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| RESEARCH ARTICLE

Effects of Biochar and Arbuscular Mycorrhizal Fungal Inoculation on Soil Phosphorus Availability and Growth of *Pisum sativum* (Dwarf Snap Pea)

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| ABSTRACT

Limited plant-available phosphorus (P) in agricultural soils and the depletion of finite phosphate rock reserves present major challenges to sustainable food production. Biochar and arbuscular mycorrhizal fungi (AMF) are independently recognised as strategies to enhance soil P availability and plant productivity, yet their combined effects in organic soils and on legume crops remain poorly understood. This study investigated the effects of wood-derived biochar applied at 2% and 10% (w/w) and commercial AMF inoculation, individually and in combination, on soil physicochemical properties, plant productivity, and AMF root colonisation in dwarf snap pea (Pisum sativum L.) grown under pot conditions in organic soil at Drew University, Madison, New Jersey, USA. Six treatments were arranged in a randomised complete block design with five replications: control (T0), AMF only (T1), 2% biochar (T2), 2% biochar + AMF (T3), 10% biochar (T4), and 10% biochar + AMF (T5). Soil moisture content, pH, available P, and AMF root colonisation differed significantly among treatments ($p \leq 0.01$), while plant biomass, shoot height, root length, and tissue phosphorus did not. The co-application of 10% biochar and AMF (T5) produced the highest available soil P (43.27 ± 2.14 ppm), significantly exceeding all other treatments including the control and single-amendment treatments. This synergistic effect was absent at the 2% biochar rate, indicating a dose-dependent threshold for the biochar-AMF interaction. AMF root colonisation increased progressively with biochar rate, peaking at 70 ± 3.8% in T5. Soil moisture was greatest under 10% biochar alone (T4; 37 ± 1.94%), while AMF-only treatment (T1) recorded the lowest pH (6.61 ± 0.05), consistent with organic acid exudation during phosphate solubilisation. The absence of significant plant growth responses is attributed to the abbreviated experimental duration relative to the cultivar's recommended maturation period. These findings demonstrate that co-application of wood-derived biochar at 10% with AMF inoculation synergistically enhances soil phosphorus availability and root colonisation in organic soil, supporting its potential as a low-input strategy for reducing synthetic P fertiliser dependency in small-scale and organic agricultural systems.

| KEYWORDS

biochar; arbuscular mycorrhizal fungi; phosphorus availability; Pisum sativum; soil amendment; organic agriculture; sustainable fertilisation

| ARTICLE INFORMATION

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1. Introduction

Biochar is a carbon-rich solid produced by the pyrolysis of organic biomass under low-oxygen conditions. Its well-documented capacity to improve soil physical and chemical properties, enhance soil biological activity, reduce contaminant availability, and sequester carbon has generated substantial interest in its use as a sustainable soil amendment (Kookana et al., 2011; Sohi et al., 2010; Lehmann et al., 2021). The physicochemical characteristics of biochar - including pH, specific surface area (SSA), cation exchange capacity (CEC), and pore structure - vary considerably with feedstock type and pyrolysis temperature, which in turn

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determines the range and magnitude of its effects on soil properties and plant productivity (Tomczyk et al., 2020; Jeffery et al., 2011).

Phosphorus (P) is an essential macronutrient for plant growth, yet only a small fraction of total soil P exists in plant-available form. The majority is fixed as insoluble compounds through reactions with iron, aluminium, or calcium depending on soil pH, rendering it inaccessible to plant roots (Jahan et al., 2025). Phosphate rock, the primary source of P fertilisers, is a finite and non-renewable resource, and current extraction rates are estimated to deplete economically viable reserves within 300 - 400 years (Glaser & Lehr, 2019; Jahan et al., 2025). Simultaneously, the over-application of synthetic P fertilisers contributes to eutrophication of water bodies and environmental degradation. Biochar has been proposed as a strategy to enhance plant-available P by modifying soil pH toward a more favourable range (6-7), increasing CEC and SSA, and supplying P directly from the ash fraction of P-rich feedstocks (Glaser & Lehr, 2019). A meta-analysis of 86 studies reported a mean increase in soil available P of 65% and plant P uptake of 55% following biochar amendment, with the greatest effects observed in acidic, fine-textured, and P-poor soils (Tesfaye et al., 2021).

Arbuscular mycorrhizal fungi (AMF) form obligate mutualistic symbioses with the roots of approximately 80% of terrestrial plant species, providing mineral nutrients - particularly phosphorus - to the host plant in exchange for photosynthetically derived carbohydrates (Smith & Read, 2008). AMF colonisation extends the effective absorptive surface area of plant roots far beyond the P-depletion zone through an extensive extraradical hyphal network, and AMF hyphae are capable of accessing P from soil micropores and biochar surfaces inaccessible to plant roots (Hammer et al., 2014). AMF also secrete phosphatase enzymes that mineralise organic P, and arbuscular structures facilitate the direct translocation of inorganic P into root cells (Li & Cai, 2021). These functional attributes make AMF a biologically sustainable complement to conventional P fertilisation.

