TITLE: Phosphorus availability to beans via interactions between mycorrhizae and biochar

SUPPLEMENTARY ONLINE INFORMATION

Authors:

Steven J. Vanek[1*] and Johannes Lehmann[1]

[1] Department of Geography, Penn State University, State College, PA USA

[2] Department of Crop and Soil Science, Cornell University, Ithaca, NY USA

* Corresponding author: sjv13@psu.edu; 607-342-5940

Table SI1. Orthogonal contrasts testing main and interaction effects of inoculation with *Glomus clarum* (+AM), P type, and P co-location with biochar, for shoot biomass, root biomass, root P concentration, and specific root length.

Orthogonal contrast number and definition	**Shoot biomass** ***(g ·pot^{-1})***	**Root biomass** ***(g ·pot^{-1})***	**Root P concentration** ***(mg P·g^{-1})***	**Specific root length (m•g^{-1})**
Main effects				
1. Main effect of *Glomus clarum* (+AM **vs.** -AM)	3.0 vs 2.9 ***	3.0 vs 2.9 ns	2.00 vs 1.29 ***	166 vs.193 ***
Main effects of added P and biochar, combining +/- AM				
2. Na-P **vs.** Fe-P and low-P treatments	4.9 vs 1.8 ***	4.9 vs 1.8 ***	1.88 vs 1.50 ***	196 vs.169 ***
3. Fe-P **vs.** Low-P chars and soil-only control	2.3 vs 1.1 ***	2.3 vs 1.1 ***	1.77 vs 1.15 ***	178 vs.160 *
4. Na-P (BEF, AFT, soil +BC) **vs.** Na-P soil	ns	4.9 vs 5.1 *	ns	ns
5. Fe-P (BEF, AFT, soil +BC) **vs.** Fe-P soil	2.5 vs 2.0 *	ns	ns	ns
6. Low-P biochars (unmodified, oxidized) **vs.** soil-only control	ns	ns	ns	ns
7. [(Aft, soil+BC) **vs.** BEF], Na-P treatments	5.7 vs 3.2 ***	5.7 vs 3.2 ***	1.94 vs 1.64 **	ns
8. [(Aft, soil+BC) **vs.** BEF], Fe-P treatments	ns	ns	ns	ns
9. Na-P AFT **vs.** Na-P soil+BC	ns	5.8 vs 5.5 *	ns	ns
10. Fe-P AFT **vs.** Fe-P soil+BC	ns	ns	ns	ns
11. Unmodified **vs.** oxidized low-P biochars	ns	ns	ns	ns
Interaction Effects	***Effect of AM in the first contrast group (difference of + vs. – AM), versus effect of-AM in the second contrast group, with contrast statistical significance***			
12. AM x [Na-P **vs**. Fe-P and low P treatments]	ns	-0.9 vs 0.7 ***	0.58 vs 0.79 ***	-17 vs. -33 *
13. AM x [Fe-P **vs**. low P treatments]	ns	ns	1.03 vs 0.48 ***	-14 vs. -59 *
14. AM x [BC **vs**. no BC], Na-P treatments	ns	ns	0.62 vs 0.48 ***	ns
15. AM x [BC **vs**. no BC], Fe-P treatments	ns	ns	0.98 vs 1.19 ***	6 vs. -76 *
16. AM x [BC **vs**. no BC], low-P treatments	ns	ns	0.45 vs 0.55 +	-65 vs. -47 *
17. AM x [AFT and soil+BC **vs**. BEF], Na-P tmts.	ns	-1.0 vs -0.8 *	ns	ns
18. AM x [AFT and soil+BC **vs**. BEF], Fe-P tmts	ns	ns	1.05 vs 0.83 **	ns
19. AM x [AFT **vs**. soil+BC], Na-P treatments	ns	ns	ns	ns
20. AM x [AFT **vs**. soil+BC], Fe-P treatments	ns	ns	ns	ns
21. AM x [unmodified **vs**. oxidized BC]	ns	ns	ns	ns

Figure SI1. Shoot and root dry biomass 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 SI1 for statistical analysis.

