

# Influence of biochar on soil nitrogen availability and maize yield in a hyper-arid Leptosol soil on the Peruvian Pacific coast

Influencia del biocarbón en la disponibilidad de nitrógeno del suelo y el rendimiento de maíz en un Leptosol hiperárido de la costa del Pacífico peruano

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## ABSTRACT

The aim of this study was to evaluate the effects of biochar on the soil nitrogen supply for maize. A field experiment was conducted across two crop cycles, with four treatments: control; urea (0.5 t.ha<sup>-1</sup>); biochar (1 t.ha<sup>-1</sup>); and a combination of urea (0.42 t.ha<sup>-1</sup>) and biochar (0.5 t.ha<sup>-1</sup>). Biochar was produced from municipal green waste using an adobe kiln and later blended with urea (75%) and clay (25%) for enrichment purposes. The changes in the supply rate of soil nitrogen and increased vegetative growth (+8% in the first crop and +41% in the second crop) and yield (+160% in the first crop and +110% in the second crop) in the maize, as a result of the combined application of biochar and urea, demonstrate the value of enriching biochar with synthetic nitrogen. Furthermore, the comparatively low maize yield when only biochar was applied suggests that enriching biochar with nitrogen fertilizer may be essential for achieving agronomic benefits with biochar, at least in the case of the low fertility Leptosol soil which was the focus of this study. These results offer an initial insight into the benefits and challenges associated with biochar application in Peru's sandy coastal soils, indicating promising effects on soil nitrogen stabilization.

**Keywords:** biochar, municipal green waste, urea, maize, nitrogen

## RESUMEN

El objetivo de este estudio fue evaluar los efectos del biocarbón en el suministro de nitrógeno del suelo para el maíz. Se realizó un experimento de campo durante dos ciclos de cultivo con cuatro tratamientos: control, urea (0,42 t.ha<sup>-1</sup>), biocarbón (1 t.ha<sup>-1</sup>), y una combinación de urea (0,42 t.ha<sup>-1</sup>) y biocarbón (0,5 t.ha<sup>-1</sup>). El biocarbón se produjo a partir de residuos verdes municipales utilizando un horno de adobe y posteriormente fue mezclado con urea (75 %) y arcilla (25 %) con fines de enriquecimiento. Los cambios en la tasa de suministro de nitrógeno del suelo y el incremento del crecimiento vegetativo (+8 % en el primer cultivo y +41 % en el segundo) y del rendimiento (+160 % en el primer cultivo y +110 % en el segundo) en el maíz, como resultado de la aplicación combinada de biocarbón y urea, demuestran el valor de enriquecer el biocarbón con nitrógeno sintético. Del mismo modo, el rendimiento comparativamente bajo del maíz cuando se aplicó biocarbón solo sugiere que enriquecer el biocarbón con fertilizantes nitrogenados puede ser esencial para obtener beneficios agronómicos. Los resultados ofrecen una primera aproximación a los beneficios y desafíos de la aplicación de biocarbón en los suelos arenosos costeros del Perú, y muestran efectos prometedores en la estabilización del nitrógeno del suelo.

**Palabras clave:** biocarbón, residuos verdes municipales, urea, maíz, nitrógeno



## Introduction

Reconciling the need for more sustainable agriculture with the need to satisfy the escalating food demands of a growing global population constitutes a major challenge (Zhang *et al.*, 2015). In this context, biochar has been proposed as a key component in an integrated strategy that could simultaneously address a wide array of current challenges: i.e., climate change, food security, waste management and renewable energy generation (Biederman and Harpole, 2013; Roberts *et al.*, 2010; Ladd *et al.*, 2025). Biochar is the solid product of the thermal degradation of biomass in an oxygen-limited environment (pyrolysis) and is distinguished from charcoal as a carbon-rich soil amendment product that can be made from sustainable feedstock (i.e., waste biomass) (Lehmann and Joseph, 2015). Biochar is a modern soil amendment technology directly inspired by 'terra preta de Índio' (Indian black earth), the thick, dark-colored, nutrient-rich, anthropic epipedon that overlies deep strongly weathered soils in the Amazon. Amazonian black earth is associated with the soil management practices of Pre-Columbian societies (Lima *et al.*, 2002). It is a product of the microbial metabolism and stabilization of ash, bones, waste biomass, manure, excrement, urine and biochar, with the latter constituting a crucial component by virtue of its stability and capacity to enrich the soil via the augmentation of cation exchange capacity (Glaser and Birk, 2012).

Biochar application in poor or weathered soils can be beneficial due to its liming effect, and also because it enhances cation exchange capacity (CEC) and water retention, thereby augmenting nutrient bioavailability, which has the potential to benefit crop yields (Fowles, 2007; Glaser *et al.*, 2002). The positive effects of biochar on crop plants are attributed to a range of factors, including improved soil biology and the physical and/or chemical improvement of soil properties (Cornelissen *et al.*, 2013; Jeffery *et al.*, 2011; Liu *et al.*, 2013). Recent studies conducted in order to assess the performance of enhanced efficiency fertilizers that use biochar have produced promising results (Chen *et al.*, 2018; Liu *et al.*, 2019; Pogorzelski *et al.*, 2020; Yao *et al.*, 2015). Furthermore, research has indicated that biochar can enhance nitrogen retention in field settings, principally through abiotic processes (Bai *et al.*, 2015; Ladd *et al.*, 2018).

