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ORIGINAL RESEARCH

Biochar and sewage sludge phosphorus fertilizer effects on phosphorus bioavailability and spinach (*Spinacia oleracea* L.) yields under no-till system in semi-arid soils

Ugele Majaule[1*], Oagile Dikinya[2], Bruno Glaser[3]

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Abstract

Purpose This field study evaluated the interactive effects of biochar (BC) and sewage sludge (SS) on P bioavailability and spinach yields for two seasons.

Method Treatments were combinations of biochar (0, 2.5 and 5 Mg ha^{-1}) and sewage sludge (0, 6 and 12 Mg ha^{-1}), or mineral fertilizer (200, 28, and 18.9 kg ha^{-1}), amended in a randomized complete block design to Luvisol and Cambisol.

Results Significant ($p < 0.05$) yield increase of 53 and 65%, respectively occurred with increasing sole biochar doses on the Luvisol. Both applied alone and in combination with BC, the high rate of SS increased ($p < 0.05$) yields on the Luvisol over two seasons. Complimentary effects of 6SS+5BC on the Luvisol showed the highest yield increase for the study period. Co-application of amendments on the Cambisol decreased ($p > 0.05$) yields compared to sole amendments. Mehlich – 3 extractable P (M3-P) in control plots (CONT) increased between seasons, presumably due to P inputs from the irrigation water. Co-amendments on the Cambisol resulted in higher M3-P increase over mineral fertilizer than on the Luvisol in both seasons. Accumulation of M3-P in control plots confounded correlations between crop yields and available P. Higher P under BC compared to SS amended soils emphasize biochar capacity to capture P from irrigation water.

Conclusion The results suggest that combined low rates of SS and BC can have significant effects on P availability and crop yields. Biochar enhanced plant P uptake, but decrease in yields with simultaneous increase in M3-P between seasons warrants further research.

Keywords Co-Application, Bioavailability, Phosphorus, Spinach, Yield

✉Ugele Majaule umajaule@gmail.com

1 Department of Agricultural Research, Ministry of Agriculture Development and Food Security, Gaborone, Botswana

2 Department of Environmental Science, University of Botswana, Gaborone, Botswana

3 Soil Biogeochemistry, Institute of Agronomy and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany

Introduction

Vegetable crop production in the semi-arid tropics is greatly constrained by multiple factors including water scarcity and widespread nutrient deficiency (Reynolds et al. 2015). Phosphorus (P) deficiency has been linked to low productivity of staple vegetable crops in the semi-arid tropics (Nziguheba et al. 2016; Kanouo et al. 2019; Roobroeck et al. 2021). Indeed, many of the vegetable crops in the Glen Valley cluster farms in Botswana have been observed to exhibit strong P deficiency, resulting in the common practice of high inputs of mineral fertilizers, sewage sludge and livestock manures, to improve soil fertility and yields.

Spinach (*Spinacia Oleracea* L.) is a highly traded and consumed vegetable crop in Botswana. The plant leaves are an important source of various minerals, antioxidant vitamins, phytochemicals and bioactive compounds with health-beneficial effects (Pandjaitan et al. 2005). Spinach plants are considered as “heavy feeder”, requiring medium to high soil fertility, although over application of N results in excessive accumulation of nitrates in the leaves. Sewage sludge can directly provide adequate P supply for plant growth (Ngole 2010), or through modifying soil microbial and physicochemical properties (Houben et al. 2019). However, sewage sludges employing highly efficient P recovery systems or chemical treatment contain low bioavailable contents of P (Wollmann et al. 2017), thus requiring high dosages to meet plant P requirement. However, sludge can contain high levels of heavy metals and salinity, which impairs plant growth. There is therefore a need to enhance P fertilizer value of sewage sludge to avoid environmental and agronomic risks from sludge-borne organic and inorganic pollutants (Domínguez et al. 2019; Romanos et al. 2021).

Increase in plant available P in biochar – amended soils is attributable to several mechanisms including the direct supply of predominantly inorganic P (Uchimiya et al. 2014; Rose et al. 2019), increase in soil pH and CEC (Nelson et al. 2011), retention of P in irrigation water, adsorption of chelates, and increasing microbial activities (Glaser and Lehr 2019). Biochar application in high-input horticultural production systems is shown to have inconsistent effects on nutrient bioavailability and crop productivity, presumably because in most cases, the soils are fertile (Boersma et al. 2017). However, in marginal soils with intrinsic low P supply capacity, biochar application could increase P availability and plant uptake from both organic and inorganic nutrient sources. However, no studies have investigated P bioavailability in wastewater-irrigated soils amended with biochar and sewage sludge. Therefore, the objectives of this study were to determine the interactive effects of biochar and sewage sludge applications on P bioavailability and spinach yields.

Materials and methods

Site location

The study was undertaken in Glen Valley horticultural cluster farm (24º 35' 55" S, 25º 57' 46" E) located about 10 km north of Gaborone, Botswana (Fig. 1). The area has different soil types, but the experimental sites were located on a Calcic Luvisol and Vertic Cambisol, in a farmer’s field. The site on the Luvisol was continuously cultivated with various vegetable crops for over 10 years with application of livestock manures, sewage sludge and mineral fertilizers, whereas the Cambisol site was fallow for over five years.

