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Effects of biochar and sewage sludge on spinach (*Spinacia oleracea* L.) yield and soil NO_3^- content in texturally different soils in Glen Valley, Botswana

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The effects of biochar and sewage sludge application on spinach (*Spinacia oleracea* L.) yield and soil NO_3^- content were investigated in typical soils of Botswana (Luvisol, Cambisol) under field conditions. Ten treatments with 3 levels of biochar (0, 2.5, 5 tons ha^{-1}) and sewage sludge (0, 6, 12 ton ha^{-1}) were applied in 2 subsequent seasons. Significant ($p < 0.05$) yield increase on the Luvisol occurred if sewage sludge was added at 12 Mg ha^{-1} with or without biochar. A combination of 6 Mg ha^{-1} sludge and 5 Mg ha^{-1} biochar application resulted in the highest crop yield over 2 seasons. On the Cambisol, only marginal yield increase occurred upon high rates of sole organic amendments and chemical fertilizer, while co-applications decreased yields. Decrease in soil NO_3^- content caused yield declines in the second season, while P uptake increased significantly ($p < 0.05$). Correlations between yields, soil NO_3^- and leaf N contents were insignificant ($p > 0.05$). On the Cambisol, a significant regression model for sludge and soil NO_3^- was determined. Therefore, one – time combined application of 6 Mg ha^{-1} sewage sludge and 5 Mg ha^{-1} on the Luvisol, and 12 Mg ha^{-1} sewage sludge are recommended for spinach production on the Luvisol and Cambisol, respectively. In subsequent seasons, crop productivity could be maintained by application of mineral N in order to mitigate over-application of P.

Key words: Biochar, sewage sludge, soil NO_3^-, luvisol, cambisol.

INTRODUCTION

Soil fertilization with sewage sludge is an effective way to recycle nutrients and combat nutrient deficiency in agricultural systems (Sharma et al., 2017). Spinach is one of the most important vegetable crops in Botswana, but good crop yields are constrained by poor soil fertility, especially N and P deficiency. Many studies have reported high spinach yield response to mineral fertilizers and sewage sludge applications (Ngole, 2010; Biemond

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et al., 1996; Wang and Li, 2004; Lefsrud et al., 2007; Stagnari et al., 2007; Rodríguez-Hidalgo et al., 2010; Türkmen* et al., 2004). These studies showed that adequate N availability from sewage sludge is critical for high quality and yields of spinach. When contents of heavy metals, pathogens, and toxic organic compounds in sludge are within the WHO limits, such as the case for the Glen Valley sludge (Ngole, 2010; Mosekiemang and Dikinya, 2012), application rates of sludge to agricultural soils is based on the crop nitrogen (N) demand (Gilmour and Skinner, 1999; Correa et al., 2006).

On dry basis, sewage sludge contains 2 – 6% total N which is predominantly organic (Rigby et al., 2016) hence the rate of N mineralization influences potential plant-available N. In sludge-amended soils, this plant-available N varies between 20 to 63% of organic N in a crop year under field conditions (Magdoff and Amadon, 1980), depending on factors such as sludge application rates, timing, climate, soil properties and moisture dynamics (Weggler-Beaton et al., 2003). Thus, the application rate of sludge should consider the sum of the inorganic N and mineralised organic N in the soil and the added sludge. However, important soil processes such as microbial-mediated immobilization, leaching, ammonia volatilization and denitrification can decrease the amount of the plant available N pool (Clough et al., 2013).

At modest sewage sludge application rates (c.a. 10 tons ha^{-1}), sludge may not provide adequate N for optimum spinach yields because it has a high N demand. In addition, spinach typically prefers NO_3^- because high concentrations of ammonium (NH_4^+) ions can be toxic and suppress both root development and plant growth (Wang et al., 2009). Thus, the relative concentrations of NO_3^- and NH_4^+ in sewage sludge, and the nitrification rates determine N uptake and productivity of spinach. But, excessive levels of NO_3^- in spinach leaves may be noxious to humans (Citak and Sonmez, 2010), while leaching of NO_3^- into groundwater is linked to methemoglobinemia or “blue-baby” syndrome in infants, cancer and spontaneous abortions (Spalding and Exner, 1993). Nitrogen availability from sewage sludge in Botswana was sparsely explored by Ngole (2010) under controlled conditions but the results were confounded by lack of mineral fertilizer comparisons.

Biochar-induced changes of soil properties such as soil pH, cation exchange capacity (CEC), moisture dynamics, and microbial activity may in turn significantly influence N transformation reactions (Nelson et al., 2011; Clough et al., 2013; Anderson et al., 2011). Besides the potential direct N supply, biochar effects on soil organic matter decomposition rates could either decrease or increase organic N mineralization from organic amendments, retention of NH_4^+ on its surfaces, and therefore, soil NO_3^- and NH_4^+ ratio. Biochar-induced N deficiency due to net N immobilization have been linked to high biochar C/N and increased microbial activities (Deenik et al., 2010). Other studies showed that such microbial N immobilization and partly due to high biochar cation exchange capacity (CEC) improved N fertilizer use efficiency and plant productivity (Chan et al., 2008; Steiner et al., 2007). Nitrification rates in biochar-amended soils can either increase or decrease due to the biochar stimulatory or inhibitory effects, respectively (Nelissen et al., 2012; Clough et al., 2013; Zackrisson et al., 1996), with significant implications for spinach growth and yields as it prefers NO_3^- compared to NH_4^+ (Wang et al., 2009).

A growing number of studies showed significant synergistic effects of biochar and N fertilizers on N use efficiency and crop yields (Chan et al., 2008; Adekiya et al., 2019; Partey et al., 2014). Contrastingly, Lentz and Ippolito (2012) found no synergistic effects of hard-wood biochar and cattle manure on corn silage yields and nutrient concentrations, except for Mn. Others reported biochar-induced N deficiency due to N immobilization or decreased nitrification rates (Zheng et al., 2013; Cayuela et al., 2013).

The contrasting results suggest that the effects of biochar on N availability is heterogeneous, depending on biochar and soil properties, application rates for both biochar and organic N fertilizers and other experimental conditions. However, information regarding the combined effects of biochar and sewage sludge fertilization on N uptake and yields of spinach is scarce. We hypothesized that synergistic effects of biochar and sewage sludge application would significantly increase NO_3^- availability and spinach yields. The objective of this study was to determine the effects of co-application of biochar and sewage sludge on soil NO_3^- content, N uptake and spinach yields in typical soils of Botswana.

MATERIALS AND METHODS

Study sites

The experiments were established at Glen Valley, Botswana. A Calcic Luvisol (henceforth called Luvisol) and Vertic Cambisol (henceforth called Cambisol) were selected for the study. Some properties of the surface soils (0–15 cm) are shown in Table 1. The Luvisol was classified as a sandy loam textural class (sand – 73.3%, clay – 16.4% and silt – 10.3%) with a bulk density of 1.6 g cm^{-3}. The Cambisol had sandy clay texture comprising of the following size fractions; sand – 48.5%, clay – 39% and silt – 12.6%), and 1.4 g cm^{-3} bulk density.

Experimental design

The experiment comprised 10 different treatments with 3 replicates and so each site comprised 30 plots (1.8 m x 1.5 m) arranged in a randomized complete block design (RCBD). Table 2 shows the treatments structure and application rates in each season. The spinach (variety; *Fordhook Giant*) crop in the first season was planted in March and harvested in April, 2018. The second harvest was done in June, 2018. Similar amendments were applied before the plots were replanted in the second season with spinach seedlings in July, 2018. Harvests 1 and 2 were done in September and October, 2018, respectively.

Table 1. Pre-crop planting soil properties and basic characteristics of sludge and biochar used in the study (n=3).

Properties	Luvisol	Cambisol	Biochar	Sewage sludge
pH ($CaCl_2$)	7.5 ± 1.5	6.8 ± 1.3	7.7 ± 1.1	6.3 ± 0.3
EC (µS/cm)	54 ± 6	80 ± 11	1124 ± 204	2270 ± 318
CEC (cmolc kg^{-1})	8.4 ± 1.1	26.2 ± 3.2	12± 2.5	38 ± 6
Organic matter (%)	1.83 ± 0.37	2.30 ± 0.55	nd	24.6 ± 2.8
Total carbon (%)	nd*	nd	65.4 ± 5.1	80.1 ± 7.9
Organic Carbon (%)	1.0±0.2	1.8±0.3	nd	nd
Ash content (%)	nd	nd	34.7 ± 3.3	19.9 ± 2.2
Total P (ppm)	103 ± 27	91.3 ± 14.3	824 ± 123	5753 ± 525
Available P (ppm)	42.3 ± 5.1	24.0 ± 4.1	51.3 ± 8.1	272 ± 38
Total N (%)	0.08 ± 0.01	0.04 ± 0.01	1.1 ± 0.6	4.5 ± 1.2
C/N ratio	12.5	45	59	17.8
Exchangeable bases (cmolc kg^{-1})				
Ca	6 ± 2.3	17.4 ± 5.6	128 ± 22	159 ± 47
Mg	2.3 ± 1	8.5 ± 3	34 ± 8	66 ± 13
Na	0.06 ± 0.01	0.17 ± 0.02	3 ± 1	13 ± 4
K	0.06 ± 0.01	0.18 ± 0.07	153 ± 13	51 ± 7
Sand (%)	73.3 ± 8	48.5 ± 5	nd	nd
Clay (%)	16.4 ± 2.9	39 ± 3	nd	nd
Silt (%)	10.3 ± 1	12.6 ± 1.4	nd	nd
Bulk density (g/cm3)	1.60 ± 0.29	1.42 ± 0.66	nd	nd

*nd – Not determined.

