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ORIGINAL ARTICLE

Mixing ratio and Nitrogen fertilization drive synergistic effects between biochar and compost

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Abstract Compost and biochar are increasingly considered to improve crop growth and soil functioning in agriculture. However, their combined application has shown contrasting results, probably resulting from the use of different biochar/compost ratios and divergent (synergistic or antagonist) impacts on nutrient availability, especially nitrogen (N). We aimed to elucidate how biochar/compost mixtures affect nutrient availability and plant growth. We hypothesised that biochar and compost will have a synergistic effect, which will depend on the biochar/compost ratio, consequently impacting nutrient uptake and biomass of plants. In this context, ryegrass was grown on agricultural soil amended with five compost/biochar ratio mixtures with and without N fertilisation. We followed soil fertility parameters, soil microbial carbon (C) and N, nutrient uptake, and plant growth. Results showed that irrespective of their ratio, biochar and compost mixtures had no effect on microbial biomass but increased soil nitrate concentration, suggesting that, despite their high C/N ratios, amendments increased N availability while preventing microbial immobilisation. Plant biomass and nutrient uptake improvements depended on the biochar/compost mixing ratio. Plant stoichiometric analysis revealed that a mixture containing less

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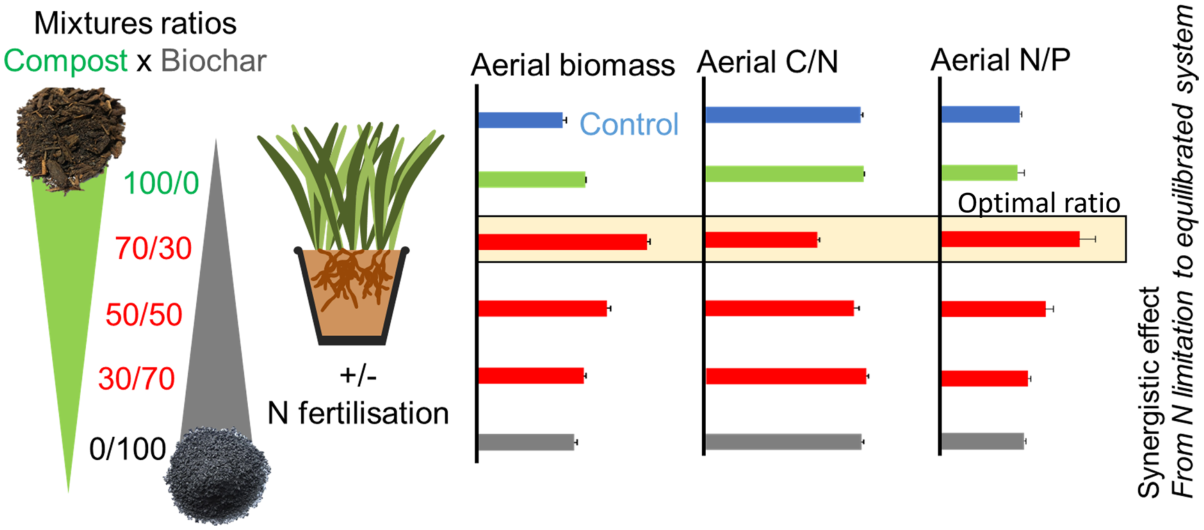
biochar than compost reduced N limitation and was even more effective in stimulating plant growth than mineral N fertiliser. The beneficial effects of biochar and compost on plant growth were strengthened when used in combination with N fertilisation. In conclusion, we demonstrated synergistic effects between biochar and compost, predominantly driven by their mixing ratio, to reduce N limitation in the soil towards a more nutrient-equilibrated system and highlighted their potential use as a sustainable alternative or supplement to mineral fertilisers.