Biochar and sewage sludge amendments

Sewage sludge was sampled from the Glen Valley Waste Water Treatment Plant (GVWWTP) where an activated system is applied for treatment of municipal wastewater (Fig. 1a). After anaerobic digestion and dewatering, sewage sludge at GVWWTP is further dried in evaporation ponds and eventually stockpiled for collection by farmers. The air-dried sludge was crushed, sieved (2 mm) and stored at room temperature (25 ºC) plastic bags. Mixed-wood chips collected from the Gaborone City Council yard was carbonized at 535 ºC for 6 h. The slow pyrolysis unit (Fig. 2a, b) with four thermocouples was developed in the engineering section under the Department of Agricultural Research, Botswana. Biochar was ground, sieved (2 mm) and stored for analysis or field application.

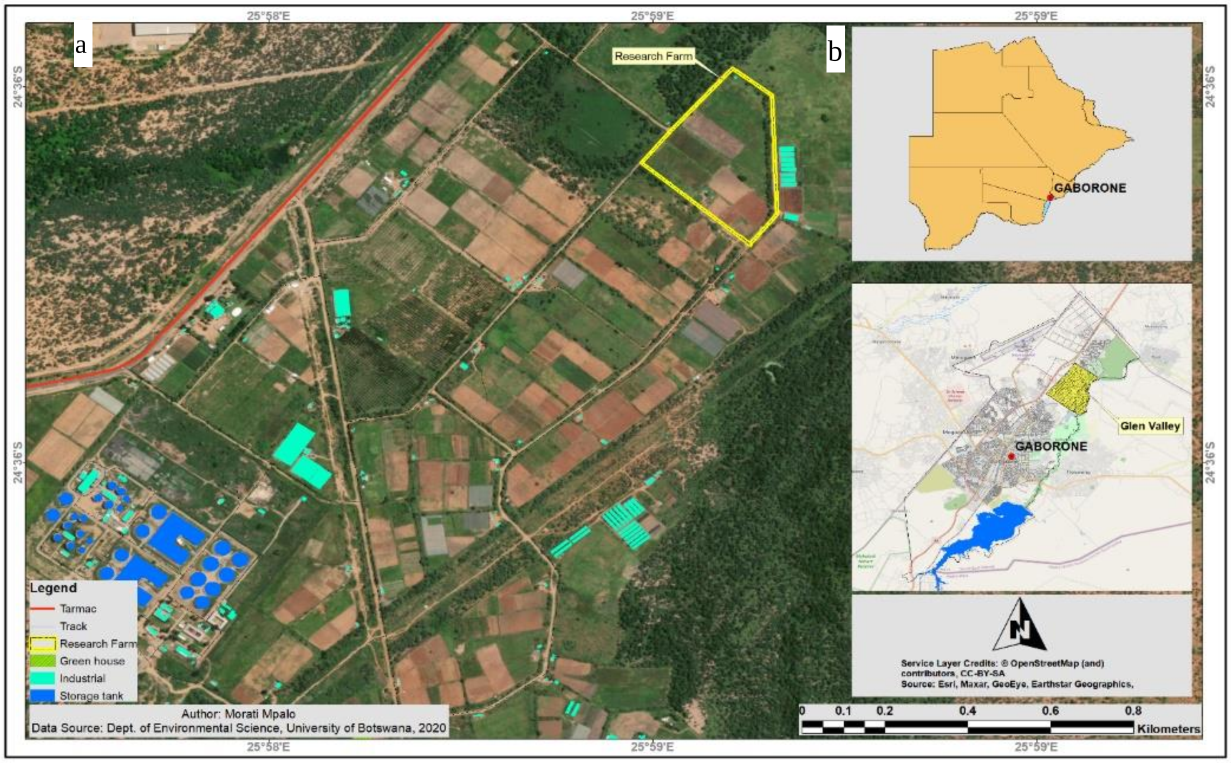


Fig. 1 a) Aerial photo of Glen Valley Waste Water Treatment Plant (GVWWTP) and study sites; b) Location map of Glen Valley, Botswana

Fig. 2 a) Biochar production, b) Biochar drying process, c) Application of amendments

Experimental design and trial management

The experiment was a randomized complete block design (RCBD) with three replicates consisting of the two soil types with substantially different textures (Luvisol and Cambisol), and two factors; biochar and sewage sludge. Three levels of biochar (0, 2.5 and 5 Mg ha^{-1}) and sewage sludge (0, 6 and 12 Mg ha^{-1}) were factorially combined to give nine treatments (including unamended control; CONT). A positive control (recommended mineral fertilizer dose (Kg ha^{-1}); N - 200, P - 28, K - 18.9) was included to compare the effectiveness of the organic amendments. The resulting ten treatments were replicated three times, thus constituting a total of 30 experimental plots per site, each measuring 1.8 m x 1.5 m (2.7 m^2). To prevent cross contamination, plots were separated by a 1 m within each replication and 2 m buffer between replications. Treatments were applied on the same plots in both cropping seasons. The treatments and their codes were as follows;

(T1) CONT – no amendment (control)

(T2) 2.5BC – 2.5 Mg ha^{-1} biochar

(T3) 5BC – 5 Mg ha^{-1} biochar

(T4) CHEM – mineral fertilizer at 200, 28, and 18.9 kg ha^{-1} $season^{-1}$ of N, P and K, respectively.