Both sites were disc ploughed to about 30 cm depth before the study. Planting rows were constructed using hand-hoes before organic amendments were incorporated and mixed into soil (15 cm). Transplanting was done one day after irrigation. Mineral fertilizer (2:3:2, 22%) was applied by banding during transplanting at 300 kg ha^{-1} (Bok et al., 2006). Urea ammonium sulphate (46% N) was top-dressed on CHEM plots at 200 kg ha^{-1} after 2 weeks of transplanting and after each harvest. The trials were drip-irrigated based on soil moisture conditions for about 2 h per irrigation. In the second season, the plots were cleared of crop residues, but the soil was not ploughed. The planting lines and treatment plots were maintained as during the first season.

Air-dried sewage sludge was collected from the stock piles at the Glen Valley Waste Water Treatment Plant. The sewage sludge was crushed and sieved (2 mm) before analysis and soil application. Biochar was produced from mixed-wood chips via a home-made slow pyrolysis unit (535ºC, 6 h). After cooling, the biochar was air-dried, then mixed thoroughly before crushing and sieving (2 mm). The properties of the biochar (BC) and sewage sludge (SS) used for this study are presented in Table 1. The sludge was enriched in N, P, Ca, Mg and Na relative to the biochar, while the K concentration of biochar was over 3–fold that of sludge (Table 2). The biochar C/N ratio was 64:1, over 3-fold that of the sewage sludge, which indicates a potential for N immobilization during labile biochar C degradation by soil microorganisms. The biochar was characterised by slightly alkaline pH (7.7), high EC (1124 µS/cm), and high contents of available Ca (128 cmol kg^{-1}) and K (153 cmol kg^{-1}), medium concentrations of Mg (34 cmol kg^{-1}) and low contents of Na (3 cmol kg^{-1}) (Table 1).

Soil sampling and analysis

Soil samples (0 – 15 cm) from each plot were collected using the composite sampling procedure at each harvesting stage. Air-dried samples were sieved < 2 mm and analysed in triplicate. Total carbon (TC) of the biochar and sewage sludge was characterized by ashing in muffle furnace at 500°C for 48 h. Exchangeable cations and soil CEC were determined using the ammonium acetate method at pH 7, using a mechanical extractor on a 2.5 g sample (van Reeuwijk, 1993). Exchangeable cations were quantified using a 4210 MP-AES (Agilent Technologies).

The pH of soil and sludge samples was potentiometrically determined in a 1:5 distilled water and 0.01 M $CaCl_2$ solution. Biochar pH was measured on 1 g of sample as described by Wang et al. (2015). The pH values were determined in biochar-to-water ratio of 1:20 (w/v) via an Orion pH meter installed with a glass electrode. Total nitrogen (TN) of soil, sewage sludge, plant and biochar samples was analysed according to the micro-Kjeldahl procedure (van Reeuwijk, 1993). Plant-available phosphorus was determined as described by Ziadi and Tran (2008). Soil bulk density (BD) was determined using 100 cm^3 soil core samplers. Soil particle-size distribution of air-dried samples was measured according to the hydrometer method (van Reeuwijk, 2002). Soil pH and EC were determined at the end of each season while soil bulk density (BD) was measured in the second season only. Soil NO_3^- was quantified according to the Cadmium reduction procedure. Briefly, 3 g thawed soil samples were extracted with 2 M KCl at the soil-to-solution ratio of 1:10 (w/v), while simultaneously determining the moisture factors. The extracts were frozen until they were required for NO_3^- analysis using a Technicon Autoanalyzer II (Technicon Cooperation).

Plant sampling and analysis

The spinach plants were grown for about 60 days from the date of

Table 2. Treatments, their application amounts per season and abbreviations.

Treatment	Sewage sludge (Mg ha^{-1})	Biochar (Mg ha^{-1})	Chemical fertilizer (kg ha^{-1})
CT (Control)*	0	0	0
2.5BC	0	2.5	0
5BC	0	5	0
CHEM (NPK)	0	0	300
6SS	6	0	0
6SS+2.5BC	6	2.5	0
6SS+5BC	6	5	0
12SS	12	0	0
12SS+2.5BC	12	2.5	0
12SS+5BC	12	5	0

*Symbols represent additions of; CT – no amendment (control); 2.5BC – 2.5 ton ha^{-1} biochar; 5BC – 5 ton/ha biochar; CHEM – NPK mineral fertilizer at 300 kg ha^{-1}; 6SS – 6 ton ha^{-1} sewage sludge; 6SS+2.5BC – 6 ton/ha sewage sludge and 2.5 ton ha^{-1} biochar; 6SS+5BC – 6 ton ha^{-1} sewage sludge and 5 ton ha^{-1} biochar; 12SS – 12 ton ha^{-1} sewage sludge; 12SS+2.5BC – 12 ton ha^{-1} sewage sludge and 2.5 ton ha^{-1} biochar; 12SS+5BC – 12 ton ha^{-1} sewage sludge and 5 ton ha^{-1} biochar.

transplanting. Harvesting was done on plot basis. Randomly selected plants were cut at about 5 cm above the soil surface on each plot. Fresh weights of leaves from each plot were recorded at each harvest stage, before oven drying and sieving (2 mm). For total content of P and bases in sludge and plant, 1.25 g of sample was wet digested in 2.5 ml of sulphuric acid-selenium mixture according to van Reeuwijk (2002). Basic cations were determined in the diluted digests via 4210 MP-AES (Agilent Technologies). In the diluted digests, P was measured spectrophotometrically by the indophenol-blue method (van Reeuwijk, 2002). Total P was measured by the method of Murphy and Riley (1962). Determination of the total content of P, K, S, Mg and Ca in biochar was done according to the modified dry-ashing method (Enders and Lehmann, 2012).

Statistical analysis

A 2-way ANOVA was conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC) with significant differences identified at 5%, unless specified otherwise. Mean differences due to treatments were evaluated using least significant difference (LSD) and were ranked according to Duncan's multiple range tests. The Pearson's correlation procedure was used to analyse relationships between variables at 5% level of significance. The values given at each entry for all the parameters, except soil pH, electrical conductivity and bulk density are the average analyses for each of the two separate harvests in each season.

RESULTS

Effects of amendments on soil NO_3^- content

The ANOVA showed that treatments and the interactions between soil type and treatments significantly ($p = 0.0001$) affected soil NO_3^- content. The unamended control on the Luvisol had significantly ($p < 0.05$) lower NO_3^- concentrations compared to the Cambisol in both seasons (Figure 1a and b).

Season 1

During the first season, the control on the Cambisol had higher NO_3^- content (13.1 mg kg^{-1}) compared to the same treatment on the Luvisol (9.2 mg kg^{-1}). These levels are consistent with commonly reported NO_3^- values (10 – 25 mg kg^{-1}) in agricultural soils (Tisdale et al., 1993). Except for application of low rate of sole sewage sludge (6SS), organic amendments significantly ($p < 0.05$) increased NO_3^- on the Luvisol while the effects were insignificant on the Cambisol during the first season. The highest NO_3^- levels during this season were caused by co-application of 6 Mg ha^{-1} sewage sludge and 5 Mg ha^{-1} biochar on the Luvisol (19.6 mg kg^{-1}) while on the Cambisol, sole sewage sludge application at 12 Mg ha^{-1} gave the highest NO_3^- level (15.1 mg kg^{-1}), which also coincided with the highest spinach yields for the respective soils.

Soil NO_3^- content insignificantly ($p > 0.05$) increased with the amount of applied soil amendments on the Luvisol during the first season (Figure 1a). The same trend was observed for sole sewage sludge on the Cambisol, while increasing biochar amount marginally decreased NO_3^- content from 14.1 to 13.2 mg kg^{-1} over the same period. On the Luvisol, sole sewage sludge application significantly increased NO_3^- content relative to the control only when applied at 12 Mg ha^{-1}, but when combined with both rates of biochar, the lower rate of sewage sludge (6 Mg ha^{-1}) resulted in a significant increase in soil NO_3^- (Figure 1a).

