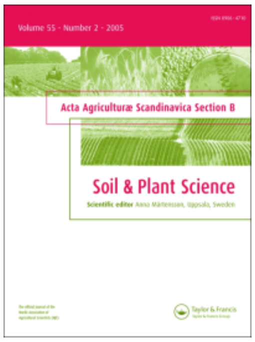

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Biological N_2 fixation, C accumulation and water-use efficiency ($\delta^{13}C$) of chickpea grown in three different soil types: response to the addition of biochar from poultry litter and acacia

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ABSTRACT

Adding biochar to soil can alter soil properties and thus affect plant growth; however, the effect of biochar on biological nitrogen fixation, carbon accumulation and water-use efficiency of chickpea in tropical soils is not fully understood. Therefore, this study assessed the efficacy of biochar derived from poultry litter (denoted as PLB) and acacia (denoted as ACB) feedstocks on biological nitrogen fixation, carbon accumulation and water-use efficiency ($\delta^{13}C$) of chickpea grown in three contrasting soils of Fernwood (Arenosol) and Griffin (Helvic Acrisol) and Pinedene (Gleyic Acrisol). The biochars were applied at the rate of 0.5%, 1% and 2% (w/w) with control (0%) and replicated four times. Chickpeas grown in PLB treatments in Griffin and Pinedene soils investigated accumulated more N and C, for greater biomass production, resulting in an average total N-fixed of 77 and 52 mg N/plant, respectively. Nitrogen fixation and carbon accumulation of chickpea increased by the addition of 0.5% PLB and ACB in the Fernwood soil. The findings of this study demonstrate the potential of improving N inputs through biological nitrogen fixation with poultry litter biochar application in soils with varying nutrient status and texture, which is important in arid environments with limited N inputs.

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Biochar; biomass; chickpea; nitrogen fixation; nodulation; nutrient availability

Introduction

The agronomic added value of leguminous plants in cropping systems lies in their ability to fix atmospheric nitrogen through the biological nitrogen fixation (BNF) process, thus reducing the use of costly nitrogen fertiliser in enhancing soil fertility (Hiama et al. 2019). Nitrogen addition to soils through BNF is considered as a cheap, sustainable and environmentally friendly compared to that supplied by synthetic N-fertilisers. However, the sustainability of BNF depends on factors such as the soil type, fertility of the soil, association between host plant and rhizobia, as well as the potential influence of and presence or absence of inputs such as fertilisers or biofertilisers in the establishment of the symbiotic legumes (Mpai and Maseko 2018). Studies have shown that the dependence on N_2 fixation and total N-fixed is generally lower when legumes are grown in nutrient-deficient soils and largely high in fertile cropping fields (Mohale et al. 2014; Güereña et al. 2015; Hiama et al. 2019). This means that nutrient availability is proportional to nitrogen fixation in legumes.

Biological N_2 fixation may be improved by applying lime to reduce soil acidity (Rondon et al. 2007; Hiama et al. 2019; Khan et al. 2020) and fertilisers to reduce key nutrient deficiencies such as phosphorus and by enhancing the potential of rhizobia-legume symbiosis (Thies and Rilling 2012). Biochar technology has been proposed as an alternative strategy to improve soil edaphic properties (Rondon et al. 2007; Mia et al. 2014), enhance soil biological processes (Lehmann and Joseph 2015) and crop performance, as also shown in South Africa (Sika and Hardie 2014; Lusiba et al. 2017; Macil et al. 2017; Lusiba et al. 2018). Evidence that the application of biochar influences the symbiotic performance of legumes and BNF has been provided (Rondon et al. 2007; Mia et al. 2018; Hiama et al. 2019; Khan et al. 2020). In addition, studies have shown that biochar is an excellent support material for Rhizobium inoculants (Quilliam et al. 2013; Macil et al. 2017).

The possible reasons behind the observed effect of biochar on BNF include increased availability of macro- and micro-nutrients such as P, B and Mo in soils (Rondon et al. 2007; Tagoe et al. 2008; Mia et al. 2014).

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Biochar's liming potential may alter soil pH and have a positive or negative effect on BNF (Lehmann et al. 2011; Khan et al. 2020). Changes in nutrient cycling processes such as N-mineralisation or immobilisation may increase or decrease the N-fixing (Bruun et al. 2011; Nelissen et al. 2012; Güereña et al. 2015). Available biochar compounds such as volatile matter (VM) may stimulate or reduce soil microbial activity, thereby affecting the amount of N-fixed by legumes (Lehmann and Joseph 2015). Although BNF is enhanced by biochar application, reported results are contradictory and inconclusive (Rondon et al. 2007; Quilliam et al. 2013; Mia et al. 2014; Güereña et al. 2015). In addition, the reported findings indicated a variation in BNF at different biochar application rates. For example, Rondon et al. (2007) reported higher BNF by common bean at 100 t/ha biochar application in a clay loam, Oxisol. Quilliam et al. (2013) reported a reduction in nodulation of red clover with elevated application rates of 25 and 50 t/ha, even though nitrogenase activity remained unchanged in a sandy clay loam, Cambisol. Güereña et al. (2015) reported higher BNF by common bean at 15 t/ha biochar application in a humic, Acrisol. This shows that BNF is largely dependent on soil texture; hence, biochar characteristics, soil nutrient status, biochar application rates, soil type and legume crop response could account for the positive and negative responses on BNF (Rondon et al. 2007;Quilliam et al. 2013; Güereña et al. 2015).

This clearly demonstrates that the reasons/mechanism associated with the change in BNF with biochar application remains hypothetical and further studies are crucial for a better understanding of the relationship between biochar properties and BNF. Generally, the knowledge about the effect of biochar (especially from poultry litter) and the dependence of grain legumes such as chickpea on N_2 fixation and their N contribution under semi-arid environment is very limited and there is no published literature currently. Investigation of such a research idea could reveal whether there is a dynamic and process interdependency between the grain legumes particularly chickpea and N-fixed through atmospheric N_2 fixation. Moreover, it is also important to understand whether N from biochar and symbiotic chickpea contributes to the N economy of different types of soils.

Chickpea (*Cicer arietinum L.*) is the third widely grown legume in the world but it is under researched and under-utilised in African countries (FAO 2014; Mpai and Maseko 2018). Chickpea was introduced in South Africa a decade ago, particularly in the provinces of Limpopo and Mpumalanga. However, poor nodulation has been reported, partly due to a lack of, or insufficient populations of effective native rhizobia in the soil, despite the use of biochar derived from *Acacia nilotica Delile and Eucalyptus obliqua* feedstocks (Macil et al. 2020, 2017). This suggests that more research is essential to investigate the effect of biochar derived from different feedstocks and application rates on BNF of chickpea in South Africa, as majority of the studies were done elsewhere using crop residues feedstocks (Rondon et al. 2007: Mia et al. 2014; Güereña et al. 2015) and hardly on animal litter feedstock (Tagoe et al. 2008). Moreover, there is a need for more studies that will evaluate the effect of biochar derived from poultry litter on BNF in tropical soils.

Although, chickpea has been shown to fix about 29–45 kg N/ha in Ethiopia (Meleta and Abera 2019), 40–50 kg N/ha in Spain (López-Bellido et al. 2011) and 47–78% N/ ha in Iran (Biabani et al. 2011), demonstrating its potential to alleviate nitrogen in environments with limited nitrogen availability and thereby contributing to soil fertility. However, agronomic factors such as crop management, soil acidity, low phosphorus availability and moisture stress, on the other hand, have the potential to limit the crop's maximum BNF and yield (Khan et al. 2020). Similarly, soil nutrient status and water deficit affect carbon nutrition in plants, as these factors can alter CO_2 fixation and overall plant growth. With increasing concerns about water scarcity threatening agricultural sustainability, it is important to include drought-tolerant legumes with high water use efficiency (WUE), such as chickpea, in low-input cropping systems such as dry environments in semi-arid areas (Mohale et al. 2014; Lusiba et al. 2018). Plant water use efficiency indicates crop productivity as well as crop water use to produce biomass and yield in water-stressed environments. Biochar has been demonstrated to improve soil moisture content and nutrient availability, hence improving root systems, increasing nutrient uptake and enhancing plant development (Tag et al. 2016). As a result, crops grown with biochar use less water to accumulate biomass in water-stressed environments (Gao et al. 2020).

The objective of this study was to assess the potential impact of biochar derived from poultry litter and acacia on biological N_2 fixation, C accumulation and WUE of chickpea grown in three contrasting soils differing in texture and fertility. We hypothesised that (i) biochar derived from poultry litter and acacia feedstocks would improve BNF, C accumulation and WUE by chickpea due a change in rhizospheric properties of the contrasting soils and thus improve biomass production. (ii) Depending on the type of biochar feedstock used and application rates, N fixation, C accumulation and WUE by chickpea will vary amongst the contrasting soils. Furthermore, (iii) due to a change in rhizospheric soil properties caused by biochar application at different rates, there is a linear relationship between the produced biomass and the total N-fixed, C accumulation and WUE of chickpea, but this could depend on the type of soil, biochar feedstock and application rates.