(T5) 6SS – 6 Mg ha^{-1} sewage sludge

(T6) 6SS+2.5BC – 6 Mg ha^{-1} sewage sludge and 2.5 Mg ha^{-1} biochar

(T7) 6SS+5BC – 6 Mg ha^{-1} sewage sludge and 5 Mg ha^{-1} biochar

(T8) 12SS – 12 Mg ha^{-1} sewage sludge

(T9) 12SS+2.5BC – 12 Mg ha^{-1} sewage sludge and 2.5 Mg ha^{-1} biochar

(T10) 12SS+5BC – 12 Mg ha^{-1} sewage sludge and 5 Mg ha^{-1} biochar

Before application, suitable doses of amendments were weighed and packed into plastic bags. Subsequently, the organic amendments were applied manually along planting lines to a depth of 0.15 m before the plots were levelled using garden rakes (Fig. 2c). Biochar and sewage sludge were applied at the beginning of each season. Mineral fertilizers were side-dressed along planting lines. Further details of the trial management are provided in Majaule et al. (2020).

Soil and plant sampling

Surface (0 – 15 cm) soil samples from each plot were collected during harvest. The soil samples were air-dried and passed through a 2 mm sieve. Spinach plants were harvested twice in a cropping season, when the leaves reached marketable size, then weighed at the laboratory to determine wet biomass yield. Air-dried leaves were further oven dried for 24 h at 70 $^{\circ}$C, ground and sieved (2 mm).

Chemical analyses

The chemical analyses of soil, organic amendments and spinach samples were carried out following standard laboratory procedures as detailed in Majaule et al. (2020). Briefly, exchangeable cations and soil CEC were determined after extracting air-dried 2.5 g samples using the ammonium acetate method at pH 7 as described by van Reeuwijk (1993). Exchangeable cations (Ca, Mg, Na, and K) were quantified using micro plasma atomic emission spectrometer (4210 MP-AES, Agilent Technologies). Soil pH was determined after shaking samples on an end-to-end shaker for 1 h in a 1: 5 distilled water and 0.01M $CaCl_2$ solution. Sewage sludge pH was determined after shaking 20 g sample in 100 ml of 0.01 M $CaCl_2$ (soil: solution 1: 5). Biochar pH was determined on a 1: 20 biochar to distilled water ratio according to Wang et al. (2015). Total N in soil, sewage sludge, plant and biochar were analysed by the micro-Kjeldahl procedure (van Reeuwijk 1993). Mehlich-3 P (M3-P) was determined according to the method of Ziadi and Tran (2008) in 1: 10 soil to KCl solution (2 M). Soil organic carbon (SOC) was determined by Walkley-Black method, whereas biochar and sewage sludge to total carbon was determined by ashing samples in muffle furnace for 48 h at 500 °C.

Total P and bases in sludge and plant were determined after wet digestion of 1.25 g sample in 2.5 ml of sulphuric acid – selenium mixture (van Reeuwijk 2002). Basic cations in digested samples were quantified using 4210 MP-AES (Agilent Technologies), while P was measured spectrophotometrically by the indophenol-blue method. Total content of P, K, S, Mg and Ca in biochar was quantified according to the modified dry-ashing method (Enders and Lehmann 2012).

Statistical analysis

Statistical analysis was done using SAS (version 9.4). Results were the means of three replicates. A two-way ANOVA was carried out to examine the effects of factors and their interactions. Significantly, different means were separated by the Duncan's multiple range tests ($p < 0.05$).

Results and discussion

Soil, biochar and sewage sludge properties

The physicochemical properties of the soils (0 – 15 cm) and organic amendments were described by Majaule et al. (2020). Soil pH was neutral in both soil types, whereas EC, clay and organic C content and CEC were higher in the Cambisol. Briefly, organic amendments, whereas EC, clay and organic C content and CEC were higher in the Cambisol. Organic amendments had neutral pH, but sewage sludge had higher levels of salinity, exchangeable Ca and Mg, total and available P, and CEC than biochar.

M3-P was deficient in the Cambisol (24 mg kg^{-1}) but adequate in the Luvisol (42.3 mg kg^{-1}) before the study. Further, total soil P was higher in the Luvisol, presumably due to the influence of previous management practices. While the Luvisol site was continuously cropped and amendment with both organic and inorganic P fertilizers, the Cambisol site was fallowed for five years. Substantially, higher clay content in the Cambisol (39%) suggest greater P sorption relative to the Luvisol. In addition, precipitation of Ca and Mg phosphates due to higher exchangeable Ca and Mg in the Cambisol may account for lower pre-crop plant available P levels.