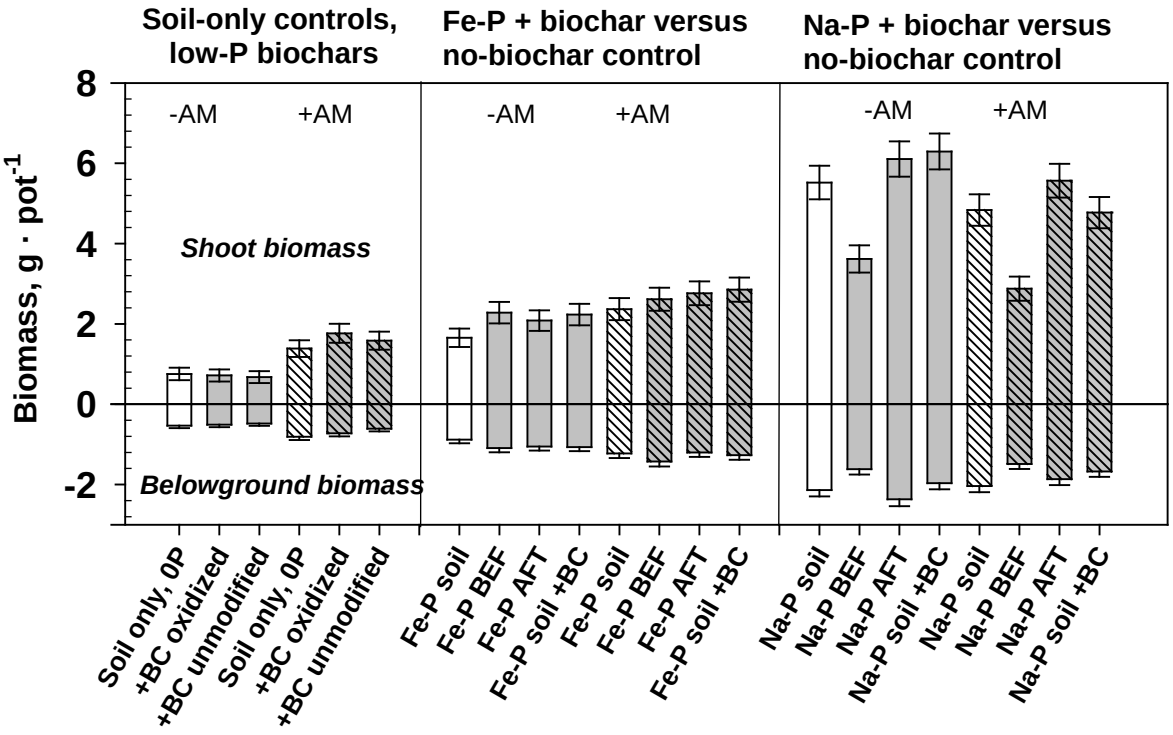
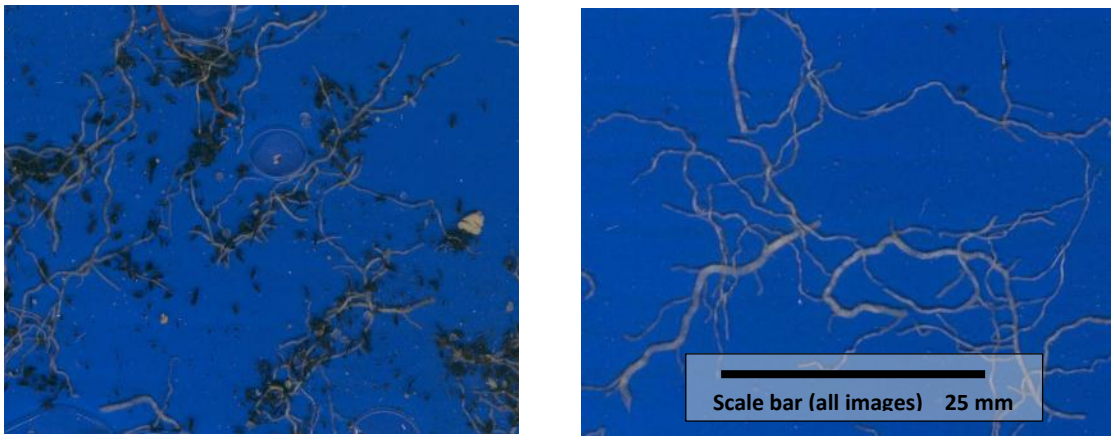


Table SI2. Results for NLFA and PLFA biomarker lipids of several indicator classes. Microbial. Zero values are listed for fatty acids that were not detectable. Table entries are mean concentration of three replicates with standard error in parentheses.