Co-application of amendments marginally increased soil NO_3^- compared to both rates of sole amendments on the Luvisol. With regard to the Cambisol, there were no significant ($p > 0.05$) treatment effects on soil NO_3^- content during the first season, but co-applications decreased soil NO_3^- content in comparison to the sole amendments and mineral fertilizer (Figure 1a). Mineral fertilizer (CHEM) did not significantly increase NO_3^- above

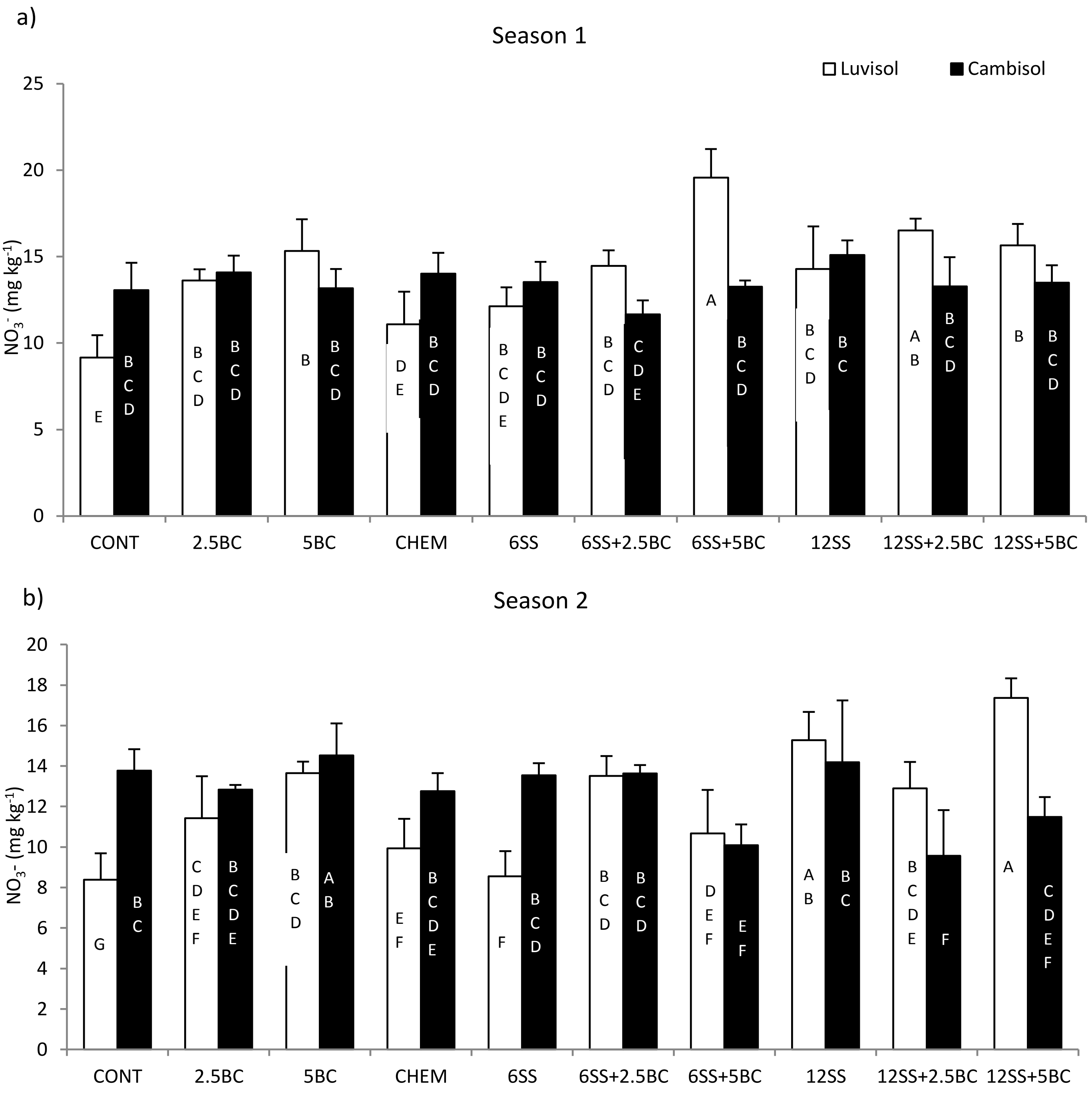

Figure 1. Soil NO_3^- content during two seasons; (a) Season 1 (March – June) and (b) Season 2 (July – Oct) 2018. Error bars denote standard error of the mean (SEM). Columns with different letters are significantly different ($p<0.05$).

the control on both soil types. Interestingly, all combinations of sewage sludge and biochar on the Luvisol significantly increased NO_3^- content relative to mineral fertilizer (Figure 1a), indicating a great potential of these organic amendments to substitute mineral fertilizers (Glaser et al., 2015; Dikinya and Mufwanzala, 2010).

Season 2

During the second season, all the treatments significantly ($p < 0.05$) increased NO_3^- content relative to the control on the Luvisol (Figure 1b). Soil NO_3^- content increased with increasing amount of each organic amendment on both soils, but the differences were only significant

Table 3. Regression functions for relationships between treatments and agronomic parameters.

Variable	Luvisol		Cambisol	
	Sewage sludge	***Biochar***	***Sewage sludge***	***Biochar***
Yield	$y = 1.9x + 17.2$	$y = 3.9x + 15.2*$	$y = 0.9x + 18.2$	$y = 0.58x + 18$
Soil NO_3^-	$y = 1.9x + 10*$	$y = 2x + 9.9$	$y = 0.9x + 13.4*$	$y = -0.8x + 13.4$
Leaf N	$y = 0.06x + 3.9$	$y = 0.08x + 3.8$	$y = 0.03x + 3.5$	$y = -0.03x + 3.5$

*Significant at p = 0.05 level.

($p < 0.05$) for sole sewage sludge on the Luvisol. As can be seen in Table 3, the regressions between NO_3^- content and sewage sludge amount on both soil types are significant ($p < 0.05$), and the regression coefficient is higher on the Luvisol. Conversely, biochar had an insignificant, but positive influence on NO_3^- content on the Luvisol, while on the Cambisol, increasing biochar amount resulted in a decrease for NO_3^-.

Soil NO_3^- content generally decreased during the second season for most of the treatments, on both soil types. Notably, substantial decrease was observed for 6 Mg ha^{-1} sewage sludge plus 5 Mg ha^{-1} biochar (19.6 to 10.7 mg kg^{-1}) on the Luvisol. With the few exceptions where NO_3^- content increased (e.g. 12SS, 12SS+5BC on the Luvisol, and CONT, 5BC, 6SS+5BC on the Cambisol; Figure 1a and b), the differences between seasons were marginal.

Effects of amendments on leaf N content

ANOVA indicated that soil type had significant ($p = 0.0004$) effects on spinach leaf N content, while the effects of treatments, and soil by treatment interactions were insignificant ($p > 0.05$). Generally, the spinach leaf N content in this study is similar to other studies (2 – 5%; Tisdale et al., 1993). In both seasons, treatments maintained statistically similar ($p > 0.05$) leaf N content compared to the control for both soil types (Table 4).

Increasing the amount of sewage sludge on the Luvisol marginally increased leaf N content during the first season, but the same trend did not occur on the Cambisol. Leaf N content generally increased in the second season for the corresponding treatments on both soils, except for co-application of 6 Mg ha^{-1} sewage sludge and 5 Mg ha^{-1} biochar on the Luvisol. Sole biochar at either rate also had no significant effects on leaf N on both soils (Table 4). In general, the effects of factors on leaf N content were marginal as shown by the small regression coefficients for both soil types (Table 3). The effects of biochar were positively related to both NO_3^- and plant leaf N contents on the Luvisol, while on the Cambisol, both parameters decreased with increasing biochar rates, as indicated by negative coefficients of the regression equations.

Effects of amendments on spinach yields

The spinach yield data for the 2 cropping seasons are presented in Figure 2. Treatment effects were significant ($p < 0.0001$; CV = 33.5%) on yield as indicated by the general ANOVA model. Spinach yields in the control plots were similar between seasons. In the first season, all treatments improved yields relative to CONT on the Luvisol, with the greatest yields resulting from 6 Mg ha^{-1} sewage sludge plus 5 Mg ha^{-1} biochar. During the second season, all organic amendments maintained higher yields than control on the Luvisol while CHEM resulted in slightly lower yields relative to the control. On the Cambisol, yields were statistically independent of treatments ($p > 0.05$).