Effects of amendments on M3-P and leaf P contents

On both soils, M3-P in the control plots (CONT) decreased below the pre-crop levels after the first season which was attributed to plant uptake (Fig. 3a, b). Subsequently, M3-P in the control plots significantly ($p < 0.05$) increased from 28.6 to 53.6 mg kg^{-1} between seasons in the Luvisol, while the increase in the Cambisol was insignificant (Fig. 3a, b). The increasing trends of M3-P between seasons followed similar trends to leaf P concentrations (Fig. 3c, d). Such increases in M3-P despite plant P uptake can be linked to either elevated P concentrations in the irrigation water (Emongor and Ramolemana 2004) or plant mobilization of soil P from sorption sites (Wollmann et al. 2017). Thus, future research needs to monitor the levels of P in the irrigation water and to also provide environmental risk assessment of P loss from the Glen Valley farming system (El-Ouahmani et al. 2021). ANOVA showed that treatment effects significantly ($p < 0.001$) affected plant available

P ($r^2 = 0.84$; CV = 36%). Further, treatments significantly ($p < 0.05$) increased plant available phosphorus on the Luvisol in both seasons, except for sole sewage sludge applied at either 6 or 12 Mg ha^{-1} (6SS and 12SS). Mineral fertilizer (CHEM) only significantly ($p < 0.05$) increased available P in the second season on the Luvisol, presumably due to higher P sorption on the Cambisol caused by higher clay contents. The Cambisol had higher levels of Ca and Mg compared to the Luvisol, and this might have increased formation of Ca-P and Mg-P precipitates, thus decreasing M3-P concentration (Kanouo et al. 2019).

Increasing application rates of sole sewage sludge insignificantly increased M3-P relative to the control on both soil types (Fig. 3a, b), with plant available P consistently higher on the Luvisol for each of the corresponding treatments. These results corroborate the report by Ngole (2010) of a higher increase in P availability in Luvisols compared to a Vertisol and Arenosol, with levels of bioavailable P increasing with sludge application rates. On both soils, sewage sludge dosages had statistically similar effects on M3-P compared to mineral P fertilizer (CHEM) in the first and second seasons (except for 6SS on the Cambisol in season 2). Previous studies also reported on the high bioavailability of sludge-borne P in the year of application, comparable to mineral P fertilizers (Siddique and Robinson 2003; Wollmann et al. 2017), mainly because sludge P is predominantly inorganic (Rigby et al. 2016). Biochar efficacy on increasing M3-P was greater on the Luvisol, following the same trend as mineral P fertilizer and sludge. Further, sole biochar applications had greater effects on increasing M3-P than sole sewage sludge on both soils, despite much lower total P application rates compared to sole sewage sludge. This is probably due to the slower-releasing pattern of P from the sewage sludge compared to biochar. In addition, ash in biochar (34.7%; Majaule et al. 2020) is a direct source of soluble inorganic P forms, and provide liming effects which in turn enhanced P bioavailability (Mosharrof et al. 2021). Moreover, the drastic increase in M3-P under biochar treated plots support suggestions from recent research on the potential of biochar for capturing nutrients in the soil, thus reducing their leaching potential and increasing plant P availability (Shepherd et al. 2017; Nobaharan et al. 2021). Our results indicate that for the Glen Valley system, sole biochar application is effective in increasing P bioavailability, thus greatly reducing the risk of adding organic and inorganic pollutants contained in the sludge.

Notably, co-amendments on both soils consistently resulted in significant ($p < 0.05$) increase in Mehlich-3 extractable P in both seasons (Fig. 3a, b). Biochar addition, alone or in combination with sludge was highly effective in increasing available P in the first season across soils, with the exception of low biochar rate (2.5BC) on the Cambisol. Bioavailable P concentration under the sole biochar treatments significantly ($p < 0.05$) increased with application rates, evidence of the significance of biochar as a P source for plant growth (Mukherjee and Zimmerman 2013). The increase in the second season across soils suggests that biochar effectively inhibited P sorption, which has been attributed to the presence of phenolic and carboxylic groups on its surfaces (Lehmann et al. 2003). Furthermore, as the soil CEC increased (Table 1), retention of cations (Ca, Mg, Na, K) and heavy metals might have reduced the propensity of these elements forming P precipitates. High levels of M3-P on both soils occurring from high rates of biochar, or combination of biochar and sewage sludge suggests that annual applications of amendments pose a risk of P loss into the environment. Instead, research must focus on maximizing biochar retention of P in these soils, or development of biochar-based technology to remove P.