Graphical abstract



Keywords Biochar · Compost · Soil fertility · Sustainable solutions · Synergism

Introduction

The exponential rise of the human population is associated with increasing consumption and demand for agricultural products (Kopittke et al. 2019). To improve yields, the use of mineral fertilisers increased exponentially throughout the world over the last decades (Savci 2012), and led to disruptions of biogeochemical cycles with deleterious environmental impacts (El-Naggar et al. 2019; Laghari et al. 2016; Tei et al. 2020). Indeed, excessive fertilisation has adverse effects on soil microbial communities (Savci 2012) and may also be a threat for water quality due to high N and P losses to aquatic systems (Savci 2012; Tei et al. 2020). Consequently, there is a need to find more sustainable and cost-effective materials to supply nutrients to plants (Igalavithana et al. 2015). One option are organic amendments, which not only supply nutrients, but also improve the overall soil quality (Diacono and Montemurro 2010; Siedt et al. 2021) and may be beneficial for soil organic carbon (SOC) sequestration (Chabbi et al. 2017; Védère et al. 2023). Among the different potential organic amendments, biochar and compost are the subject of many studies due to their ability to improve soil fertility and the SOC content simulaneously.

Compost is a stabilised product, rich in organic matter, resulting from the microbial degradation of organic wastes (Diacono and Montemurro 2010; Kammann et al. 2016). The composting process reduces the amount of organic wastes as the majority is mineralised and the resulting material can be used for soil amendment. However, at the end of the process, not all materials are composted, and compost refusals are leftovers, which still need to be disposed. To further reduce waste in the context of a circular economy, these composting refusals can be pyrolyzed to produce biochar in a cost-effective and sustainable process, which, to our knowledge, is a so far neglected possibility. Biochar is a stable and carbon-rich product, which has a high surface area and porosity, and is used as a soil amendment in degraded environments to improve soil physical and chemical properties (Chen et al. 2019; Karim et al. 2022). However, as biochar and compost are derived from a wide variety of precursor materials, they are characterized by contrasting properties (Abbott et al. 2018).

Previous studies hypothesised that combining nutrient rich compost with biochar will induce synergistic effects on the soil quality, nutrient supply, microbial activity and ultimately plant growth (Kammann et al. 2016; Radin et al. 2018). Indeed, compost contains organic matter, can be rich in nutrients and stimulate microbial activity. However, it is easily decomposable and subject to rapid loss. On the other hand, biochar is a more stable product with a low nutrient content but with high sorption potential able to store nutrients and supply them to plants during their growth. Biochar, as a very porous material, can also serve as a habitat for microorganisms (Chen et al. 2019). Previous studies applying biochar and compost in combination showed contrasting results, with positive, neutral or negative effects of the combined treatments compared to the single ones. More precisely, a higher plant biomass and nutritious state and higher organic carbon was found by Abbas et al. (2020) when applying a combination of biochar and compost at a 1:1 ratio, whereas Doan et al. (2015) applied a mixture of biochar and compost at a ratio 1:3 and found a higher soil N content and maize yield than the single amendments. No difference between the single and combined biochar/compost treatments were observed in two studies with mixing ratios ranging between 1/3 and 1/5 with ryegrass and lettuce (Aubertin et al. 2021; Trupiano et al. 2017). Finally, negative effects of the mixture on plant biomass, height, leaf area and nutritious state were observed at a mixing ratio of 1:1.2 (Libutti and Revelli 2021; Seehausen et al. 2017). These studies showed that there is no clear trend on the best ratio between biochar and compost, and potential synergism, to improve soil properties and plant growth. Furthermore, most of the cited studies tested only one biochar/compost ratio. The potential synergistic effects between biochar and compost may be highly dependent on the ratio between recalcitrant and labile carbon (C) compounds and thus the effect of different proportions of biochar and compost in the mixtures needs to be evaluated. In fact, one of the possible limitations of the use of biochar could be its potential negative effect on plant N availability, although it was shown to have beneficial effects on K availability (Nobile et al. 2022). When applied to soil, due to its high proportion of C relative to N, biochar can induce a microbial immobilisation of N (Abbas et al. 2020; Chen et al. 2021; Schofield et al. 2019) even though this effect depends on the type of biochar and its C mineralisation potential (Nguyen et al. 2017). As a result, additional mineral N fertilisation may be required when biochar is added to the soil (1) to avoid N deficiency in plants due to N immobilisation by microorganisms and (2) to sustain crop production (Iglesias-Jimenez and Alvarez 1993; Li et al. 2022). On the other hand, Dakora and Phillips (2002) showed that biochar C/N ratio was a poor predictor of soil N mineralisation and that, unexpectedly, biochar tended to raise N mineralisation potential in soil by inducing a priming effect.