The changes in crop yield and soil NO_3^- between seasons followed contrasting trends on the different soil types. An insignificantly negative correlation ($p = 0.43$: $r^2 = -0.22$) between yield and NO_3^- was determined on the Luvisol, while the correlations on the Cambisol was positive and significant ($p < 0.05$: $r^2 = 0.57$). Decreasing yield during the second season on the Luvisol coincided with decreasing soil NO_3^- content (except 12SS, 12SS+5BC), but marginal yields increases on the Cambisol followed an increasing trend of soil NO_3^- content.

Considering other plant nutrients, the decline in crop yield in the second season as already highlighted above corresponded with increasing content of both leaf P (Table 4) and available P (Table 5). Other studies (Bhattacharjee et al., 1998; Tisdale et al., 1993; Türkmen* et al., 2004) have reported higher spinach leaf mineral composition than observed in our study; hence, this could be a contributing factor to the decreased yields in the second season.

DISCUSSION

Effects of amendments on NO_3^- availability and leaf N content

Soil NO_3^- content in the control was significantly lower than the Luvisol compared to the Cambisol (Figure 1a and b), which confirms the lower N availability in this soil,

Table 4. Effects of amendments on leaf nutrient contents [mg kg^{-1}] of spinach in (A) season 1 and (B) season 2.

Treatment	N		P		Ca		K		Mg	
	A	B	A	B	A	B	A	B	A	B
Luvisol										
CONT	3.07±0.46A	4.99±0.31A	2068±392ABCD	2154±399IJ	55±9AB	94±8B	534±111EF	437±56C	80±6BC	95±8BC
2.5BC	2.78±0.00A	5.23±0.40A	2275±402AB	3297±393DEFG	75±10AB	100±9B	677±127BCDEF	532±73ABC	85±8ABC	96±8BC
5BC	3.12±0.34A	5.44±0.10A	1780±403ABCD	3525±394CDEF	57±8AB	110±10AB	532±54F	559±90ABC	71±9BC	105±89ABC
CHEM	2.78±0.10A	6.21±0.73A	1823±399ABCD	2514±401HIJ	68±7AB	124±13AB	672±72BCDEF	538±110ABC	83±7ABC	131±12AB
6SS	2.91±0.34A	6.28±0.67A	1900±393ABCD	3128±399EFGH	66±8AB	113±13AB	687±98BCDEF	591±59ABC	77±6BC	107±10ABC
6SS+2.5BC	2.63±0.12A	5.05±0.39A	1727±394ABCD	3751±397CDE	72±9AB	111±10AB	693±83BCD	560±92ABC	102±10AB	105±9ABC
6SS+5BC	3.98±0.39A	6.01±0.64A	2002±401ABCD	5136±389A	74±8AB	141±14A	663±129CDEF	652±110AB	91±8ABC	139±13A
12SS	3.32±0.08A	5.47±0.33A	1779±392ABCD	2818±395FGHI	60±7AB	116±14AB	546±96DEF	556±89ABC	82±7ABC	103±13ABC
12SS+2.5BC	2.75±0.00A	5.54±0.07A	1762±399ABCD	2657±392GHI	64±9AB	118±10AB	727±145BC	587±85ABC	97±9ABC	110±9ABC
12SS+5BC	2.84±0.14A	5.74±0.21A	2300±395A	1772±390J	67±8AB	110±10AB	693±123BCD	554±106ABC	89±9ABC	109±9ABC
Cambisol										
CONT	3.70±0.34A	3.84±0.31A	1423±407CD	2747±405GHI	69±8AB	100±9B	826±153AB	605±124AB	86±6ABC	93±9C
2.5BC	3.81±0.44A	3.34±0.25A	1721±386ABCD	3897±402BCD	76±9AB	96±12B	758±120BC	608±119AB	96±9ABC	102±10ABC
5BC	4.12±0.32A	3.61±0.59A	1858±368ABCD	4653±402AB	68±8AB	96±9B	786±97ABC	566±104ABC	80±8ABC	96±10BC
CHEM	3.99±0.00A	3.17±0.40A	2285±397AB	3585±398CDE	61±8AB	109±9AB	737±88BC	675±98A	64±9C	110±10.6ABC
6SS	3.76±0.10A	3.30±0.40A	1299±375D	3575±374CDEF	62±6AB	95±8B	829±134AB	592±104ABC	83±5ABC	96±10BC
6SS+2.5BC	4.04±0.00A	3.54±0.43A	1660±367ABCD	4028±409BCD	71±8AB	112±12AB	717±119BC	633±110AB	83±9ABC	101±8ABC
6SS+5BC	3.90±0.15A	3.54±0.07A	1608±399ABCD	4008±402BCD	65±7AB	102±9B	745±99BC	506±94BC	77±8BC	99±9.7BC
12SS	3.92±0.10A	3.78±0.13A	1883±405ABCD	4210±408BC	54±6B	110±9AB	691±68BCDE	644±98AB	86±7ABC	94±7.9BC
12SS+2.5BC	3.59±0.23A	3.50±0.33A	1488±407BCD	3333±376DEFG	92±6A	121±11AB	924±113A	643±116AB	118±10A	117±12.5ABC
12SS+5BC	3.87±0.10A	3.35±0.53A	2194±386ABC	5200±409A	71±8AB	100±9B	754.6±104BC	612±145AB	62±6C	92±8.9C

*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; $p<0.05$).

and is possibly due to the effects of past management practices and variability in the soil textural properties (Table 1). The Luvisol site was continuously cropped for the previous five years before the inception of the experiments, while the Cambisol was fallow during that time. Thus, exhaustion of mineral N by the crops preceding the trial on the Luvisol may have accounted for the comparatively lower NO_3^- content. Comparatively high soil NO_3^- content in the control of the Cambisol could also be explained by greater mineralization of N from soil organic matter, which was higher on this soil type (Table 4).

In addition, the Luvisol and Cambisol had sandy loam and sandy clay textures, respectively. Therefore, the potential movement of soil NO_3^- that is mineralized from native organic N or contained in the irrigation water down the profile (below the root zone) is higher on the Luvisol. As a result of these differences in NO_3^- content in the control plots, the increase in soil NO_3^- due to application of amendments was greater on the Luvisol.