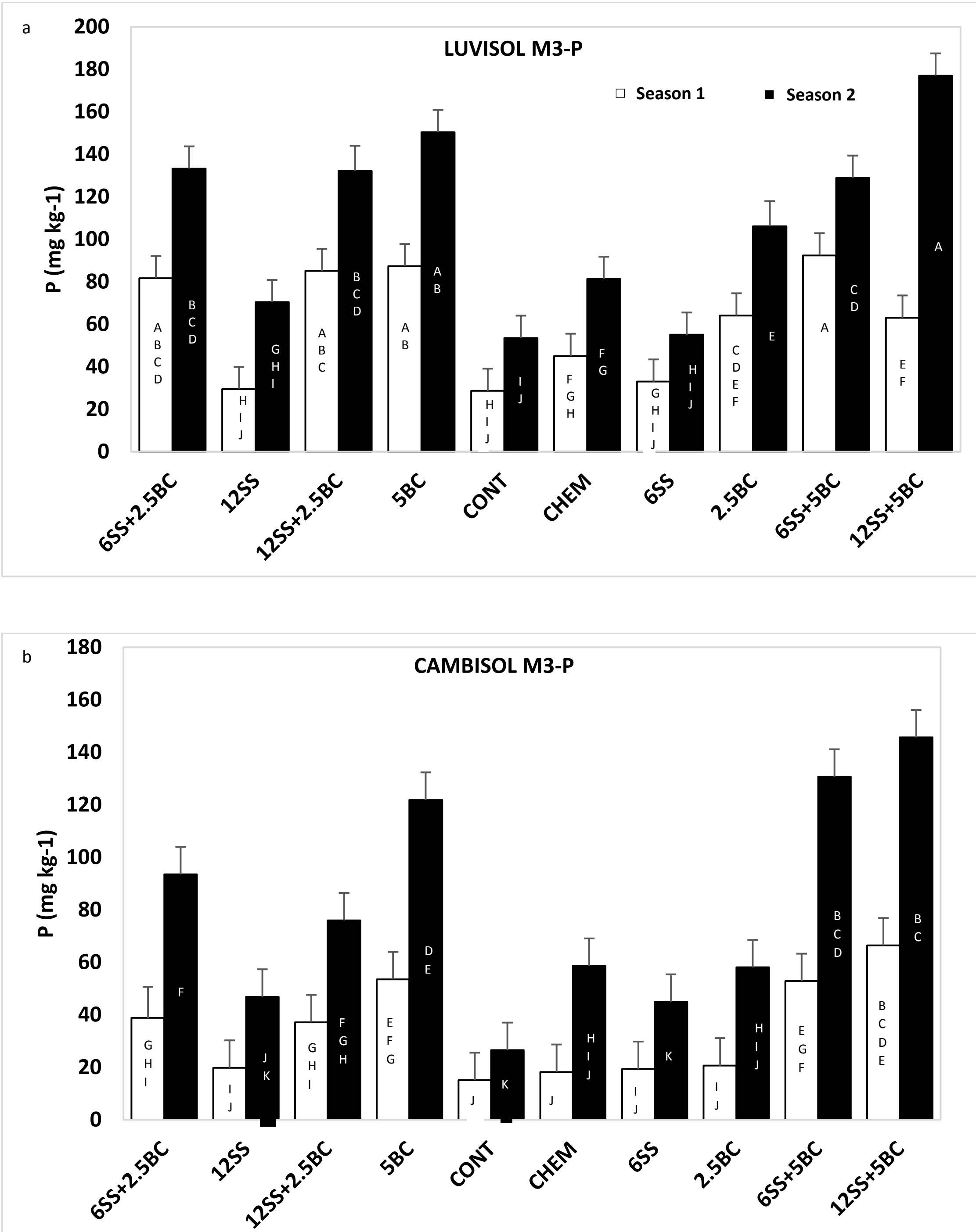
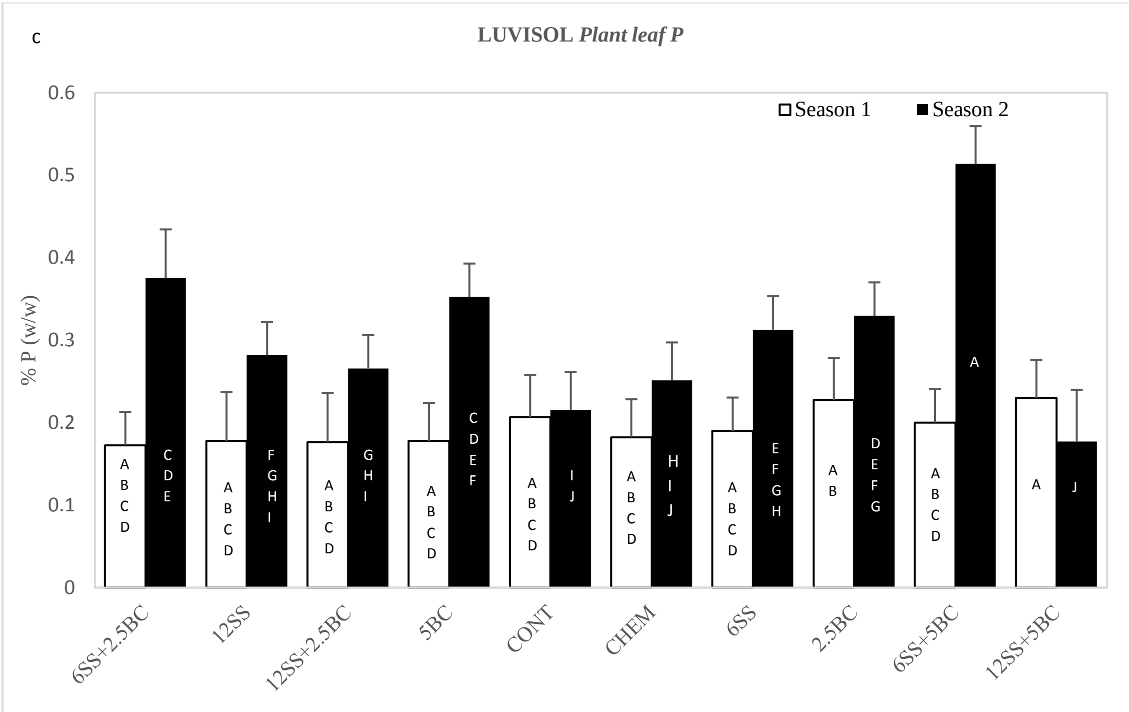