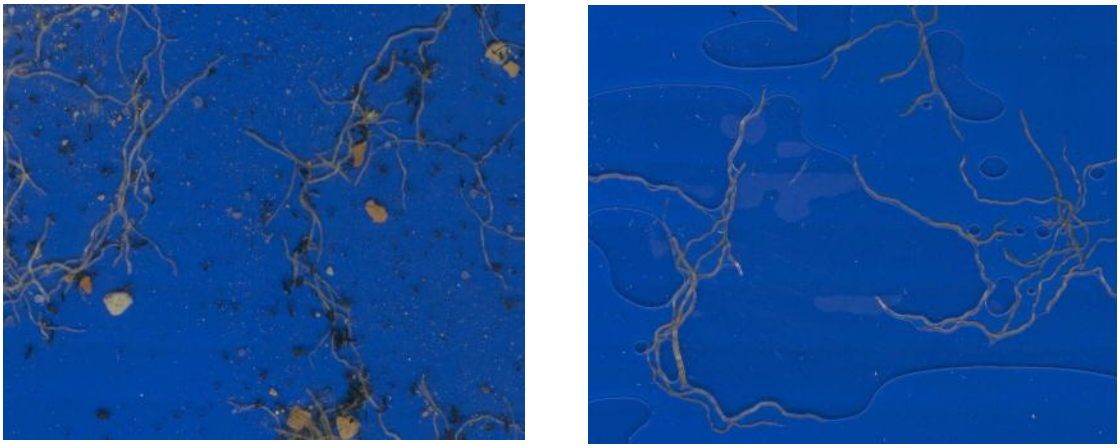
		Fe-P AFT	Na-P AFT	Fe-P Soil+BC	Na-P soil+BC	Fe-P soil	Na-P Soil	Unamended soil, 0P	Unmodified biochar	Oxidized biochar
Fatty Acid	**Microbial guild**	**Neutral Lipid Fatty Acid, NLFA (nmol g^{-1})**								
15:0 Anteiso	GM+	**0.26** (0.26)	**0.24** (0.24)	**0.20** (0.20)	**0.23** (0.23)	0.0 (0.0)	0.0 (0.0)	**0.11 (0.11)**	0.0 (0.0)	0.0 (0.0)
15:0 ISO	Gm+	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.00 (0.00)	0.0 (0.0)	0.0 (0.0)
16:0 10 Methyl	Actino-mycete	**0.83** (0.83)	**0.70** (0.70)	**0.52** (0.52)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.00 (0.00)	0.0 (0.0)	0.0 (0.0)
16:1 w5c	AMF	**12.23** (3.99)	**2.01** (0.17)	**6.61** (2.15)	**3.58** (0.34)	**12.01** (0.12)	**2.26** (0.28)	**7.25 (1.96)**	**9.10** (1.31)	**14.22** (2.63)
16:1 w7c	Gm-	**6.37** (6.37)	**0.70** (0.70)	**2.11** (2.11)	**0.96** (0.96)	**0.25** (0.25)	**0.0** (0.0)	0.00 (0.00)	**2.58** (2.58)	**4.36** (4.36)
17:0 CYCLO	Gm-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.00 (0.00)	0.0 (0.0)	0.0 (0.0)
17:0 ISO	Gm+	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.00 (0.00)	0.0 (0.0)	0.0 (0.0)
17:1 Anteiso	Gm+	**0.41** (0.41)	**0.67** (0.34)	**0.38** (0.19)	**0.69** (0.35)	**0.85** (0.14)	**1.05** (0.06)	**0.52 (0.02)**	**0.53** (0.34)	**0.52** (0.26)
18:1 w7c	Gm-, Methano-troph	**1.23** (0.37)	**0.00** (0.00)	**0.66** (0.34)	**0.22** (0.22)	**1.38** (0.01)	**0.44** (0.07)	**0.90 (0.24)**	**1.09** (0.15)	**1.66** (0.27)
18:1 w9c	Fungi	**1.02** (0.15)	**0.47** (0.02)	**1.21** (0.26)	**0.80** (0.41)	**1.84** (0.42)	**0.62** (0.05)	**0.69 (0.09)**	**0.95** (0.13)	**1.05** (0.05)
18:2 w6,9c	Fungi	**0.46** (0.23)	0.0 (0.0)	**0.21** (0.21)	**0.30** (0.30)	**0.92** (0.64)	**0.39** (0.00)	**0.22 (0.02)**	**0.23** (0.23)	**0.38** (0.19)
19:0 CYCLO	Gm-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	**0.43** (0.22)	**0.11 (0.11)**	0.0 (0.0)	0.0 (0.0)
		Phospholipid Fatty Acid, PLFA (nmol g^{-1})								
15:0 Anteiso	GM+	**0.14** (0.04)	**0.21** (0.06)	**0.15** (0.03)	**0.19** (0.01)	**0.15** (0.01)	**0.19** (0.08)	**0.15** (0.01)	**0.07** (0.04)	**0.11** (0.02)
15:0 ISO	Gm+	**0.16** (0.03)	**0.16** (0.04)	**0.15** (0.02)	**0.19** (0.01)	**0.15** (0.03)	**0.14** (0.06)	**0.16** (0.01)	**0.14** (0.08)	**0.11** (0.02)
16:0 10 Methyl	Actino-mycete	**0.02** (0.02)	**0.03** (0.03)	**0.07** (0.04)	**0.07** (0.03)	**0.12** (0.03)	**0.10** (0.04)	**0.11** (0.01)	**0.00** (0.00)	**0.07** (0.04)
16:1 w5c	AMF	**0.20** (0.07)	**0.06** (0.02)	**0.14** (0.03)	**0.10** (0.00)	**0.23** (0.07)	**0.04** (0.03)	**0.16** (0.02)	**0.13** (0.00)	**0.16** (0.03)
16:1 w7c	Gm-	**0.07** (0.04)	**0.07** (0.04)	**0.09** (0.01)	**0.10** (0.01)	**0.12** (0.01)	**0.09** (0.04)	**0.08** (0.00)	**0.03** (0.03)	**0.05** (0.03)
17:0 CYCLO	Gm-	**0.08** (0.04)	**0.10** (0.03)	**0.09** (0.01)	**0.12** (0.01)	**0.10** (0.03)	**0.04** (0.02)	**0.06** (0.01)	**0.04** (0.04)	**0.09** (0.01)
17:0 ISO	Gm+	0.0 (0.0)	0.0 (0.0)	**0.04** (0.02)	**0.04** (0.02)	**0.03** (0.03)	**0.28** (0.23)	**0.05** (0.02)	0.0 (0.0)	0.0 (0.0)
17:1 Anteiso	Gm+	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	**0.05** (0.05)	**0.09** (0.04)	**0.04** (0.02)	0.0 (0.0)	0.0 (0.0)