Materials and methods

Study site and farmers cropping history

A pot experiment was carried out in a naturally ventilated tunnel house at the Experimental Farm located at 22°58.08′S and 30°26.40′E and 595 m above sea level, at the University of Venda, Thohoyandou, Limpopo Province, South Africa. Soil samples used in this study were collected from three smallholder farmer's fields at Dopeni village approximately 38.9 km away from Thohoyandou town. Farm A, B and C are located at 22° 92.43′ S & 30° 22.06′E; 22° 92.84′S & 30° 23.24 E; and 22° 93.70′S & 30° 24.58′E, respectively. The cropping history of these three farms includes continuous cropping with limited application of animal manure (poultry and cow dung), and NPK fertiliser 3:2:1 which is applied once per annum before planting maize. The main cropping practice is intercropping of maize with groundnuts (farm A and B), maize with sweet potato and butternut squash (Farm C).

Soil sampling and analysis

Prior to soil sample collection for the pot experiment, soil profiles were excavated in the farmer's field. The soils were classified as Fernwood, Griffin and Pinedene according to Fey (2010) and as Arenosol, Gleyic Acrisol and Helvic Acrisol, respectively, according to FAO (2014). Thereafter, a composite soil sample was randomly collected at the depth of 0–30 cm. The samples were crushed and sieved through a 2 mm sieve for analysis of soil texture using the hydrometer method (Bouyoucos, 1962). Selected chemical properties (pH, P, K, Mg, Ca, B, Mo and NO_3^-) that influence nitrogen fixation by legumes were assessed. Soil pH was determined in 1:2.5 ratio of soil: water (w/v) as outlined in (Peech, 1965). Exchangeable cations (Ca^{2+} & Mg^{2+}) were determined using the ammonium acetate extraction procedure (Peech, 1965). Available P was extracted using the Bray 1 method (Bray and Kurtz, 1945). Nitrate nitrogen (NO_3^-) in the soil solution was measured using the colorimetric method (Bremmer and Mulvaney, 1982). Concentration of B and Mo was measured by atomic absorption spectrophotometer following the Mehlich 3 extraction method (Mehlich, 1984). Table 1 shows the results of the chemical analysis of the soils prior to the potting experiment.

Production and characterisation of biochar

Fresh poultry litter consisting of a mixture of poultry manure and sawdust as bedding was collected at the University of Venda poultry houses. The poultry litter was air dried in an open space, and then broken into small chunks for pyrolysis. Poultry litter biochar was produced in a kiln furnace with height and width 1.4 and 1.3 m, respectively, by pyrolysing the litter at a temperature of 550°C for 60 min under limited oxygen condition. Ready-made acacia biochar produced at temperature of 550°C was purchased from Lanstar (Pty) (Ltd). The poultry and acacia biochar were crushed and sieved (2.00 mm) for further analysis. The biochars were analysed for pH, Ca, Mg, K, P, Mo and B. Proximate analysis such as fixed carbon, moisture content, ash content and volatile matter were analysed according to the modified analysis by the American Society for Testing and Materials (ASTM D1762-84 (2001) as described by Aller et al. (2017)). The results of the chemical analysis of the biochars are presented in (Table 2).

Table 1. Soil physiochemical characteristics of three different soil types used in the study.

		Chemical Characteristics		
Parameters	Units	Fernwood soil (Arenosol)	Griffin soil (Helvic Acrisol)	Pinedene soil (Gleyic Acrisol)
Clay	%	10	38	22
Sand	%	84	44	57
Silt	%	6	18	21
Textural class	–	Loamy sand	Clay loam	Sandy clay loam
pH	–	4.1	5.5	3.8
P	g/kg	0.01	0.01	0.03
Ca	g/kg	0.26	1.08	0.40
Mg	g/kg	0.14	0.39	0.28
B	g/kg	0.02	0.04	0.05
Mo	g/kg	0.04	0.05	0.08
NO_3^-	g/kg	0.01	0.02	0.01

Experimental setup and design

The experiment consisted of three types of soils namely Fernwood, Griffin and Pinedene; two types of biochars, poultry litter (PLB) and acacia (ACB) as well as four application rates which were 0% (control), 0.5%, 1% and 2% w/w which is equivalent to 10, 20 and 40 t/ha, respectively. The treatments were arranged in a completely randomised design with four replicates. Pots with a diameter of 25 cm and height of 25 cm were filled with 4 kg of air-dried sieved soil. The poultry and acacia biochar were applied according to the stated application rates and thoroughly mixed with the soil in a bucket before being transferred to the pot. Starter superphosphate fertiliser (10.5% P) was applied uniformly in all pots at approximately 2 g P/pot (equivalent to 60 kg P/ha). Prior to planting, the pots were watered to 60% field capacity. Chickpea was used as a test crop. Approximately, 450 g seeds of desi cultivar were inoculated with bradyrhizobium *spp cicer* containing 5×10^8 bacterial cells per gram and soaked in a mixture of 2.25 g bradyrhizobium powder and planted immediately. Four seeds were planted into each pot. Ten days after emergence (DAE), the plants were thinned to two plants per pot. Every three days, the pots (pot + soil mixture + water) were weighed and watered when necessary to targeted 60% field capacity. Non-nitrogen fixing crop amaranthus (*Amaranthus retroflexus*) was used as a reference crop to estimate the percentage of N derived from the atmosphere by chickpea. The amaranthus was planted using the same soil and biochar treatments.

Postharvest plant and soil analysis

At flowering stage, approximately 65 days after emergence, chickpea and reference crops amaranthus were harvested and each separated into shoot and root. Approximately, 20 g of rhizosphere soil was shaken from the root and transferred to sampling bags. The soils were analysed for selected chemical properties (pH, available P, Ca, K, Mg, B, Mo and NO_3^-) as described in Section 'Soil sampling and analysis'. The roots were placed in paper sample bags and transferred to the laboratory where the chickpea roots were washed with tap water. Thereafter, nodules were detached from the roots, counted and oven-dried at 60°C for 48 h for nodulation assessment. Shoot and root samples of chickpea and amaranthus were oven-dried at 65°C for 48 h to a constant weight and then weighed to determine shoot and root dry weights. The dried chickpea and amaranthus shoot samples were immediately ground to fine powder. The samples were stored in a tight zip lock plastic bag and taken for analysis of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotopes as well as shoot N concentration using the 1 M HCl digestion method and the inductively couple plasma spectrophotometer.

Table 2. Selected chemical properties of poultry litter and acacia biochar used in the study.

		Biochar feedstocks	
Parameters	Units	Poultry litter (PLB)	Acacia (ACB)
pH		10.3	9.2
Ca	g/kg	32.0	18.4
Mg	g/kg	16.2	2.2
K	g/kg	40.1	5.1
P	g/kg	24.0	0.5
Fixed Carbon	g/kg	730	732
Total C	g/kg	56.0	56.7
B	g/kg	0.06	0.02
Mo	g/kg	0.01	0.01
Ash content	g/kg	170	164
Volatile matter	g/kg	30	39
Moisture content	g/kg	60	68

Analysis of $^{15}N/^{14}N$ isotopic ratio

Finely ground plant materials were analysed for isotope ^{15}N and ^{13}C composition at the stable light isotope unit, University of Cape Town. Approximately, 1.2 mg samples of both chickpea and amaranthus were weighed into tin capsules, and analysed for %N and $^{15}N/^{14}N$ ratio using a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finnigan MAT252 mass spectrometer via Conflo II open-split device. The ^{15}N natural abundance is expressed as δ (delta) notation expressed as the deviation of the ^{15}N natural abundance of the sample from atmospheric (atm) N_2 (0.36637 atom % ^{15}N). The isotopic composition ($\delta^{15}N$) was measured as described by Unkovich and Baldock (2008) using the following equation:

$$\delta^{15}N(o/oo) = \frac{[^{15}N/^{14}N]_{\text{sample}} - [^{15}N/^{14}N]_{\text{standard}}}{[^{15}N/^{14}N]_{\text{standard}}} \times 1000 \quad (1)$$

where $^{15}N/^{14}N_{sample}$ is the abundance ratio of ^{15}N and ^{14}N of the chickpea plant or reference crop amaranthus; while $^{15}N/^{14}N_{standard}$ is the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

Percent N derived from the atmosphere (% Ndfa)

The percentage of N derived from symbiotic nitrogen fixation from atmospheric N_2 was estimated using the following equation (Shearer and Kohl 1986; Unkovich and Baldock 2008):

$$\%Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{ref}}{\delta^{15}N_{ref} - B} \times 100 \tag{2}$$

where $\delta^{15}N_{ref}$ is the mean value of the ^{15}N natural abundance of non-fixing reference plants (amaranthus) dependent on soil N; $\delta^{15}N_{leg}$ is the ^{15}N natural abundance value for the N_2-fixing chickpea crop and B is the ^{15}N natural abundance of chickpea plants depending solely on N_2 fixation for their N nutrition. The B value used in this study was −2.00 (Unkovich and Baldock 2008).