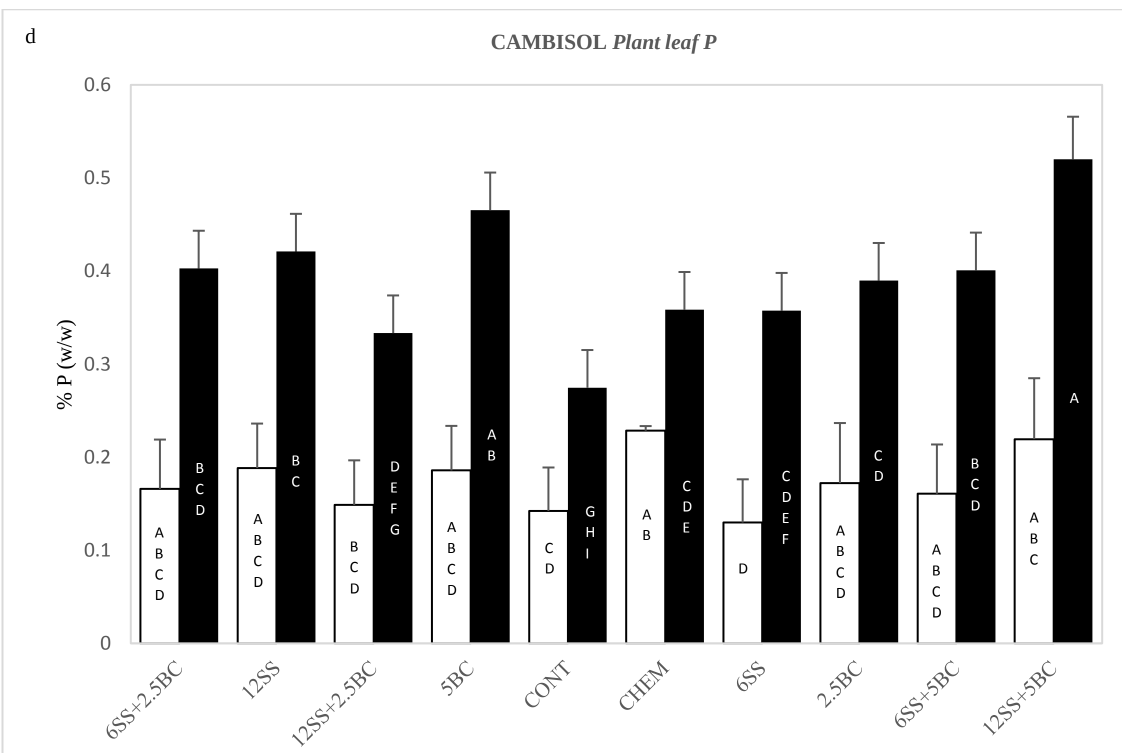


