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil - the role of soil aeration. Soil Biol Biochem 51: 125–134

Chacon N, Silver WL, Dubinsky EA, Cusack DF (2006) Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. Biogeochemistry 78:67–84

Chakraborty D, Garg RN, Tomar RK, Dwivedi BS, Aggarwal P, Singh R, Behera UK, Thangasamy A, Singh D (2010) Soil physical quality as influenced by long-term application of fertilizers and manure under maize-wheat system. Soil Sci 175:128–136

Cui M, Caldwell MM (1996) Facilitation of plant phosphate acquisition by arbuscular mycorrhizas from enriched soil patches.1. Roots and hyphae exploiting the same soil volume. New Phytol 133:453–460

Cui HJ, Wang MK, Fu ML, 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

Deenik JL, Diarra A, Uehara G, Campbell S, Sumiyoshi Y, Antal MJ (2011) Charcoal ash and volatile matter effects on soil properties and plant growth in an acid ultisol. Soil Sci 176: 336–345

Dempster DN, Gleeson DB, Solaiman ZM, Jones DL, Murphy DV (2012) Decreased soil microbial biomass and nitrogen mineralisation with eucalyptus biochar addition to a coarse textured soil. Plant Soil 354:311–324

Elmer WH, Pignatello JJ (2011) Effect of biochar amendments on mycorrhizal associations and fusarium crown and root rot of asparagus in replant soils. Plant Dis 95:960–966

Enders A, Lehmann J (2012) Comparison of wet-digestion and dry-ashing methods for total elemental analysis of biochar. Commun Soil Sci Plan 43:1042–1052

Erich MS, Fitzgerald CB, Porter GA (2002) The effect of organic amendments on phosphorus chemistry in a potato cropping system. Agr Ecosyst Environ 88:79–88

Gerke J (2010) Humic (organic matter)-Al(Fe)-phosphate complexes: an underestimated phosphate form in soils and source of plant-available phosphate. Soil Sci 175:417–425

Gichangi EM, Mnkeni PNS, Brookes PC (2010) Goat manure application improves phosphate fertilizer effectiveness through enhanced biological cycling of phosphorus. Soil Sci Plant Nutr 56:853–860

Gollner MJ, Wagentristl H, Liebhard P, Friedel JK (2011) Yield and arbuscular mycorrhiza of winter rye in a 40-years fertilisation trial. Agron Sustain Dev 31:373–378

Gregorich EG, Wen G, Voroney RP, Kachanoski RG (1990) Calibration of a rapid direct chloroform extraction method for measuring soil microbial biomass-C. Soil Biol Biochem 22: 1009–1011

Guinel FC, Geil RD (2002) A model for the development of the rhizobial and arbuscular mycorrhizal symbioses in legumes and its use to understand the roles of ethylene in the establishment of these two symbioses. Can J Bot 80:695–720

Guppy CN, Menzies NW, Moody PW, Blamey FPC (2005) Competitive sorption reactions between phosphorus and organic matter in soil: a review. Aust J Soil Res 43:189–202

Haynes RJ, Mokolobate MS (2001) Amelioration of al toxicity and p deficiency in acid soils by additions of organic