In this study, we aimed at gaining insight into the impact of biochar and compost mixed at different ratios on nutrient availability and plant growth. For this purpose, we investigated the effect of five biochar/compost mixtures on (i) soil chemical properties, (ii) nutrient availability, and (iii) plant growth, under two N fertilisation regimes. We monitored aboveground ryegrass growth, and above- and belowground nutrition status of the plants after three harvests. We hypothesised that (i) biochar/compost mixtures will show a synergistic effect leading to a greater improvement of soil fertility and plant growth than the two amendments applied alone, and (ii) the intensity of the synergistic effect will depend on the ratio between biochar and compost and the nitrogen fertilisation.

Materials and methods

Soil and amendments

Soil was collected from an experimental site based at the UniLaSalle campus, in Beauvais (Oise, France) at the following GPS coordinates: 49°25′49″ N, 2°04′51″ E. It was classified as a silt loam Haplic Luvisol. Samples were taken at 0–10 cm depth and sieved at 5 mm.

Table 1 Initial properties of the soil and amendment used in the experimentation

	Units (dry weight)	Soil	Compost	Biochar
Clay	%	20.5	–	–
Fine silt	%	26.4	–	–
Coarse silt	%	43.0	–	–
Fine sand	%	7.47	–	–
Coarse sand	%	2.60	–	–
pH (H_2O)[a]	–	7.93	8.00	11.4
Total carbonates[b]	%	0.80	–	–
Organic carbon[c]	g kg^{-1}	10.2	292	316
Cation exchange capacity[d]	cmolc kg^{-1}	109	34.9	8.50
Total nitrogen[e]	g kg^{-1}	1.13	27.0	0.84
Total phosphorus	g kg^{-1}	0.57	4.20	3.71
Total potassium	g kg^{-1}	14.3	17.0	17.9
Total magnesium	g kg^{-1}	3.20	2.80	5.07
Total calcium	g kg^{-1}	7.77	29.0	34.0
Total sodium	g kg^{-1}	5.85	–	–

[a] 1:5 ratio NF ISO 10390, [b] NF ISO 10693, [c] dry combustion NF ISO 14235, [d] Metson method NFX 31-130, [e] Dumas method

Data from Nobile et al. (2022)

The compost was a green waste compost (grass, poplar, and conifer branches) sampled at the platform of Fertivert (Seine-Maritime, France), being the result of a 4-month-thermophilic phase and 2-month-maturation process.

The non-composted residues generated by the composting process were used to produce biochar. Those residues were predominantly made of the non-composted tree branches. Pyrolysis took place in an industrial pyrolysis reactor (Biogreen® Pyrolysis Technology, ETIA, Oise, Haut-de-France, France) without oxygen, at 450 °C for 10 min.

Initial soil, compost, and biochar characteristics have been previously assessed (Nobile et al. 2022, 2020; Védère et al. 2023) and are presented in Table 1.

Experimental design

A two factorial pot experiment was performed using the soil, the two amendments, and a N fertiliser solution. The first factor was "amendment" and the second was "fertilisation".

For the "amendment" factor, biochar and compost were applied together in the soil at five different mixing ratios (Table S1): (i) BC100, corresponded to pure biochar (ii) BC70CP30, corresponded to a combination of biochar and compost at a ratio of 70:30 (on a dry weight (dw) basis) (iii) BC50CP50, corresponded to a combination of biochar and compost at a ratio of 50:50 (on a dw basis) (iv) BC30CP70 corresponded to a combination of biochar and compost at a ratio of 30:70 (on a dw basis) and (v) CP100, corresponded to pure compost. Amendment application rate was 10 t ha^{-1} in every treatment (considering an application at 5 cm). A control without amendment was also prepared. Considering the properties of the amendments, the mixtures added different quantities of N, P and K: (i) BC100 added 8.4 kg ha^{-1} N, 37 kg ha^{-1} P, and 179 kg ha^{-1} K; (ii) BC70CP30 added 87 kg ha^{-1} N, 39 kg ha^{-1} P and 176 kg ha^{-1} K; (iii) BC50CP50 added 139 kg ha^{-1} N, 40 kg ha^{-1} P and 174 kg ha^{-1} K; (iv) BC30CP70 added 191 kg ha^{-1} N, 41 kg ha^{-1} P and 173 kg ha^{-1} K; and (v) CP100 added 270 kg ha^{-1} N, 42 kg ha^{-1} P and 170 kg ha^{-1} K. Based on these values, the treatment CP100 presents the highest nutrient content and should thus lead to the highest biomass production, if no synergism occurs between biochar and compost.

