J. Agric. Biol. 03(2) 2025. 71-81

Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

Research Article

Biochar and Phosphate-Solubilizing Bacteria Enhance Maize Growth and Soil Fertility

Muhammad Yasir[1], Muhammad Baqir Hussain[1*], Suleman Haider Shah[1,4], Ghulam Murtaza[1], Wazir Ahmed[1], Shahid Iqbal[2], Sajid Hussain[3]

[1] Department of Soil and Environmental Sciences, MNS University of Agriculture, Multan, Pakistan.
[2] Institute of Plant Breeding and Biotechnology, MNS University of Agriculture, Multan, Pakistan.
[3] Soil and Water Testing Laboratory, Fatima Fertilizer Company, Multan, Pakistan.
[4] Department of Agriculture (Extension and Adaptive Research), Government of Punjab, Murree, Pakistan.

ABSTRACT

Efficient phosphorus (P) management is essential for sustainable maize (*Zea mays* L.) production, particularly in phosphorus-deficient calcareous soils where P fixation limits plant growth. In current study, the effects of integrated application of phosphate-solubilizing bacteria (PSB) and biochar (BC) with two P fertilizers; Diammonium phosphate (DAP) and Nitrophos (NP) in eight treatments with three replications were evaluated on maize growth in a lathhouse pot experiment under a completely randomized design (CRD) arrangement. The integrated application of BC and PSB with DAP significantly enhanced maize growth and soil fertility compared to NP and control treatments. BC+PSB+DAP increased shoot fresh weight by 81%, root dry weight by 130%, and plant height by 55%. Physiological traits improved significantly, with photosynthetic rate, stomatal conductance, and water use efficiency increasing by 48%, 60%, and 33%, respectively. Soil organic matter increased by 36%, nitrogen by 40%, and phosphorus availability by 48%. These results demonstrate the potential of BC and PSB integration, particularly with DAP, to enhance phosphorus use efficiency, reduce reliance on synthetic fertilizers, and improve maize productivity in calcareous soils.

Keywords: Biochar, diammonium phosphate, Maize productivity, phosphate-solubilizing bacteria, phosphorus use efficiency, soil fertility, sustainable agriculture.

Correspondence
Muhammad Baqir Hussain
baqir.hussain@mnsuam.edu.pk

Article History
Received: June 22, 2025
Accepted: September 12, 2025
Published: October 03, 2025

INTRODUCTION

Maize (*Zea mays* L.) has a prime economic important use. It serves both as a cereal and as a fodder. Maize (*Zea mays* L.) is the 3rd most important cereal crop in Pakistan after wheat and rice, serving as both a staple food and fodder. It contributes 0.7% to the Gross Domestic Product (GDP) and 3.2% to agriculture value addition. During 2022-2023, maize cultivation area was 1.418 million hectare and production 10.635 million tons in Pakistan (Pakistan Economic Survey 2022-23, 2023). Despite increased output, the yield was less than anticipated because of ineffective nutritional management and a deficiency in essential nutrients (Baligar et al., 2001). In order to ensure that plants have access to the desired nutrients, it is important to maximize soil nutritional status and availability. Maize (*Zea mays* L.) is a monoecious annual plant of the maideas or maiden-grass family, and it has 2n chromosomes in its cells. There are more countries growing maize than any other crop in the world, after wheat and rice. Apart from Antarctica, virtually all parts of the world cultivate it. In order to thrive, it requires specific water and climatic conditions. A temperature range of 15 to 20 °C is essential for the plant to germinate

https://doi.org/10.55627/agribiol.003.02.1408

(Adom et al., 2022). Additionally, maize is used as a food and raw material for industrial purposes. A greater proportion of grain in industrialized countries is used as livestock feed and as industrial raw material. The majority of maize grown in developing countries is used for human consumption, although it is increasingly being used for animal feed as well. PSBs enhance phosphorus solubilization by producing organic acids such as gluconic, citric, and oxalic acids, which mobilize otherwise insoluble phosphates (Khan et al., 2009). PSBs improve crop growth in maize crops by increasing height of shoot, dry biomass of root and shoot, grain yield and overall Phosphorus intake. PSB release organic acid such as (*pseudomonas prosekii* produce 2-3 dimethyl fumaric acid, *pseudomonas fluorescens* produce gluconic acid-format acid, propane di- acids, *Erwinia rhapontici*, *Bacillus subtilis, Pseudomonas chlororaphis* acetic acid-propionic acid- 2-keto-gluconic acid- gluconic acid). *Bacillus* strains release acetic acid, citric acid and oxalic acids. Because PSB and fertilizers combination with high return products makes it more cost effective. These findings show that integrating PSB into fertilizers would be a suitable addition for the application of chemical phosphate fertilizer in sustainable agriculture systems. These PSB have potential to increase maize crop growth by 15%. They improve root development after amending the soil with various fertilizers such as DAP, NP and Urea. When employing local PSB in conjunction with chemical fertilizers the dose required is lowered by 20-35% (Sundara et al*.,* 2002). Phosphorus is a critical macronutrient for plant development, influencing three primary functions: seed germination, energy transfer through ATP, and root development. Its availability supports vigorous early seedling growth, enhances photosynthesis and metabolic activity, and promotes stronger root systems that improve nutrient and water uptake (Zhu and Smith, 2001; Rychter and Rao, 2005; Malhotra et al., 2018). In phosphorus-deficient soils, maize exhibits stunted growth, reduced biomass, and lower productivity, underscoring the need for efficient P management strategies.

However, compost is a decay product of plants, produced by bacteria as part of the breakdown process (Keener et al., 2000) is a rich source of organic matter for improving physio-chemical characteristics of soil and the soil health status. It helps to aerate the soil, improve soil texture and helps the soil to store more water, and serves a good source of nutrients like nitrogen, phosphorus and potassium (Bertran et al., 2004).