The co-application of biochar and AMF has attracted increasing research attention as a combined strategy to promote soil health and crop productivity without reliance on synthetic inputs. Biochar’s porous architecture provides protected microhabitats for AMF hyphae and propagules, while its alkaline pH and improved moisture retention create conditions conducive to fungal colonisation (Lehmann et al., 2011; Warnock et al., 2010). Studies on rice and cereal crops have demonstrated that the combined treatment significantly outperforms either amendment applied alone in terms of plant biomass, nutrient uptake, and root colonisation rates (Mulyadi & Jiang, 2023; Delroy et al., 2025). Recent evidence further indicates that the biochar - AMF combination stabilises soil carbon through a fungal-mediated sequestration pathway, enhancing both soil fertility and climate change mitigation potential (Mason et al., 2025). However, the interactive effects of biochar rate and AMF inoculation remain poorly characterised for legume crops and organic soils, where native P availability and microbial community composition may produce distinct outcomes from those reported for cereals.

While individual effects of biochar and AMF on soil properties and plant growth have been widely studied, comparatively little research has examined their combined effects in organic soils typical of urban or peri-urban farming contexts. The present study was therefore designed to evaluate the effects of two biochar application rates (2% and 10% w/w) and AMF inoculation, applied individually and in combination, on (i) soil physicochemical properties including available phosphorus, (ii) plant productivity parameters, and (iii) AMF root colonisation in *Pisum sativum* (dwarf snap pea) grown in organic soil under pot conditions. This research contributes to the growing evidence base for sustainable, low-input soil management strategies suitable for small-scale and organic agricultural systems.

2. Materials and Methods

2.1 Site Description and Experimental Design

A pot experiment was conducted between June and July 2024 at a fenced outdoor area adjacent to the Hall of Sciences building at Drew University, Madison, New Jersey, USA (Figure 1).

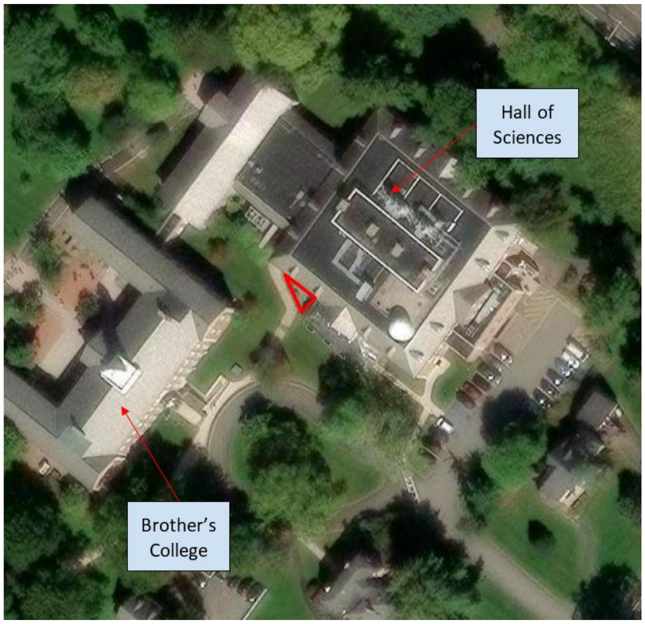

Figure 1. ArcGIS Satellite Map of the experimental location at Drew University, Madison, NJ. The red triangle located directly next to the Hall of Sciences indicates the location of the testing site. Brother's College and the Hall of Sciences are both labeled as notable landmarks.

Treatments were arranged in a randomized complete block design (RCBD) with five replications per treatment. Six treatments were evaluated: T0 = control (no amendment); T1 = AMF inoculant only; T2 = 2% (w/w) biochar; T3 = 2% biochar + AMF; T4 = 10% biochar; T5 = 10% biochar + AMF. All pots received water every other day except on days of rainfall.

2.2 Biochar and Arbuscular Mycorrhizal Fungi (AMF) Treatments

Wakefield Premium Organic BioChar (wood-derived) was used for all biochar treatments and was obtained commercially. Selected physicochemical properties of the biochar, determined by ASTM (2007) proximate analysis for wood charcoals (Drew University, NJ), BET surface analysis (Particle Technology Labs, IL) and CHNOS elemental analysis (GalBraith, TN) are presented in Table 1. AMF inoculant was obtained commercially and applied at the manufacturer's recommended rate.

Table 1. Selected physicochemical properties of the biochar used as treatments for the pot experiments.