For the "fertilisation" factor, half of the pots were fertilised (treatments noted Fert+) with a N solution, in the form of ammonium-nitrate, corresponding to 70 N units.ha^{-1}, while distilled water was applied to the other half (treatments noted Fert−). N fertiliser was applied after amendments were mixed with the soil and substrates were placed in the pots, and one week before sowing.

In total, this two factorial design had 12 treatments (Table S1) and all treatments were repeated five times. Plastic pots (8 cm diameter, 7 cm height) were filled with 450 g of soil (dw basis) and the amendments and were randomly arranged in a greenhouse with controlled conditions (photoperiod 16 h light/8 h dark, light intensity of 10 W m^{-2}, temperature of 21 °C). Before sowing, soils with and without amendments were equilibrated during one week at 80% water holding capacity.

After substrate equilibration, a subsample of soil was taken in three replicates and was analysed (Centre provincial de l´Agriculture et de la Ruralité, La Hulpe, Belgium) for pH (in KCl, ISO 10390), total N (Dumas method), ammonium-N and nitrate-N contents (KCl extraction), available P and K concentrations (ammonium acetate + EDTA extraction), organic C content (dry combustion NF ISO 14235) and C/N ratio. Then, 0.5 g of seeds of ryegrass (*Lolium multiflorum*) were sown in each pot and plants were allowed to grow for 3 months (13 weeks). During those 13 weeks, substrates were maintained at 80% water holding capacity through regular watering (every 2 days) based on mass loss and thus no leaching was observed.

Soil pore water sampling and analysis

Soil pore water (SPW) was sampled (8 mL volume) with a rhizon sampler (model MOM, Solutions Technologiques pour l´Environnement, Reignac sur Indre, France) placed into the soil at the beginning of the experiment. Sampling had been done before (T0) and 4, 8 and 13 weeks after sowing (T4, T8 and T13, respectively) by applying a pressure on the soil moisture sampler using vacuum tubes (Solutions Technologiques pour l´Environnement,Reignac sur Indre, France). pH was immediately measured with a pH meter (Metler Toledo, Seven Easy).

Plant harvest and analysis

Each month after the start of the experiment, the aerial parts of the plants were harvested by cutting all the biomass at 1 cm above the soil level. Plant shoots were dried at 50 °C for 2 days and weighed to determine their dry biomass.

At the end of the experiment (i.e., 13 weeks after sowing), plants were harvested and, following the removal of the aerial part of the plants, soil and roots were gently separated. Roots were washed with water, dried for two days at 50 °C and then carefully manually cleaned in order to remove all the possible remaining soil, compost and biochar particles. Root biomass could not be determined due to a too important biomass loss during the harvest.

Shoot (from the three harvests) and root (from the last harvest) materials were grounded in order to analyse their C and N content using an elemental analyser (Vario Isotope Select, Elementar, Hanau, Germany) and acid digested in a microwave in order to determine their P and K concentrations using inductively coupled plasma mass spectrometry (Thermo Scientific iCAP 6000 Series). Using those concentrations, C/N and N/P ratios were calculated.

Soil sampling and analysis

Once plants were harvested and roots removed from soil, the soil was sampled and separated in two subsamples. The first sub-sample was kept at − 20 °C (to conserve the chemical state of the substrates) until further analysis. The second sub-sample was stored at 4 °C and microbial biomass was extracted within 48 h.

Similarly to the analysis at sowing time, the soils sampled at the end of the experiment and stored at − 20 °C were analysed for pH, electrical conductivity, total N, ammonium-N content, nitrate-N content, available P, and K concentrations, organic C content and C/N ratio. The methods used were the same as described previously.