Biochar and PSB can work together to boost nutrient availability. Both are capable of providing phosphorus to the soil. Maize plant height and nutritional contents are positively affected by PSB inoculation, biochar incorporation, and combination. Crop yields have benefited from biochar production and soil amendment with biochar (Hussain et al., 2017; Ali et al., 2023).

The effects of PSB and BC have been widely reported independently on plant P however, few studies have reported their interactive effect on plant growth and P alkaline calcareous soils, where P deficiency is a common problem. Biochar contains carbon in two forms, recalcitrant and labile, and is composed of mineral nutrients that make it a beneficial resource for enhancing soil microbial activities. Biochar addition to soil may improve microbial-linked soil physicochemical and biological properties (Siddiqui et al*.,* 2016). Biochar serves as a P activator by providing a favorable habitat in the soil for solubilizing organic and inorganic forms of P. Microbes such as bacteria living in the porous structure of biochar remain safe from predators like protozoa. This results in the more efficient transformation of nutrients. Applying biochar with P-fertilizer increases microbial biomass carbon (Mahmoud et al., 2019). An increase in soil P availability is greater with the combined use of biochar and PSB than with biochar and PSB alone (Rafique et al., 2017). Previously, Wei et al. (2016) showed that combining biochar and PSB during composting improves the inorganic P content, known as plant accessibility, in the final composts. Rafique et al., (2017) observed a beneficial impact of sawdust biochar and PSB on maize plant height and nutrient absorption in another investigation. Soil biochar application may boost microbial biomass because it consists of labile C components, un pyrolyzed feedstock, better soil nutrient availability (N, P, Ca, and K), toxic chemical adsorption, and improved soil water quality (Lehmann et al., 2011).

Biochar is a carbonaceous solid product obtained from the thermochemical pyrolysis of organic material. It is a stable form of carbon and does not decompose easily when applied to soil. In the process, biomass is thermochemically converted into biochar without oxygen (Cha et al*.,* 2016). The finished product has enough carbon to boost soil health. Biochar is another good organic matter source that has been demonstrated to promote soil health and structure, retain nutrients, reduce CO_2 emissions, increase soil water holding capacity (WHC) and stabilize SOM (Das and Ghosh 2020). Biochar stabilizes the soil by binding the soil particles and minimizing the repulsive forces among them (Yang et al*.,* 2021). The use of biochar increases the health of soil and ensures nutrient availability to plants promoting plant development and yield. Over the last four decades, biochar is used to improve soil quality by aiding in water retention and drainage as well as lowering soil acidity in European soils (Verma et al., 2012). Biochar is a natural fertilizer that improves soil nutrients while preventing evaporation. Biochar aids in enhancing the health of soil by preserving soluble nutrients (Ali et al., 2023).

While the independent benefits of biochar and PSB are well documented, their combined impact with chemical fertilizers on maize growth and soil fertility under calcareous conditions remains underexplored. This study aimed to (i) evaluate the influence of BC and PSB integration with mineral fertilizers on maize productivity, (ii) assess effects on soil properties and nutrient availability, and (iii) determine phosphorus use efficiency under these treatments.

MATERIALS AND METHODS

Study Site and Experimental Setup

This study was conducted at MNS University of Agriculture, Multan (30.145811° N, 71.443470° E), located in a semi-arid region of Pakistan, to evaluate the effects of compost, biochar, and phosphate-solubilizing bacteria (PSB) on maize growth and nutrient uptake. The experiment was conducted inside a lathhouse, and the soil was collected from the university's research area. The soil was air-dried, ground, sieved (2 mm), and homogenized before use. A completely randomized design (CRD) with three replications was employed, and eight treatments were applied as described below (Ryan et al., 2001). The treatments included different combinations of biochar, PSB, diammonium phosphate (DAP), and nitrophos (NP) at the following rates:

Biochar (7 g/pot equivalent to 2000 kg/ha)
DAP (0.8 g/pot equivalent to 228 kg/ha)
NP (0.3 g/pot equivalent to 86 kg/ha)
PSB (50 ml/kg seed)

A total of 24 pots were used, with each pot containing 7 kg of soil.

Soil and Plant Analysis

Soil samples were collected at the end of the experiment to analyze physio-chemical properties, including organic matter, nitrogen, and phosphorus. Organic matter was quantified using the Walkley-Black method (Walkley and Black, 1934). Nitrogen content was determined using the Kjeldahl method (AOAC Official Method 978.04), where plant samples were digested with sulfuric acid and catalyst mixtures, followed by distillation and titration. Extractable phosphorus was measured using Olsen's method (Olsen et al., 1954) and spectrophotometer readings at 882 nm.

Plant growth traits such as shoot height, chlorophyll content, fresh and dry biomass of shoot and root, and nitrogen (N) and phosphorus (P) content were recorded. Chlorophyll content was measured using the SPAD chlorophyll meter (Konica Minolta, Japan) as per standard procedures (Grzeszczuk, 2020).

Physiological Measurements

Physiological parameters, including photosynthetic rate, transpiration rate, water use efficiency, and stomatal and sub-stomatal conductance, were measured using a CIRAS system (CIRAS-3 Portable Photosynthetic System, PP Systems, Amesbury, MA, USA) under full sunlight conditions. These parameters were recorded following the method described by Liu et al. (2015) and Krzyżak et al. (2023).

Photosynthetic rate was measured under ambient CO_2 conditions (360 ppm). Transpiration rate and stomatal conductance were assessed using a leaf chamber attached to the CIRAS system. Water use efficiency (WUE) was calculated as the ratio of photosynthetic rate to transpiration rate.

Statistical Analysis

Data were statistically analyzed using SPSS software version 20, following a completely randomized design (CRD). The significance of differences among treatments was determined using Analysis of Variance (ANOVA), and treatment means were compared using the Least Significant Difference (LSD) test at a 5% probability level (Steel et al., 1997).