Parameters	Mean Values
Moisture (%)	30.29 ± 0.85
Volatile Matter (%)	27.82 ± 0.47
Ash (%)	40.37 ± 0.91
Fixed Carbon (%)	31.81 ± 0.50
pH	8.8 ± 0.04

SSA[§] (m^2/g)	355.93
TPV[¶] (cm^3/g)	0.05
Average pore size (nm)	5.95
Phosphorus (%)	0.17 ± 0.006
Carbon (%)	31.2 ± 1.81
H/C[‡]	0.13
O/C[#]	1.22

[§]SSA = Specific surface area
[¶]TPV = Total pore volume
(SSA and TPV is done my BET Surface Analyzer)
[‡]H/C = Ratio of hydrogen and carbon
[#]O/C = Ratio of oxygen and carbon
(Elemental analysis is done by a CHNOS analyzer)
Numbers are mean ± standard error

2.3 Soil Collection and Preparation

Soil was collected from Grow It Green Urban Organic Farm, Morristown, New Jersey, USA (Figure 2), from a section where no crops were actively grown and no synthetic fertilizers or pesticides had been applied.

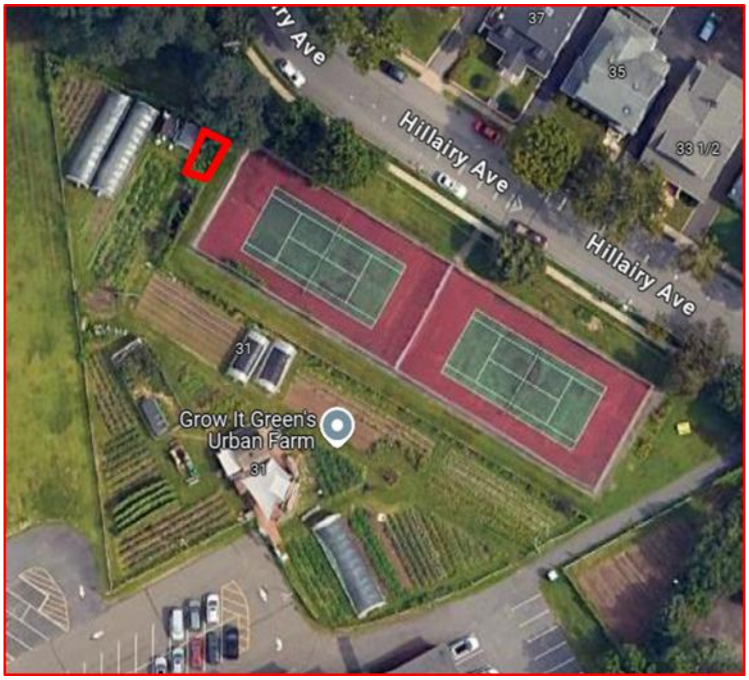

Figure 2. Site of Soil Collection. The site where soil was collected at Grow It Green Urban Organic Farm in Morristown, NJ. Indicated by a bold red shape in the upper left corner.

Selected background properties of the collected soil are presented in Table 2. Soil was lightly packed into 2-gallon nursery pots (7.6 L). Soil was homogenized and amended with biochar at either 2% or 10% (w/w) prior to packing and AMF applied after. All pots except T0 received a dairy manure-based compost as a starter fertilizer, applied at the manufacturer's recommended rate at the time of planting.

Table 2. Characteristics of Soil used in this study

Parameters	Mean Values
pH[†]	7.2 ± 0.04
OM (%)[§]	17.34 ± 0.55
Moisture (%)[‡]	25.05 ± 1.80
Sand (%)[¶]	65.42
Silt (%)[¶]	14.17
Clay (%)[¶]	20.42
Texture[¤]	Sandy Clay Loam

[†] pH is measured in 1:2 soil and deionized water slurry (w/v)
[§] OM = Organic matter content was done by loss in ignition method
[‡] Moisture content is measured by gravimetric method
[¶] Particle size distribution (sand, silt and clay) was done by hydrometer method
Texture[¤] is determined using the USDA Soil textural triangle
Numbers are mean ± standard error

2.4 Pot Plant Experiment and Parameter Analysis

Dwarf Snap Pea (*Pisum sativum* L.) was used as the test plant. Three seeds per pot were sown approximately 2.5 cm deep in June 2024; seedlings were thinned to one per pot after emergence. Soil and plant parameters were measured at the termination of the experiment.

2.4.1 Soil Parameters

Gravimetric soil moisture content was determined by drying soil samples at 105°C for 24 hrs (Gardner, 1986). Soil pH was measured in a 1:2 (w/v) soil-to-deionized water suspension using a Denver Instruments glass electrode pH meter (McLean, 1982). Soil organic matter (SOM) was determined by loss on ignition (LOI) at 550°C for 5 hrs in a muffle furnace, calculated as the difference in dry weight before and after combustion (Heiri et al., 2001). Available soil phosphorus was extracted with 0.5 M sodium bicarbonate (pH 8.5) and measured colorimetrically (Olsen et al., 1954).

2.4.2 Plant Parameters

Plant parameters were measured following methods adapted from Gravel et al. (2013) and Liu et al. (2019). After uprooting, shoot height and root length were measured. Roots were thoroughly washed to remove soil. All plant material was oven-dried at 70°C for 72 hrs to estimate shoot and root dry biomass. Total phosphorus in shoots and roots was determined colorimetrically by the molybdate-blue method (Murphy & Riley, 1962) following nitric acid–perchloric acid wet digestion.