As a primary nutrient, nitrogen is essential for plant growth and commonly functions as a limiting resource, with the potential to limit productivity, hence the extensive use of nitrogen fertilizers to increase production across a variety of agricultural systems (Bernhard, 2010). Around 48% of the nitrogen absorbed in the three major cereal crops (corn, rice and wheat) is supplied by synthetic nitrogen fertilizers (Ladha *et al.*, 2016). At the same time, a much smaller percentage (33%) of the synthetic nitrogen applied to agricultural fields actually achieves the aim of being absorbed by the target crop (Raun and Johnson, 1999). Nitrogen loss pathways include ammonia volatilization (Beusen *et al.*, 2008), nitrate leaching and surface runoff (Khan and Mohammad, 2014), dissolved organic nitrogen (DON) leaching (van Kessel *et al.*, 2009), and nitrous oxide emissions (Signor and Cerri, 2013). Therefore, considerable potential exists for improvements in the management of nitrogen within agricultural systems.

Given the growing body of evidence indicating the potential of biochar to reduce nutrient stress and improve crop yields, particularly in the case of tropical and degraded soils (Jeffery *et al.*, 2017), it would seem reasonable to hypothesize that biochar may increase nitrogen efficiency and maize yields in the low-fertility coastal soils of Peru (MINAM, 2016). Few studies have measured the variability of in-season nitrogen availability in soils amended with biochar (Martinsen *et al.*, 2014; Pandit *et al.*, 2018) and, therefore, the aim of this study was to assess the effect of biochar on the nitrogen supply to maize grown in the sandy Leptosol found in the province of Lima, across two crop cycles.

The aim of the study was to evaluate the following hypotheses:

- 1) That biochar stabilizes nitrogen in soil, thereby increasing plant nitrogen availability.
- 2) That biochar increases maize growth and yield through enhanced plant nitrogen uptake.

## Materials and methods

### Soil and biochar

A superficial layer (at a depth ranging from 0 to 30 cm) of Leptosol soil (IUSS Working Group WRB, 2015) was collected from a coastal desert area in the province

**Table 1.** Chemical and physical properties of the soil mixture

	pH	EC	Sand	Slit	Clay	Texture	OM	C
		dS.m-1	%				%	
Soil mixture	7.29	19.92	89.12	8	2.88	Sandy	10.38	6.02
	ANC	P (available)	K	CEC	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
	% CaCO <sub>3</sub> eq	ppm		emol.c.kg-1				
Soil mixture	1.27	23.4	180	24	15.56	7.86	0.17	0.41

Note. EC: Electrical conductivity; OM: Organic matter; ANC: Acid-neutralizing capacity; CEC: Cation exchange capacity.

of Lima, Peru (11°77' S, 77°15' W). This material was mixed with a compost-based substrate (2:1) (w/w) and then analyzed for its chemical properties. The soil mixture used in this experiment was found to be slightly alkaline (pH 7.29), with a high-CEC (6.05 cmol<sub>c</sub>.kg<sup>-1</sup> extracted with 1 N NH<sub>4</sub>CH<sub>3</sub>CO<sub>2</sub> at pH 7), strongly saline (19.92 dS.m<sup>-1</sup> with 1:1 soil-water solution), and 90% sand (Table 1).

The biochar was produced via slow pyrolysis at temperatures ranging from 450°C to 650°C for two days, using a 2-m diameter adobe brick kiln (Brown, 2009). The biochar feedstock was composed of a) green waste, from the pruning of *Ficus benjamina*, *Schinus terebinthifolius* and *Schinus molle* (94%); b) compost, produced from a mixture of green waste and cow manure (1:1) that was composted in trenches over a period of 3 months (4%); c) ground igneous rock (2%).

As detailed in their study by Joseph *et al.* (2013), a portion of the biochar produced (25%) was blended with clay (5%) and uncoated granular urea (70%), and then stored in a sealed container for 4 weeks, in order to ensure the integration of substrates before its incorporation into the soil. The treated biochar was analyzed for its chemical properties by the New South Wales Department of Primary Industries (DPI) (Sydney, Australia) (Table 2)

### Mesocosm experimental design

The experiment was conducted under field conditions, organized in accordance with a randomized complete block design. The experimental treatments were replicated in polyvinyl chloride tubes (diameter: 6 inches; length: 50 cm) buried in the ground to a depth of 45 cm. The four treatments were as follows:

- (i) Soil mixture only (CC)
- (ii) Soil mixture + urea at 0.42 ton/ha rate (FO)
- (iii) Soil mixture + biochar at 1 ton/ha rate (BO)
- (iv) Soil mixture + biochar at 0.5 ton/ha rate + urea at 0.42 ton/ha rate (BF)

Biochar was added to the soil mixtures in the tubes, with application rates of 2.02 g per tube (BO) and 1.01 g per tube (BF). The biochar application rate was selected based

on those detailed in similar studies (Liu *et al.*, 2013; Yang *et al.*, 2015). For each treatment, there were 10 replicates. In total, 40 tubes were placed in a fenced plot, at the same location where the soil had been collected. The experiment was conducted within the Antonio Raimondi National Ecological Park (PENAR). The average temperature was 23.4 ± 1.6 °C (average minimum temperature 20.7 °C and average maximum temperature 27.5 °C) during the first growing season, and 20.3 ± 1.7 °C (average minimum temperature 18.4 °C and average maximum temperature 23.0 °C) during the second growing season (SENAMHI, 2016).