RESULTS

The integrated application of biochar (BC) and phosphate-solubilizing bacteria (PSB) with diammonium phosphate (DAP) or nitrophos (NP) significantly enhanced soil fertility, maize growth, and physiological performance. Soil analysis revealed that BC+PSB+DAP treatment increased organic matter by 36%, nitrogen availability by 40%, and phosphorus availability by 48% compared to the control ($P<0.05$). PSB inoculation alone elevated soil phosphorus content by 35–40%, while BC further amplified these effects by improving microbial activity and nutrient retention. DAP outperformed NP in alkaline soil conditions, with BC+PSB+DAP showing the highest phosphorus solubilization efficiency. Maize growth attributes were markedly improved under BC+PSB+DAP, with 81% and 130% increases in shoot fresh and root dry weights, respectively, alongside a 55% rise in plant height (Table 1). PSB+DAP treatments also enhanced root biomass by 12% compared to NP-based treatments. Chlorophyll content (SPAD) increased by 33–38% under PSB+DAP and BC+DAP, indicating superior nitrogen assimilation. Physiological traits demonstrated pronounced improvements: photosynthetic rate surged by 48%, stomatal conductance by 60%, and water use efficiency (WUE) by 33% under BC+PSB+DAP (Fig. 2). Transpiration rate at 22% in PSB+NP treatments, highlighting microbial-mediated water regulation. Plant nutrient uptake mirrored soil trends, with BC+PSB+DAP elevating nitrogen and phosphorus concentrations in tissues by 32% and 40%, respectively (Fig. 3). PSB inoculation alone boosted phosphorus uptake by 35–40%, while BC integration further stabilized nutrient availability. ANOVA confirmed significant differences ($P<0.05$) among treatments, with BC+PSB+DAP consistently outperforming other combinations.

Table 1. Physical and chemical characteristics of biochar and soil

			Soil	
Parameters	**Units**	**Biochar**	**Pre-sowing**	**Post-harvest**
Organic Matter	%	0.64	0.65	0.85
EC	dS/m	1.91	0.82	1.86
pH	-	7.4	8.45	8.56
Total N	%	0.0545	0.035	2–3
Available P	mg/kg	7.1	8	12
Available K	mg/kg	85	30	45

Soil Properties

Soil Nitrogen

The inoculation of PSB significantly increased soil nitrogen compared to the control (Fig. 1(A)). The highest nitrogen concentration was observed in the PSB-treated soils, showing a 30-40% increase compared to the control, which had the lowest nitrogen levels. Similarly, BC combined with PSB and NP also resulted in a significant increase in nitrogen content. DAP treatment alone also significantly raised soil nitrogen levels, highlighting the positive effect of integrated treatments on nitrogen availability.

Soil Phosphorus

Phosphorus concentrations in soil were significantly affected by PSB inoculation (Fig. 1(B)). The highest phosphorus content was observed under PSB inoculation, with a 48% increase in phosphorus availability compared to the control. In contrast, the control treatment, with no PSB inoculation, showed the lowest phosphorus levels. Additionally, the combination of PSB with DAP exhibited significantly higher phosphorus availability than the control. BC+PSB+DAP also demonstrated improved phosphorus levels, but NP treatment showed the least improvement.

Soil Organic Matter Content

The application of PSB significantly enhanced organic matter content in the soil, as shown in Fig. 1(C). Compared to the control, the PSB-treated soils exhibited a noticeable increase in organic matter content, with BC+PSB+DAP treatment showing the most significant improvement, increasing organic matter by 30% to 36%. Similarly, biochar with NP treatment resulted in a significant increase in organic matter, showing a 30% increase over the control. These results suggest that PSB inoculation and biochar incorporation improve soil quality, potentially by enhancing microbial activity and nutrient cycling.

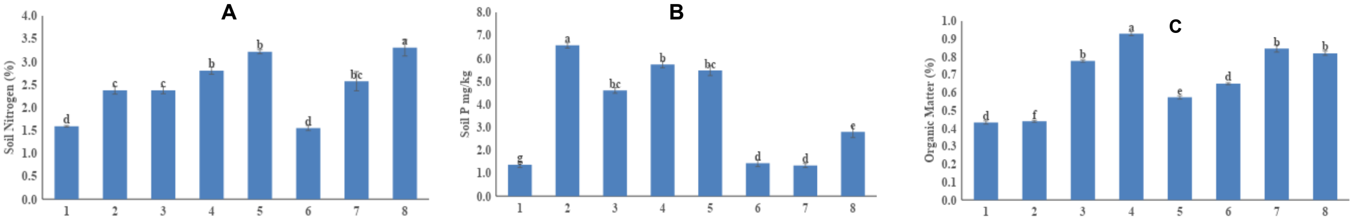

Figure 1. Effect of biochar and phosphate solubilizing bacteria with diammonium phosphate and nitrophos on soil nitrogen (A), soil P (B) and organic matter (C) The bars sharing similar letters are statistically non-significant to each other at $p \leq 0.05$.

Plant Growth and Physiological Responses

Biomass and Plant Height

The combined application of BC and PSB significantly improved plant growth parameters compared to the control. Shoot fresh weight was highest in the BC+PSB+DAP (T7) treatment, reaching 14.10 g, a 65% increase over the control (T1), and 81% higher than the NP treatments (T2) (Table 2). Similarly, root dry weight under BC+PSB+DAP (T7) showed a 130% increase compared to the control, reaching 2.45 g, while root fresh weight was highest under BC+NP (T6) at 4.67 g, which was significantly higher than all other treatments ($p < 0.05$).