2.5 Estimation of Arbuscular Mycorrhizal Fungi (AMF) Root Colonization

AMF root colonization was assessed using a modified method (McGonigle et al., 1990). Fine root segments (n = 25 per plant) were cleared in 10% KOH at 70°C for 2 hrs, rinsed with deionized water, and stained with 0.5% Trypan blue in lactoglycerol at 70°C for 30 min. Stained roots were examined under a compound microscope and scored for colonization based on the presence of hyphae, vesicles, or arbuscules. The percentage AMF colonization was calculated as:

$$\text{AMF Root Colonization (\%)} = \frac{\text{Number of Colonized Roots}}{25} \text{x } 100\%$$

2.6 Statistical Analysis

All data are presented as means with standard errors. One-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics version 28 to assess treatment effects on soil properties, plant productivity, and AMF colonization. Mean separation was carried out using Tukey–Kramer post hoc tests. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

An overview of statistically significant treatment effects is provided in Table 3. Soil moisture content ($p = 0.01$), soil pH ($p < 0.001$), available soil phosphorus ($p = 0.01$), and AMF root colonization ($p < 0.001$) differed significantly among treatments. Plant growth parameters (shoot height, root length, shoot mass, root mass) and soil organic matter content did not differ significantly at $p < 0.05$.

Table 3. Parameters showing statistically significant differences among treatments ($p < 0.05$).

Parameter	p-value	Key findings
Soil moisture content	0.01	Highest moisture in T4 (10% biochar, 37 ± 1.94%); significantly higher than T0, T2, and T3
Soil pH	<0.001	Lowest pH in T1 (AMF, 6.61 ± 0.05); significantly different from T0, T3, T4, and T5
Available soil phosphorus (P)	0.01	Highest P in T5 (10% biochar + AMF, 43.27 ± 2.14 ppm); significantly higher than all other treatments
AMF root colonization	<0.001	All treatments significantly higher than T0; T5 showed greatest colonization (70 ± 3.8%)

T0 = Control; T1 = AMF; T2 = 2% biochar; T3 = 2% biochar + AMF; T4 = 10% biochar; T5 = 10% biochar + AMF. AMF = Arbuscular Mycorrhizal Fungi.

3.1 Effects of Treatments on Soil Parameters

3.1.1 Soil Moisture Content

The treatments significantly influenced the moisture content in soil ($p = 0.01$; Figure 3).

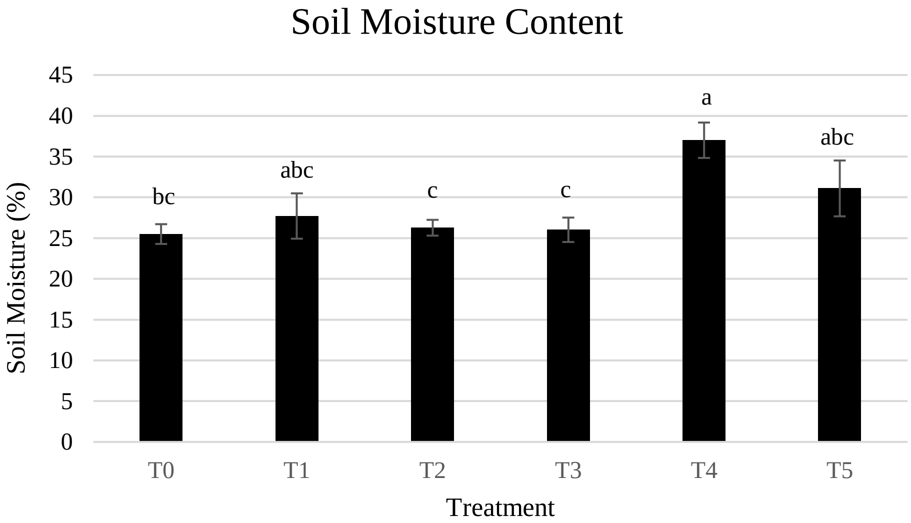

Figure 3. Effects of different treatments on soil moisture content, as represented by means with standard error. T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$.

Figure 3 shows that T4 (10% biochar) had the highest moisture content (37 ± 1.94 %), which was significantly greater than T0 (Control), T2 (2% biochar), and T3 (2% biochar + AMF). T5 (10% biochar + AMF) did not differ significantly from any other treatment. Treatments receiving 2% biochar or AMF alone showed intermediate moisture values that were not significantly different from T0.

The elevated moisture content under T4 is consistent with the known capacity of biochar to reduce bulk density and increase soil porosity, thereby enhancing water retention (Seyedsadr et al., 2022). Biochar pore structure, in particular total pore volume and average pore size, directly governs water-holding capacity by creating additional storage space for soil water (Tomczyk et al., 2020). The comparatively lower moisture content in T5 relative to T4, despite receiving the same biochar rate, may reflect AMF-mediated water uptake. AMF hyphae actively draw soil water for hyphal transport and host-plant delivery (Pauwels et al., 2023), which could reduce residual soil moisture even when water retention capacity is high. This interaction warrants further investigation under field-scale conditions.