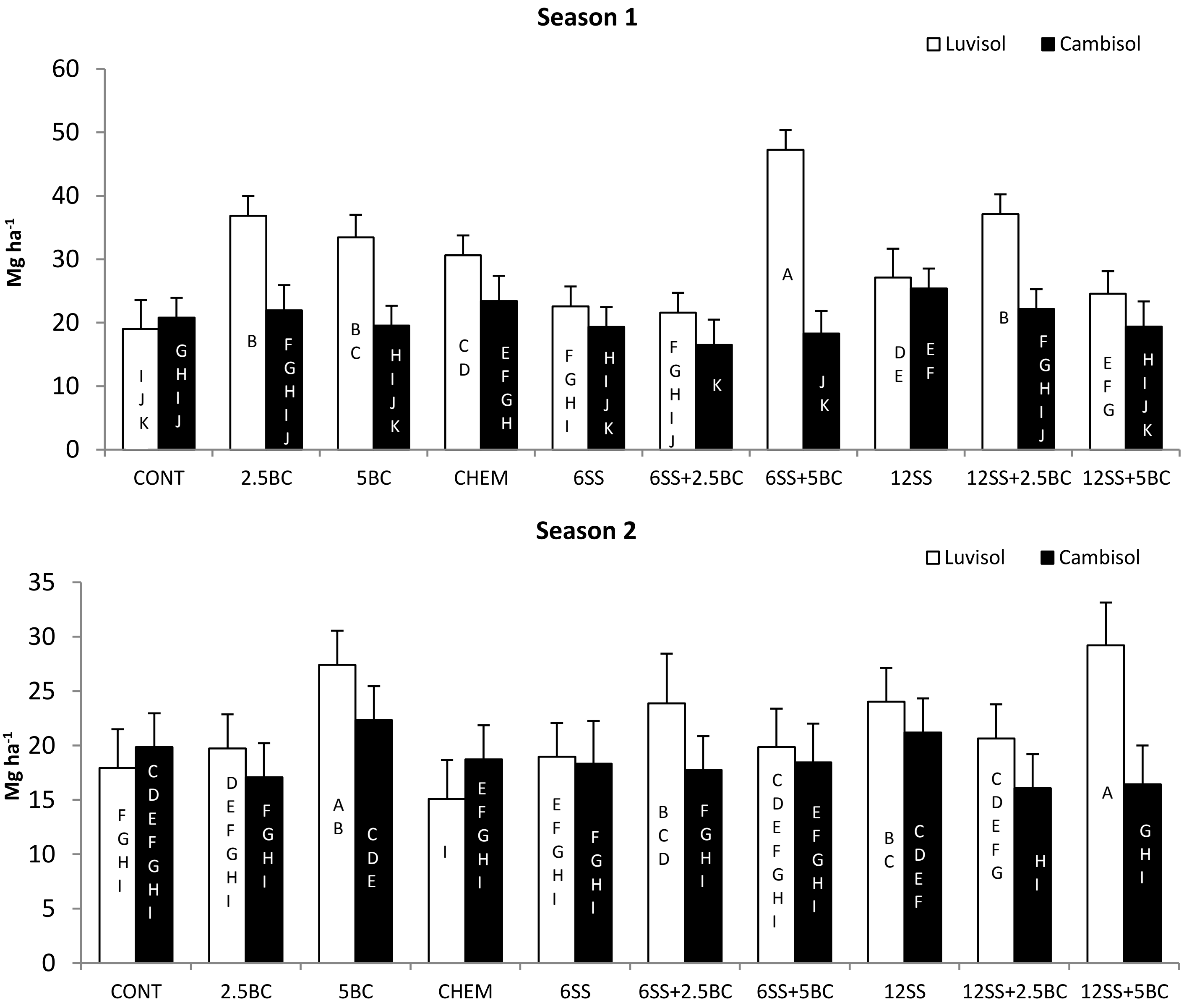

Figure 2. Spinach yields during two seasons; Season 1 (March – June) and Season 2 (July – Oct) 2018. Error bars denote standard error. Columns with different letters are significantly different ($p<0.05$).

Furthermore, organic amendments were more effective in increasing soil NO_3^- relative to application of mineral fertilizer on the Luvisol. In particular, significant treatment effects were detected when biochar was co-amended with sewage sludge compared to sole amendments on the Luvisol, whereas the opposite effects were determined on the Cambisol. These findings can be attributed to a number of reasons. Soil-specific effects of biochar on organic matter decomposition (priming), with potentially greater organic N mineralization on the sandy loam textured Luvisol is expected to play a significant role. The higher clay (39%) and native organic matter (2.3%) contents in the Cambisol could result in occlusion of biochar particles (Zackrisson et al., 1996; Wardle et al., 2008; Brodowski et al., 2006), thus restricting the interactions between biochar and sewage sludge particles. The complexation of humus by clay fraction has been linked to reduced soil C and N mineralization (Amlinger et al., 2003). Therefore, greater biochar and sewage sludge interactions on the Luvisol presumably enhanced mineralization of sludge-borne organic N and consequently higher nitrification of NH_4^+ into NO_3^-. This effect is well known and can be explained by the supply of nutrients, reduced soil bulk density and improved aeration, optimum soil pH and potential biochar sorption of nitrification inhibitory compounds such as terpenes

Table 5. Physicochemical properties of the sites after season 1 (A) and season 2 (B).

Treatment	pH ($CaCl_2$)		Available P (mg kg^{-1})		Organic C (%)		CEC (cmolc kg^{-1})	
	A	B	A	B	A	B	A	B
Luvisol								
CONT	7.7± 1.1A	7.5±2.3AB	28.6±3.3HIJ	53.6±7.1IJ	1.0±0.1DE	0.4±0.0G	8.7±3.1D	8.5±1.6E
2.5BC	7.5±2.3BCD	7.4±1.7ABC	64±8.1CDEF	106±9.8E	1.3±0.3ABCDE	1.0±0.2DEFG	7±2.7D	11±2.1DE
5BC	7.3±1.4D	7.1±0.9EF	87±7.3AB	150±12.6AB	1.0±0.1CDE	2.0±0.6BCD	9±3.1D	15±1.7D
CHEM	7.6±2.1ABC	7.6±1.1A	45± 4.5FGH	81±10.2FG	1.2±0.2ABCDE	0.4±0.0G	8±1.9D	8.5±1.1E
6SS	7.7±1.8A	7.5±1.0AB	33±3.2GHIJ	55±6.5HIJ	1.1±0.1CDE	0.5±0.0FG	7.9±2.3D	7.5±2.1E
6SS+2.5BC	7.3±1.5D	7.3±1.6CDE	81.7±5.4ABCD	133±12.6BCD	1.1±0.1BCDE	1.1±0.1DEFG	10±1.8D	11.5±3.6DE
6SS+5BC	7.4±1.2CD	7.3±2.0BCD	92±6.6A	128.9±13CD	1.5±0.2ABCDE	1.5±0.2CDEFG	8.6±1.4D	10±2.2DE
12SS	7.6±2.3AB	7.5±2.6AB	29.5±5.1HIJ	70±8.9GHI	0.9±0.1E	0.8±0.1EFG	9.2±2.9D	10.5±2.6DE
12SS+2.5BC	7.3±1.5D	7.4±1.9BCD	85±7.9ABC	132±11.2JBCD	1.2±0.4ABCDE	1.2±0.3DEFG	8±2.1D	11±3.4DE
12SS+5BC	7.4±1.8D	7.2±2.2DE	63±5.8DEF	177±22.4A	1.3±0.2ABCDE	2.5±0.5BC	8.4±1.7D	12.7±2.7DE
Cambisol								
CONT	6.7±1.6HI	6.9±1.6GH	15±1.3J	26.5±3.2K	1.7±0.4ABCDE	1.6±0.6BCDEF	26±4.9BC	27.5±4.6ABC
2.5BC	6.9±1.1FGH	6.8±2.1H	20.6±5.2IJ	58±6.6HIJ	1.9±0.3ABCDE	2.6±0.8B	30.6±3.2AB	30±3.5AB
5BC	6.5±1.8I	6.6±1.1I	53±7.9EFG	121.9±19.3DE	2±0.2ABCD	2.6±0.7B	26.5±2.9ABC	29±6.3ABC
CHEM	6.9±2.5FG	6.9±1.0GH	18±4.2IJ	58.6±5.5HIJ	1.8±0.3ABCD	2±0.4BCD	31.8±2.6A	31.8±6.5A
6SS	6.9±1.9FGH	6.9±1.7GH	19±5.4IJ	44.9±3.9JK	2.2±0.5AB	2.1±0.8BCD	31±4.1AB	31±3.9AB
6SS+2.5BC	6.5±0.8I	7±2.1FGH	38.8±6.6GHI	93.5±7.7F	2.1±0.4ABC	2.4±0.4BC	27.9±5.2ABC	32±2.7A
6SS+5BC	6.8±1.3GH	6.8±1.8HI	52.8±8.1EFG	130.7±10.6BCD	2.1±0.5ABC	2.1±0.6BCD	29.7±4.9AB	31±6.2AB
12SS	7±1.8E	7±1.4FG	19.7±3.2IJ	46.8±4.7JK	1.9±0.3ABCDE	1.8±0.5BCDE	24±3.3C	23.6±4.2C
12SS+2.5	7±1.1EF	6.9±1.3GH	37±4.8GHI	75.9±7.2FGH	1.9±0.4ABCDE	1.7±0.4BCDE	27.7±2.8ABC	26±4.4ABC
12SS+5BC	7±1.7EF	6.9±1.6GH	66±8.6BCDE	145.7±10.6BC	2.3±0.6A	3.7±1.1A	29.8±3.3AB	31±2.9AB
				Exchangeable cations (cmolc kg^{-1})				
	Ca		Mg		Na		K	
	A	B	A	B	A	B	A	B
Luvisol								
CONT	106±24C	117±14CD	40±5.5FGH	33.9±2.9G	1.0±0.1CDE	1.4±0.4CDE	1.0±0.0A	1.0±0.1AB
2.5BC	119±28ABC	124±12CD	50.5±6.7DEFG	35.9±4.3G	1.0±0.0CDE	1.4±0.1CDE	1.1±0.0A	1.1±0.1AB
5BC	130±23ABC	120±17CD	55±8.3DEF	33.9±2.7G	1.1±0.0BCDE	0.9±0.3E	1.1±0.2A	0.8±0.0B
CHEM	99±17C	108±10D	38.7±4.4GH	31.6±5.6G	1.0±0.0CDE	1.25±0.1DE	1.0±0.1A	1.0±0.2AB
6SS	102.5±24C	115±16CD	38±5.6H	31.8±3.9G	1.0±0.1CDE	0.9±0.1E	1.0±0.0A	0.9±0.1AB
6SS+2.5BC	104.9±16C	127±11CD	39±6.7GH	42±4.1G	0.9±0.1DE	1.3±0.2DE	1.0±0.0A	1.0±0.1AB
6SS+5BC	119±19.4ABC	121±20CD	48.7±8.2EFGH	34±5.2G	0.9±0.0DE	1.1±0.1DE	1.0±0.3A	1.0±0.0AB

Table 5. contd.