Plant height was most significantly improved in PSB+NP (T4), with a 55% increase compared to the control (135.17 cm vs. 120.07 cm), and leaf number was highest in PSB+NP (T4) with 12 leaves per plant, compared to 9 leaves in the control. Plants treated with BC+PSB+DAP (T7) showed the lowest height (102.00 cm), significantly lower than PSB+DAP (T3) and BC+PSB+NP (T8), which had heights of 132.33 cm and 125.83 cm, respectively.

These results suggest that treatments involving PSB and NP significantly enhance root growth and plant height, while biochar with PSB and DAP enhances overall biomass production.

Table 2. Effect of Biochar and Phosphate Solubilizing Bacteria with Diammonium Phosphate and Nitrophos on maize plant growth.

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Plant height (cm)
T_1: DAP	21.83 c	7.17 bc	3.57 b	0.96 b	135.17 c
T_2: NP	30.10 c	12.67 f	4.47 b	2.45 b	120.07 d
T_3: PSB+DAP	17.17 a	6.77 a	3.40 a	0.99 a	132.33 a
T_4 : PSB+NP	17.7 b	3.80 b	3.27 e	0.51 b	138.67 a
T_5 : BC + DAP	14.10 e	4.40 c	2.17 bc	0.38 a	125.83 b
T_6 : BC+NP	23.27 de	5.23 bc	4.67 f	0.93 bc	133.33 bc
T_7: BC+PSB+DAP	14.10 e	4.40 d	2.17 cd	0.95 c	102.00 ab
T_8: BC+PSB+NP	24.07 cd	4.27 d	1.67 b	0.79 d	115.00 h

The treatment means sharing similar letters are not significantly different from each other according to LSD at $P \leq 0.05$

Photosynthetic Rate

The results revealed significant differences in photosynthetic rate between treatments and the control (Fig. 2(a)). PSB inoculation alone significantly increased the photosynthetic rate, with a 27% to 48% increase compared to the control ($p < 0.05$). The BC+PSB+DAP (T7) treatment showed a 27% improvement over the control, while BC with NP (T6) resulted in an 8.7% to 18.7% increase. The enhanced photosynthesis observed with PSB and biochar treatments suggests that the increased microbial activity in the root zone, facilitated by PSB, likely improved nutrient uptake and photosynthetic efficiency.

Transpiration Rate

Significant differences were observed in transpiration rate across treatments (Fig. 2(b)). PSB+NP (T4) exhibited a 22% improvement over the control, while PSB+DAP (T3) resulted in a 14% increase compared to the control. The combined BC+PSB+DAP (T7) treatment showed a 10% improvement over the control. These results suggest that PSB inoculation enhances transpiration efficiency, likely through improved stomatal regulation and microbial activity in the root zone.

Water Use Efficiency (WUE)

Water use efficiency was significantly improved by PSB inoculation (Fig. 2(c)). PSB+NP (T4) treatment showed a 33% increase in WUE compared to the control, while PSB+DAP (T3) showed a 7% increase. BC+PSB+DAP (T7) and BC+PSB+NP (T8) both exhibited similar results, with improvements ranging from 0% to 7% over the control. These results indicate that PSB inoculation enhances water use efficiency, likely through better water regulation in the root zone, which was further supported by biochar's role in improving soil water retention.

Stomatal Conductance

Stomatal conductance was significantly higher in all treatments compared to the control (Fig. 2(d)). The highest increase was observed in BC+NP (T6), which showed a 33% to 60% improvement over the control. Similarly, BC+PSB+DAP (T7) showed a 34% increase compared to the control, indicating that PSB inoculation, combined with biochar, enhances stomatal function and improves water and gas exchange. The results suggest that biochar enhances stomatal conductance, possibly by improving root health and nutrient availability in the soil.

Sub-Stomatal Conductance

The analysis of sub-stomatal conductance showed significant improvements in all treatments compared to the control (Fig. 2(e)). The BC+PSB+DAP (T7) treatment showed a 70% increase, while NP+PSB (T4) resulted in a 43% increase over the control. The increase in sub-stomatal conductance under PSB inoculation suggests a more efficient internal gas exchange, likely facilitated by better root-zone microbial activity and enhanced nutrient availability.

Chlorophyll content (SPAD value)

Chlorophyll content (SPAD value) was significantly influenced by PSB inoculation (Fig. 2(f)). ANOVA revealed that PSB inoculation alone significantly increased chlorophyll content compared to the control. The highest chlorophyll content was observed in PSB+DAP (T3), with an increase of 33% to 38% compared to the control. Similarly, BC+DAP (T5) showed a 27% increase in chlorophyll content over the control, while BC+PSB (T7) also demonstrated an improvement, with a 22% to 25% increase. PSB with NP (T4) resulted in a 18% to 20% increase in chlorophyll compared to the control.