3.1.2 Soil pH

Treatment effects on soil pH were highly significant ($p < 0.001$; Figure 4).

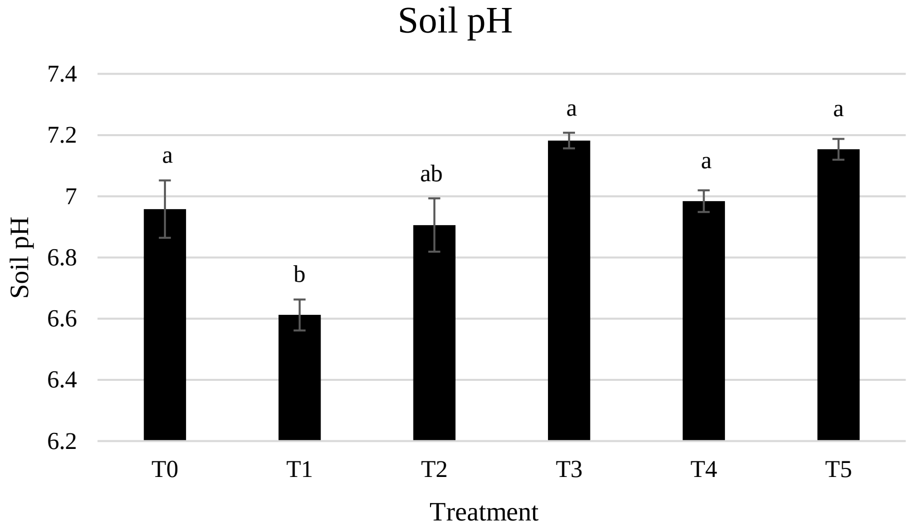

Figure 4. Effects of treatments on Soil pH, represented as means with standard error. T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$.

T1 (AMF) had the lowest pH (6.61 ± 0.05), which was significantly lower than T0, T3, T4, and T5. T3 (2% biochar + AMF) recorded the highest mean pH (7.18 ± 0.03), though it did not differ significantly from the biochar-only treatments. All treatment pH values fell within the range considered agronomically suitable for most crops (6.0 - 7.5; Brady & Weil, 2017).

The acidification observed in T1 is consistent with the release of organic acids by AMF during phosphate solubilisation. AMF produce and exude low molecular weight organic acids to convert insoluble phosphorus into plant-available orthophosphate forms, a process that concurrently lowers rhizosphere pH (Liu et al., 2020). In T1, the absence of biochar - which typically carries an alkaline pH due to the concentration of base cations in ash during pyrolysis (Tomczyk et al., 2020) - meant that no buffering capacity was available to offset this acidification. The comparatively higher pH in biochar-amended treatments (T2 - T5) likely reflects the inherently alkaline nature of most biochars, a pattern previously documented across a range of feedstocks and pyrolysis temperatures (Gaffar et al., 2021). The pH depression in T1 also has implications for available phosphorus; at pH values below 6.5, phosphorus fixation by iron and aluminium increases, reducing plant availability (Preston, 2019; Cerozi & Fitzsimmons, 2016), which may partially explain why T1 did not show the highest soil phosphorus despite supporting AMF activity.

3.1.3 Soil Organic Matter

No significant differences in soil organic matter (SOM) were detected among treatments (Figure 5).

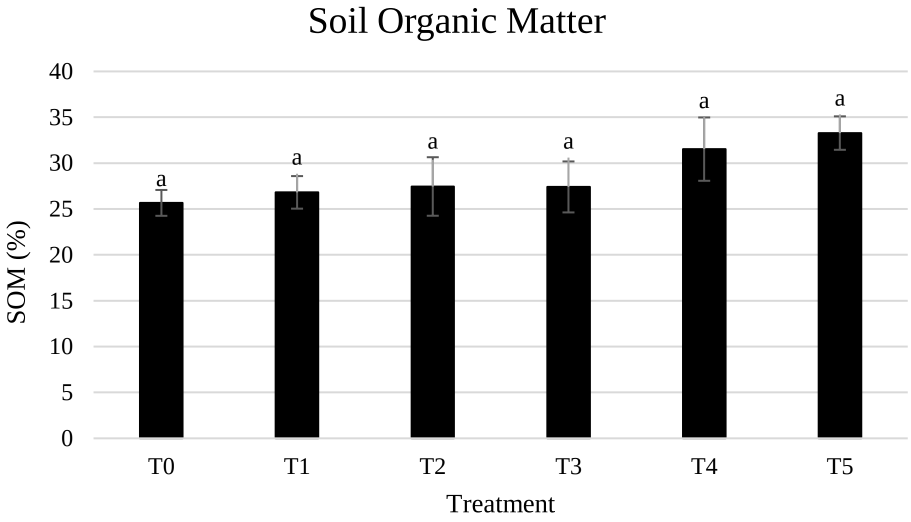

Figure 5. Effects of treatments on Soil Organic Matter, represented as means with standard errors. Treatments are labeled as follows: T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at p<0.05.