18:1 w7c	Gm-, Methano-troph	**0.33** (0.11)	**0.32** (0.08)	**0.30** (0.05)	**0.31** (0.07)	**0.31** (0.10)	**0.25** (0.11)	**0.22** (0.01)	**0.18** (0.04)	**0.21** (0.02)
18:1 w9c	Fungi	**0.17** (0.03)	**0.12** (0.07)	**0.15** (0.02)	**0.18** (0.03)	**0.14** (0.04)	**0.12** (0.05)	**0.13** (0.00)	**0.12** (0.02)	**0.12** (0.01)
18:2 w6,9c	Fungi	**0.18** (0.03)	**0.18** (0.06)	**0.21** (0.07)	**0.17** (0.01)	**0.09** (0.03)	**0.06** (0.02)	**0.10** (0.03)	**0.10** (0.02)	**0.09** (0.02)
19:0 CYCLO	Gm-	**0.12** (0.04)	**0.18** (0.05)	**0.12** (0.02)	**0.17** (0.04)	**0.15** (0.06)	**0.16** (0.07)	**0.13** (0.01)	**0.08** (0.03)	**0.11** (0.02)

+ AM **-AM**



Fe-P Before (+AM on left and –AM on right)



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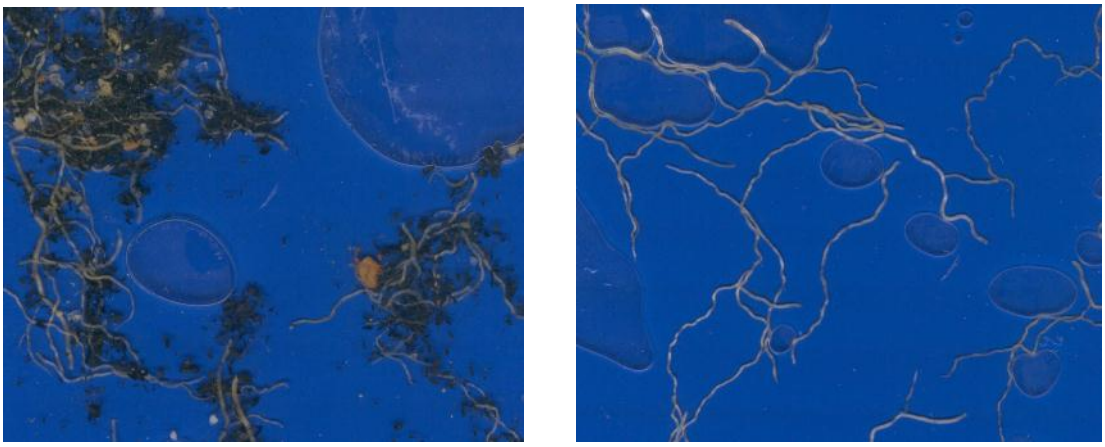
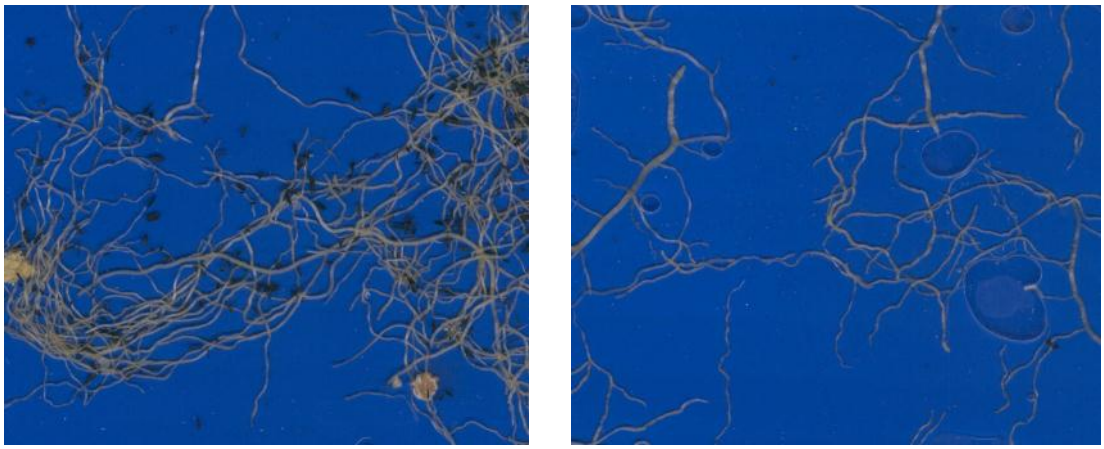
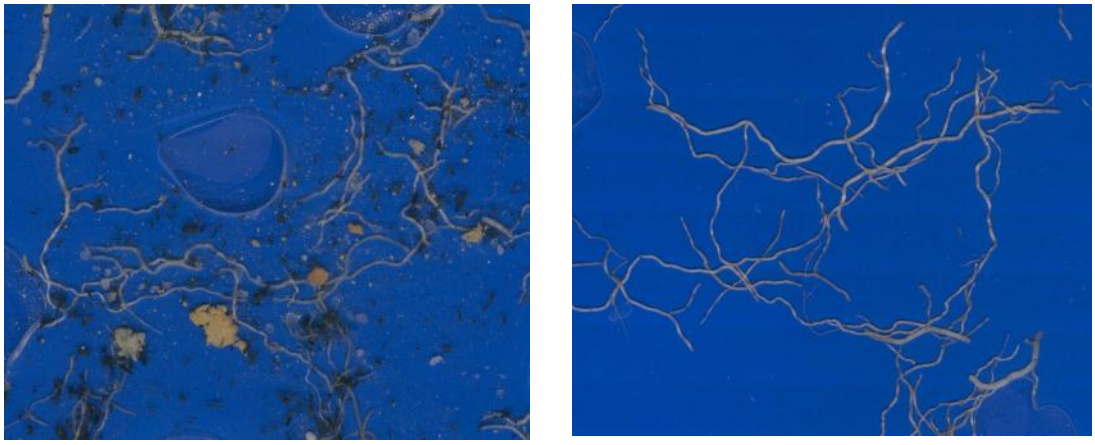