### Soil nitrogen availability

Plant Root Simulator (PRS)<sup>TM</sup> probes (Western Ag Group, Saskatoon, Canada) were used to measure soil nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) supply rates. One pair of cation and anion probes per tube was inserted into the soil in 5 randomly selected tubes per treatment (20 in total). Three separate deployments of the PRS probes were performed per crop cycle (6 in total). After each deployment, the probes were removed and replaced with a new set of PRS probes in the same experimental tubes. The timing and duration for each of the six probe deployments were as follows:

First crop cycle:

- Period A: 8 days, before sowing
- Period B: 31 days, after sowing
- Period C: 57 days, after urea addition

Second crop cycle:

- Period D: 7 days before sowing
- Period E: 74 days, covering the first half of the cycle
- Period F: 74 days, covering the second half of the cycle

Upon removal from the tubes, the probes were cleaned and rinsed with deionized water and sent to Western Ag Innovations for analysis. The probes were extracted with 0.5 mol.dm<sup>-3</sup> HCl and subjected to colorimetric analysis for NO<sub>3</sub>-N and NH<sub>4</sub>-N, employing an automated flow injection analysis (FIA) system.

**Table 2.** Chemical and physical properties of the treated biochar

	pH	CE	C	N	C/N	Al	Ca	Fe	K	Na	P	S	Mg
	(CaCl <sub>2</sub> )	dS.m <sup>-1</sup>	%			%							
Biochar	7.4	1.9	36.09	0.85	42.46	0.13	1.2	0.75	0.7	0.083	0.26	0.11	0.26
	ANC	As	B	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Se	Zn
	% CaCO <sub>3</sub> eq	ppm											
Biochar	2.7	<5.0	9.6	<0.3	4.8	4.4	12	460	1.3	2.6	4	<4	72

Note. EC: Electrical conductivity; ANC: Acid-neutralizing capacity.



## Cultivation of *Zea mays* L.

Flint maize (*Zea mays* L. var. *indurata* [Sturtev.]) was cultivated across two crop cycles. The first crop was grown from January to April 2015 and the second crop from August 2015 to January 2016. The second crop cycle was longer, due to slower development attributable to the winter temperatures (FAO, 2015). In each tube, two seeds were sown 2 cm below the surface. On Day 31 of the first crop cycle, FO and BF treatment tubes were top dressed with uncoated granular urea (0.42 g N per pot). Equivalent to 230 kg/ha, this application rate was based on the nitrogen rates recommended by the University of Minnesota guidelines (Kaiser et al., 2016). No supplementary soil amendment was applied subsequently; the same soil was maintained in the tubes for the second crop cycle, in order to evaluate the residual effects of the treatments. Irrigation was provided as required in order to maintain soil moisture and prevent water stress.

## Plant measurements

On Day 1 of the tasseling stage (VT) (Reitsma et al., 2009), the height (measured from the stem base to the tassel tip) and the number of leaves of each plant were recorded and one fully expanded leaf from the lower third of each plant was harvested. The leaf area of the collected leaf samples was determined using Image J software (National Institute of Mental Health, Bethesda, Maryland, USA), then dried in an oven at 67°C for three days and weighed in order to determine its dry weight. The specific leaf area (SLA) is the fresh leaf area divided by its dry mass (Poorter et al., 2012).

Whole plants were harvested at the end of each growing season and separated into roots, stems, leaves and corn, dried in an oven at 67°C for three days, and weighed in order to determine total plant biomass and biomass allocation. Total plant biomass was determined by adding together the dry weights of these components, and biomass allocation was calculated as the mass fraction of each part relative to the total biomass. The first crop cycle was interrupted at the blister stage (R2) due to the presence of *Spodoptera frugiperda*, a chronic pest whose caterpillars damage plant tissue through chewing (Flanders et al., 2007). An estimate of the probable yield at the maturity stage (R6) was calculated based on the study by Reitsma et al. (2009).

Thereafter, leaf nitrogen concentration (LNC) was determined for the plants in the second crop cycle using the Dumas combustion method, employing a LECO C/N auto-analyzer, in order to determine leaf nitrogen uptake at the end of the crop cycle.

## Statistical analysis

One plant per tube was selected for analysis. Shapiro-Wilk, Pearson's chi-squared and Kolmogorov-Smirnov tests were performed to ensure normal distribution

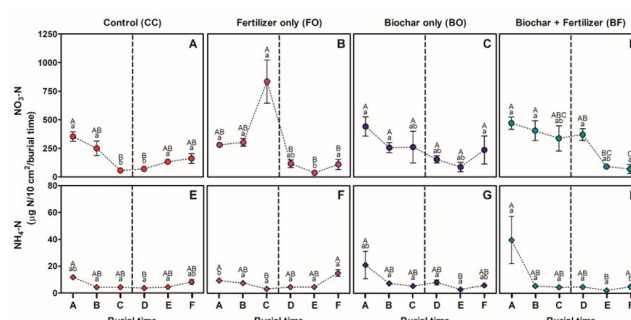
and homogeneous variances. Data conforming to these assumptions was analyzed through a one-way analysis of variance (ANOVA) and analyzed using Tukey's Honest Significant Difference (HSD) test, in order to assess any significant differences. Nonparametric data was analyzed using the Kruskal-Wallis and Wilcoxon tests, followed by the Dunn's test, in order to assess significant any differences. Results at  $p < 0.05$  are considered significant. All statistical analysis was conducted using JMP 15 statistical software (SAS Institute, Cary, NC, USA).