Shoot nitrogen content was determined as the product of shoot N concentration (% N) and shoot dry weight (mg/plant) of chickpea plant as described by Pausch et al. (1996)

$$\text{Shoot N(mg N/plant)} = (\%N \times \text{shoots dry weight})/100 \tag{3}$$

Total N-fixed was calculated as described by (Maskey et al. 2001)

$$\text{N-fixed(mg N/plant)} = \%Ndfa \text{ x shoot N} \tag{4}$$

Soil N uptake was calculated by computing the difference between shoot N and N-fixed

$$\text{Soil N uptake(mg N/plant)} = \text{Shoot N} - \text{total N-fixed} \tag{5}$$

Analysis of shoot $^{13}C/^{12}C$ isotopic ratio

Studies have shown that $\delta^{13}C$ can be used as an indicator of water-use efficiency especially in C3 plants (Farquhar et al. 1982). Low ^{13}C discrimination (less negative $\delta^{13}C$ values) implies greater water-use efficiency during photosynthesis, while high ^{13}C discrimination (more negative $\delta^{13}C$ values) implies low water-use efficiency. This forms the basis for using shoot $\delta^{13}C$ as a tool for measuring water-use efficiency in chickpea as a C3 plant. Shoot samples of chickpea of approximately 3 mg were weighed into a tin capsule and run on a mass spectrometer, as described for $^{15}N/^{14}N$ isotopic ratio. The ratio of $^{13}C/^{12}C$ in each sample was used to calculate the ^{13}C natural abundance or $\delta^{13}C$ (‰) as described by Farquhar et al. (1989):

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1 \right] \times 1000 \tag{6}$$

where $^{13}C/^{12}C_{sample}$ is the isotopic ratio of the chickpea sample and $^{13}C/^{12}C_{standard}$ (0.0112372), is the isotopic ratio of PDV, a universally accepted standard from Vienna Pee-Dee Belemnite (Craig 1957). The carbon concentration (%C) per plant was obtained directly from mass spectrometric analysis. The shoot C content/plant was calculated as the product of C concentration (% C) and shoot dry matter weight (mg/plant).

$$\text{Shoot C (mg C/plant)} = (\%C \times \text{shoots dry weight})/100 \tag{7}$$

Statistical analysis

The Kolmogorov–Smirnov test was used to determine the normality of the data. The normally distributed data (pH, K, Mg, Ca, B, Mo and NO_3^-) and plant parameters (N concentration, N content, $\delta^{15}N$, Ndfa, N-fixed, soil N uptake, C content, C/N ratio and $\delta^{13}C$) were analysed using the Generalised Linear Mixed Model in R software version 3.5.2. Tukey's honest significant difference (HSD) test was used for treatment mean separations with the threshold probability level set at $P \leq 0.05$. Pearson's correlation analysis was performed using Minitab version 19 to assess the correlation between soil parameters and chickpea biomass, BNF attributes and C accumulation of chickpea. Principal component analysis using XLSTART software was done to assess the variability and relationship that exist among the biomass, BNF attributes and C accumulation of chickpea with the biochar treatments and soil types (see Supplementary Figure S2). This was followed by plotting the graphs and significant linear regression of N-fixed with shoot dry weight, C content and N content using Sigmaplot 14.

Results

Effect of biochar on selected chemical properties (pH, K, Mg, Ca, B, Mo and NO_3^-)

In the Fernwood, Griffin and Pinedene soils, PLB and ACB had a significant effect on pH, P, K and Mg, but only on Ca in the Griffin soil. Application of PLB and ACB at different rates did not affect nitrate, B or Mo in any soil type (Table 3). Application of PLB and ACB increased soil pH in the Fernwood and Griffin soils as compared to the control, but pH was considerably higher when PLB was applied at 1% and 2% in all soil types in treatments FPL1, FPL2, GPL1, GPL2, PPL1 and PPL2 (Table 3). Available P varied significantly in all soil types, with PLB application at 2% resulting in increased available P in the Fernwood, Griffin and Pinedene soils in treatments FPL2, GPL2 and PPL2, respectively. In the Fernwood soil, 1% PLB and ACB in treatments FPL1 and FAC1 increased available P compared to the control. Similarly, when 1% PLB was applied to the Fernwood, Griffin and Pinedene soils, K and Mg concentrations increased significantly in treatments FPL1, GPL1 and PPL1. Application of PLB and ACB, on the other hand, had no significant effect on Ca concentration in the Fernwood and Griffin soils, although ACB application at 2% in treatment GPL2 increased Ca concentration in the Griffin soil. Nitrate (NO_3^-) concentration increased significantly when 0.5% PLB were applied in treatments FPL0.5 and PPL0.5 in the Fernwood and Griffin soils but decreased with higher levels of PLB and ACB. Application of PLB and ACB had no effect on Mo and B concentrations in the Fernwood, Griffin and Pinedene soils (Table 3).

Effect of biochar on nodulation and BNF attributes of chickpea

Application of PLB and ACB at different rates significantly affected shoot $\delta^{15}N$, %Ndfa and total N fixed in the Fernwood soil, %Ndfa, total N fixed, soil N uptake in the Griffin soil as well as total N fixed, soil N uptake and shoot N concentration and shoot N content in the Griffin soil (Table 4). Chickpea shoot $\delta^{15}N$ values varied when PLB and ACB was applied in the Fernwood soil, with chickpea grown in ACB treatments having the lowest values. In the Griffin and Pinedene soils, however, chickpea shoot $\delta^{15}N$ did not differ depending on whether PLB or ACB was applied. Application of PLB and ACB had a significant effect on %Ndfa of chickpea grown in the Fernwood and Griffin soils. In the Fernwood soil, %Ndfa of chickpea ranged from 3–21% in PLB treatments and 22–30% in ACB treatments, whereas in the Griffin soil, %Ndfa by chickpea ranged from 31–42% and 26–30% in PLB and ACB treatments, respectively (Table 4). On the contrary, PLB and ACB application had no effect on the %Ndfa of chickpea grown in Pinedene soil. In all the soil types, total N-fixed and soil N uptake by chickpea varied in all PLB and ACB treatments.

Chickpea N fixation decreased from 14 to 1 mg N/plant as PLB levels increased, whereas soil N uptake increased from 64 to 81 mg N/plant in the Fernwood soil, while treatment FPL0.5 had higher total N-fixed than the other treatments. The total N-fixed in ACB treatments ranged from 18 to 37 mg N/plant, with treatment FAC0.5 having the most N-fixed. In contrast, chickpea grown in the Griffin soil fixed more N, resulting in lower soil N uptake when PLB was applied, and the opposite was possible when ACB was applied. Chickpea grown in treatment GPL2 fixed the most N (90 mg N/plant), whereas chickpea grown in treatment GAC2 only fixed 17 mg N/plant. When 1% of either PLB (treatment GPL1) or ACB (treatment GAC1) was applied, soil N uptake decreased from 52 to 40 mg N/plant and increased from 52 to 137 mg N/plant, respectively. Conversely, the total N-fixed and soil N uptake of chickpea grown in Pinedene soil were higher in PLB treatments than ACB treatments. However,

Table 3. Selected soil chemical properties of Fernwood, Griffin and Pinedene soils after PLB and ACB application at 0.5, 1, 2% and control (0%).

Treatments	pH	Bray-*P*	K	Mg	Ca	NO_3^-	Mo	B
	mg/kg							
Fernwood soil								
FC0	4.8c	18.0e	14.5e	135.5b	308a	52.6a	0.5a	0.1a
FPL0.5	5.6b	57.8c	123.3	185.8b	398a	62.0a	0.5a	0.1a
FPL1	6.2a	105.0b	213.5b	207.2ab	403a	51.1a	0.5a	0.1a
FPL2	6.9a	161.5a	548.5a	357.0a	604a	47.7a	0.4a	0.1a
FAC0.5	5.0b	29.5d	18.8e	144.8b	382a	55.6a	0.4a	0.1a
FAC 1	5.3b	33.0d	28.5d	151.5b	399a	26.6a	0.5a	0.1a
FAC2	5.7ab	43.0c	46.0c	1485.0b	492a	15.9d	0.6a	0.1a
Griffin soil								
GC0	6.4c	13.6c	257.0c	508.9b	1253b	36.6a	0.5a	0.1a
GPL0.5	6.8b	38.5b	450.0b	557.0b	1271b	52.4a	0.2a	0.2a
GPL1	7.1a	68.5b	686.5b	603.5ab	1334ab	50.9a	0.7a	0.1a
GPL2	7.2a	86.3a	782.3a	628.8a	1327ab	50.5a	0.5a	0.2a
GAC0.5	6.6b	23.0c	275.3c	491.0b	1210b	22.1a	1.3a	0.2a
GAC1	6.7b	15.0c	257.3c	444.0c	1180c	23.1a	0.2a	0.1a
GAC2	6.9ab	16.5c	304.0b	509.2b	1469a	21.6a	0.6a	0.3a
Pinedene soil								
PC0	4.2b	18.5c	38.3c	214.4b	420a	41.3a	0.5a	0.1a
PPL0.5	4.5b	40.3b	168.8b	300.5b	481a	58.8a	0.6a	0.3a
PPL1	5.0a	73.3ab	295.5b	337.0ab	578a	47.1a	0.3a	0.1a
PPL2	5.4a	80.8a	451.3a	343.8a	513a	41.5a	0.4a	0.2a
PAC0.5	4.1b	31.0b	43.3c	202.2b	480a	26.8a	0.6a	0.1a
PAC1	4.2b	20.3b	55.3c	236.8b	512a	23.6a	0.2a	0.2a
PAC2	4.4b	16.0c	65.0c	232.2b	521a	25.1a	0.9a	0.1a

Values with dissimilar letters in a column the means are significant different at $P < 0.05$ and values with similar letters are non-significant. Abbreviations: FC0 – Fernwood control; GCO – Griffin control; PCO – Pinedene control, FPL – Fernwood soil with poultry litter, FAC – Fernwood soil with acacia biochar; GPL – Griffin soil with poultry litter; GAC – Griffin soil with acacia biochar; PPL – Pinedene soil with poultry biochar; PAC – Pinedene soil with acacia biochar; 0; 0.5; 1 & 2 refer to application rates in %.