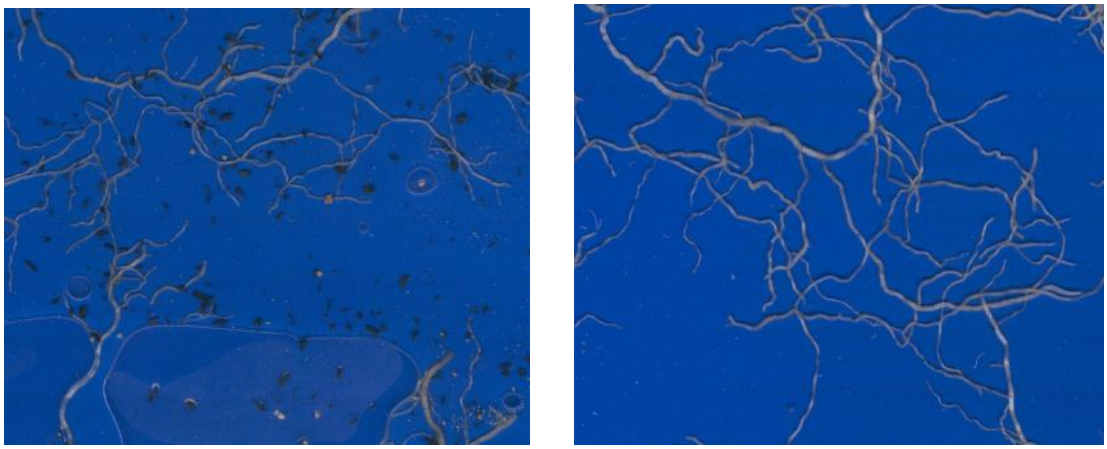
Fe-P After (+AM on left and –AM on right)



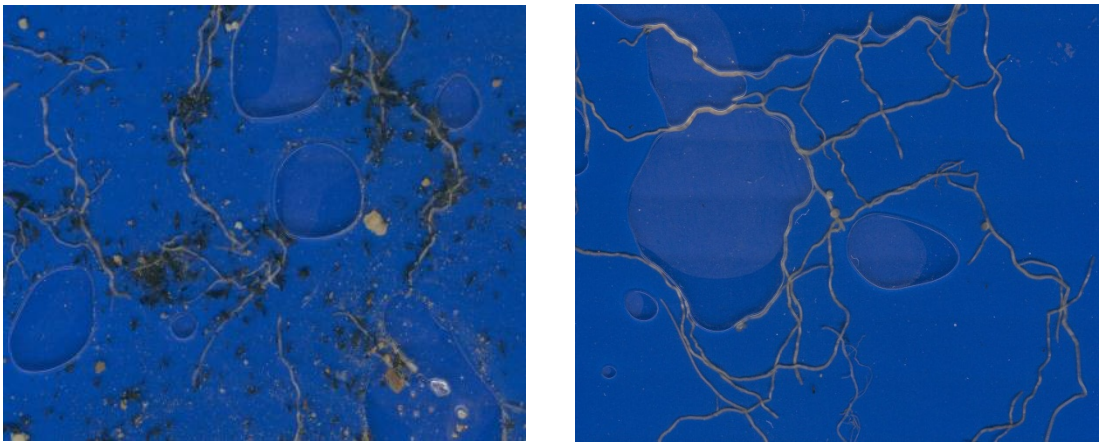
Na-P after (+AM on left and –AM on right)