## Results

### Soil nitrogen supply

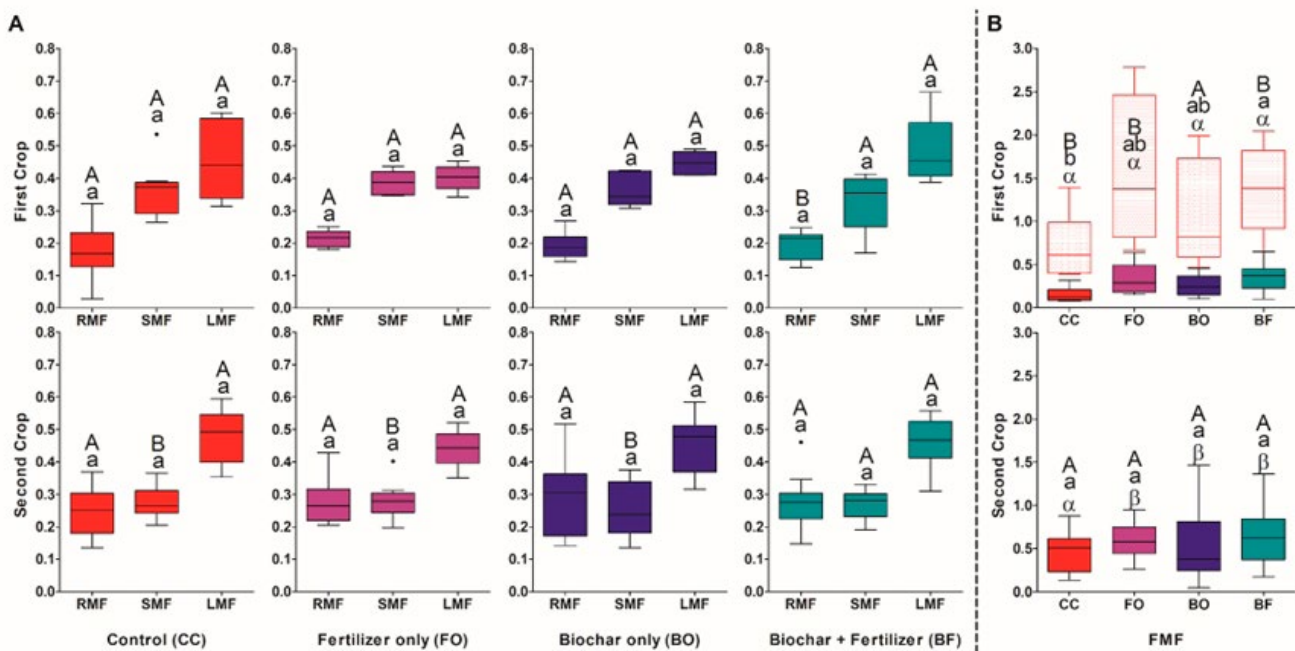
The addition of fertilizer significantly increased the soil nitrate ( $\text{NO}_3\text{-N}$ ) supply rate during the first crop cycle. The fertilizer-only (FO) treatment peaked during period C at  $833.93 \pm 420.02$ , a rate significantly higher than the  $56.37 \pm 35.85$  observed in the control (CC) treatment ( $p = 0.0033$ ). During the second crop cycle, the biochar + fertilizer (BF) displayed a significantly higher supply rate in period D ( $370.30 \pm 116.30$ ) compared to the control ( $69.76 \pm 19.56$ ;  $p = 0.0028$ ). This period was also marked by a significant decline in the FO treatment, which dropped from its peak to  $116.69 \pm 74.77$  ( $p = 0.0267$ ). Later in the second cycle (during period E), the supply rate in the control treatment ( $132.17 \pm 50.52$ ) was found to be significantly higher than in the FO treatment ( $36.32 \pm 14.41$ ;  $p = 0.0099$ ).

The supply rates for ammonium ( $\text{NH}_4\text{-N}$ ) showed different trends. In period A of the first crop cycle, the rate in the BF treatment ( $39.61 \pm 39.26$ ) was significantly higher than in the FO treatment ( $9.44 \pm 2.29$ ;  $p = 0.0233$ ). This trend was reversed by the end of the experiment, when the BF treatment rate was significantly lower than that of the FO treatment in period F of the second crop cycle ( $4.80 \pm 1.98$  vs.  $15.12 \pm 5.46$ , respectively;  $p = 0.0166$ ) (Figure 1).



**Figure 1.** Soil nitrogen supply rates over two crop cycles as affected by the addition of biochar and fertilizer to a sandy soil. Treatments included an unamended control (CC), fertilizer only (FO), biochar only (BO), and biochar plus fertilizer (BF). Error bars represent the standard error of the mean (SEM,  $n=5$ ). Different lowercase letters indicate significant differences among treatments at each time point, while different uppercase letters denote significant differences between crop cycles for a given treatment ( $p < 0.05$ ). PRS™ probe burial times for the first crop cycle were: A=8 days, B=31 days, and C=57 days; and for the second crop cycle: D=7 days, E=74 days, and F=74 days.