residues: a critical review of the phenomenon and the mechanisms involved. Nutr Cycl Agroecosyst 59:47–63
Hetrick BAD (1991) Mycorrhizas and root architecture. Experientia 47:355–362
Hetrick BAD, Wilson GWT, Todd TC (1992) Relationships of mycorrhizal symbiosis, rooting strategy, and phenology among tallgrass prairie forbs. Can J Bot 70:1521–1528
Hue NV (1991) Effects of organic-acids anions on p-sorption and phytoavailability in soils with different mineralogies. Soil Sci 152:463–471
Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytol 185:631–647
Joseph SD, Camps-Arbestain M, Lin Y, Munroe P, Chia CH, Hook J, van Zwieten L, Kimber S, Cowie A, Singh BP, Lehmann J, Foidl N, Smernik RJ, Amonette JE (2010) An investigation into the reactions of biochar in soil. Aust J Soil Res 48:501–515
Kalra YP (1998) Handbook of reference methods for plant analysis. CRC Press, Boca Raton
Kimetu JM, Lehmann J, Ngoze SO, Mugendi DN, Kinyangi JM, Riha S, Verchot L, Recha JW, Pell AN (2008) Reversibility of soil productivity decline with organic matter of differing quality along a degradation gradient. Ecosystems 11:726–739
Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect va mycorrhizas. Mycol Res 92:486–488
Kudeyarova AY (2010) Chemisorption of phosphate ions and destruction of organomineral sorbents in acid soils. Eurasian Soil Sci 43:635–650
Kuzyakov Y, Bogomolova I, Glaser B (2014) Biochar stability in soil: decomposition during 8 years and transformation as assessed by compound-specific 14c analysis. Soil Biol Biochem 70:229–236
Larsen J, Olsson PA, Jakobsen I (1998) The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the saprotrophic fungus *Fusarium culmorum* in root-free soil. Mycol Res 102:1491–1496
LeCroy C, Masiello CA, Rudgers JA, Hockaday WC, Silberg JJ (2013) Nitrogen, biochar, and mycorrhizae: alteration of the symbiosis and oxidation of the char surface. Soil Biol Biochem 58:248–254
Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota – a review. Soil Biol Biochem 43:1812–1836
Lekberg Y, Hammer EC, Olsson PA (2010) Plants as resource islands and storage units–adopting the mycocentric view of arbuscular mycorrhizal networks. FEMS Microbiol Ecol 74: 336–345
Lineweaver H, Burk D (1934) The determination of enzyme dissociation constants. J Am Chem Soc 56(3):658–666. doi: 10.1021/ja01318a036
Lobartini JC, Tan KH, Pape C (1998) Dissolution of aluminum and iron phosphate by humic acids. Commun Soil Sci Plan 29:535–544
Lynch J, Brown KM (1997) Ethylene and plant responses to nutritional stress. Physiol Plant 100:613–619
Miyauchi MYH, Lima DS, Nogueira MA, Lovato GM, Murate LS, Cruz MF, Ferreira JM, Zangaro W, Andrade G (2008) Interactions between diazotrophic bacteria and mycorrhizal fungus in maize genotypes. Sci Agric 65:525–531
Morales MM, Comerford N, Guerrini IA, Falcão NPS, Reeves JB (2013) Sorption and desorption of phosphate on biochar and biochar-soil mixtures. Soil Use Manag 29:306–314
Mukherjee A, Zimmerman AR (2013) Organic carbon and nutrient release from a range of laboratory-produced biochars and biochar–soil mixtures. Geoderma 193–194:122–130
Nzanza B, Marais D, Soundy P (2012) Effect of arbuscular mycorrhizal fungal inoculation and biochar amendment on growth and yield of tomato. Int J Agric Biol 14:965–969
Ostonen I, Puttsepp U, Biel C, Alberton O, Bakker MR, Lohmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as an indicator of environmental change. Plant Biosyst 141: 426–442
Pang JY, Ryan MH, Tibbett M, Cawthray GR, Siddique KHM, Bolland MDA, Denton MD, Lambers H (2010) Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. Plant Soil 331:241–255
Quilliam RS, Marsden KA, Gertler C, Rousk J, DeLuca TH, Jones DL (2012a) Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. Agric Ecosyst Environ 158:192–199
Quilliam RS, DeLuca TH, Jones DL (2012b) Biochar application reduces nodulation but increases nitrogenase activity in clover. Plant Soil 366:83–92
Richardson AE, Lynch JP, Ryan PR, Delhaize E, Smith FA, Smith SE, Harvey PR, Ryan MH, Veneklaas EJ, Lambers H, Oberson A, Culvenor RA, Simpson RJ (2011) Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349:121–156
Richter DD, Markewitz D, Wells CG, Allen HL, April R, Heine PR, Urrego B (1994) Soil chemical-change during 3 decades in an old-field loblolly-pine (*Pinus-taeda* L.) ecosystem. Ecology 75:1463–1473
Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to IMAGE-J: 25 years of image analysis. Nat Methods 9:671–675
Schnoor TK, Martensson LM, Olsson PA (2011) Soil disturbance alters plant community composition and decreases mycorrhizal carbon allocation in a sandy grassland. Oecologia 167:809–819
Schomberg HH, Gaskin JW, Harris K, Das KC, Novak JM, Busscher WJ, Watts DW, Woodroof RH, Lima IM, Ahmedna M, Rehrah D, Xing BS (2012) Influence of biochar on nitrogen fractions in a coastal plain soil. J Environ Qual 41:1087–1095
Seguel A, Cumming JR, Klugh-Stewart K, Cornejo P, Borie F (2013) The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. Mycorrhiza 23: 167–183
Sheng M, Tang M, Chen H, Yang BW, Zhang FF, Huang YH (2009) Influence of arbuscular mycorrhizae on the root system of maize plants under salt stress. Can J of Microbiol 55: 879–886
Shi Z, Wang F, Zhang C, Yang Z (2011) Exploitation of phosphorus patches with different phosphorus enrichment by three arbuscular mycorrhizal fungi. J Plant Nutr 34:1096–1106
Silva GL, Lima HV, Campanha MM, Gilkes RJ, Oliveira TS (2011) Soil physical quality of luvisols under agroforestry, natural vegetation and conventional crop management systems in the Brazilian semi-arid region. Geoderma 167–68:61–70

Simojoki A (2001) Morphological responses of barley roots to soil compaction and modified supply of oxygen. Agr Food Sci Finl 10:45–52

Snapp S, Koide R, Lynch J (1995) Exploitation of localized phosphorus-patches by common bean roots. Plant Soil 177: 211–218

Spokas KA, Baker JM, Reicosky DC (2010) Ethylene: potential key for biochar amendment impacts. Plant Soil 333:443–452

Useche A, Shipley B (2010) Plasticity in relative growth rate after a reduction in nitrogen availability is related to root morphological and physiological responses. Ann Bot-Lond 106:617–625

Van Aarle IM, Olsson PA (2003) Fungal lipid accumulation and development of mycelial structures by two arbuscular mycorrhizal fungi. Appl Environ Microbiol 69:6762–6767

Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol 127:390–397

Warnock DD, Lehmann J, Kuyper TW, Rillig MC (2007) Mycorrhizal responses to biochar in soil – concepts and mechanisms. Plant Soil 300:9–20

Warnock DD, Mummey DL, McBride B, Major J, Lehmann J, Rillig MC (2010) Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. Appl Soil Ecol 46:450–456

Woolf D, Amonette JE, Street-Perrott FA, Lehmann J, Joseph S (2010) Sustainable biochar to mitigate global climate change. Nat Comm 1:56. doi:10.1038/ncomms1053

Xu G, Wei LL, Sun JN, Shao HB, Chang SX (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

Yan XL, Lynch JP, Beebe SE (1996) Utilization of phosphorus substrates by contrasting common bean genotypes. Crop Sci 36:936–941

Zaifnejad M, Clark RB, Sullivan CY (1997) Aluminum and water stress effects on growth and proline of sorghum. J Plant Physiol 150:338–344

Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environ Sci Technol 44:1295–1301

Zobel RW, Alloush GA, Belesky DP (2006) Differential root morphology response to no versus high phosphorus, in three hydroponically grown forage chicory cultivars. Environ Exp Bot 57:201–208