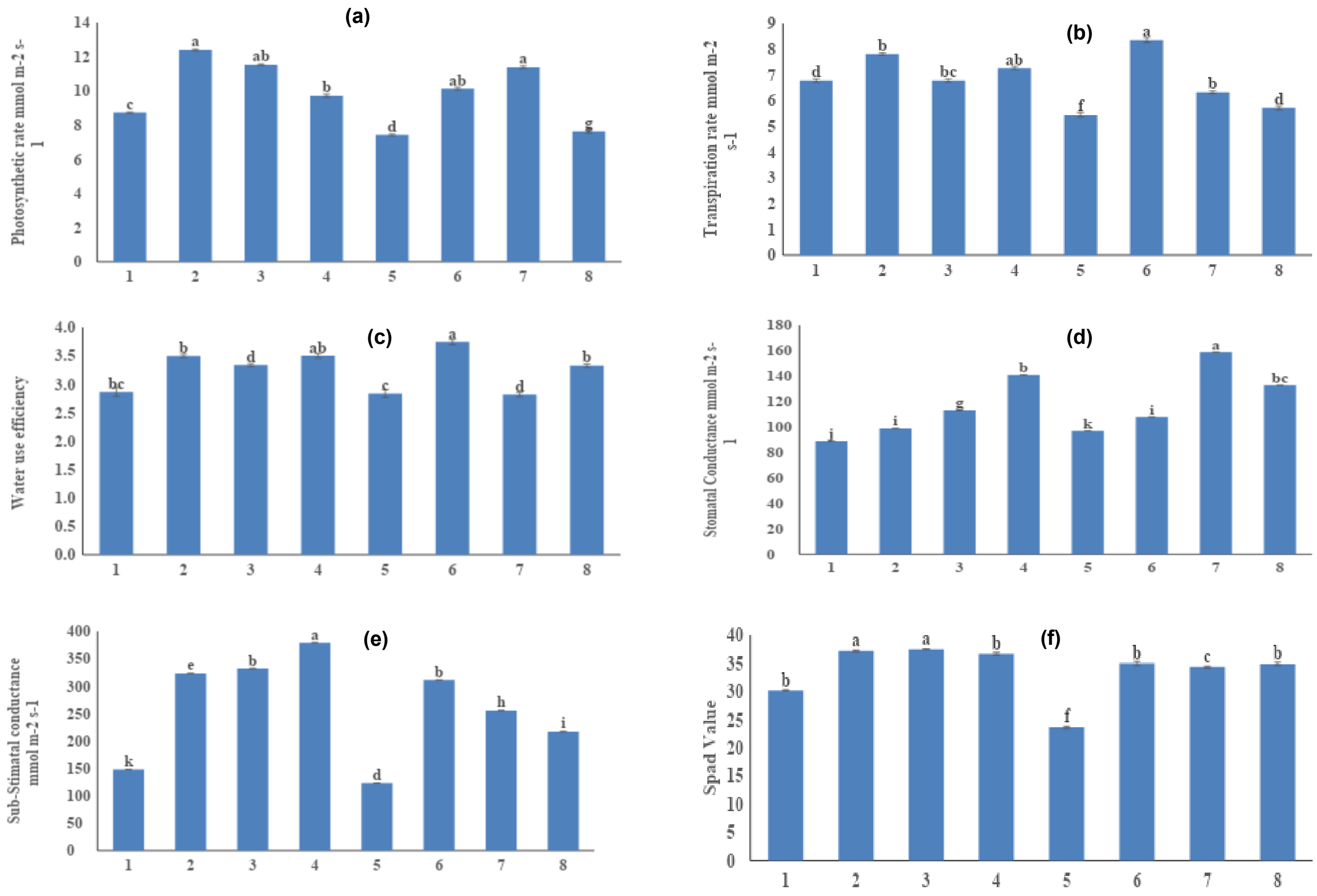

Figure 2. Effect of biochar and phosphate solubilizing bacteria with diammonium phosphate and nitrophos on photosynthetic rate (a), transpiration rate (b), water use efficiency (c), stomatal conductance (d) and sub-stomatal conductance (e). The bars sharing similar letters are statistically non-significant to each other at p ≤ 0.05.

https://doi.org/10.55627/agribiol.003.02.1408

Plant Nutrient Uptake

Nitrogen Content in Plants

Results revealed significant differences in nitrogen concentration in maize plants across treatments (Fig. 3(a)). PSB inoculation significantly increased nitrogen content in maize compared to the control ($p < 0.05$). PSB-treated plants exhibited a 20% to 32% increase in nitrogen concentration, while biochar (BC) treated with DAP (T5) resulted in a 15% to 20% increase compared to the control. BC and PSB treated with NP (T6) also significantly enhanced nitrogen uptake, with a 8.7% to 18.7% increase over the control. These results indicate that PSB inoculation plays a significant role in improving nitrogen uptake in maize plants.

Phosphorus Content in Plants

Significant differences in phosphorus concentration in maize plants were observed across treatments (Fig. 3.(b)). PSB inoculation significantly improved phosphorus content in plants compared to the control, with the highest increase observed in PSB+DAP (T3), showing a 35% to 40% increase. NP inoculated with PSB (T4) resulted in a 25% increase in phosphorus concentration compared to the control ($p < 0.05$). Similar improvements were noted in other treatments, with biochar (BC) enhancing phosphorus uptake when combined with PSB, further highlighting the synergistic effect of biochar and PSB in phosphorus solubilization.

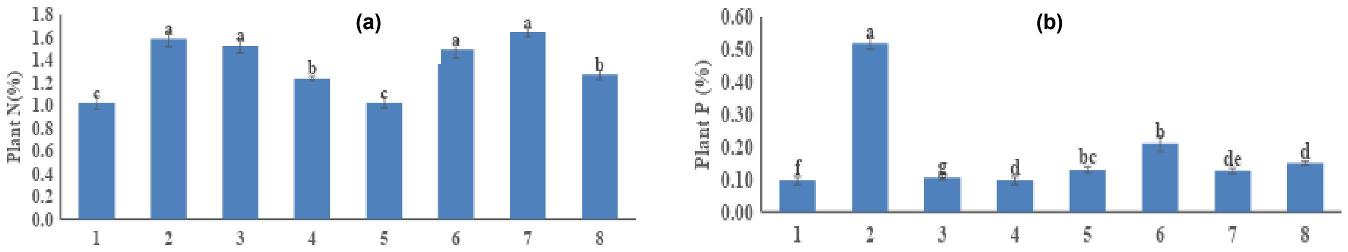

Figure 3. Effect of biochar and phosphate solubilizing bacteria with diammonium phosphate and nitrophos on plant N (a) and plant P (b). The bars sharing similar letters are statistically non-significant to each other at $p \le 0.05$.