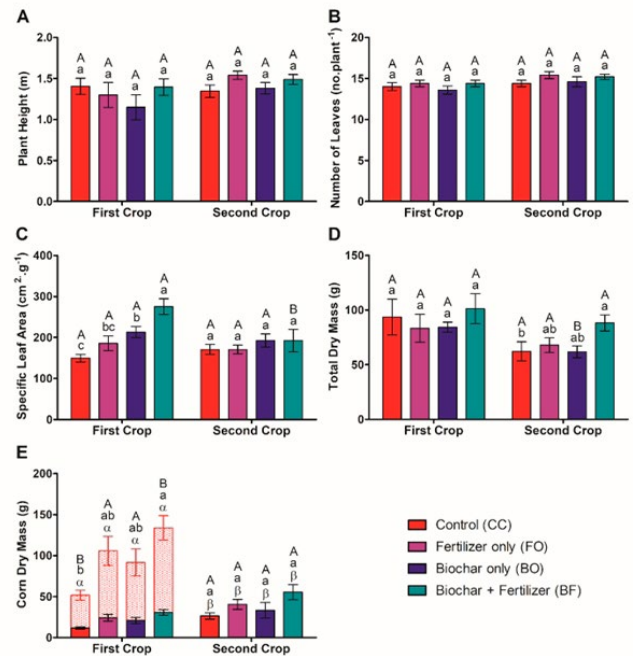
**Figure 2.** Biomass allocation of the two maize crops as affected by the addition of biochar and fertilizer. A) Biomass fraction allocated to leaves (LMF), stems (SMF), and roots (RMF). B) Fruit mass fraction (FMF, solid boxes) and estimated corn dry mass (g, hatched boxes). The hatched boxplots in B) represent the estimated yield at maturity (R6) for the first crop, calculated from post-harvest plant size data to account for insect damage. For panel A, uppercase letters denote significant differences among biomass components (RMF, SMF, LMF) within each treatment. For panel B, lowercase letters denote significant differences among treatments within a crop cycle. For both panels, Greek letters denote significant differences between the first and second crop for the same component and treatment ( $p < 0.05$ )

### Biomass allocation

Although there were no significant differences across the treatments with regard to root mass fraction (RMF), stem mass fraction (SMF), and leaf mass fraction (LMF), SMF was found to have significantly decreased for CC across the crop cycles (from  $0.37 \pm 0.08$  to  $0.28 \pm 0.05$ ;  $p$  value: 0.0113), FO (from  $0.38 \pm 0.04$  to  $0.28 \pm 0.05$ ;  $p$  value: 0.0022), BO (from  $0.36 \pm 0.05$  to  $0.26 \pm 0.09$ ;  $p$  value: 0.0177) and RMF was found to have significantly increased for BF (from  $0.19 \pm 0.04$  to  $0.27 \pm 0.09$ ;  $p$  value: 0.0199) from the first to the second crop cycle (Figure 2A). The fruit mass fraction (FMF) ( $0.35 \pm 0.17$ ) of the first crop cycle in BF ( $0.15 \pm 0.08$ ) was significantly higher than in CC ( $p$  value: 0.0159). Across the crop cycles, FMF was significantly higher during the first crop cycle for CC ( $p$  value: 0.0051), FO ( $p$  value: 0.0332) and BF ( $p$  value: 0.0294). Similarly, the FMF of the first crop was significantly higher for FO (from  $1.42 \pm 0.82$  to  $0.59 \pm 0.20$ ;  $p$  value: 0.0120), BO (from  $1.12 \pm 0.56$  to  $0.53 \pm 0.44$ ;  $p$  value: 0.0346) and BF (from  $1.51 \pm 0.72$  to  $0.66 \pm 0.36$ ;  $p$  value: 0.0039) than for the second crop (Figure 2B).

### Plant growth and yield

The SLA of the first crop in the BF treatment ( $275.19 \pm 60.89$ ) was significantly higher than in the CC treatment ( $149.71 \pm 28.70$ ;  $p < 0.001$ ), FO ( $185.70 \pm 39.56$ ;  $p$  value: 0.0101), BO ( $212.92 \pm 43.35$ ;  $p$  value: 0.0241) and BF of the second crop ( $192.10 \pm 86.45$ ;  $p$  value: 0.0230), while the BO



**Figure 3.** Plant measurements of the two maize crops as affected by the addition of biochar and fertilizer. Bars represent means  $\pm$  SEM. The hatched bars in E) represent the estimated yield at maturity (R6) for the first crop based on post-harvest plant size data. Lowercase letters denote significant differences among treatments within each crop cycle. Uppercase and Greek letters denote significant differences between the first and second crop cycle for the same treatment ( $p < 0.05$ )

( $212.92 \pm 43.35$ ) of the first crop was significantly higher than in the CC (p value: 0.0051) (Figure 3C). There was no significant difference among treatments and between crops with regard to plant height and number of leaves (Figure 3A, 3B).

The total dry mass of the second crop in the BF treatment was significantly higher than in the CC treatment (from  $93.46 \pm 46.62$  to  $101.11 \pm 41$ ; p value: 0.00131), while the BO treatment was found to have significantly decreased from the first crop (from  $84.25 \pm 11.35$  to  $61.80 \pm 17.14$ ; p value: 0.0132) (Figure 3D). Biochar and fertilizer amendments in the BF treatment resulted in greater plant biomass in the CC, FO and BO treatments, by 8.2%, 21.4% and 20% respectively, in the first crop, and by 41.4%, 29.8% and 42.6% in the second crop.

The corn dry mass in the BF treatment was significantly higher than in the CC treatment for the first crop cycle ( $11.84 \pm 3.91$  vs  $30.78 \pm 10.28$ ; p value: 0.0015). Also, in the CC (p value: 0.0051) and BF (p value: 0.0373) treatments significant differences between the crop cycles were found. Furthermore, the predicted corn dry mass of the first crop was significantly higher for the CC treatment (from  $51.30 \pm 16.94$  to  $26.46 \pm 12.33$ ; p value: 0.0024), FO

(from  $105.3 \pm 39.37$  to  $40.61 \pm 18.82$ ; p value: 0.0007), BO (from  $91.22 \pm 39.74$  to  $33.5 \pm 29.83$ ; p value: 0.0108) and BF (from  $133.37 \pm 44.53$  to  $55.51 \pm 29.30$ ; p value: 0.0003) than for the second crop cycle (Figure 3E). In the biochar and fertilizer amended tubes (BF), the corn mass was higher than in the CC, FO and BO treatments by 160%, 26.7% and 46.2%, respectively for the first crop cycle, and by 109.8%, 36.7% and 65.7% for the second crop cycle.