Numerically, T4 (31.53 ± 3.45%) and T5 (33.28 ± 1.82%) showed the highest SOM values, indicating that biochar addition preserves or augments SOM through physical protection of native organic carbon within biochar pores (Ernest et al., 2024). Longer-term studies would be required to detect statistically significant changes in SOM under these treatment regimes.

3.1.4 Available Soil Phosphorus

Available soil phosphorus differed significantly among treatments (p = 0.01; Figure 6).

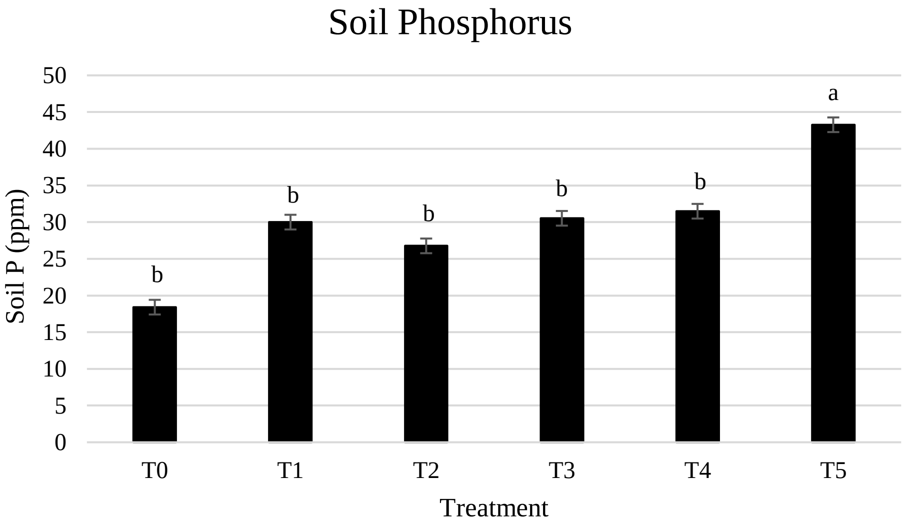

Figure 6. Effects of Treatments on Available Soil Phosphorus. Data presented as means with standard errors. Treatments are labeled as follows: T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at p<0.05.

T5 (10% biochar + AMF) yielded the highest available phosphorus (43.27 ± 2.14 ppm), which was significantly greater than all other treatments including T0 (lowest). T1, T2, T3, and T4 did not differ significantly from each other or from T0.

The significantly elevated phosphorus under T5 suggests a synergistic interaction between high-rate biochar and AMF inoculation. Biochar enhances phosphorus availability through multiple mechanisms - it raises soil pH to a range favourable for phosphorus solubilisation, provides sorption sites that reduce phosphorus fixation, and supplies phosphorus directly from the ash fraction of certain feedstocks (Glaser & Lehr, 2019; Li & Cai, 2021). AMF further increases phosphorus acquisition by extending hyphal networks beyond the root depletion zone and actively solubilising inorganic phosphorus (Li & Cai, 2021). The fact that T3 (2% biochar + AMF) did not yield significantly higher phosphorus than T0 suggests that a threshold biochar application rate is necessary for the synergistic effect to manifest, underscoring the dose-dependent nature of biochar-AMF interactions. This finding corroborates Mulyadi and Jiang (2023), who reported that co-application of biochar and AMF produced significantly greater soil phosphorus availability than either amendment applied alone, particularly at higher biochar concentrations.

3.2 Effects of Treatments on Plant Productivity

3.2.1 Shoot Height and Root Length

Neither shoot height nor root length differed significantly among treatments (Figure 7; p > 0.05).