Fe-P soil + biochar (+AM on left and –AM on right)



Na-P soil + biochar (+AM on left and –AM on right)



Unmodified biochar (+AM on left and –AM on right)

Figure SI2. Illustrations of root scans showing hyphal connection between roots and char in *Glomus clarum* –inoculated +AM treatments (left), and virtual lack thereof in corresponding uninoculated –AM treatments (right). All scan images were cropped from larger, full size scans and represent 51 x 42 mm of scan area. Scale bar shown is 25 mm, for all images.

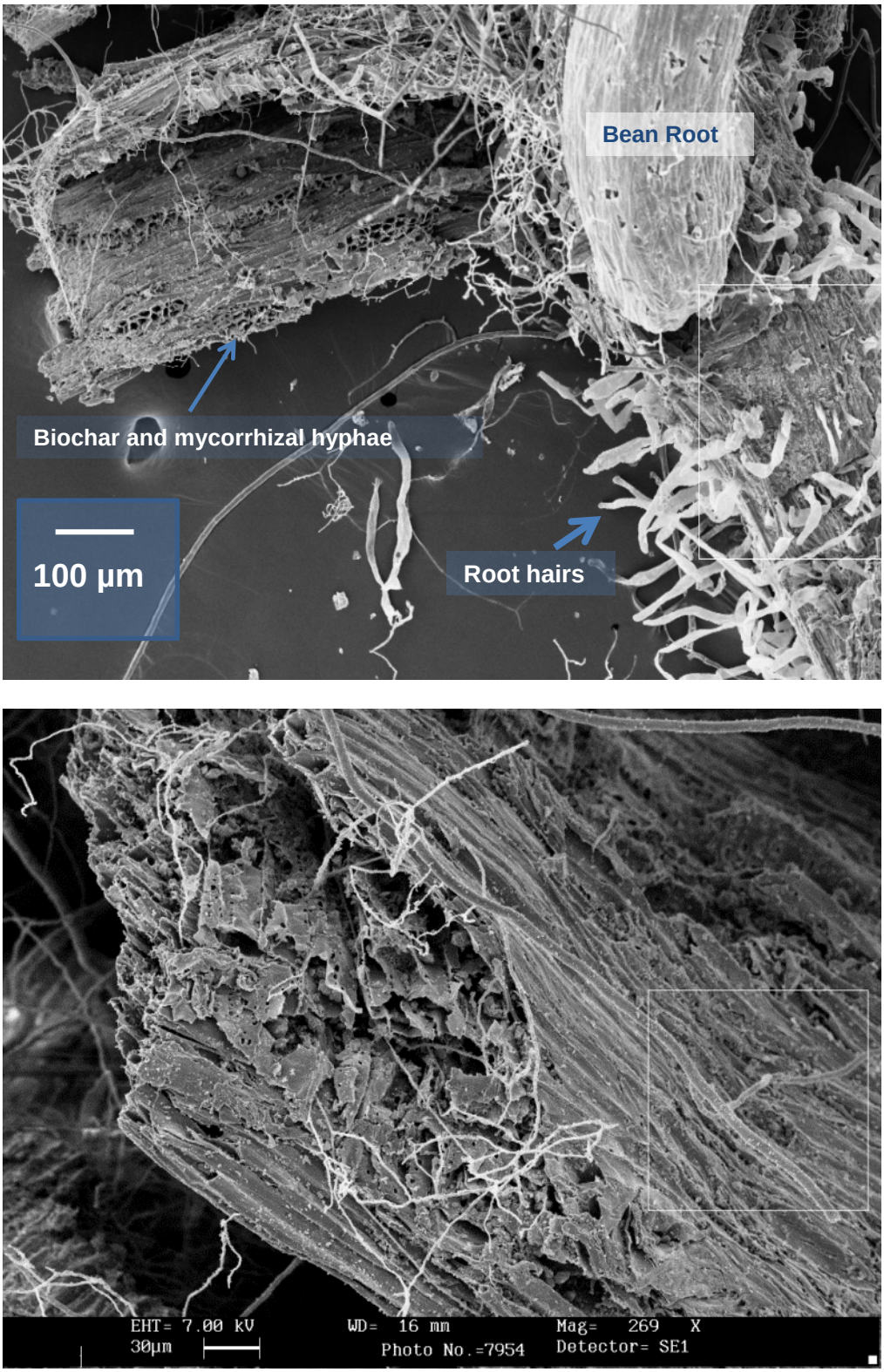


Figure SI3. Above: SEM image of Bean root (diameter ~200 µm), root hairs (diameter ~5-15 µm), and mycorrhizal hyphae (diameter 2-10 µm) juxtaposed against a hardwood biochar particle. **Below**: Close-up of mycorrhizal exploration of a biochar particle (30µm scale bar at lower left).