The LNC ranged from 0.48 to 0.91%, with an outlier of 1.2% in the case of the CC treatment. No significant differences were found among the treatments (Figure 4A). Leaf N uptake in the BF treatment was significantly higher than for the CC treatment (p value: 0.0407) (Figure 4B).

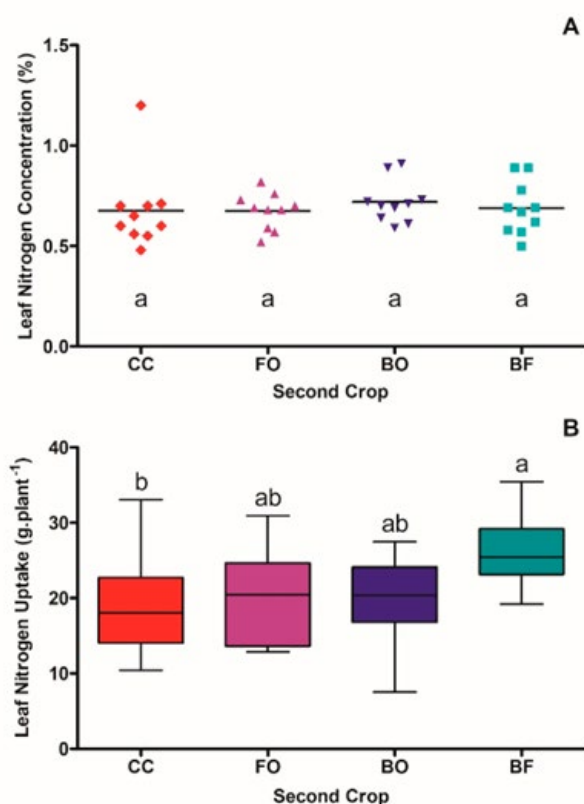
## Discussion

### Soil nitrogen availability

Ammonium values were low compared to nitrate values, probably due to the dominance of nitrification (Fenn and Poth, 2001) across both the crop cycles. Warm temperatures and moderately alkaline soil are optimal for higher nitrification rates (Vaccari *et al.*, 2005). Moreover, urea is utilized as a source of N by nitrifying microorganisms, which accounts for the rapid nitrification of urea-N in soils (Marsh *et al.*, 2005).

Higher  $\text{NH}_4\text{-N}$  values for both the BO and BF treatments in period A may be attributable to the enrichment of the biochar, if we remember that Joseph *et al.* (2013) argued that the biochar matrix reacts with clay and chemical fertilizer, and Spokas *et al.* (2012) reported that carboxyl groups on the biochar surface may lead to the formation of ammonium salts or amides, facilitating its uptake by plants. Ammonia-N adsorbed by biochar is bioavailable in the soil matrix (Taghizadeh-Toosi *et al.*, 2012). Also,  $\text{NH}_4^+$  linked to clay may have provided a temporary sink for plant-available N that would otherwise have been lost to  $\text{NH}_3$  volatilization (Nieder *et al.*, 2011). Both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  values decreased as the growing seasons progressed, presumably due to plant uptake (Bloom *et al.*, 2012).

Plant uptake was probably responsible for most of the reduction over time in the  $\text{NO}_3^-$  supply (Gastal and Lemaire, 2002; Rozas *et al.*, 2004), along with leaching due to irrigation (Lehmann and Schroth, 2003). Slower rates of nitrate loss in the BO and BF treatments compared to the CC treatments suggest that the biochar may have reduced nitrate leaching (Knowles *et al.*, 2011; Yao *et al.*, 2012). The contrast between the FO and BF treatments after fertilization indicates that biochar can retain soluble  $\text{NO}_3^-$  in its porous structure or via electrostatic interactions resulting from functional groups on the biochar surface (Glaser *et al.*, 2002; Taghizadeh-Toosi *et al.*, 2012; Prendergast-Miller *et al.*, 2014). In their work, Clough *et al.* (2013) mentioned that  $\text{NO}_3^-$  could be desorbed by water infiltration since sorption is weak, resulting in an increased residence time in the soil for  $\text{NO}_3^-$ . This is particularly important in the case of sandy or coarse soils



**Figure 4.** A) Leaf nitrogen concentration (LNC) and B) leaf nitrogen uptake (LNU) at the end of the second crop cycle as affected by the addition of biochar and fertilizer to sandy soil. Significant differences among treatments are represented by lowercase letters (p<0.05). Solid lines in A) represent means. In B), the horizontal line within each box represents the median

amended with biochar, which benefit from water retention (Borchard *et al.*, 2019). In addition, the presence of biochar may also affect anion exchange capacity (AEC), which may improve nitrate retention (Lawrinenko and Laird, 2015); however, biochar aging leads to a substantial reduction of this property, due to the disappearance of positive surface charge in the biochar (Cheng *et al.*, 2008). These results highlight the need for longer term experiments.

Furthermore, it is important to point out that biochar may have caused a reduction in  $\text{NH}_3$  volatilization because the biochar absorbed water-soluble  $\text{NH}_4^+$  and  $\text{NH}_3$  (Steiner *et al.*, 2010), while at the same time high soil CEC (Zhenghu and Honglang, 2000) is associated with a fraction of  $\text{NH}_4^+$  that is not instantaneously accessible for nitrification (Ulyett *et al.*, 2014). Rozas *et al.* (2004) reported a 7% N loss by volatilization in an acidic soil where 210 kg N/ha rate was applied. On the other hand, soil pH may have promoted loss via gaseous pathways (Rochette *et al.*, 2013) due to the fact that urea is hydrolyzed under moist warm conditions (Lei *et al.*, 2017); however, the soil pH experiment probably decreased nitrification, an acidifying process, which might have counteracted evaporative losses to some degree (Fageria *et al.*, 2010). In any case, our data does not allow for a clear discrimination between leached and volatilized nitrogen.