DISCUSSION

The present study highlights the synergistic effects of biochar (BC) and phosphate-solubilizing bacteria (PSB) in combination with diammonium phosphate (DAP) and nitrophos (NP) on maize productivity and soil health in phosphorus-deficient calcareous soils. The combined application of BC+PSB+DAP consistently outperformed all other treatments, suggesting that integrated nutrient management strategies can effectively address the limitations of phosphorus availability in alkaline soils. The increased availability of nitrogen and phosphorus in the BC+PSB+DAP treatment can be attributed to the complementary functions of biochar and microbial inoculants. Biochar provides a porous habitat that enhances microbial colonization and survival, protecting PSB from environmental stress and predators like protozoa (Lehmann et al., 2011; Siddiqui et al., 2016). In turn, PSB increase the bioavailability of phosphorus through acidification, chelation, and organic acid production processes well-documented in strains such as *Pseudomonas fluorescens* and *Bacillus subtilis* (Khan et al., 2009; Malhotra et al., 2018). These mechanisms align with the observed 48% increase in available phosphorus and 40% increase in nitrogen in soil under BC+PSB+DAP treatment. Moreover, the enhancement of soil organic matter in BC-treated pots reflects the biochar's role in stabilizing carbon and improving soil structure (Das and Ghosh, 2020). Higher microbial biomass likely stimulated nutrient cycling and retention, particularly of ammonium and phosphate ions, reducing leaching losses and boosting nutrient use efficiency (Mahmoud et al., 2019; Rafique et al., 2017). These soil improvements are critical in calcareous systems where phosphorus fixation severely limits plant uptake.

Growth-related improvements in maize under integrated treatments such as increased shoot and root biomass, plant height, and SPAD chlorophyll content further support the hypothesis of improved nutrient acquisition and assimilation. PSB and biochar likely promoted better root architecture, enhancing the root surface area for nutrient absorption. These findings are consistent with those of Laghari et al. (2016) and Chauhan et al. (2016), who reported improved biomass production and photosynthetic performance in crops treated with biochar and beneficial microbes. The enhanced physiological performance evident through higher photosynthetic rates, stomatal conductance, water use efficiency (WUE), and sub-stomatal conductance indicates more efficient carbon assimilation and water regulation under integrated treatments. Improved gas exchange is directly linked to optimal nitrogen and phosphorus nutrition, affecting chlorophyll synthesis, ATP generation, and enzymatic activity in the Calvin cycle (Rychter and Rao, 2005). Notably, PSB-inoculated plants showed increased stomatal activity even under NP treatments, suggesting PSB's role in modulating stress responses and improving water balance, potentially through auxin and cytokinin production (Lugtenberg and Kamilova, 2009). Plant tissue analysis further confirmed increased nutrient uptake under PSB and BC-treated soils, with nitrogen and phosphorus concentrations in leaves and stems significantly higher than the control. This reinforces the role of PSB in mobilizing insoluble phosphorus and biochar to retain nutrients within the rhizosphere. The effectiveness of DAP over NP is likely due to its better solubility and compatibility with microbial metabolism in alkaline environments, where phosphorus tends to form insoluble calcium-phosphates (Rose et al., 2012).

Despite these promising results, certain limitations must be acknowledged. The study was conducted in a controlled pot environment over a short duration. While useful for mechanistic insights, these conditions may not fully represent field-scale dynamics, such as weather variability, microbial community shifts, and long-term soil fertility trends. Therefore, field trials under diverse soil types and cropping systems are essential to validate the scalability and consistency of these findings. In summary, the integrated application of BC and PSB with DAP improves phosphorus solubilization and microbial activity and significantly enhances maize growth, physiological function, and nutrient uptake. These benefits, driven by biological and physicochemical processes, underline the potential of integrated nutrient management in promoting sustainable maize cultivation, especially in phosphorus-deficient calcareous soils. Future research should focus on field-scale validation, microbial community profiling, and optimizing application rates across soil textures to refine these sustainable strategies further.

CONCLUSIONS

The integration of biochar (BC) and phosphate-solubilizing bacteria (PSB) with diammonium phosphate (DAP) and nitrophos (NP) demonstrated a synergistic enhancement in maize growth, nutrient uptake, and soil fertility under phosphorus-deficient calcareous soil conditions. The combined application of BC+PSB+DAP emerged as the most effective treatment, significantly improving maize growth attributes, including shoot fresh weight (81%), root dry weight (130%), and plant height (55%) compared to the control. Physiological parameters such as photosynthetic rate (48%), stomatal conductance (60%), and water use efficiency (33%) were also markedly elevated, underscoring the role of enhanced nutrient availability and microbial activity in optimizing plant metabolic processes. Soil health indicators revealed increases in organic matter (36%), nitrogen (40%), and phosphorus availability (48%) under BC+PSB+DAP, attributed to capacity of biochar to stabilize soil structure, retain nutrients, and microbial proliferation, alongside PSB's phosphorus-solubilizing efficacy. DAP exhibited superior compatibility with BC and PSB over NP, likely due to its efficient phosphorus release in alkaline soils. This synergy not only amplified nutrient accessibility but also reduced dependency on synthetic inputs, aligning with sustainable agricultural goals. PSB inoculation alone improved phosphorus uptake by 35–40%, while biochar further amplified these benefits by enhancing microbial habitat and nutrient retention. The study validates the hypothesis that integrated nutrient management surpasses isolated applications, offering a pragmatic strategy to address phosphorus fixation challenges in calcareous soils.

These findings advocate for adopting BC+PSB+DAP as a cost-effective, eco-friendly approach to maize productivity and soil resilience. However, long-term field trials are essential to evaluate the persistence of these effects, microbial community dynamics, and scalability across diverse agroecosystems. Future research should also optimize application rates for varying soil textures and cropping regimes to refine sustainable phosphorus management frameworks. By bridging organic amendments with microbial inoculants, this study paves the way for transformative agricultural practices that harmonize productivity with the environment.