In addition, the soils were also analysed for microbial biomass C and N, using the chloroform fumigation method (Vance et al. 1987): 6 g of chloroform fumigated and non-fumigated soil were extracted with 40 mL of a K_2SO_4 solution (0.05 M). After 30 min, solutions were centrifuged and filtered (0.45 µm). The K_2SO_4 extracts were frozen, freeze dried and their C and N content was determined using an elemental analyser (Vario Isotope Select, Elementar, Hanau, Germany). The difference between fumigated and non-fumigated soils was used as the microbial C or N flush, and converted to microbial biomass C and N, using the following equations (Beck et al. 1997; Lovell et al. 1995):

$$\text{Microbial C} = \text{C flush} \times 2.22 \qquad (1)$$

$$\text{Microbial N} = \text{N flush} / 0.5 \quad (2)$$

Statistical analysis

All data were analysed using the R software, version 3.5.1. First, the normality of the data was evaluated using the Shapiro test. Thereafter, the homogeneity of variance was assessed using either the Bartlett test (for normal data) or the Fligner test (for non-normal data). Finally, means were compared using the Anova test, when data distributions were parametric, or the Kruskal–Wallis test, when data distributions were not parametric, followed by a post-hoc test, i.e., Tukey test or Dunn test, respectively. In addition, the effect of amendment, fertilisation and amendment*fertilisation was assessed using two-way Anova or Adonis tests. Difference was considered significant at $p < 0.05$.

Results

Soil properties and pore water pH

Following amendment applications, pH, total N, nitrate–N, organic C, C/N, available P and K increased, while ammonium-N content decreased (Table 2 and Table S2). Nitrogen fertilisation only affected nitrate–N concentration, which increased in the fertilised pots compared to non-fertilised ones. Finally, the interaction amendment*fertilisation only had a significant effect on the ammonium-N concentration. The highest values were observed when BC100/-Fert was applied for pH, organic C, C/N and available P, when CP100/-Fert was applied in the case of total N and available K, while the ratio BC50CP50/Fert+ showed the highest nitrate-N concentration.

After the end of the experiment, amendment application had a significant effect on all soil chemical properties, except pH and nitrate-N content (Table 3 and Table S2). All those affected soil properties (total N, ammonium-N, organic C, C/N, available P and K) were increased by amendment application, except ammonium-N content. OC and C/N ratios showed no differences between the different biochar-compost mixtures. BC100 treatments showed the highest available P, CP100 the highest total N and both treatments had similar and high available K concentrations compared to the controls and mixtures.

At the end of the experiment, microbial biomass C (MBC) and N (MBN) were 0.21 and 0.024 mg g^{-1}, respectively, on CT/Fert− (Figure S1a and 1b) and they were not affected by any of the amendments.

Before the start of the experimentation, the pH measured in soil pore water (SPW) of the control (CT/Fert−) was 7.61 (Figure S2), and increased at the latter sampling times to reach a value of 8.69. Amendments significantly affected SPW pH in the first three samplings but had no effect at the harvest time (T13) (Table S2). Amendments tended to decrease SPW pH at T0 (values ranging from 7.08 to 7.53) and T8 (values ranging from 7.80 and 8.45), while an increase was noted at T4 (values between 7.61 and 7.95). Fertilisation only affected SPW pH at the initial time, and induced a reduction of SPW pH, which was less strong when amendments were applied. The interaction amendment*fertilisation had a significant effect on SPW pH at all sampling times except the T0.

Plant biomass

After each harvest, the plant biomass in the control treatment were respectively 1.18, 0.42 and 0.51 g (Fig. 1). Plant biomass was significantly affected by amendment and fertilisation (Table S3). Plant biomass was positively and significantly affected by the interaction of amendments with fertiliser addition during the first and second harvest. All the organic amendments, independently of the fertiliser application, increased the plant aerial biomass following the order: CT < CP100 < BC100 < BC70CP30 < BC50CP50 < BC30CP70 during the two first harvests. After the third harvest the addition of amendment only significantly increased the biomass in the case of BC30CP70 and CP100 treatments, irrespective of the presence or the absence of N fertiliser. The positive effect of fertiliser addition was observed after the first and the second harvest (except for CP100). At the last havest only the BC30CP70/Fert+ treatment showed an effect. The highest biomass was always recorded for the BC30CP70/Fert+ treatment with 3.01, 1.21 and 0.72 g after the three harvests.