Fig. 3 *M-3 P* (a, b) and *Plant leaf P* concentrations (c, d) across of all treatments over the two seasons; March - April and July - Sep 2018

Error bars denote standard error of the mean (SEM). Columns with different letters are significantly different ($p < 0.05$).





Continued **Fig. 3** *M-3 P* (a, b) and *Plant leaf P* concentrations (c, d) across of all treatments over the two seasons; March - April and July - Sep 2018

Error bars denote standard error of the mean (SEM). Columns with different letters are significantly different ($p < 0.05$).

Table 1 Selected soil chemical properties of the sites after Season 1 (A) and Season 2 (B)

Treatments	pH ($CaCl_2$)		CEC (cmolc kg^{-1})		Exchangeable cations (cmolc kg^{-1})			
LUVISOL					Ca		Mg	
	A	B	A	B	A	B	A	B
CONT	7.7± 1.1A	7.5±2.3AB	8.7±3.1D	8.5±1.6E	106±24C	117±14CD	40±5.5FGH	33.9±2.9G
2.5BC	7.5±2.3BCD	7.4±1.7ABC	7±2.7D	11±2.1DE	119±28ABC	124±12CD	50.5±6.7DEFG	35.9±4.3G
5BC	7.3±1.4D	7.1±0.9EF	9±3.1D	15±1.7D	130±23ABC	120±17CD	55±8.3DEF	33.9±2.7G
CHEM	7.6±2.1ABC	7.6±1.1A	8±1.9D	8.5±1.1E	99±17C	108±10D	38.7±4.4GH	31.6±5.6G
6SS	7.7±1.8A	7.5±1.0AB	7.9±2.3D	7.5±2.1E	102.5±24C	115±16CD	38±5.6H	31.8±3.9G
6SS+2.5BC	7.3±1.5D	7.3±1.6CDE	10±1.8D	11.5±3.6DE	104.9±16C	127±11CD	39±6.7GH	42±4.1G
6SS+5BC	7.4±1.2CD	7.3±2.0BCD	8.6±1.4D	10±2.2DE	119±19.4ABC	121±20CD	48.7±8.2EFGH	34±5.2G
12SS	7.6±2.3AB	7.5±2.6AB	9.2±2.9D	10.5±2.6DE	122±17ABC	132±11CD	52±5.9DEF	35.9±3.9G
12SS+2.5BC	7.3±1.5D	7.4±1.9BCD	8±2.1D	11±3.4DE	109±19BC	125±10CD	44.5±5.1FGH	32.9±5.7G
12SS+5BC	7.4±1.8D	7.2±2.2DE	8.4±1.7D	12.7±2.7DE	116±18ABC	156±15BCD	48±6.3EFGH	41±6.2G
CAMBISOL								
	A	B	A	B	A	B	A	B
CONT	6.7±1.6HI	6.9±1.6GH	26±4.9BC	27.5±4.6ABC	127±22ABC	153±24BCD	62±6.9CD	76±7.4CDEF
2.5BC	6.9±1.1FGH	6.8±2.1H	30.6±3.2AB	30±3.5AB	144±1.9ABC	190±29AB	72±5.4BC	92.5±8.3AB
5BC	6.5±1.8I	6.6±1.1I	26.5±2.9ABC	29±6.3ABC	118±17ABC	162±32ABC	56.9±6.6DE	72.7±6.5EF
CHEM	6.9±2.5FG	6.9±1.0GH	31.8±2.6A	31.8±6.5A	166±21A	200±18AB	85.7±7.8A	95±8.3A
6SS	6.9±1.9FGH	6.9±1.7GH	31±4.1AB	31±3.9AB	158±21AB	188±20AB	80.9±10.2AB	85±7.9ABCD
6SS+2.5BC	6.5±0.8I	7±2.1FGH	27.9±5.2ABC	32±2.7A	165±17AB	189±31AB	81.7±8.7AB	82±10.6CDE
6SS+5BC	6.8±1.3GH	6.8±1.8HI	29.7±4.9AB	31±6.2AB	150±23ABC	202±35AB	74±8.3ABC	92±7.8AB
12SS	7±1.8E	7±1.4FG	24±3.3C	23.6±4.2C	114±19ABC	162±22ABC	55.5±6.9DEF	68±6.9F
12SS+2.5BC	7±1.1EF	6.9±1.3GH	27.7±2.8ABC	26±4.4ABC	134±18ABC	167±22ABC	62±9.3CD	74.8±5.2DEF
12SS+5BC	7±1.7EF	6.9±1.6GH	29.8±3.3AB	31±2.9AB	151±20ABC	213±24A	71±8.4BC	88±10.1ABC

*Values followed by different letters in the same column for each season are significantly different ($p<0.05$), given error is standard error ($n=3$; $p < 0.05$).

from the wastewater during treatment processes (Zheng et al. 2019; Nobaharan et al. 2021). The resultant P – rich biochar would then constitute a valuable fertilizer material for application to the agricultural soils. On the other hand, as biochar anion exchange capacity (AEC) is reported to decrease over time, long term studies are required to elucidate how long the observed beneficial effects of biochar on P availability could last (Wang et al. 2020). Mehlich-3 P followed a similar trend as leaf P concentration; it substantially increased between seasons and was also greater on the Cambisol. Leaf P content in the control plots did not change significantly on the Luvisol between seasons, but significantly ($p < 0.05$) increased on the Cambisol in the second season, as both available P and soil pH increased (Fig. 3b; Table 1). Furthermore, application of 5 Mg ha^{-1} biochar was equally competitive with combined high rates of organic amendments in increasing leaf P close to the sufficiency level on the Cambisol only. On the other hand, the critical range of leaf P concentration on the Luvisol was only observed in the second season for co-application of 6 Mg ha^{-1} and 5 Mg ha^{-1} of sludge and biochar, respectively.

Effects of amendments on spinach yields

Across the two seasons, there was greater response of both yield and P availability to organic amendments on the Luvisol than on the Cambisol. On the Luvisol, the mean yields for the two seasons significantly ($p < 0.05$) increased above the control as follows; 6SS+5BC (33.6), 5BC (30.4), 12SS+2.5BC (28.9), 2.5BC (28.3), 12SS+5BC (26.9) and 12SS (25.6). Therefore, mean yield data for the study period shows significant synergistic effects of biochar and sewage sludge on the Luvisol for most of the treatments but no significant complimentary effects on the Cambisol. The high clay contents in the Cambisol are presumed to have limited interactions between biochar and sewage sludge, thus, restricting the synergistic effects on this soil type (Greenberg et al. 2019). Therefore, co-composting of biochar with sewage sludge prior to soil application (Zhang et al. 2014) offers a viable alternative to increase benefits of co-application on clayey soils. Similarly, the mean yields for the two seasons on the Cambisol were influenced the treatments, with the majority of the treatments showing yield decline compared to the unamended control (CONT). Further, the yield data demonstrated that the organic amendments generally had greater effects than mineral fertilizer on spinach yields on the Luvisol, and equally effective as the mineral fertilizer on the Cambisol (Fig. 4a, b). Khaliq et al. (2017) also reported that sewage sludge compost increased radish and bean yields than inorganic fertilizers on a soil irrigated with groundwater and sewage sludge treated wastewater. The results of our study also corroborate earlier reports that biochar application confers more benefits on poor soils (Boersma et al. 2017; Agegnehu et al. 2017). Therefore, future research using other soil types with contrasting chemical and mineralogical properties, e.g. Arenosols which are prevalent in Glen Valley might reveal more agronomic benefits of biochar addition (Biederman and Harpole 2013). The yield decrease for most treatments in the second season (Fig. 4a, b) concided with increase in M3-P (Fig. 3a, b), showing that biochar and sewage sludge cannot provide sustained nutrient requirements for plant growth. Other studies have reported delayed crop yield response to biochar, but these findings were based on rainfed systems (Major et al. 2010). A related report based on the same study showed significant decrease in soil N in the second season, which linked to the yield decline (Majaule et al. 2020). This suggests that rather than annual applications of biochar and sewage sludge, N fertilizers are required to augement the organic amendents.