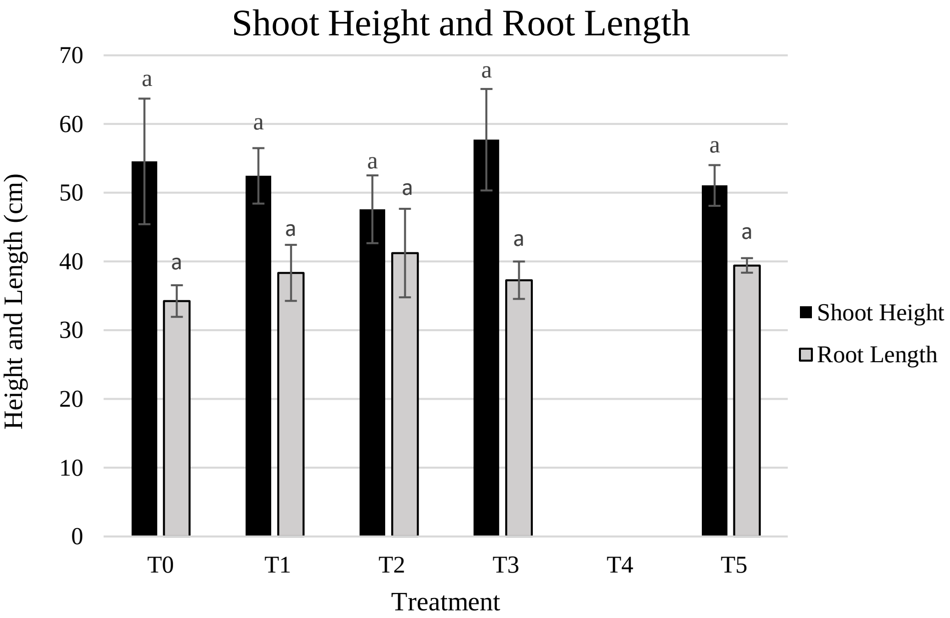

Figure 7. Effects of Treatments on Shoot Height and Root Length. Data represented by means and standard error. Treatments are labeled as follows: T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at p<0.05. No data shown for T4 as plants could not be recovered.

The absence of significant treatment effects on these parameters is consistent with findings from comparable pot and field studies by Jones et al. (2012) who found no significant biochar effect on plant growth parameters in a three-year field trial. Numerically, T3 (2% biochar + AMF) produced the tallest shoots (57.72 ± 7.38cm) and T2 (2% biochar) the longest roots (41.23 ± 4.9cm). Plants in T5 (10% biochar + AMF) had a shorter average shoot height (51.07 ± 2.96cm) than T3, suggesting that high biochar concentrations may not translate to improved above-ground growth, even when combined with AMF. This may reflect the high volatile matter content or elevated pH of some biochars, which can suppress nitrogen availability and impair early plant growth (Deenik et al., 2010). The 40-day growing period, shorter than the recommended 65-day maturation for the cultivar used, limits interpretation of these data and likely contributed to the absence of statistically detectable treatment effects.

3.2.2 Plant Shoot and Root Mass

As shown in Table 4, shoot and root mass did not differ significantly among treatments. These results are consistent with previous reports showing that biochar amendments do not always increase plant biomass under pot conditions, particularly over short growing periods (Jones et al., 2012).

Table 4. Effects of Treatments on Plant Shoot and Root Mass.

Treatment	Shoot Mass (g)	Root Mass (g)
T0	1.76 ± 0.28^a	0.20 ± 0.07^a
T1	1.83 ± 0.60^a	0.3 ± 0.06^a
T2	1.70 ± 0.44^a	0.40 ± 0.11^a
T3	1.55 ± 0.39^a	0.26 ± 0.09^a
T4	-	-
T5	1.29 ± 0.21^a	0.56 ± 0.17^a

Values represented as means with standard error. T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$. No data shown for T4 as plants could not be recovered.

Numerically, T1 (AMF) produced the greatest shoot mass (1.83 ± 0.60g), while T5 (10% biochar + AMF) had the lowest (1.29 ± 0.21g), suggesting that high biochar rates may have a minor suppressive effect on above-ground biomass. Conversely, root mass was highest in T5 (0.56 ± 0.17g), indicating a possible shift in biomass allocation toward root growth at high biochar rates. Such a shoot-to-root ratio shift can reflect enhanced nutrient foraging under conditions where above-ground growth is constrained (Lopez et al., 2023). Overall, additional replication and a full growing season are needed to draw firm conclusions about treatment effects on plant biomass.

3.2.3 Shoot and Root Phosphorus

No significant differences in root or shoot phosphorus content were detected among treatments (Table 5).

Table 5. Effects of Treatments on Root and Shoot Phosphorus.

Treatment	Shoot P (%)	Root P (%)
T0	0.02 ± 0.01^a	0.38 ± 0.03^a
T1	0.05 ± 0.01^a	0.19 ± 0.03^a
T2	0.08 ± 0.01^a	0.16 ± 0.04^a
T3	0.06 ± 0.01^a	0.14 ± 0.03^a
T4	-	-
T5	0.07 ± 0.02^a	0.08 ± 0.04^a

Values represented as means with standard error. Treatments are labeled as follows: T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$. No data shown for T4 as plants could not be recovered.

This result must be interpreted in the context of the experimental timeline - because plants did not reach a uniform developmental stage, differential nutrient partitioning among organs may have introduced variability that obscured treatment effects (Bender et al., 2015). Numerically, T2 (2% biochar) had the highest shoot phosphorus (0.08%), while T0 had the lowest (0.02%). Root phosphorus was highest in T0 (0.38 ± 0.03%) and declined progressively across treatments, with T5 showing the lowest root P (0.08 ± 0.04%). The elevated root phosphorus in T0 may reflect phosphorus accumulation in root tissue in the absence of efficient shoot-directed transport, which is facilitated by AMF colonization. In AMF-treated plants, phosphorus is preferentially translocated via fungal hyphae to the shoot, potentially explaining the trend of lower root P and higher shoot P observed in AMF-amended treatments. This interpretation aligns with the role of AMF in optimising phosphorus use efficiency through hyphal translocation rather than root tissue accumulation (Li & Cai, 2021).