12SS	122±17ABC	132±11CD	52±5.9DEF	35.9±3.9G	1.0±0.0CDE	1.5±0.1BCDE	1.2±0.2A	1.1±0.1AB
12SS+2.5BC	109±19BC	125±10CD	44.5±5.1FGH	32.9±5.7G	1.3±0.2ABCDE	1.2±0.3DE	1.2±0.2A	0.9±0.1AB
12SS+5BC	116±18ABC	156±15BCD	48±6.3EFGH	41±6.2G	0.8±0.1E	1.7±0.1ABCDE	1.1±0.1A	1.1±0.2AB
Cambisol								
CONT	127±22ABC	153±24BCD	62±6.9CD	76±7.4CDEF	1.3±0.2ABCDE	1.8±0.2ABCD	1.2±0.2A	1.4±0.3AB
2.5BC	144±1.9ABC	190±29AB	72±5.4BC	92.5±8.3AB	1.7±0.0ABCD	1.9±0.1ABCD	1.2±0.1A	1.6±0.1AB
5BC	118±17ABC	162±32ABC	56.9±6.6DE	72.7±6.5EF	1.3±0.1ABCDE	1.7±0.1ABCDE	1.1±0.1A	1.4±0.1AB
CHEM	166±21A	200±18AB	85.7±7.8A	95±8.3A	2±0.2A	2.2±0.3ABC	1.4±0.1A	1.7±0.4A
6SS	158±21AB	188±20AB	80.9±10.2AB	85±7.9ABCD	1.8±0.1ABC	1.9±0.2ABCD	1.5±0.2A	1.7±0.3A
6SS+2.5BC	165±17AB	189±31AB	81.7±8.7AB	82±10.6CDE	1.9±0.2AB	2.2±0.2ABC	1.3±0.1A	1.5±0.1AB
6SS+5BC	150±23ABC	202±35AB	74±8.3ABC	92±7.8AB	1.7±0.1ABC	2.2±0.1AB	1.2±0.0A	1.5±0.2AB
12SS	114±19ABC	162±22ABC	55.5±6.9DEF	68±6.9F	1.1±0.0BCDE	1.7±0.3ABCDE	1.3±0.2A	1.5±0.3AB
12SS+2.5	134±18ABC	167±22ABC	62±9.3CD	74.8±5.2DEF	1.2±0.2ABCDE	1.7±0.2ABCDE	1.2±0.2A	1.4±0.4AB
12SS+5BC	151±20ABC	213±24A	71±8.4BC	88±10.1ABC	1.7±0.1ABC	2.3±0.4A	1.3±0.1A	1.7±0.5A

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

(Zackrisson et al., 1996).

Furthermore, biochar has greater effects on improvement of water retention on sandy soils than clay soils (Biederman and Harpole, 2013). This effect can result in higher responses in microbial decomposition and mineralization of sludge N on the Luvisol. The expected improvement in water retention could reduce leaching losses of soil NO_3^-, and this effect is likely to be more pronounced on the Luvisol because of its coarse texture, hence co-application of amendments had more NO_3^- content compared to sole sewage sludge treatments (Figure 1a and b).

Soil NO_3^- content generally decreased for most treatments in the second season. Significant ($p < 0.05$) differences were determined for application of sole sewage sludge at 6 Mg ha^{-1} on the Luvisol, or in combination with 5 Mg ha^{-1} on both soils. During the first season, both the highest leaf N (Table 5) and yields (Figure 2a) on the Luvisol were determined for combination of 6 Mg ha^{-1} sewage sludge plus 5 Mg ha^{-1} biochar. Thus, the significant decrease in NO_3^- content in the second season for 6SS+5BC could be a direct result of greater plant N assimilation which accounted for high crop yields during the first season. On the Cambisol, the decrease in soil NO_3^- content was also statistically significant for co-application of 2.5 Mg ha^{-1} biochar and 12 Mg ha^{-1} sludge (Figure 1b).

Such decreasing trends in NO_3^- content could be attributed to several factors including the variability between agro-climatic conditions between seasons (Figure 3a and b) as shown by the statistically significant ($p < 0.05$) seasonal effects from the ANOVA. Total rainfall in the first and second seasons as determined from a nearby weather station at Sebele was 160 and 24 mm, respectively. The mean minimum and maximum temperature was 16.2 and 31.3ºC, respectively during the first month (March) after application of organic amendments. On the other hand, these attributes were 4.5 and 21.6ºC in the second season.

Several studies have been conducted which indicate that under warm moist conditions, sewage sludge organic N mineralization is more than in low temperatures (Sierra et al., 2001; Magdoff and Amadon, 1980; Barbarika et al., 1985). N mineralization rates in the first month after sewage sludge application is critical as it precedes the period of high spinach N demand and is usually supplied via top dressing with mineral N fertilizers. Thus, the warmer and humid climatic conditions in the first season potentially contributed to the higher N mineralization of sludge N resulting in high crop yields than in the following season. Furthermore, these results indicate that a significant proportion of the sewage

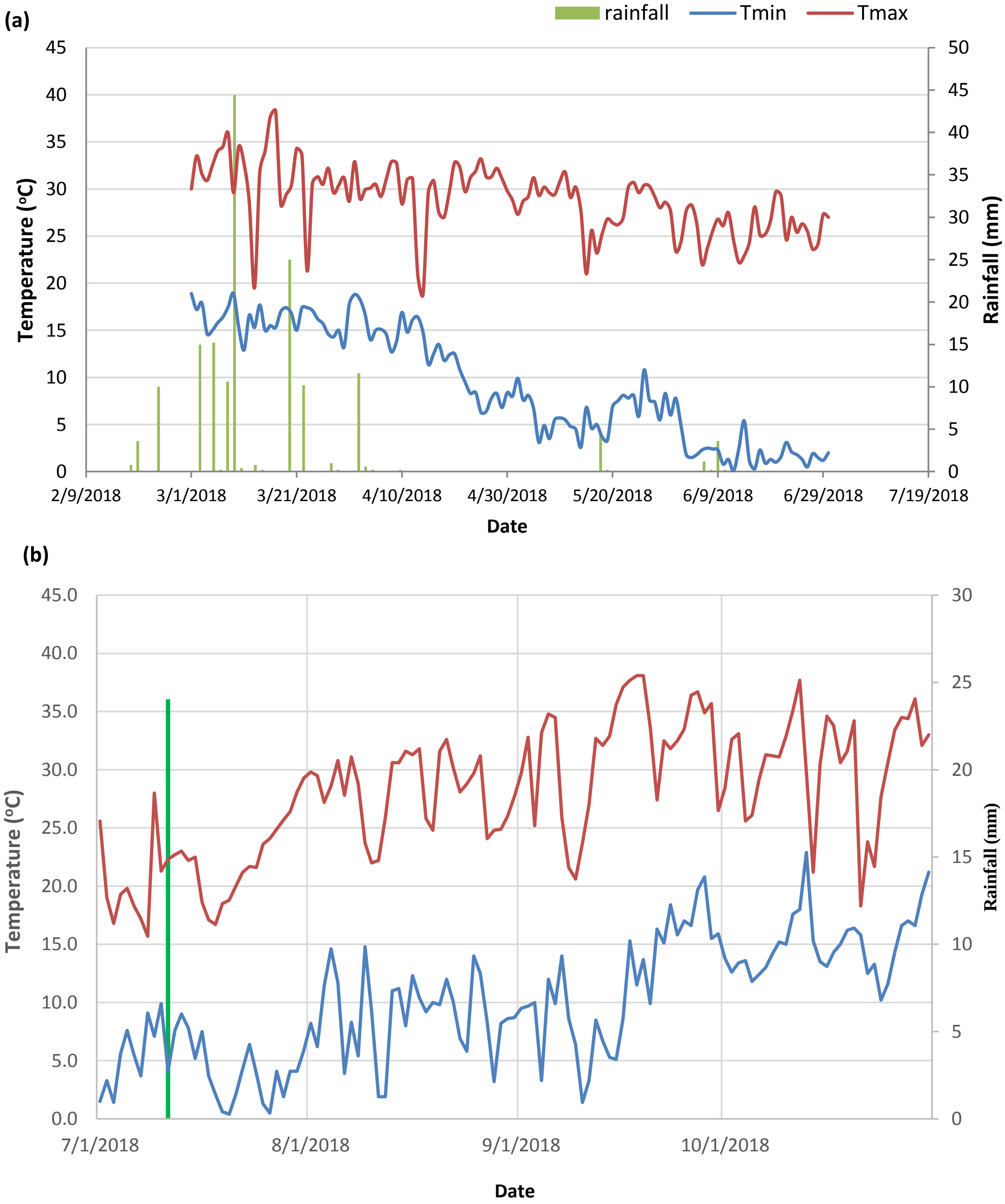

Figure 3. Precipitation and temperature variability during (a) season 1 and (b) season 2. TMPmax; Maximum temperature, TMPMin; Minimum temperature.

sludge used in this study was organic (Magdoff and Amadon, 1980) which required mineralization before plants N assimilation.