Table 2 Chemical properties of the different substrates at the begininng of the experiment

Amendment	Fertilization	pH_{KCl}	Total nitrogen (g kg^{-1})	Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Organic carbon (g kg^{-1})	C/N	Available [P] (g kg^{-1})	Available [K] (g kg^{-1})	Available [Mg] (mg kg^{-1})	Available [Ca] (mg kg^{-1})
CT	Fert−	7.51 ± 0.04^{bcde}	1.1 ± 0.0^{c}	2.70 ± 0.43^{ab}	13 ± 8^{f}	10.31 ± 0.04^{bc}	9.59 ± 0.23^{ac}	77 ± 8^{def}	147 ± 4^{f}	84 ± 2^{e}	4081 ± 239^{a}
	Fert+	7.49 ± 0.01^{de}	1.1 ± 0.1^{bc}	1.26 ± 0.32^{c}	54 ± 4^{cd}	9.89 ± 0.39^{c}	$8.66\ 0.32^{c}$	64 ± 4^{f}	125 ± 10^{f}	82 ± 4^{e}	3930 ± 65^{a}
BC100	Fert−	7.57 ± 0.00^{a}	1.3 ± 0.0^{ab}	1.26 ± 0.10^{c}	44 ± 4^{de}	16.18 ± 1.53^{a}	12.22 ± 1.04^{b}	109 ± 3^{a}	186 ± 3^{d}	94 ± 1^{d}	4294 ± 130^{a}
	Fert+	7.57 ± 0.01^{ab}	1.3 ± 0.0^{ab}	1.75 ± 0.20^{bc}	65 ± 3^{bcd}	14.59 ± 0.13^{a}	11.03 ± 0.10^{ab}	105 ± 1^{ab}	188 ± 1^{de}	96 ± 1^{d}	4402 ± 42^{a}
BC70CP30	Fert−	7.57 ± 0.01^{ab}	1.4 ± 0.0^{a}	1.95 ± 0.14^{abc}	55 ± 3^{cd}	14.65 ± 0.65^{a}	10.82 ± 0.34^{ab}	102 ± 2^{abc}	203 ± 1^{cde}	102 ± 1^{bcd}	4174 ± 79^{a}
	Fert+	7.52 ± 0.02^{cde}	1.4 ± 0.0^{a}	2.07 ± 0.07^{abc}	79 ± 1^{b}	14.24 ± 0.70^{a}	10.53 ± 0.20^{ab}	99 ± 4^{abc}	199 ± 4^{cde}	101 ± 1^{cd}	4179 ± 45^{a}
BC50CP50	Fert−	7.52 ± 0.00^{cde}	1.3 ± 0.0^{ab}	1.39 ± 0.09^{c}	72 ± 5^{bc}	13.15 ± 0.56^{abc}	10.16 ± 0.26^{ac}	86 ± 4^{cde}	191 ± 7^{cde}	103 ± 2^{bcd}	4259 ± 141^{a}
	Fert+	7.48 ± 0.02^{e}	1.4 ± 0.0^{a}	1.94 ± 0.17^{abc}	119 ± 3^{a}	13.81 ± 0.52^{a}	10.08 ± 0.15^{ac}	91 ± 1^{bcd}	199 ± 1^{cde}	107 ± 2^{abc}	4388 ± 193^{a}
BC30CP70	Fert−	7.54 ± 0.00^{abcd}	1.3 ± 0.1^{ab}	2.88 ± 0.11^{ab}	22 ± 1^{ef}	13.52 ± 0.88^{ab}	10.10 ± 0.22^{ac}	76 ± 4^{def}	213 ± 9^{bcd}	102 ± 2^{bcd}	4032 ± 77^{a}
	Fert+	7.54 ± 0.01^{abcd}	1.4 ± 0.1^{a}	2.33 ± 0.41^{abc}	54 ± 2^{cd}	14.63 ± 0.67^{a}	10.60 ± 0.20^{ab}	77 ± 2^{def}	217 ± 4^{bc}	104 ± 1^{bcd}	4316 ± 149^{a}
CP100	Fert−	7.53 ± 0.01^{abcde}	1.5 ± 0.0^{a}	2.82 ± 0.28^{ab}	31 ± 6^{ef}	14.15 ± 0.43^{a}	9.60 ± 0.05^{ac}	77 ± 1^{def}	244 ± 5^{a}	114 ± 2^{a}	4167 ± 120^{a}
	Fert+	7.56 ± 0.02^{abc}	1.5 ± 0.0^{a}	3.15 ± 0.31^{a}	64 ± 5^{bcd}	14.41 ± 0.05^{a}	9.72 ± 0.15^{ac}	73 ± 1^{ef}	234 ± 3^{ab}	111 ± 1^{ab}	4306 ± 73^{a}