Table 4. Effect of PLB and ACB biochar at different rates on nodulation, N uptake and total N-fixed of chickpea grown in the Fernwood, Griffin and Pinedene soils.

Treatments	δ^{15}N (‰)	Ndfa (%)	Total N-fixed (mg N/plant)	Soil N uptake (mg N/plant)	N concentration (%)	N content (mg/plant)
Fernwood soil						
FC0	7.5b	18.3b	14.0b	64.4a	1.2a	78.4a
FPL0.5	7.5ab	21.3b	15.3b	53.4a	1.2a	87.9a
FPL1	8.8a	6.7c	6.0c	78.2a	1.3a	68.7a
FPL2	9.6a	2.8c	0.5c	81.9a	1.7a	78.7a
FAC0.5	2.6c	30.9a	36.7a	48.4a	1.4a	70.1a
FAC1	6.8ab	25.7ab	25.3ab	71.5a	1.3a	92.1a
FAC2	7.1b	22.3ab	17.6c	50.3a	1.5a	67.0a
Griffin soil						
GC0	6.0a	36.3b	29.5c	52.3b	1.1a	88.5a
GPL0.5	6.0a	32.4c	70.8b	53.1b	1.3a	168.4a
GPL1	6.8a	31.6c	71.5b	40.1b	1.5a	184.8a
GPL2	6.4a	42.2a	89.6a	50.3b	1.7a	183.4a
GAC0.5	6.0a	30.8c	22.9bc	116.1a	1.4a	78.1a
GAC1	6.0a	26.4c	21.3c	137.4a	1.2a	57.4a
GAC2	4.8a	30.6c	17.9b	126a	1.1a	86.8a
Pinedene soil						
PC0	7.0a	23.6a	39.1b	126.4b	2.9c	158.9b
PPL0.5	7.8a	16.5a	52.3ab	324.5a	2.7c	395.5a
PPL1	7.2a	22.6a	47.4ab	259.1a	2.8c	330.6a
PPL2	7.2a	19.9a	57.4a	313.5a	3.0c	403.5a
PAC0.5	7.4a	20.0a	37.8c	143.3b	2.9c	181.2b
PAC1	7.5a	20.9a	41.8b	174.3b	3.4b	216.1b
PAC2	7.0a	23.4a	47.5ab	154.1b	3.7a	201.7b

Values with dissimilar letters in a column the means are significant different at $P < 0.05$ and values with similar letters are non-significant. Abbreviations: %Ndfa amount of nitrogen derived from the atmosphere; N – nitrogen; FC0 – Fernwood control; GCO – Griffin control; PCO – Pinedene control, FPL – Fernwood soil with poultry litter, FAC – Fernwood soil with acacia biochar; GPL – Griffin soil with poultry litter; GAC – Griffin soil with acacia biochar; PPL – Pinedene soil with poultry biochar; PAC – Pinedene soil with acacia biochar; 0; 0.5; 1 & 2 refer to application rates in %.

the total N-fixed and soil N uptake followed a similar pattern, treatments with the most N-fixed having higher soil N uptake. In PLB treatments, the average N-fixed was 53 mg N/plant and soil N uptake was 299 mg N/plant, while in ACB treatments, N-fixed and soil N uptake were 43 and 156 mg N/plant, respectively (Table 4). Similarly, chickpea shoot N concentration and uptake were higher in treatments with high N-fixed, especially at 2% PLB or ACB (treatment PPL2 and PAC2).

Effect of biochar application on δ^{13}C, C content and C/N ratio of chickpea

The effect of biochar on water-use efficiency (δ^{13}C) and C accumulation (shoot C content and C/N ratio) of chickpea in different soil types is presented in (Table 5). When PLB and ACB were applied at different rates in the Fernwood, Griffin and Pinedene soils, the values of shoot δ^{13}C, C accumulation and shoot C/N ratio varied, in such that in all soil types, shoot δ^{13}C values were lower and more negative in PLB treatments, but higher and less negative in ACB treatments (Table 5). Higher levels of PLB resulted in higher shoot δ^{13}C in Fernwood soils, but at 2% ACB shoot δ^{13}C increased to −28.7 ‰ (treatment FAC2) when compared to the control (treatment FC0). Similarly, in the Griffin and Pinedene soils, chickpea shoot δ^{13}C values decreased with increased PLB levels and increased with increased ACB levels, with the highest shoot δ^{13}C value in treatments GAC2 (−28.3‰) and PAC2 (−27.8‰), respectively. Chickpea grown with PBL and ACB at different rates had no change in shoot C concentration as well as shoot C/N ratio in the Fernwood soil. When PLB and ACB were applied at different rates in the Griffin and Pinedene soils, however, shoot C content and C/N ratio of chickpea were significant. Shoot C content of chickpea grown in the Griffin and Pinedene soils was higher at 0.5% PLB (GPL0.5 and PPL0.5) and ACB (GAC 0.5 and PAC0.5) treatments, but decreased in all PLB and ACB treatments, with higher biochar application in both soils. Similarly, in PLB treatments in the Fernwood soil and ACB treatments in the Pinedene soil, the shoot C/N ratio of chickpea was lower than the control treatments but shoot C/N values decreased with higher levels of biochar application. On the other hand, chickpea shoot C/N ratios were higher in ACB treatments in the Griffin soil and in PLB treatments in the Pinedene soil than the control treatment, but C/N values decreased with higher ACB application rates in both soils (Table 5).

Pearson's correlation analysis

Total N-fixed by chickpea positively correlated with shoot dry weight, N content and soil N uptake, with Parsons' coefficients (r) of 0.79, 0.92 and 0.86, respectively (Table 6). Figure 1 shows that increased shoot biomass and N content resulted in higher N-fixed irrespective of PLB or ACB application (Figure 1(a, d)). On the other hand, PLB

Table 5. Effect of PLB and ACB on shoot C concentration, C content, C/N ratio and water-use efficiency (WUE) of chickpea in the Fernwood, Griffin and Pinedene soils.

Treatment	$\delta^{13}C$ (‰)	C concentration (%)	C content (mg C/plant)	C/N ratio (g/g)
Fernwood soil				
FC0	−29.9ab	41.1a	1774a	43.3a
FPL0.5	−29.7ab	40.9a	2711a	36.9a
FPL1	−30.0ab	40.3a	2744a	39.9a
FPL2	−30.1c	39.7a	2075a	29.79a
FAC0.5	−29.2b	41.1a	2921a	34.2a
FAC1	−29.4ab	40.5a	3017a	30.9a
FAC2	−28.7a	40.7a	2777a	32.3a
Griffin soil				
GC0	−29.6ab	41.1a	2404bc	26.9b
GPL0.5	−29.5ab	42.3a	6656a	16.7c
GPL1	−29.3ab	40.6a	4430ab	14.6c
GPL2	−29.6ab	41.5a	3751ab	13.6c
GAC0.5	−30.4c	40.7a	2886bc	38.3ab
GAC1	−29.0b	40.7a	2299bc	31.7b
GAC2	−28.3ab	40.2a	1530c	25.5c
Pinedene soil				
PC0	−29.1b	40.8a	2448c	29.4b
PPL0.5	−29.9ab	40.4a	5136ab	46.1a
PPL1	−30.4c	39.6a	4977ab	41.42ab
PPL2	−30.4c	39.5a	4489ab	36.4ab
PAC0.5	−28.6ab	41.4a	3244bc	18.6c
PAC1	−28.3ab	42.7a	2458c	17.8c
PAC2	−27.8a	42.4a	1635c	17c

Values with dissimilar letters in a column the means are significant different at $P < 0.05$ and values with similar letters are non-significant. Abbreviations: FC0 – Fernwood control; GCO – Griffin control; PCO – Pinedene control, FPL – Fernwood soil with poultry litter, FAC – Fernwood soil with acacia biochar; GPL – Griffin soil with poultry litter; GAC – Griffin soil with acacia biochar; PPL – Pinedene soil with poultry biochar; PAC – Pinedene soil with acacia biochar; 0, 0.5, 1, & 2 refer to application rates in %.

application increased shoot dry weight, total N-fixed and N content, resulting in higher C accumulation in chickpea shoots (Figure 2). This was demonstrated by the significant correlation between shoot dry weight with N and C content. Interestingly, the %Ndfa negatively correlated with shoot $\delta^{15}N$ ($r = -0.80$) and not with total N-fixed. The change in soil properties (soil pH, P, NO_3^-, K, B, Mo) caused by biochar application directly and indirectly attributed to the increase in total N-fixed. The total N-fixed was significantly correlated with the change in soil P and soil NO_3^-, with r values of 0.77 and 0.82, respectively, while Parsons' coefficients (r) for N content were 0.87 and 0.72, respectively. Similarly, shoot dry weight and %Ndfa, correlated significantly with soil NO_3^-, while soil N uptake had a strong relationship with soil P and NO_3^- (Table 6).