ACKNOWLEDGEMENTS

Not applicable

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

REFERENCES

Adnan, M., Fahad, S., Zamin, M., et al 2020. Coupling phosphate-solubilizing bacteria with phosphorus supplements improve maize phosphorus acquisition and growth under lime induced salinity stress. *Plants.* 9:900

Adom, K.K., Liu, R.H. 2002. Antioxidant activity of grains. *J. Agric. Food Chem.* 50(21):6182–6187

Ahemad, M. 2014. Phosphate-solubilizing bacteria-assisted phytoremediation of metalliferous soils: a review. *3 Biotech.* 4:111–121

Ali, S., Uddin, S., Ullah, O., et al 2012. Yield and yield components of maize response to compost and fertilizer-nitrogen. *Food Sci. Qual. Manag.* 38:39–44

Ali, S., Rasheed, R., Qazi, M., et al 2023. Enhancing rhizosphere bacterial activity against bacterial wilt of tomato (Ralstonia solanacearum) using biochar. *J Agric Appl Biol*, *1*(2), 61-69.

Amanullah, Khan, I., Jan, A., et al 2015. Compost and nitrogen management influence productivity of spring maize (Zea mays L.) under deep and conventional tillage systems in semi-arid regions. *Commun. Soil Sci. Plant Anal.* 46:1566–1578

AOAC. 2005. Official Method 978.04. In: Official Methods of Analysis of AOAC International. 18th ed. AOAC International, Gaithersburg, MD.

Assuero, S.G., Mollier, A., Pellerin, S., et al 2004. The decrease in growth of phosphorus deficient maize leaves is related to a lower cell production. *Plant Cell Environ.* 27:887–895

Baligar, V.C., Fageria, N.K., He, Z.L., et al 2001. Nutrient use efficiency in plants. *Commun. Soil Sci. Plant Anal.* 32(7–8):921–950

Bertran, E., Sort, X., Soliva, M., et al 2004. Composting winery waste: sludges and grape stalks. *Bioresour. Technol.* 95(2):203–208

Bertrand, I., Hinsinger, P., Jaillard, B., et al 1999a. Dynamics of phosphorus in the rhizosphere of maize and rape grown on synthetic, phosphated calcite and goethite. *Plant Soil.* 211:111–119

Blanco-Vargas, A., Chacón-Buitrago, M.A., Quintero-Duque, M.C., et al 2022. Production of pine sawdust biochar supporting phosphate-solubilizing bacteria as an alternative bioinoculant in Allium cepa L. culture. *Sci. Rep.* 12:12276

Cha, J., Park, S., Jung, S.H., et al 2016. Production and utilization of biochar: a review. *J. Ind. Eng. Chem.* 40:1–15

Chauhan, A.K., Maheshwari, D.K., Kim, K., et al 2016. Termitarium-inhabiting Bacillus endophyticus TSH42 and Bacillus cereus TSH77 colonizing Curcuma longa L.: isolation, characterization, and evaluation of their biocontrol and plant-growth-promoting activities. *Can. J. Microbiol.* 62:880–892

Das, S.K., Ghosh, G.K. 2020. Soil health management through low cost biochar technology. pp. 193–206. In: Biochar applications in agriculture and environment management. Springer, Singapore

De Tender, C., Haegeman, A., Vandecasteele, B., et al 2016. Dynamics in the strawberry rhizosphere microbiome in response to biochar and Botrytis cinerea leaf infection. *Front. Microbiol.* 7:2062

Dey, G., Banerjee, P., Sharma, R.K., et al 2021. Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate-solubilizing bacteria—a review. *Agronomy.* 11:1552

Eghball, B., Power, J.F. 1999. Phosphorus- and nitrogen-based manure and compost applications corn production and soil phosphorus. *Soil Sci. Soc. Am. J.* 63(4):895–901

Estrada-Bonilla, G.A., Durrer, A., Cardoso, E.J.B.N., et al 2021. Use of compost and phosphate-solubilizing bacteria affect sugarcane mineral nutrition, phosphorus availability, and the soil bacterial community. *Appl. Soil Ecol.* 157:103760

Grzeszczuk, M. 2020. Analysis of chlorophyll and carotenoid content. *Int. J. Agron.* 2020:4159186

Hussain, M., Farooq, M., Nawaz, A., et al 2017. Biochar for crop production: potential benefits and risks. *J. Soil Sci.* 17:685–716

Keener, H.M., Dick, W.A., Hoitink, H.A., et al 2000. Composting and beneficial utilization of composted by-product materials. pp. 315–341. In: Land application of agricultural, industrial, and municipal by-products. SSSA, Madison, WI

Khan, A.A., Jilani, G., Akhtar, M.S., et al 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *J. Agric. Biol. Sci.* 1(1):48–58

Kim, K.Y., Jordan, D., McDonald, G.A., et al 1998. Enterobacter agglomerans, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. *Soil Biol. Biochem.* 30:995–1003

Krzyżak, A., Górka, P., Szumny, A. 2023. Electrolyte leakage method for membrane stability analysis in plants under stress conditions. *Environ. Exp. Bot.* 194:104799

Laghari, M., Hu, Z.Q., Mirjat, M.S., et al 2016. Fast pyrolysis biochar from sawdust improves the quality of desert soils and enhances plant growth. *J. Sci. Food Agric.* 96:199–206

Lee, K.K., Mok, I.K., Yoon, M.H., et al 2012. Mechanisms of phosphate solubilization by PSB (phosphate-solubilizing bacteria) in soil. *Korean J. Soil Sci. Fertil.* 45:169–176

Lehmann, J., da Silva Jr, J.P., Steiner, C., et al 2011. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant Soil.* 249:343–357

Li, M., Cai, L. 2021a. Biochar and arbuscular mycorrhizal fungi play different roles in enabling maize to uptake phosphorus. *Sustainability.* 13:3244