Methods and results for Ergosterol analysis of whole soil with biochar, and biochar particles

Ergosterol in soil and associated with biochar particles

We used ergosterol as a fungal biomarker extracted from ground soil+biochar, from biochar separated from soil, and from +AM treatments with biochar addition. After frozen storage at -15°C, ~100 mL soil samples were lyophilized (Kinetics dura-dry MP, Kinetics Systems, Fremont, CA, USA), and immediately processed for ergosterol content. Biochar separation on freeze-dried samples was accomplished by flotation in distilled water as follows: 70 ± 5 g of soil was repeatedly rinsed and the supernatant decanted through a 37-µm sieve until all visible biochar particles were recovered on the sieve. 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. Biochar from the 74-µm sieve was then washed into a 100-mL graduated cylinder while the 53-µm fraction was retained for ignition (550°C) and biochar mass was calculated by difference (described below). Hyphae in the graduated cylinder supernatant (thus not associated with biochar) were decanted using agitation for ten seconds with a hand-held blender followed by two minutes of biochar settling to the bottom. After three decants, the supernatant was inspected for floating hyphae in a beaker using a stereomicroscope, and rinsing was continued until visible floating hyphae were absent, and hyphae attached to biochar extended into the solution less than half the biochar particle diameter. The washed biochar sample with attached and internal hyphae was then filtered on an ashed, weighed glass fiber filter, a small aliquot (57±16 mg dry wt ± S.D.) removed for moisture determination using a microbalance and drying oven (24h at 105°C), and the remainder (281±52 mg) extracted immediately with methanol for ergosterol content (described below). Meanwhile, unrecovered biochar on the 53-µm sieve and removed with the supernatant during pouring off of hyphae (88±29 mg) was estimated by filtering through the same glass filter used for the extracted sample, which was dried at 105°C, ignited at 550°C, and reweighed to determine the dry mass of biochar by difference.

The ergosterol extraction method was modified from Djajakirana et al. (1996) with the addition of 7-dehydrocholesterol (7-DHC) internal standard to control for biochar and soil sorption of ergosterol. Biochar was taken from the procedure above, while freeze-dried soil was ground in a mortar before extraction of 2 g soil. After addition of a weighed amount (≈1.00 mL) of a 20-mg•L-1 7-DHC methanol solution (≈20 µg 7-DHC) , samples were shaken in 40 mL methanol for 30 minutes in capped, amber glass tubes previously washed and ashed at 500°C for 2h to destroy organic residues. Extracts were glass-fiber filtered (0.7 µm nominal), and methanol removed using a rotary evaporator (Buchi, Flawil, Switzerland) at 45°C. The residue was dissolved in 1 mL methanol and analyzed for 7-dehydrocholesterol (7-DHC) and ergosterol via HPLC using an isocratic flow of pure methanol carrier at 200 µl•min-1 for 15 minutes. The injection volume of sample was 10 µL and the column was a 150 x 2.00 mm, reverse-phase C-18 column with 3µm particle size (Gemini-NX, Phenomenex, Torrens, CA). Eluted peaks were detected using ACPI ionization in positive mode and mass spectrometry with m/Z=379 for ergosterol and m/Z=367 for 7-DHC. Peaks were automatically detected with a signal:noise criterion of >3. Peaks were automatically detected with a signal:noise criterion of >3. A standard curve was developed relating eluted peak areas of known samples to their concentration ratios of ergosterol to7-DHC (Erg:7-DHC). Ergosterol was varied in the standards and 7-DHC was fixed at the spike amount of 20 µg. Mass of ergosterol in the extracted sample was then calculated as:

$$erg_{sample} = K_{calib} \cdot (Erg{:}7\text{-}DHC) \cdot D_{spike},$$

with K_{calib} as the slope of the calibration curve (=1.115) and D_{spike} the 7-DHC spike amount from the weighed spike solution added. We divided erg_{sample} by the initial dry mass of soil or biochar to yield ergosterol concentration. The procedure was validated using frozen and lyophilized garden soil, and the Calhoun forest soil fortified with yeast, yielding values representative of the literature (Djajakirana et al. 1996). The proportion of ergosterol associated with biochar, $X_{erg \cdot BC,}$ was then calculated as:

$$X_{erg \cdot BC} = ([erg_{bc}] \cdot (bc_{ext}+bc_{recov}+bc_{moisture}) / m_{s\text{-}bc})/[erg_{soil}],$$

where [erg_{bc}] and [erg_{soil}] are concentrations of ergosterol in biochar and soil, respectively, (bc_{ext}+ bc_{recov}+$bc_{moisture}$) is the sum of the biochar mass recovered from extraction, moisture determination, and ashing of filters with residual biochar, and $m_{s\text{-}bc}$ is the mass of soil washed and decanted to float out biochar. $X_{erg\cdot BC}$ was compared to 5%, the expected ratio if ergosterol is homogeneously distributed between biochar and soil based on the 5% volume addition of biochar to the soil.