Discussion

Effect biochar on biological N fixation of chickpea in three contrasting soils

Chickpea N_2 fixation varied across the three soil types, depending on the type of biochar used and the rate at

Table 6. Pearson's correlation (*r*) among plant growth, BNF and WUE attributes of chickpea grown.

Variables	Shoot Dwt mg/plant	% N concentration	N content mg/plant	C content mg/plant	% Ndfa	$\delta^{15}N$ ‰	Soil N uptake	$\delta^{13}C$ ‰	C/N ratio g/g
N-fixed	**0.794*****	0.600***	**0.920*****	0.5139*	0.4187***		**0.864*****		−0.506***
%Ndfa		0.2090*	0.3397***			**−0.806*****			
$\delta^{15}N$					**−0.806*****				
$\delta^{13}C$	0.2011*	−0.4253*	0.696*						
N content	**0.621*****	0.5435***				−0.3252**			
C content	**0.9921*****		0.5204**						
%C			0.2648***				0.490*		
C/N ratio		−0.871	−0.5251***					0.5235**	−0.4574*
Soil N uptake	0.533*	**0.756*****	**0.985****						**−0.709****
Variables	Soil pH	Soil P	Soil NO_3^-	Soil K	Soil B	Soil Mo			
N-fixed	**0.901*****	**0.775*****	**0.822*****	0.2041*	−0.697*				
N content		**0.877*****	**0.720*****	0.246*					
Shoot Dwt		0.504*	**0.777****	0.635	−0.482*				
%Ndfa	0.2678*	−0.5555**	0.646*	0.3672***	0.2062*	−0.2425*			
$\delta^{15}N$	−0.2625*	0.5117**		−0.3598***	−0.1956*	0.2391*			
Soil N uptake		**0.879*****	0.646*						

Values with asterisk symbol represents significant correlation at *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.
Bold values represent high correlation, Dwt – Dry weight and C/N ratio by weight.

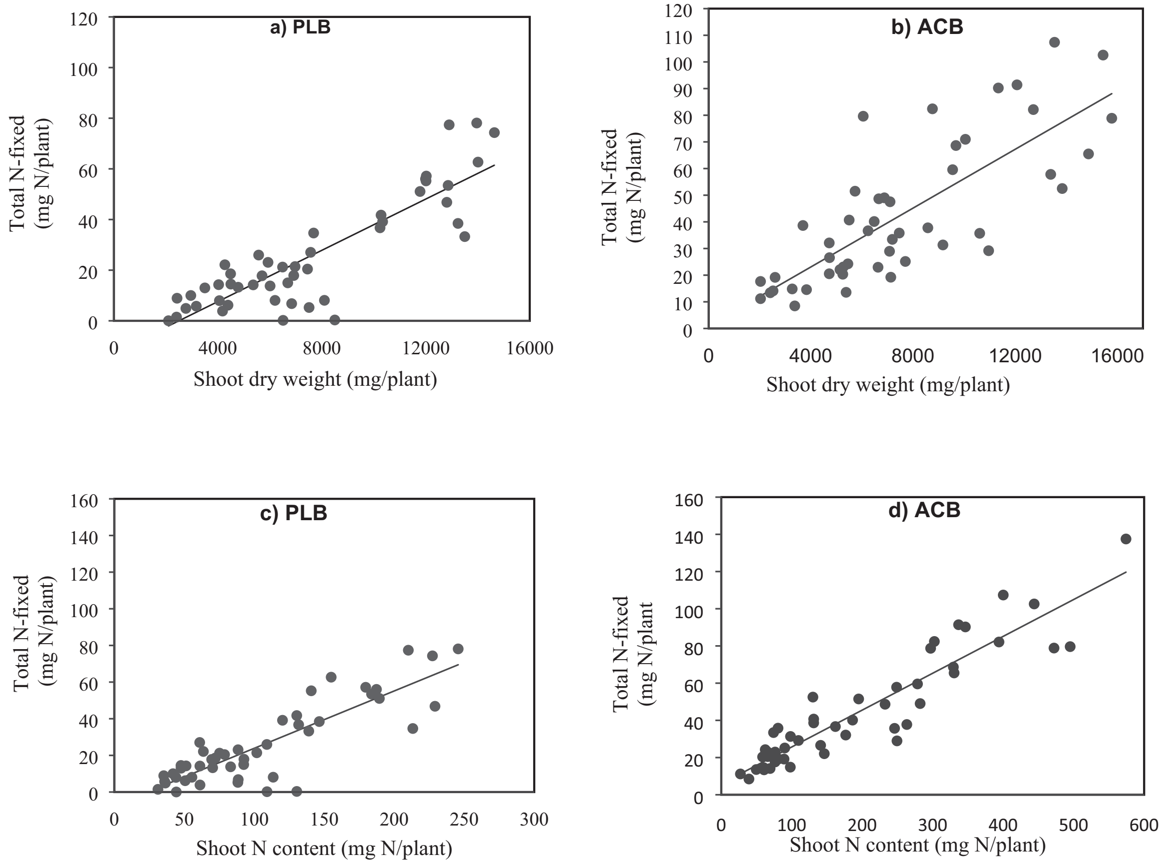

Figure 1. Relationship between the total N-fixed with shoot dry weight (a & b) and N content (c & d) of chickpea as influenced by PLB and ACB application. PLB – poultry litter biochar and ACB – acacia biochar.

which the biochar was applied. As shown in (Table 4), chickpea grown in the Fernwood, loamy sand soil derived more N from the atmosphere (%Ndfa) at 0.5% PLB (21%) and at 0.5 ACB (30%), resulting in higher total N-fixed (15 mg N/plant and 37 mg N/plant, respectively). On the other hand, chickpea grown in PLB treatments in the Griffin, clay loam soil derived more N from the atmosphere (35% on average), resulting in higher total N-fixed (77 mg N/plant on average), however, as ACB application rates increased, so did the amount of nitrogen derived from the atmosphere, resulting in a 30% decrease in total N-fixed. The low N-fixed values reported in this study when ACB was applied in the Griffin soil (Table 4) is in contrary to findings reported by (Rondon et al. 2007; Horel et al. 2018; Mia et al. 2018) in a clay loam textured soil.

Despite the lower amount of nitrogen derived from the atmosphere (20% on average) in PLB and ACB treatments than the control in the Pinedene, sandy clay loam soil, chickpea grown had higher total N-fixed (57 and 48 mg N/plant) at 2% PLB and ACB respectively, due to higher biomass produced. Similar findings were reported by Mia et al. (2018) who found that applying 20 t/ha of eucalyptus-wood biochar to a sandy clay loam soil reduced the amount of nitrogen derived from the atmosphere by 73%, but total N-fixed of red clover was compensated at this rate by increased biomass production. The presence of biochar derived from plant materials has been shown to increase the amount of nitrogen derived from the atmosphere, resulting in higher total N-fixed (Rondon et al. 2007; Quilliam et al. 2013; Mia et al. 2014; Güereña et al. 2015) but the effect of PLB biochar on BNF is scarcely documented (Tagoe et al. 2008). The total N-fixed values of chickpea reported in this study are consistent with previous findings using biochar (Khan et al. 2020) and without biochar (López-Bellido et al. 2011; Meleta and Abera 2019).

Higher total N-fixed in the Griffin and Pinedene soils was attributed to increased biomass production of chickpea grown with PLB application, resulting in higher N-fixed. This was demonstrated by the strong and positive relationship between total N-fixed and shoot dry weight ($r = 0.79$) as shown in (Table 6). As indicated in (Figure 1 (a, b)) applying PLB and ACB in the studied soils enhanced biomass production, consequently increased the amount of N-fixed by chickpea, which is comparable to the findings by Mia et al. (2014). Higher production of