Liu, X., Zhou, H., Wu, X. 2015. Measurement of photosynthesis and stomatal conductance using the CIRAS-3 system. *J. Plant Physiol.* 187:98–104

Lugtenberg, B., Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63:541–556

Mahmoud, E., Ibrahim, M., Abd El-Rahman, L., et al 2019. Effects of biochar and phosphorus fertilizers on phosphorus fractions, wheat yield and microbial biomass carbon in Vertic Torrifluvents. *Commun. Soil Sci. Plant Anal.* 50:362–372

Malhotra, H.S., Sharma, R., Pandey, R., et al 2018. Phosphorus nutrition, plant growth in response to deficiency and excess. *Plant Nutr. Abiotic Stress Tol.* 123:171–190

Marchetti, R., Castelli, F., Orsi, A., et al 2012. Biochar from swine manure solids: Influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate incorporation. *Ital. J. Agron.*. 7: 189–195.

Nadeem, M.A., Mollier, C., Morel, A., et al 2012. Seed phosphorus remobilization is not a major limiting step for phosphorus nutrition during early growth of maize. *J. Plant Nutr. Soil Sci.*. 175: 805–809.

Olsen, S.R., Cole, C.V., Watanabe, F.S., et al 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. United States Department of Agriculture Circular No. 939.

Olsson, P.A., Rahm, J., Aliasgharzad, N., et al 2010. Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiol. Ecol.*. 72: 125–131.

Ouattara, V.T., Konate, Z., Messoum, G.F., et al 2018. Effects of organo-phosphate fertilization on fertility of organic matter and the adsorbent complex on ferralsol in cocoa trees in the region of Divo. *Int. J. Biol. Chem. Sci.*. 12: 2901–2921.

Pakistan Economic Survey 2022-23. 2023. Finance Division, Government of Pakistan. p. 467.

Piccolo, A., Spaccini, R., Nieder, R., et al 2004. Sequestration of a biologically labile organic carbon in soils by humified organic matter. *Clim. Change*. 67: 329–343.

Pouya, B.M., Soma, M.D., Gnankambary, Z., et al 2022. Effects of organo-phosphate fertilizers on soil characteristics and yields of sorghum, maize and cowpea in the Eastern region of Burkina Faso. [Journal/Publisher info missing].

Rafique, M., Sultan, T., Ortas, I., et al 2017. Enhancement of maize plant growth with inoculation of phosphate-solubilizing bacteria and biochar amendment in soil. *Soil Sci. Plant Nutr.*. 63: 460–469.

Rose, T.J., Pariasca-Tanaka, M., Mori, A., et al 2012. Seeds of doubt: re-assessing the impact of grain P concentrations on seedling vigor. *J. Plant Nutr. Soil Sci.*. 175: 799–804.

Ryan, J., Estefan, G., Rashid, A. 2001. Soil and Plant Analysis Laboratory Manual, 2nd edn. International Center for Agricultural Research in the Dry Areas (ICARDA).

Ryan, M.H., McInerney, J.K., Record, I.R., et al 2008. Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *J. Sci. Food Agric.* 88: 1208–1216.

Rychter, A.M., Rao, I.M. 2005. Role of phosphorus in photosynthetic carbon metabolism. pp. 1–27. In: Pessarakli, M. [ed.]. *Handbook of Photosynthesis*. 2nd edn. CRC Press, Boca Raton.

Siddiqui, A., Nazeer, R.S., Piracha, M.A., et al 2016. The production of biochar and its possible effects on soil properties and phosphate solubilizing bacteria. *J. Appl. Agric. Biotechnol*. 1: 27–40.

Sgroy, V., Cassán, F., Masciarelli, O., et al 2009. Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera. *Appl. Microbiol. Biotechnol.*. 85: 371–381.

Shah, S.H., Hussain, M.B., Zahir, Z.A., et al 2022. Thermal plasticity and cotton production enhancing attributes of phosphate-solubilizing bacteria from cotton rhizosphere. *J. Soil Sci. Plant Nutr.*. 22: 3885–3900.

Shanta, N., Schwinghamer, T., Backer, R., et al 2016. Biochar and plant growth promoting rhizobacteria effects on switchgrass (Panicum virgatum cv. cave-in-rock) for biomass production in southern Québec depend on soil type and location. *Biomass Bioenerg.*. 95: 167–173.

Shen, J., Yuan, L., Zhang, J., et al 2011. Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156: 997–1005.

Steel, R.G.D., Torrie, J.H., Dickey, D.A. 1997. *Principles and Procedures of Statistics: A Biometrical Approach*. 3rd edn. McGraw-Hill, Inc.

Sundara, B., Natarajan, V., Hari, K., et al 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *Field Crops Res.* 77: 43–49.

Verma, B.C., Mandal, S., Choudhury, B.U., et al n.d. Application of biochar in agriculture to improve soil. *Soil Air Water*. 40: 1093–1098.

Walkley, A., Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37: 29–38.

Wei, Y., Zhao, Y., Wang, H., et al 2016. An optimized regulating method for composting phosphorus fractions transformation based on biochar addition and phosphate-solubilizing bacteria inoculation. *Bioresour. Technol.* 221: 139–146.

Yang, F., Sui, L., Tang, C., et al 2021. Sustainable advances on phosphorus utilization in soil via addition of biochar and humic substances. *Sci. Total Environ.* 768: 145106.

Zhang, Y., Li, C., Wang, Y., et al 2016. Maize yield and soil fertility with combined use of compost and inorganic fertilizers on a calcareous soil on the North China Plain. *Soil Tillage Res*. 155: 85–94.

Zhu, Y.G., Smith, S.E. 2001. Seed phosphorus (P) content affects growth and P uptake of wheat plants and their association with arbuscular mycorrhizal (AM) fungi. *Plant Soil*. 231: 105–112.