Results: Ergosterol in biochar as a proportion of whole soil ergosterol

Biochar was preferentially enriched with ergosterol in treatments with co-located P. For the BEF and AFT treatments where the two P sources was added to biochar rather than to soil adjacent to biochar, as well as the ambient and oxidized biochars, the fraction of ergosterol found in biochar vs. the total soil+biochar content was statistically greater than 5%, the expected value if fungi and thus ergosterol were homogeneously distributed between biochar and soil volumes (Fig. SI3). Co-location of added P and biochar increased the proportion of ergosterol found in the biochar from 9% for soil+BC treatments to 24% for BEF and AFT treatments (Fig. 3b; $p<0.001$ for a statistical contrast combining P sources and comparing [BEF and AFT] to [soil +BC] treatments).

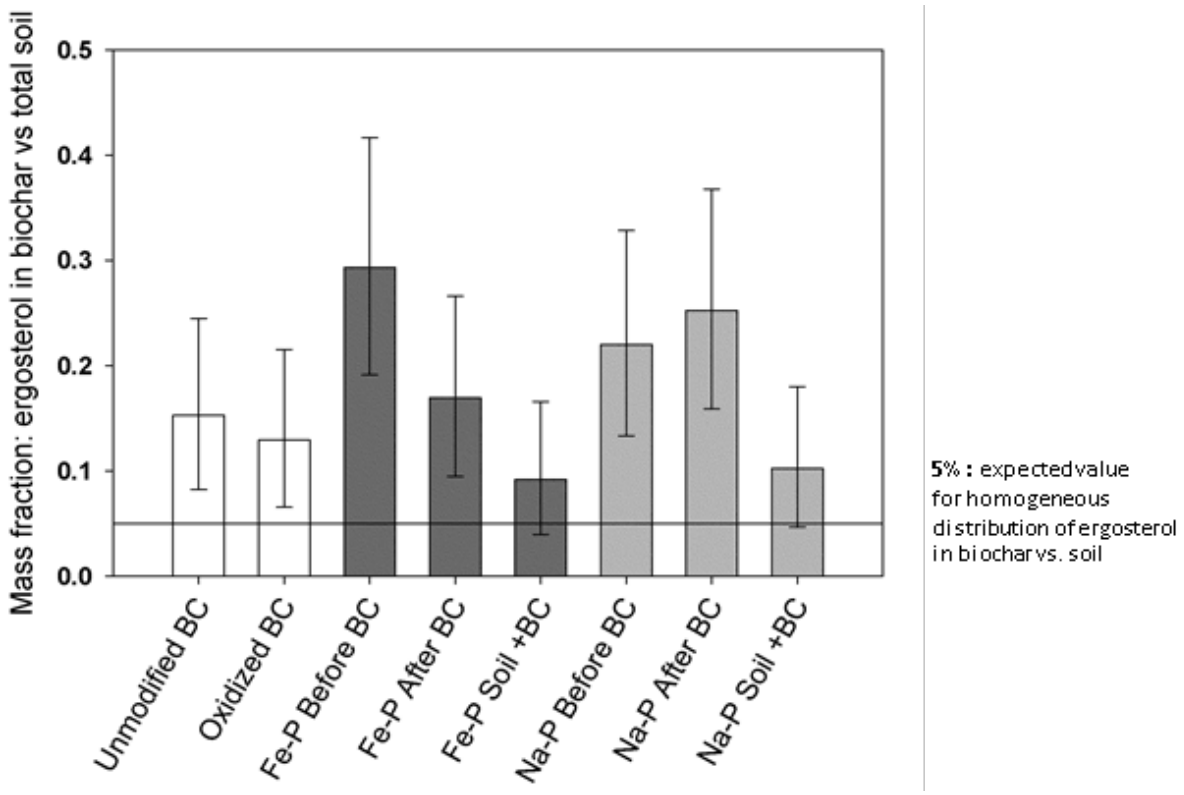


Figure SI4. Proportion of ergosterol found within or on biochar particles in different treatments versus whole ground soil+biochars from the same treatment. Error bars show the 95% confidence interval for each mean. The reference line at 5% is the expected value for ergosterol proportion associated with biochar particles if ergosterol were homogeneously distributed between soil and biochar.