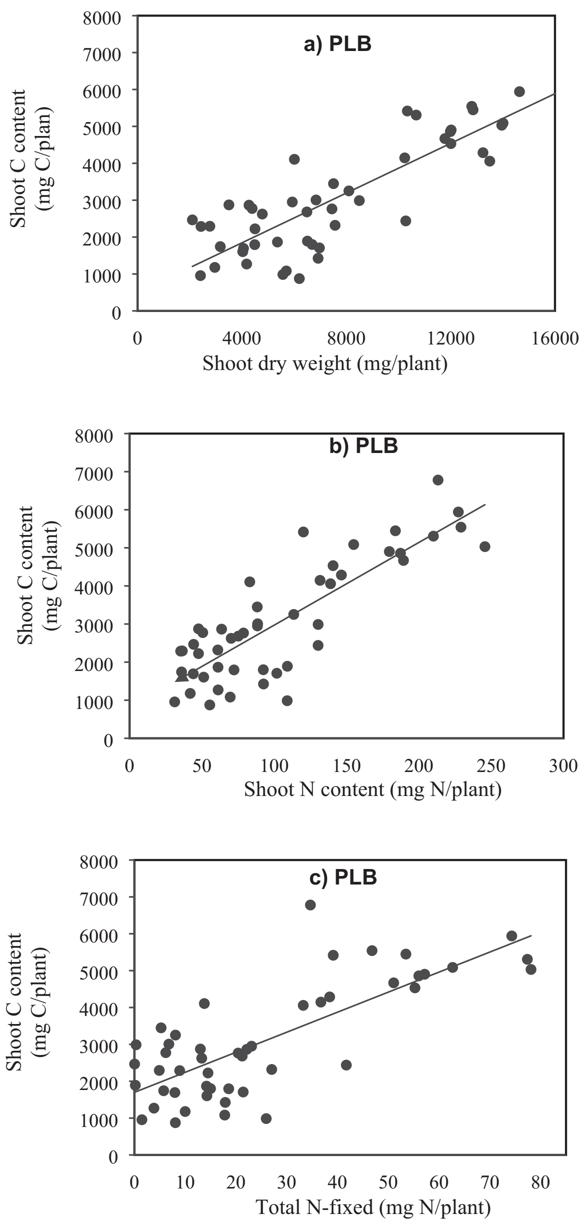

Figure 2. Relationship between shoot C content and shoot dry weight (a); total N-fixed (b) and shoot N content (c) of chickpea as influenced by PLB and ACB application. PLB- poultry litter biochar; ACB-acacia biochar.

chickpea shoot biomass in the presence of PLB in the Griffin and Pinedene soils was demonstrated by (Lusiba et al. 2021) as well as the application of PLB to other crops (Rajkovich et al. 2012; Macdonald et al. 2014). Other than %Ndfa and biomass production, the N concentration in legumes affects total N-fixed (Mia et al. 2018). Total N-fixed of chickpea increased in magnitude where N content was high, especially when 2% PLB and ACB was applied in the Griffin and Pinedene soils (Table 4). The Pearson correlation (Table 6) showed that shoot N concentration significantly correlated with total N-fixed ($r = 0.60$), whereas shoot N content strongly correlated with total N-fixed ($r = 0.92$). On the other hand, the PCA analysis (supplementary file Figure S1) revealed that the variation in total N-fixed in the Griffin soil was largely due to N concentration and N content; however, N content was only significant in the Pinedene soil (Table 6). The relatively lower dependence of chickpea on BNF for its N nutrition as demonstrated in this study (3% in the Fernwood soil; 17% in the Pinedene soil and 26% in the Griffin soil) as shown in (Table 4) is similar to the findings by (Makhura et al. 2015).

Biochar changes soil chemical properties thus enhance BNF

In this study, changes in rhizospheric soil pH and nutrient availability caused by biochar application at various rates were hypothesised to improve chickpea growth and nodulation, resulting in higher total N-fixed. However, this effect varied depending on the application of PLB or ACB in the Fernwood, Griffin and Pinedene soils. Indeed, higher total N-fixed by chickpea in this study was attributed to increased soil pH with PLB and ACB application (Table 4), supporting the findings by (Rondon et al. 2007; Quilliam et al. 2013; Mia et al. 2014; Güereña et al. 2015; Khan et al. 2020; Farhangi-Abriz et al. 2022). Chickpea grown at pH levels ranging from 5.0 to 7.0 had higher total N-fixed (Tables 4 and 5), because N_2 fixing bacteria thrive in the pH range of 6.5–7.0, it's possible that PLB application created a favourable environment for bacteria at this pH range, which is consistent with the findings by (Yin et al. 2021; Farhangi-Abriz et al. 2022). Given that the chickpea was inoculated with Rhizobium in this study, PLB biochar application may have aided the rhizobia's survival, reproduction and competitiveness allowing the rhizobia to efficiently colonise the crop and result in greater N fixed (Khan et al. 2020). Furthermore, increasing soil pH with biochar application improves nutrient bioavailability, such as P, which stimulates root growth and legume nodulation, contributing to increased BNF (Xiu et al. 2021; Farhangi-Abriz et al. 2022). Increased BNF in several legumes, including soybean (Tagoe et al. 2008), common bean (Rondon et al. 2007) and cowpea (Hiama et al. 2019) has been linked to increased available P with biochar application (Sun et al. 2020). This suggests that biochar, particularly biochar derived from poultry litter, has the potential to lime acid soils, improving rhizospheric conditions for soil organisms like bacteria to thrive and function, as well as contributing to improved soil quality.

Application of PLB and ACB at different rates had no effect on chickpea nodulation in the Fernwood, Griffin and Pinedene soils, contrary to the findings of Mia et al. (2014); Torabian et al. (2018); Hiama et al. (2019); Macil et al (2017); Xiu et al. (2021). However, when PLB was applied to the Griffin and Pinedene soils, chickpeas derived more N from the atmosphere and increased total N fixed than the Fernwood soil. Chickpea grown in the Fernwood and Pinedene soils with ACB application, on the other hand, had a higher amount of N derived from the atmosphere and total N fixed (Table 4). This indicates that Rhizobium-inoculated chickpea may have formed effective nodules as a result of increased biomass, releasing more assimilates to the rhizobia and thus affecting nitrogen fixation (Mia et al. 2018). The low total N fixed at high PLB application rates (1 and 2%) in the Fernwood soil and high ACB application rates in the Griffin soil could be due to high soil N uptake by the crop (Table 4), which is consistent with the findings of Quilliam et al. (2013); Khan et al. (2020) who demonstrated that high N concentration in the soil inhibits the effectiveness of nodules, reducing the maximum BNF of legumes.

Treatments with higher available P, K, Mg and NO_3^- contributed significantly to the increase in total N-fixed by chickpea in the Griffin and Pinedene soils (Table 4). This was demonstrated by the significant and positive correlation between soil pH, P, K, Mg NO_3^- with total N-fixed, where ($r > 0.80$) in the Pinedene and Griffin soils (Table 6). Application of PLB increased soil pH and nutrient availability such as P, K and Mg, which resulted in increased shoot biomass (Lusiba et al. 2021) and N accumulation (Table 4), which attributed to higher BNF by chickpea in the Griffin and Pinedene soils, as there was a linear relationship between N uptake and BNF, as well as biomass and BNF (Figure 1). In contrast, enhanced available P, K and Mg by biochar application in the Fernwood soil did not results in an increase in total N-fixed as observed by the negative correlation (Supplementary Figure S1), suggesting that soil pH was the only most attributing factor to the increased total N-fixed in the Fernwood soil. These findings are consistent with previous reports (Horel et al. 2018; Torabian et al. 2018; Farhangi-Abriz et al. 2022) however, they contradict results reported by Mia et al. (2018); Rondon et al. (2007). In this study, soil B concentration in the soil negatively correlated with %Ndfa and total N-fixed, while shoot B concentration correlated positively with total N-fixed in the Fernwood soil (Supplementary Figure S1), indicating that higher soil B concentration due to biochar application is likely to inhibit total N fixation by chickpea, contradicting the findings by Mia et al. (2014); Rondon et al. (2007). Furthermore, when PLB or ACB was applied at different rates in the Fernwood, Griffin and Pinedene soils, the concentration of B and Mo was not significantly different.

The findings of this study have shown for the first time that the use of biochar derived from poultry litter, in a clay textured soil such as the Griffin and Pinedene with adequate nutrient availability (Table 1) could improve BNF by chickpea. The greater increase in total N-fixed by chickpea grown in the Griffin and Pinedene soils than the Fernwood soil when PLB was applied was linked to the fertility and texture of the soils. The Griffin and Pinedene soils had higher nutrient availability than the Fernwood soil which contained the lowest concentrations of nutrients after biochar application (Table 3). The effect of biochar acting as a liming agent, supplying and enhancing nutrients to soils has been reported elsewhere (Ding et al. 2016; Wang et al. 2015), thus contributing to enhanced legume crop growth and higher BNF (Hiama et al. 2019; Khan et al. 2020; Farhangi-Abriz et al. 2022). Moreover, this study demonstrated that the application of biochar derived from poultry litter feedstock as low as 10 t/ha and as high as 40 t/ha has the potential to improve BNF by chickpea in slightly acid, fertile soils such as the Griffin and acidic soils such as the Pinedene soil. However, application of poultry litter and acacia at 10 t/ha could possibly enhance BNF in low fertile sandy loam soils such as the Fernwood soil. Very acid soils, such as the Pinedene soils, will, on the other hand, require higher application rates of PLB or ACB at 2 t/ha to raise the pH of the soil, improve nutrients and thus improve BNF. The results of this study show potential for increasing N inputs through BNF with biochar application in acid sandy and clay textured soils, which is important for smallholder farmers who practice crop rotation with limited N inputs. Since this was a pot study with controlled moisture and temperature, whereas in the field conditions, temperature and moisture fluctuate and treatment behaviour on smallholder farms varies under dryland conditions. The findings of this study could be used as a reference point for future field investigations on the effect of poultry litter and acacia biochar and nodulation, BNF, as well as the relationship between BNF with changing soil properties on inoculated chickpea using different application rates in semi-arid environments.