Moreover, it is worth considering that before the trial was established in March 2018, the land was disc ploughed whereas prior to the second season, reduced tillage was applied to retain the planting lines and plots. The differences in mechanical aeration of the soil due to tillage possibly contributed to higher decomposition of sewage sludge and biodegradable biochar C in the first season, leading to accumulation of N into the labile microbial N pool. Although N immobilization can result in reduced yields (Deenik et al., 2010), in a wet season such as the March – June period in this study, it probably mitigated NO_3^- leaching potential, which possibly resulted in higher N efficiency. However, from a long-term field trial in Gottingen, Hoffmann et al. (1997) concluded that minimal tillage systems increased N – net mineralisation compared with conventional tillage systems, mainly due to the greater microbial biomass under reduced tillage. More research is therefore required to determine the factor(s) with overriding effects on sewage sludge N mineralization under Botswana conditions for development of sewage sludge management guidelines on agricultural soils.

There was insignificant correlation ($p > 0.05$) between soil NO_3^- and leaf N contents. While soil NO_3^- generally decreased among the majority of treatments in the second season, leaf N increased for all the treatments (Table 5). This disparity can be attributed to the dilution effects of greater yields in the first season. Furthermore, lower crop yields in the second season probably increased leaf N content to sufficiency levels (3%) and above compared to the first season.

Effects of amendments on spinach yield

Table 3 shows that applications of sole organic amendments had greater effects on spinach yields on the Luvisol than on the Cambisol as shown by the higher slope of the regression equations. Moreover, application of biochar on the Luvisol significantly ($p < 0.05$) increased spinach yield, while the relationship on the Cambisol was insignificant. Crop yields across the two seasons also indicate greater yield response on the sandy loam textured Luvisol, and these responses were better than under mineral fertilizer (Figure 2a and b). Contrastingly, yield responses to organic amendments on the Cambisol were similar to mineral fertilizer. Studies on biochar effects on spinach productivity are lacking, but the results of this study support findings by Boersma et al. (2017). In their study, *Eucalyptus polybractea* biochar application to a fertile red Ferrosol did not increase yields of several vegetable crops. These results demonstrate the greater prospects of organic amendments in improving the crop productivity of degraded sandy soils which are prevalent in the tropics.

Mean yield data across the two seasons (data not shown) indicates significant synergistic effects of biochar and sewage sludge on the Luvisol for most of the treatments but no significant complimentary effects on the Cambisol. That there was significant ($p < 0.05$) yield increase from combined application of high rates of amendments on the Luvisol in the second season, while yield declined for the majority of the treatments (Figure 2a), is evidence of the synergistic effects of the amendments on the relatively infertile soil. Contrasting results were reported in a comparative short-term pot study in Zimbabwe by Gwenzi et al. (2016). Working on a clayey soil, the authors reported synergistic effects of sewage sludge and its biochar amended at 15 Mg ha^{-1} in increasing maize biomass yields, while sole biochar application without mineral fertilizer was less effective in increasing biomass yields. In our study, sole biochar application on the clay soil had more positive influence on yields than combination of biochar and sewage sludge. The difference in performance of amendments with their results for a soil type with similar texture to the Cambisol can be attributed to the variability in experimental conditions (Glaser et al., 2015).

Crop yields were statistically independent from soil NO_3^- content ($p > 0.05$), which presumably was caused by high coefficient of variation of the yield data (CV = 33.5%). Nonetheless, the decrease in yields on the Luvisol closely followed the same trend as soil NO_3^- and leaf P contents between seasons (Figure 1a and Table 5), while leaf N increased. These data demonstrate that soil NO_3^- content was the limiting factor for yields in this study because available P increased above the critical range of 45 – 50 ppm (Ziadi and Tran, 2008) between seasons. Comparison of yield data between the two soil types indicates that there are more beneficial effects of application of organic amendments on the Luvisol than on the Cambisol.

Distinctly, 12SS+5BC consistently increased soil NO_3^- and plant N contents, and yields ($p < 0.05$) between seasons on the Luvisol (Figures 1 and 2). The average yields for 12SS+5BC (26.9) was less than that for 6SS+5BC (33.6 ton ha^{-1}), hence there is no added benefit of increasing the amount of sewage sludge combined with 5 Mg ha^{-1} biochar from 6 to 12 ton ha^{-1}. Also, available P increased well above the critical level to 177 mg kg^{-1} under 12SS+5BC (Table 3) on the Luvisol. Although P is not toxic and less mobile compared to NO_3^- in the environment, its potential loss into the nearby Notwane River could result in eutrophication and degradation of the aquatic life.

Biotic and abiotic oxidation of biochar surfaces increases the CEC (Liang et al., 2006; Glaser et al., 2000; Wiedner et al., 2015), and this presumably retained significant levels of ammonium (NH_4^+), thus suppressing nitrification (Clough et al., 2013; Nelson et al., 2011). This effect is hypothetically greater on the Cambisol due to its high clay content (Amlinger et al., 2003). Spinach typically prefers NO_3^- to NH_4^+ (Wang et al., 2009). Thus, in maintaining a relatively small pool of NO_3^-, formation and assimilation by spinach is suppressed, which could account for the yield declines in the second season for most treatments on both soils, specifically the biochar-amended treatments, because elevated levels of NH_4^+ can be toxic to aerobic plants and suppress both root development and plant growth (Wang et al., 2009; Deenik et al., 2010). Therefore, the decline in the yields under co-applications (Figure 3b) might be attributed to NH_4^+ toxicity in biochar treated plots, since the other nutrients were in adequate supply (Table 3). Biochar addition of 5 Mg ha^{-1} on the Luvisol significantly reduced spinach yields (mean = 23 Mg ha^{-1}) compared to application of biochar at 2.5 ton ha^{-1} (mean = 28 Mg ha^{-1}), emphasizing the possibility of greater NH_4^+ accumulation under higher sole biochar application.

High yields for a combination of intermediate sewage sludge (6 Mg ha^{-1}) and biochar applications (5 Mg ha^{-1}) during the first season depleted soil NO_3^-, leading to decreased yields the following season. This hypothesis and the likely low mineralization rates of sludge organic N during second season due to cooler temperatures played a significant role in decreasing yields relative to the first season. Since available P was in adequate supply, crop yields on the Luvisol could be sustained by additional mineral N fertiliser instead of annual addition of sewage sludge to prevent excessive levels of P in the environment. This is true across all the treatments because P reached the critical level on this soil type for most of the treatments.

Conclusion

Overall, the results showed that co-application of biochar-sewage sludge had statistically similar effects on soil NO_3^- content on both soil types. Further, the improvement in N bioavailability and yields was greater on the Luvisol, while on the Cambisol, the effects of organic amendments were similar to mineral fertilizer. The decline in spinach yields in the second season was linked to the decrease in soil NO_3^- because biochar – sewage sludge addition supplied adequate P for spinach growth and leaf P reached sufficiency levels in the second season. Available P, SOC, CEC, soil bulk density, exchangeable bases all improved due to organic amendments. However, leaf micronutrients levels were comparatively lower than those reported for spinach in other studies, which should be the subject of future research. Therefore, Glen Valley farmers can reduce their fertilizer costs by using 5 Mg ha^{-1} of biochar on both soils, or combined application of 6 Mg ha^{-1} sewage sludge plus 5 Mg ha^{-1} biochar on the Luvisol. However, to prevent excess application of P, one-time application of organic amendments followed by mineral N fertilizer is necessary to maintain crop yields.

CONFLICTS OF INTERESTS

The authors have not declared any conflicts of interests.

REFERENCES

Adekiya AO, Agbede TM, Aboyeji CM, Dunsin O, Simeon VT (2019). Effects of biochar and poultry manure on soil characteristics and the yield of radish. Scientia Horticulturae 243:457-463.

Amlinger F, Götz B, Dreher P, Geszti J, Weissteiner C (2003). Nitrogen in biowaste and yard waste compost: dynamics of mobilisation and availability—a review. European Journal of Soil Biology 39(3):107-116.

Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR (2011). Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54(5-6):309-320.

Barbarika A, Sikora LJ, Colacicco D (1985). Factors Affecting the Mineralization of Nitrogen in Sewage Sludge Applied to Soils. Soil Science Society of America Journal 49(6):1403-1406.

Bhattacharjee S, Dasgupta P, Paul AR, Ghosal S, Padhi KK, Pandey LP (1998). Mineral element composition of spinach. Journal of the Science of Food and Agriculture 77(4):456-458.

Biederman LA, Harpole WS (2013). Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy 5(2):202-214.

Biemond H, Vos J, Struik P (1996). Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 3. Spinach. NJAS Wageningen Journal of Life Sciences 44(3):227-239.