The prominent spike in nitrate supply rate in period C of the FO treatment (Figure 1B) constitutes a striking result, given that most of the N uptake would occur at this stage of crop development (Butzen, 2011).  $\text{NH}_4^+$  from urea addition may have affected  $\text{K}^+$ , as a result of cation competition favorable to the former which at the same time suppressed  $\text{NO}_3^-$  (Bar-Tal, 2011); chlorosis in these plants is a sign of potassium deficiency (Murrell, 2010). The nitrate available during this peak in availability was almost certainly lost via denitrification once the first crop had completed its cycle (Ghosh *et al.*, 2017), given that the termination of the experiment precluded the possibility of N loss by leaching or crop removal. Values of N availability in the BF treatment tubes, compared to those of the FO treatment, point to biochar's role in avoiding this loss pathway, by lowering  $\text{N}_2\text{O}$  emissions resulting from  $\text{NO}_3^-$ -adsorption, and also because biochar is known to improve soil aeration (Taghizadeh-Toosi *et al.*, 2011; van Zwieten *et al.*, 2010a).

Maize plants absorb around 63% of their nitrogen requirement by the time the flowering stage (R1) begins (DeBruin and Butzen, 2014). This may explain to some degree the increase in soil N availability from period E to F in the second crop cycle. Nevertheless, there was a steady decline in the BF treatment (Figure 1D) suggesting a higher N uptake (Saarnio *et al.*, 2013). Phosphorus (Kloss *et al.*, 2014) and sulfur (Churka Blum *et al.*, 2013) sources available in biochar help reduce  $\text{NO}_3^-$  accumulation in soil by promoting N absorption (Fan *et al.*, 2003; Jamal *et al.*, 2010). Also, the decrease in nutrients susceptible to

leaching and which had been removed by the experimental maize plants, such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  (Lehmann and Schroth, 2003), may have led to a reduction in plant N uptake capacity (Grzebisz *et al.*, 2013; Mohamed, 2012; Ortas, 2018). This was not the case in the BO and BF treatments, due to the nutrient retention and the provision of nutrients associated with biochar (Ippolito *et al.*, 2015; Kloss *et al.*, 2014; Lehmann *et al.*, 2003; van Zwieten *et al.*, 2010b). Biochar aging tends to lower AEC, while increasing biochar CEC, due to the formation of carboxylic groups and other oxygenated functional groups on the biochar surface, which enables the maintaining of soil fertility (Cheng *et al.*, 2006; Cheng and Lehmann, 2009). Higher rates of biochar application, as in the BO treatment, constitute a potential risk of available phosphorus restriction in soil, as well as exchangeable  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Deenik *et al.*, 2010; Hailegnaw *et al.*, 2019; Parvage *et al.*, 2013). In their work, Deenik *et al.* (2010) stated that, in the absence of N supplements, biochar had a mild influence on micronutrient uptake, compared with non-amended soil. Importantly, in maize crops N fertilization can increase the uptake of essential nutrients (Bruns and Ebelhar, 2006; Pasley *et al.*, 2019).

### Biomass allocation

The fruit mass fraction (FMF) values for the first crop mirror the results of Olmo and Villar (2019). In their study, they concluded that the strong influence of biochar on soil properties leads to a positive effect on the FMF, yield and aboveground biomass of different species. In common with our own study, they also found that the fractions of stems and leaves displayed no significant effects (Figure 2B). Up to 60% of the total N in grain is absorbed prior to anthesis. Nitrogen is transferred from the vegetative tissues to the ears of maize during the grain-filling stage (Lafitte, 2001; Rodríguez Bragado *et al.*, 2014). The absence of such an effect in the FMF in the second crop cycle could be a response to lower N availability (Mu *et al.*, 2016), with the biochar possibly acting as a buffer under these conditions. A decrease in N supply reduces dry matter partitioning to grain during the grain-filling period (Andrade *et al.*, 2002). Additionally, it was observed that the stem mass fraction (SMF) in the second crop was lower, whereas the root mass fraction (RMF) was higher (Figure 2A). This shift in biomass allocation is a typical indicator of several factors, including nutrient limitation, lower temperatures and stem flexure due to wind exposure (Anten *et al.*, 2005; Poorter and Nagel, 2000; Poorter *et al.*, 2012). The effect of salinity on allocation is minimal (Poorter *et al.*, 2012).

### Plant growth and yield

Specific leaf area (SLA) is linked to soil nutrient and water availability (Reich *et al.*, 2003; Wright *et al.*, 2001). A plastic response to increasing levels of N fertilization has been observed in herbaceous plants, including Poaceae species (Knops and Reinhart, 2000; Meziane and Shipley,





2001). In previous studies, maize has been shown to display higher SLA values when soil N availability is higher throughout the crop season (Amanullah *et al.*, 2007). Consistent with this finding, the SLA values in the BF treatment during the first crop cycle may be attributable to the effects of biochar on this trait (Albuquerque *et al.* 2014; Olmo and Villar, 2019). In contrast, the significantly lower SLA in the BF treatment during the second crop cycle was expected, given that there was no additional amendment of the soil (Figure 3C). Overall similarity in SLA values across the crop cycles may have been attributable, in part, to the fact that lower levels of insolation lead to a higher SLA during winter (Danalatos *et al.*, 1994; Liu *et al.*, 2016). We therefore suggest that the significantly different SLA values in the BF treatment tubes is primarily a response to N availability. As for the number of leaves and plant height, the absence of significant differences can be traced back to previous studies, where sole nitrogen fertilization was inferior to NPK applications, especially where N was supplied as urea (Amin, 2011; Gungula *et al.*, 2005). Nevertheless, growth and yield did show improvement (Imran *et al.*, 2015; Karasu, 2012).