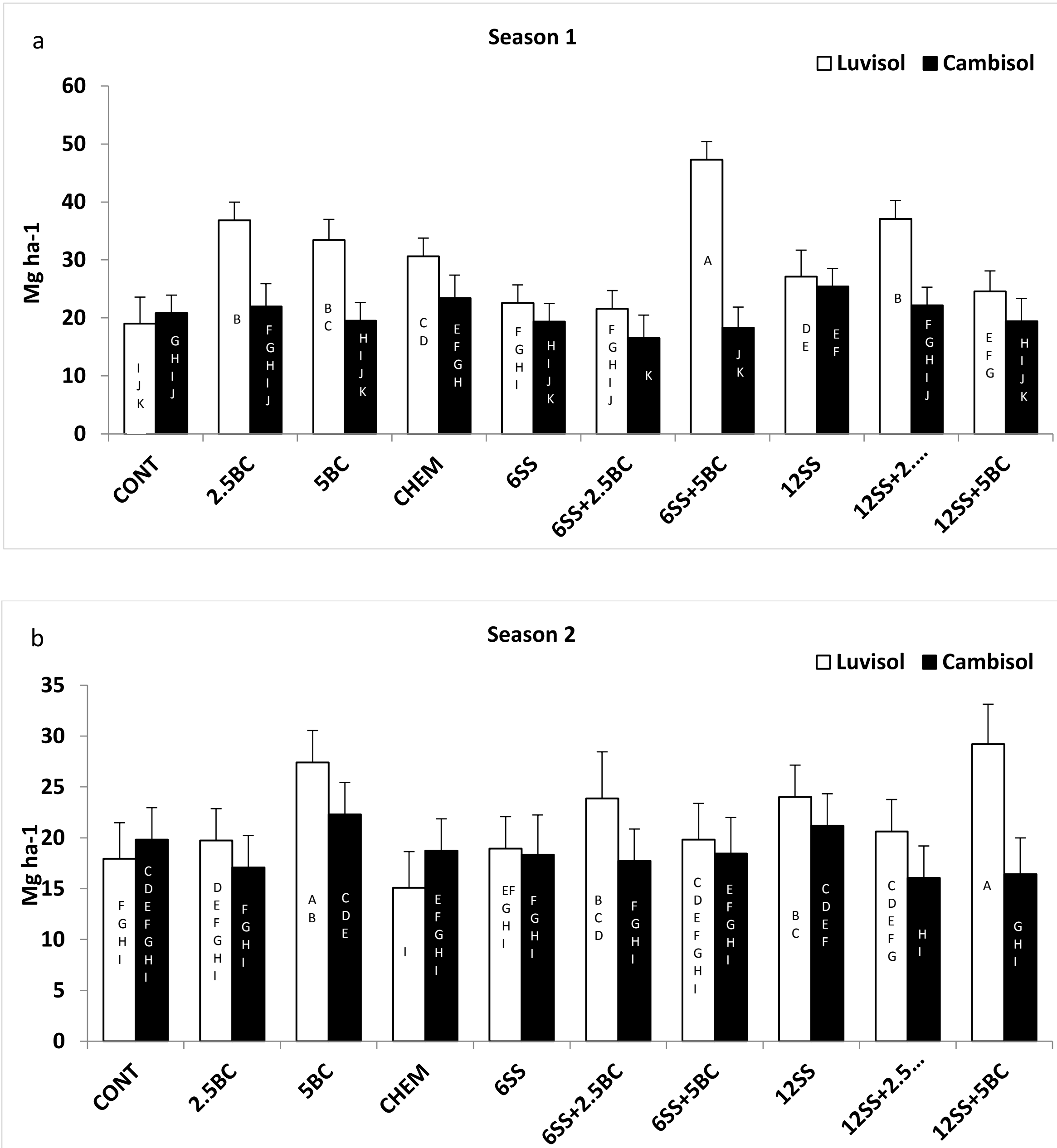


Fig. 4 Spinach yields during two seasons; Season 1 (March – June) and Season 2 (July – Oct) 2018
Error bars denote standard error of the mean (SEM). Columns with different letters are significantly different ($p < 0.05$).

Conclusion

This study has demonstrated that sole sewage sludge and biochar had inconsistent effects on plant P availability and crop yields across soil types. Combined application of amendments on the Luvisol resulted in greater yield response probably due to the lower fertility of this soil type. Yield increase in the first season is presumed to have depleted N, leading to crop yield decrease in the second season for most treatments. The interactive effects of these organic amendments and N fertilizer require further investigation, as well as the potential of biochar to capture P in irrigation wastewater warrants further research.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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