Boersma M, Wrobel-Tobiszewska A, Murphy L, Eyles A (2017). Impact of biochar application on the productivity of a temperate vegetable cropping system. New Zealand Journal of Crop and Horticultural Science 45(4):277-288.

Bok I, Madisa M, Machacha D, Moamogwe M, More K (2006). Manual for vegetable production in Botswana. Ministry of Agriculture, Gaborone.

Brodowski S, John B, Flessa H, Amelung W (2006). Aggregate-occluded black carbon in soil. European Journal of Soil Science 57(4):539-546.

Cayuela ML, Sánchez-Monedero MA, Roig A, Hanley K, Enders A, Lehmann J (2013). Biochar and denitrification in soils: when, how much and why does biochar reduce N 2 O emissions? Scientific Reports 3:1732.

Chan KY, Van Zwieten L, Meszaros I, Downie A, Joseph S (2008). Agronomic values of greenwaste biochar as a soil amendment. Soil Research 45(8):629-634.

Citak S, Sonmez S (2010). Effects of conventional and organic fertilization on spinach (Spinacea oleracea L.) growth, yield, vitamin C and nitrate concentration during two successive seasons. Scientia horticulturae 126(4):415-420.

Clough T, Condron L, Kammann C, Müller C (2013). A Review of Biochar and Soil Nitrogen Dynamics. Agronomy 3(2):275-293.

Correa R, White R, Weatherley A (2006). Risk of nitrate leaching from two soils amended with biosolids. Water Resources 33(4):453-462.

Deenik JL, McClellan T, Uehara G, Antal MJ, Campbell S (2010). Charcoal volatile matter content influences plant growth and soil nitrogen transformations. Soil Science Society of America Journal 74(4):1259-1270.

Dikinya O, Mufwanzala N (2010). Chicken manure-enhanced soil fertility and productivity: Effects of application rates. Journal of Soil Science and Environmental Management 1(3):46-54.

Enders A, Lehmann J (2012). Comparison of wet-digestion and dry-ashing methods for total elemental analysis of biochar. Communications in soil science and plant analysis 43(7):1042-1052.

Gilmour JT, Skinner V (1999). Predicting plant available nitrogen in land-applied biosolids. Journal of Environmental Quality 28(4):1122-1126.

Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W (2000). Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. Organic Geochemistry 31(7-8):669-678.

Glaser B, Wiedner K, Seelig S, Schmidt H-P, Gerber H (2015). Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. Agronomy for Sustainable Development 35 (2):667-678.

Gwenzi W, Muzava M, Mapanda F, Tauro TP (2016). Comparative short-term effects of sewage sludge and its biochar on soil properties, maize growth and uptake of nutrients on a tropical clay soil in Zimbabwe. Journal of Integrative Agriculture 15(6):1395-1406.

Hoffmann C, Platte H, Lickfett T, Koch HJ (1997). Microbial biomass and N mineralization in relation to N supply of sugar beet under reduced tillage. Zeitschrift für Pflanzenernährung und Bodenkunde 160(2):187-193.

Lefsrud MG, Kopsell DA, Kopsell DE (2007). Nitrogen levels influence biomass, elemental accumulations, and pigment concentrations in spinach. Journal of Plant Nutrition 30(2):171-185.

Lentz R, Ippolito J (2012). Biochar and manure affect calcareous soil and corn silage nutrient concentrations and uptake. Journal of Environmental Quality 41(4):1033-1043.

Liang B, Lehmann J, Solomon D, Kinyangi J, Grossman J, O'neill B, Skjemstad J, Thies J, Luizao F, Petersen J (2006). Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal 70(5):1719-1730.

Magdoff F, Amadon J (1980). Nitrogen Availability from Sewage Sludge 1. Journal of Environmental Quality 9 (3):451-455.

Mosekiemang T, Dikinya O (2012). Efficiency of chelating agents in retaining sludge-borne heavy metals in intensively applied agricultural soils. International Journal of Environmental Science and Technology 9(1):129-134.

Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36.

Nelissen V, Rütting T, Huygens D, Staelens J, Ruysschaert G, Boeckx P (2012). Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biology and Biochemistry 55:20-27.

Nelson N, Agudelo S, Yuan W, Gan J (2011). Nitrogen and phosphorus availability in biochar-amended soils. Soil Science 176(5):218-226.

Ngole V (2010). Variations in sludge effects on selected properties of four soil types and vegetable yield. African Journal of Agricultural Research 5(23):3279-3290.

Partey ST, Preziosi RF, Robson GD (2014). Short-term interactive effects of biochar, green manure, and inorganic fertilizer on soil properties and agronomic characteristics of maize. Agricultural Research 3(2):128-136.

Rigby H, Clarke BO, Pritchard DL, Meehan B, Beshah F, Smith SR, Porter NA (2016). A critical review of nitrogen mineralization in biosolids-amended soil, the associated fertilizer value for crop production and potential for emissions to the environment. Science of the Total Environment 541:1310-1338.

Rodríguez-Hidalgo S, Artés-Hernández F, Gómez PA, Fernández JA, Artés F (2010). Quality of fresh-cut baby spinach grown under a floating trays system as affected by nitrogen fertilisation and innovative packaging treatments. Journal of the Science of Food and Agriculture 90(6):1089-1097.

Sharma B, Sarkar A, Singh P, Singh RP (2017). Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. Waste Management 64:117-132.

Sierra J, Fontaine S, Desfontaines L (2001). Factors controlling N mineralization, nitrification, and nitrogen losses in an Oxisol amended with sewage sludge. Soil Research 39(3):519-534.

Spalding RF, Exner ME (1993). Occurrence of nitrate in groundwater-a review. Journal of Environmental Quality 22(3):392-402.

Stagnari F, Di Bitetto V, Pisante M (2007). Effects of N fertilizers and rates on yield, safety and nutrients in processing spinach genotypes. Scientia Horticulturae 114(4):225-233.

Steiner C, Teixeira WG, Lehmann J, Nehls T, de Macêdo JLV, Blum WEH, Zech W (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant and Soil 291(1-2):275-290.

Tisdale SL, Nelson WL, Beaton JD, Havlin JL (1993) Soil fertility and fertilizers. 5th ed. edn. Macmillan.

Türkmen* Ö, Sensoy S, Dursun A, Turan M (2004). Sewage sludge as a substitute for mineral fertilization of spinach (Spinacia oleraceae L.) at two growing periods. Acta Agriculturae Scandinavica, Section B-Soil and Plant Science 54(2):102-107.

van Reeuwijk L (1993) Cation exchange capacity (CEC) and exchangeable bases (ammonium acetate method). In: Centre ISRaI (ed) Procedures for soil analysis. 6th edn. International Soil Reference and Information Centre, Wageningen, pp. 9.1-9.11.

van Reeuwijk L (2002) Particle Size Analysis. In: Technical Paper 9: Procedures for Soil Analysis International Soil Reference and Information Centre, Wageningen, The Netherlands, pp. 3-9.

Wang J, Zhou Y, Zhou C, Shen Q, Putheti R (2009). Effects of NH4+-N/NO_3--N ratios on growth, nitrate uptake and organic acid levels of spinach (Spinacia oleracea L.). African Journal of Biotechnology 8(15).

Wang S, Gao B, Zimmerman AR, Li Y, Ma L, Harris WG, Migliaccio KW (2015). Physicochemical and sorptive properties of biochars derived from woody and herbaceous biomass. Chemosphere 134:257-262.

Wang Z, Li S (2004). Effects of nitrogen and phosphorus fertilization on plant growth and nitrate accumulation in vegetables. Journal of Plant Nutrition 27(3):539-556.

Wardle DA, Nilsson M-C, Zackrisson O (2008). Fire-derived charcoal causes loss of forest humus. Science 320(5876):629-629.

Weggler-Beaton K, Graham RD, McLaughlin MJ (2003). The influence of low rates of air-dried biosolids on yield and phosphorus and zinc nutrition of wheat (Triticum durum) and barley (Hordeum vulgare). Soil Research 41(2):293-308.

Wiedner K, Fischer D, Walther S, Criscuoli I, Favilli F, Nelle O, Glaser B (2015). Acceleration of biochar surface oxidation during composting? Journal of Agricultural and Food Chemistry 63(15):3830-3837.

Zackrisson O, Nilsson M-C, Wardle DA (1996). Key ecological function of charcoal from wildfire in the Boreal forest. Oikos:10-19.

Zheng H, Wang Z, Deng X, Herbert S, Xing B (2013). Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. Geoderma 206:32-39.

Ziadi N, Tran TS (2008) Mehlich 3-Extractable Elements. In: Carter MR, Gregorich EG (eds) Soil sampling and methods of analysis. 2nd edn. CRC Press, Florida, pp. 81-88.