In the second crop, total dry mass in the BF treatment was noticeably higher than in the other treatments, particularly when compared to the control (CC) treatment (Figure 3D). Several studies have reported greater plant growth responses when biochar is combined with fertilizers, suggesting a synergistic relationship (Chan *et al.*, 2007; Deenik *et al.*, 2010; Lehmann *et al.*, 2003; Steiner *et al.*, 2007). Moreover, Widowati *et al.* (2012) asserted that a higher nutrient availability due to biochar and fertilizer application resulted in a significantly higher second crop biomass in comparison to untreated soils, based on a pot experiment over two successive maize seasons, conducted in order to study the residual effect of biochar on plant development. Conversely, growth in the FO treatment of the first crop was 10.9% lower than in the CC treatment.  $\text{NH}_3/\text{NH}_4^+$  toxicity may disturb root growth and induce chlorosis, although under normal growing conditions they are converted to  $\text{NO}_3^-$  by nitrifiers, and accumulation in the substrate is rare (Britto and Kronzucker, 2002; Mattson, 2011). This is consistent with the PRS data (Figure 1). Similarly, growth in the BO treatment was 9.9% lower than in the CC treatment of the first crop, and 0.8% in the second crop. The ability of biochar to retain nutrients has been discussed previously, and such retention can limit plant availability (Atkinson *et al.*, 2010; Kammann *et al.*, 2015). This may have been the case in the extremely poor soil studied in this experiment. Furthermore, the presence of bioavailable polycyclic aromatic hydrocarbons (PAHs), a pyrolysis sub-product, in biochar may have affected seedling growth (Baek *et al.*, 2004; Hale *et al.*, 2012; Rogovska *et al.*, 2012). The fact that these detrimental impacts were not observed in BF treatment points to the conclusion that low rates of biochar application (as used here), coupled with suitable

fertilization, can neutralize potentially adverse impacts (Rajkovich *et al.*, 2012). Biodegradation of PAHs is a potential explanation for the mitigated negative effects observed in the biochar-only (BO) treatment during the second crop cycle (Couling *et al.*, 2010).

The corn dry mass in the BF treatment was superior during both crop cycles and was significantly higher than in the CC treatment tubes during the first crop cycle (Figure 3E). This finding is consistent with the results from a biochar composite reported by Joseph *et al.* (2013), which showed a significant improvement for rice yield. Furthermore, it aligns with numerous studies attributing the improved yield of maize from combined biochar and fertilizer applications to enhanced water holding capacity and increased crop nutrient availability (Agegnehu *et al.*, 2016; Jeffery *et al.*, 2011; Omara *et al.*, 2020). The negative effects of biochar when applied alone appear to be short term, as evidenced by the higher maize yield in the BO treatment during the second crop cycle. Potentially, this recovery may be due to the breakdown of organic phytotoxic compounds by soil microbes (Borchard *et al.*, 2014) or the stabilization of a transiently high C:N ratio, leading to N immobilization (Kloss *et al.*, 2014). Collectively, these findings indicate that enriching biochar with fertilizers and/or minerals is essential for achieving its full agronomic potential.

The superior growth and yield of maize in the BF treatment was reflected in the physiology of the plants, as evidenced by a significantly higher leaf N uptake during the second crop cycle. This increased plant uptake, combined with the comparatively lower soil N supply in the BF treatment versus the control, suggests that the biochar enhanced nitrogen use efficiency by limiting wasteful N losses. This mechanism enables plants to absorb and store nitrogen when it is abundant, for later use under conditions of nitrogen limitation (Plénet and Lemaire, 1999). However, the similarity in LNC values (Figure 4A) indicates that N consumption in the BF treatment plants may have reached a physiological peak (Galindo *et al.*, 2019). This suggests that further investigation into crop N status, using leaf N data, could help to more precisely determine in-season nitrogen requirements (Zhao *et al.*, 2017). Ultimately, the residual effects of the soil amendments on nitrogen availability highlight the importance of enriching biochar with nutrients such as N, in order to maximize its agronomic benefits.

It is also important to note that high soil salinity is challenging for agriculture, as it is responsible for lower nutrient uptake and reduced grain yield (Farooq *et al.*, 2015). Limited N uptake and reduced rates of N mineralization under saline conditions (Irshad *et al.*, 2005) may account for the findings in the FO treatment in period A and the CC treatment in period D, where values increased despite crop removal and irrigation (Figure 1A, 1B). Previous studies have indicated that biochar may alleviate salt



stress by regulating plant responses to salinity and reducing Na<sup>+</sup> uptake in favor of K<sup>+</sup>, while also increasing nutrient uptake and subsequent crop development (Ali *et al.*, 2017), although high rates of biochar application would be required in order to mitigate salt stress (Thomas *et al.*, 2013). While the limits of biochar benefits will need to be defined for different environments and land uses, the co-application of biochar with nutrients such as N can increase significantly the agronomic benefits of biochar.

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## Authorship contribution

CC was responsible for the gathering of data and the writing of the article; LMLU was responsible for the writing and editing of the article; BL was responsible for the conception of the study, and subsequent guidance.

## Conflicts of interest

The authors have no potential conflicts of interest to declare.



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