Effect of biochar application on C accumulation and WUE of chickpea

Shoot C concentration was invariable to the application of PLB and ACB at different application rates. Although, the lack of differences in shoot % C is intriguing, it is not surprising because this trend has been shown in different plant genotypes and treatments (Pule-Meulenberg et al. 2011; Yahaya et al. 2019). Despite being similar, on average, shoot % C values of chickpea ranged from 39 to 43% with the application of PLB or ACB in the three types of soils (Table 5). These values are interesting because normally, the expected shoot C concentration in legumes should be about 30% as estimated by (Sprent et al. 1996). However, studies by (Mohale et al. 2014; Mapope and Dakora 2016; Maseko and Dakora 2016) reported greater shoot %C in various legumes. According to Post et al. (2007), shoot % C values of legumes that are above 30% and/or 35%, could be an indication of high lipid distribution within the plant organ. However, in this study, lipid distribution in shoot of the selected chickpea cultivar was not determined.

The results of this study confirmed the relationship between C content and shoot dry weight (Figure 2). In the Fernwood soil, chickpea shoot dry weight was higher at 0.5% and least at 2% in PLB and ACB amended soils, respectively. Application of 0.5–2% PLB in the Griffin soil resulted in higher shoot dry weight, therefore greater C content. Meanwhile, 1% ACB resulted in the lowest growth and C accumulation. Application of 1 and 2% PLB in the Pinedene soil, chickpea had the largest shoot dry weight and the second-highest C content while the addition of 2% ACB decreased growth and C accumulation. C accumulation by chickpea grown in the Fernwood and Griffin soil was largely associated with shoot dry weight and where growth was greater, it contributed to a larger N content and C assimilation. The direct relationship between shoot dry weight and C content was confirmed by a positive and significant linear relationship (Figure 2(a)). Mapope and Dakora (2016) reported similar findings, in which shoot C content positively correlated with soybean shoot biomass. Therefore, the results of this study confirmed that C content is associated with the biomass which is mainly derived from photosynthetically fixed carbon. In addition to biomass, lower soil nitrate and ammonium increased chickpea C accumulation in the Fernwood soil (Supplementary Figure S1), whereas higher N-fixed and soil N uptake, as well as higher soil P, K and Mg, increased chickpea C accumulation in the Griffin soil (Supplementary Figure S2). The linear relationship between C content and N content, as well as total N-fixed, demonstrates the increase in C accumulation with high shoot N accumulation (Figure 2(b, c)). In the Pinedene soil, C accumulation of chickpea decreased as the crop accumulated more N, P, B and Fe, whereas an increase in soil pH, Ca and Mo uptake resulted in higher C accumulation (Supplementary Figure S3).

Shoot $\delta^{13}C$ of chickpea varied significantly between soil types, biochar feedstock and application rates. Interestingly, the most water-use by chickpea was observed in soils amended with ACB across the soil types. This is because chickpea grown in the Pinedene soil (−27.8 ‰) had the highest $\delta^{13}C$ at 2% ACB, followed by Griffin soil (−28.3 ‰) and Fernwood soil (−28.7‰). This study agrees that the type of biochar influence the discrimination of ^{13}C differently. In general, like the C/N ratio, shoot $\delta^{13}C$ is affected by the fertility status of the soil (Mohale et al. 2014). It is intriguing that chickpea grown in the acidic, fertile Pinedene soil was more water-use efficient but did not translate to higher biomass especially when ACB was applied than that grown in the slightly acid Fernwood and Griffin soils. Despite lower shoot biomass, chickpea used less water in the Pinedene soil due to increased soil pH and P, as well as higher N uptake in the soil and C accumulation in the shoots (Supplementary Figure S3). Furthermore, Chickpea grown in the Griffin and Pinedene soils, where both total N-fixed and N content were higher, had a higher WUE at 2% ACB than chickpea grown in the Fernwood soil, where N content was higher. Chickpea in the Griffin soil produced more biomass, accumulated more N and had a lower shoot C/N ratio, all of which contributed to higher WUE (Supplementary Figure S2). Mohale et al. (2014) reported a significant positive correlation between shoot $\delta^{13}C$ with total N-fixed, N content, shoot dry weight and C content of Bambara groundnut. However, in this study, shoot $\delta^{13}C$ did not correlate with total N-fixed, but correlated positively with shoot dry weight, C content and N content (Table 3). This suggests that there is a close link between WUE and BNF as well as photosynthetic activity by legumes, therefore improved WUE supports N and C assimilation in legumes. Future field studies are needed to identify the relationship between BNF and WUE of chickpea using various biochar feedstocks and application rates in dry-land conditions under semi-arid regions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

Aller D, Bakshi S, Laird DA. 2017. Modified method for proximate analysis of biochars. J Analyt Appl Pyrolysis. 124:335–342. doi: 10.1016/j.jaap.2017.01.012.

Biabani A, Carpenter-Boggs L, Coyne CJ, Taylor L, Smith JL, Higgins S. 2011. Nitrogen fixation potential in global chickpea mini-core collection. Biol Fertil Soils. 47:679–685. doi: 10.1007/s00374-011-0574-0.

Bouyoucos GJ. 1962. Hydrometer method improved for making particle size analyses of soils. Agron J. 54:464–465. doi: 10.2134/agronj1962.00021962005400050028x

Bray RH, Kurtz LT. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39–46. doi: 10.1097/00010694-194501000-00006

Bremmer JM, Mulvaney CS. 1982. Nitrogen–Total. In Page AL, Miller RH, Keeney DR, editor. Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd ed. Agronomy 9. Madison, Wisconsin: American Soc Agron Soil Sci; p.595–624.

Bruun EW, Müller-Stöver D, Ambus P, Hauggaard-Nielsen H. 2011. Application of biochar to soil and N_2O emissions: potential effects of blending fast-pyrolysis biochar with anaerobically digested slurry. Euro J Soil Sci. 62:581–589. doi: 10.1111/j.1365-2389.2011.01377.x.

Craig H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta. 12:133–149.

Ding Y, Liu Y, Liu S, Li Z, Tan X, Huang X, Zeng G, Zhou L, Zheng B. 2016. Biochar to improve soil fertility. A review. Agron Sustain Develop. 36:1–8. doi: 10.1007/s13593-016-0372-z

Farhangi-Abriz S, Ghassemi-Golezani K, Torabian S, Qin R. 2022. A meta-analysis to estimate the potential of biochar in improving nitrogen fixation and plant biomass of legumes. Biomass Convers Biorefin. 1:11. doi: 10.1007/s13399-022-02530-0.

Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Funct Plant Biol. 9:121–137.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. Ann Rev Plant Biol. 40: 503–537.

Fey M. 2010. Soils of South Africa. Cape Town: Cambridge University Press; p. 287.

Food and Agriculture Organization (FAO) of the United Nations. 2014. World reference base for soil resources report 106, Rome, Italy; p. 85–144.

Gao Y, Shao G, Lu J, Zhang K, Wu S, Wang Z. 2020 Effects of biochar application on crop water use efficiency depend on experimental conditions: A meta-analysis. Field Crops Res. 249:107763. doi: 10.1016/j.fcr.2020.107763

Güereña DT, Lehmann J, Thies JE, Enders A, Karanja N, Neufeldt H. 2015. Partitioning the contributions of biochar properties to enhanced biological nitrogen fixation in common bean (*Phaseolus vulgaris*). Biol Fert Soils. 51:479–491. doi: 10.1007/s00374-014-0990-z.

Hiama PD, Ewusi-Mensah N, Logah V. 2019. Nutrient uptake and biological nitrogen fixation in cowpea under biochar-phosphorus interaction. J Animal Plant Sci. 29:1654–1663.

Horel A, Potyó I, Szili-Kovacs T, Molnar S. 2018. Potential nitrogen fixation changes under different land uses as influenced by seasons and biochar amendments. Arabian J Geoscience. 11:1–0. doi: 10.1007/s12517-018-3916-5

Khan S, Shah Z, Mian IA, Dawar K, Tariq M, Khan B, Mussarat M, Amin H, Ismail M, Ali S, et al. 2020. Soil fertility, N_2 fixation and yield of chickpea as influenced by long-term biochar application under mung–chickpea cropping system. Sustainability. 12:9008. doi: 10.3390/su12219008.

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D. 2011. Biochar effects on soil biota–a review. Soil Biol Biochem. 43:1812–1836. doi: 10.1016/j.soilbio.2011.04.022.

Lehmann J, Joseph S. 2015. Biochar for environmental management: science, technology and implementation. Routledge, London; p. 448.

López-Bellido RJ, López-Bellido L, Benítez-Vega J, Muñoz-Romero V, López-Bellido FJ, Redondo R. 2011. Chickpea and faba bean nitrogen fixation in a Mediterranean rainfed Vertisol: Effect of the tillage system. European J Agron. 34:222–230. doi: 10.1016/j.eja.2011.01.005

Lusiba S, Odhiambo J, Ogola J. 2017. Effect of biochar and phosphorus fertilizer application on soil fertility: soil physical and chemical properties. Arch Agron Soil Sci. 63:477–490. doi: 10.1080/03650340.2016.1218477.