3.3 Estimation of Root Colonization by Arbuscular Mycorrhizal Fungi (AMF)

The effects of the five treatments on AMF colonization within the roots were determined and are discussed below.

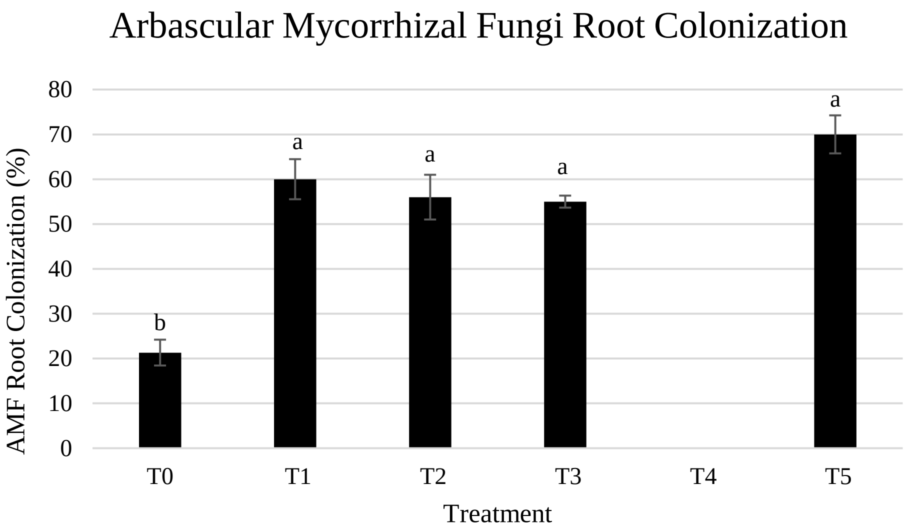

Figure 8. Effects of Treatments on AMF Colonization in Fine Roots, represented as means with standard error. Treatments are labeled as follows: T0 = Control, T1= AMF, T2 = 2% biochar, T3 = AMF and 2% biochar, T4 = 10% biochar, T5 = AMF and 10% biochar. Similar letters indicate not significantly different at p<0.05. No data shown for T4 as plants could not be recovered.

AMF root colonization differed highly significantly among treatments ($p < 0.001$; Figure 8). All four treatments that received AMF inoculant (T1, T3, and T5) and the unamended control (T0) followed a clear gradient. T5 (10% biochar + AMF) showed the highest colonization (70 ± 3.8%), which was significantly greater than T0 (lowest colonization). T0 showed the lowest colonization, while T1, T3, and T5 all differed significantly from T0 but not from each other.

The progressive increase in root colonization with increasing biochar rate (T1 < T3 < T5) is consistent with the facilitating role of biochar pore structure in providing protected habitat for AMF hyphae and propagules, as well as in improving nutrient availability that stimulates fungal proliferation (Gaffar et al., 2021). Ji et al. (2023) similarly reported that combined biochar and AMF application significantly increased root colonization compared to AMF alone, particularly at higher biochar concentrations, supporting the dose-dependent pattern observed here. The non-significant residual colonization in T0 and T2 (biochar only, no AMF inoculant) likely represents indigenous AMF populations present in the organic farm soil, whose activity was modestly enhanced by biochar-mediated improvements in soil structure and nutrient availability. AMF hyphae are capable of accessing phosphorus from biochar pores inaccessible to plant roots (Hammer et al., 2014), and their enhanced colonization in T5 likely underpins the significantly greater available soil phosphorus recorded for that treatment (Table 3). The practical implication is that AMF inoculation combined with a biochar application rate of 10% maximises colonization and thereby soil phosphorus availability for plant uptake.

4. Conclusion

This pot study evaluated the individual and combined effects of biochar (2% and 10% w/w) and AMF inoculation on soil properties, plant productivity, and root colonisation in *Pisum sativum*. Soil moisture, pH, available phosphorus, and AMF root colonisation differed significantly among treatments, while plant biomass and tissue phosphorus did not. The most notable outcome was a synergistic increase in plant-available phosphorus under co-application of 10% biochar and AMF (T5; 43.27 ± 2.14 ppm), significantly exceeding all other treatments including AMF or biochar applied alone. This effect was absent at the 2% biochar rate, indicating a dose-dependent threshold for the biochar–AMF interaction. AMF root colonisation increased progressively with biochar rate, peaking at 70 ± 3.8% in T5, consistent with biochar pores providing protected habitat for fungal hyphae and the enhanced colonisation facilitating greater P acquisition.

The absence of significant plant growth responses is attributed primarily to the abbreviated experimental duration (40 days versus the 65-day recommended maturation period), which precluded full expression of treatment effects. Future studies should extend the growing period, incorporate field-scale trials, and evaluate multiple biochar feedstocks and pyrolysis temperatures to identify the properties most critical for promoting AMF activity and phosphorus availability. Overall, the results support the co-application of wood-derived biochar at 10% with AMF inoculation as a promising low-input strategy for enhancing soil phosphorus availability in organic agricultural systems.

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