Lusiba S, Odhiambo J, Ogola J. 2018. Growth, yield and water use efficiency of chickpea (*Cicer arietinum*): response to biochar and phosphorus fertilizer application. Arch Agron Soil Sci. 64:819–833. doi: 10.1080/03650340.2017.1407027.

Lusiba SG, Odhiambo JJO, Adeleke R, Maseko ST. 2021. The potential of biochar to enhance concentration and utilization of selected macro and micro- nutrients for chickpea (*Cicer arietinum*) grown in three contrasting soils. Rhizosphere. 17:100289. doi: 10.1016/j.rhisph.2020.100289

Macdonald LM, Farrell M, Van Zwieten L, Krull ES. 2014. Plant growth responses to biochar addition: an Australian soils perspective. Biol Fert Soils. 50:1035–1045. doi: 10.1007/s00374-014-0921-z.

Macil PJ, Ochanda JBO, Odhiambo JJO, Lusiba SG. 2017. The response of some physiological traits of chickpea (*Cicer arietinum L.*) to biochar and phosphorus fertilizer application. Legume Res. 40. doi: 10.18805/lr.v0i0.7290.

Macil PJ, Ogola JB, Odhiambo JJ. 2020. Response of soil pH and nodulation of three chickpea genotypes to biochar and rhizobium inoculation. Commun Soil Sci Plant Analysis. 51:2377–87. doi: 10.1080/00103624.2020.1836204

Makhura MP, Mathews C, Dakora FD. 2015. Estimating N_2 fixation in 26 chickpeas (*Cicer arentinum L.*) genotypes using ^{15}N natural abundance: effect of genotype × environment interaction. South Afr J Bot. 98:210. doi: 10.1016/Fj.sajb.2015.03.155.

Mapope N, Dakora FD. 2016. N_2 fixation, carbon accumulation, and plant water relations in soybean (Glycine max L. merrill) varieties sampled from farmers' fields in South Africa, measured using ^{15}N and ^{13}C natural abundance. Agric Ecosyst Environ. 221:174–186. doi: 10.1016/j.agee.2016.01.023.

Maseko ST, Dakora FD. 2016. Nitrogen nutrition, carbon accumulation and $\delta^{13}C$ of Cyclopia and Aspalathus species in different settings of the cape fynbos. South Afr J Plant Ecol. 9:586–595. doi: 10.1093/jpe/rtv078.

Maskey SL, Bhattarai S, Peoples MB, Herridge DF. 2001. On-farm measurements of nitrogen fixation by winter and summer legumes in the hill and terai regions of Nepal. Field Crops Res. 70:209–221. doi: 10.1016/S0378-4290(01)00140-X.

Meleta T, Abera G. 2019. Effects of rhizobium inoculation and phosphorus fertiliser rates on growth, yield and yield components of chickpea (*Cicer arietinum L.)* at goro, bale zone, oromia regional state. Inter J Appl Agric Sci. 5:62–70. doi: 10.15580/GJAS.2019.4.101419186

Mehlich A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun Soil Sci Plant Analysis. 15:1409–1416. doi: 10.1080/00103628409367568

Mia S, van Groenigen JW, van de Voorde TFJ, Oram NJ, Bezemer TM, Mommer L, Jeffery S. 2014. Biochar application rate affects biological nitrogen fixation in red clover conditional on potassium availability. Agric Ecosyst Environ. 191:83–91. doi: 10.1016/j.agee.2014.03.011.

Mia S, Dijkstra FA, Singh B. 2018. Enhanced biological nitrogen fixation and competitive advantage of legumes in mixed pastures diminish with biochar aging. Plant Soil. 424:639–651. doi: 10.1007/s11104-018-3562-4.

Mohale KC, Belane AK, Dakora FD. 2014. Symbiotic N nutrition, C assimilation, and plant water use efficiency in Bambara groundnut (*Vigna subterranea L. Verdc*) grown in farmers' fields in South Africa, measured using ^{15}N and ^{13}C natural abundance. Biol Fertil Soils. 50:307–319. doi: 10.1007/s00374-013-0841-3.

Mpai T, Maseko ST. 2018. Possible benefits and challenges associated with production of chickpea in inland South Africa. Acta Agric Scand Sect B Soil Plant Sci. 68:479–488. doi: 10.1080/09064710.2018.1437926.

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 Biol Biochem. 55:20–27. doi: 10.1016/j.soilbio.2012.05.019.

Pausch RC, Mulchi CL, Lee EH, Meisinger JJ. 1996. Use of ^{13}C and ^{15}N isotopes to investigate O3 effects on C and N metabolism in soybeans. Part II. Nitrogen uptake, fixation, and partitioning. Agric Ecosyst Environ. 60:61–69. doi: 10.1016/S0167-8809(96)01062-6.

Peech M. 1965. Hydrogen-ion activity. methods of soil analysis: part 2 chemical and microbiological properties; p. 914-926. doi: 10.2134/agronmonogr9.2.c9

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia. 152:179–189. doi: 10.1007/s00442-006-0630-x.

Pule-Meulenberg F, Gyogluu C, Naab J, Dakora FD. 2011. Symbiotic N nutrition, bradyrhizobial biodiversity and photosynthetic functioning of six inoculated promiscuous-nodulating soybean genotypes. J Plant Physiol. 168:540–548. doi: 10.1016/j.jplph.2010.08.019.

Quilliam RS, DeLuca TH, Jones DL. 2013. Biochar application reduces nodulation but increases nitrogenase activity in clover. Plant Soil. 366:83–92. doi: 10.1007/s11104-012-1411-4.

Rajkovich S, Enders A, Hanley K, Hyland C, Zimmerman AR, Lehmann J. 2012. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biol Fert Soils. 48:271–284. doi: 10.1007/s00374-011-0624-7.

Rondon MA, Lehmann J, Ramírez J, Hurtado M. 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris L.*) increases with bio-char additions. Biol Fert Soils. 43:699–708. doi: 10.1007/s00374-006-0152-z.

Shearer G, Kohl DH. 1986. N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Funct Plant Biol. 13:699–756. doi: 10.1071/PP9860699.

Sika MP, Hardie AG. 2014. Effect of pine wood biochar on ammonium nitrate leaching and availability in a South African sandy soil. Eur J Soil Sci. 65:113–119. doi: 10.1111/ejss.12082.

Sprent JI, Geoghegan IE, Whitty PW, James EK. 1996. Natural abundance of ^{15}N and ^{13}C in nodulated legumes and other plants in the Cerrado and neighbouring regions of Brazil. Oecologia. 105:440–446. doi: 10.1007/bf00330006.

Sun Q, Liu Y, Liu H, Dumroese RK. 2020. Interaction of biochar type and rhizobia inoculation increases the growth and biological nitrogen fixation of *Robinia pseudoacacia* seedlings. Forests. 11:711.

Tag AT, Duman G, Ucar S, Yanik J. 2016. Effects of feedstock type and pyrolysis temperature on potential applications of biochar. J Analyt Appl Pyrolysis. 120:200–6. doi: 10.1016/j.jaap.2016.05.006

Tagoe SO, Horiuchi T, Matsui T. 2008. Effects of carbonized and dried chicken manures on the growth, yield, and N content of soybean. Plant Soil. 306:211–220. doi: 10.1007/s11104-008-9573-9.

Thies E, Rilling M. 2012. Characteristics of biochar: biological properties. In: Lehmann J, Joseph S, editor. Biochar for environmental management. Science and technology. London: Earthscan; p. 86–102.

Torabian S, Farhangi-Abriz S, Rathjen J. 2018. Biochar and lignite affect H+- ATPase and H+-PPase activities in root tonoplast and nutrient contents of mung bean under salt stress. Plant Physiol Biochem. 129:141–149. doi: 10.1016/j.plaphy.2018.05.030.

Unkovich M, Baldock J. 2008. Measurement of a symbiotic N_2 fixation in Australian agriculture. Soil Biol Biochem. 40:2915–2921. doi: 10.1016/j.soilbio.2008.08.021.

Wang Y, Lin Y, Chiu PC, Imhoff PT, Guo M. 2015. Phosphorus release behaviors of poultry litter biochar as a soil amendment. Sci Total Environ. 512:454–463. doi: 10.1016/j.scitotenv.2015.01.093

Xiu L, Zhang W, Wu D, Sun Y, Zhang H, Gu W, Wang Y, Meng J, Chen W. 2021. Biochar can improve biological nitrogen fixation by altering the root growth strategy of soybean in Albic soil. Sci Total Environ. 773:144564. doi: 10.1016/j.scitotenv.2020.144564.

Yahaya D, Denwar N, Mohammed M, Blair MW. 2019. Screening cowpea (*Vigna unguiculata (L.) Walp.*) genotypes for enhanced N_2 fixation and water use efficiency under field conditions in Ghana. Am J Plant Sci. 10:640–658. doi: 10.4236/ajps.2019.104047.

Yin S, Suo F, Kong Q, You X, Zhang X, Yuan Y, Yu X, Cheng Y, Sun R, Zheng H, Zhang C. 2021. Biochar enhanced growth and biological nitrogen fixation of wild soybean (*Glycine max subsp. soja Siebold & Zucc.*) in a coastal soil of China. Agric. 11:1246. doi: 10.3390/agriculture11121246.