CT = non-amended soil, BC100 = soil amended with biochar, BC70CP30 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); BC50CP50 = soil amended with a biochar:compost mixture in the ratio 50:50 (on a dry weight basis); BC30CP70 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); CP100 = soil amended with compost. All amendments were added at a dose of 10 t ha^{-1} in total. Fert− = no application of a nitrogen fertilization; Fert+ = application of a nitrogen fertilization. Letters indicate a significant difference ($p < 0.05$) (n = 3)

Table 3 Chemical properties of the different substrates at the end of the experiment

Amendment	Fertilization	pH_{KCl}	Total nitrogen (g kg^{-1})	Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Organic carbon (g kg^{-1})	C/N	Available [P] (g kg^{-1})	Available [K] (g kg^{-1})	Available [Mg] (mg kg^{-1})	Available [Ca] (mg kg^{-1})
CT	Fert−	7.48 ± 0.04	1.3 ± 0.0^{de}	1.43 ± 0.23^{abc}	0.25 ± 0.04^{ab}	10.8 ± 0.4^{bc}	8.35 ± 0.27^{cd}	62 ± 1^{fg}	108 ± 2^{g}	74 ± 3^{e}	3246 ± 41^{d}
	Fert+	7.48 ± 0.04	1.3 ± 0.0^{e}	0.90 ± 0.16^{cd}	0.18 ± 0.01^{ab}	10.4 ± 0.3^{c}	8.10 ± 0.15^{d}	53 ± 1^{g}	92 ± 2^{h}	72 ± 2^{e}	3334 ± 43^{cd}
BC100	Fert−	7.56 ± 0.03	1.4 ± 0.0^{cde}	0.84 ± 0.11^{d}	0.24 ± 0.03^{ab}	14.3 ± 0.6^{a}	10.05 ± 0.22^{ab}	114 ± 3^{a}	148 ± 2^{ab}	90 ± 0^{b}	3640 ± 48^{abc}
	Fert+	7.58 ± 0.02	1.4 ± 0.0^{cde}	$0.87 \pm 0.0.08^{d}$	0.23 ± 0.02^{ab}	14.6 ± 0.8^{a}	10.29 ± 0.30^{a}	103 ± 3^{b}	128 ± 2^{cde}	90 ± 0^{b}	3688 ± 44^{a}
BC70CP30	Fert−	7.58 ± 0.02	1.4 ± 0.0^{cde}	1.10 ± 0.09^{bcd}	0.18 ± 0.02^{ab}	13.9 ± 0.5^{a}	9.79 ± 0.32^{abc}	94 ± 4^{bc}	140 ± 5^{bc}	94 ± 3^{b}	3674 ± 94^{ab}
	Fert+	7.56 ± 0.03	1.5 ± 0.0^{abc}	1.29 ± 0.06^{abcd}	0.26 ± 0.03^{ab}	14.7 ± 0.7^{a}	9.66 ± 0.28^{abcd}	94 ± 2^{bc}	124 ± 3^{def}	92 ± 2^{b}	3526 ± 56^{abcd}
BC50CP50	Fert−	7.54 ± 0.04	1.5 ± 0.0^{abc}	1.06 ± 0.08^{cd}	0.24 ± 0.03^{ab}	13.5 ± 0.5^{ab}	9.01 ± 0.11^{abcd}	86 ± 2^{cde}	134 ± 3^{bcde}	90 ± 0^{b}	3486 ± 56^{abcd}
	Fert+	7.52 ± 0.07	1.4 ± 0.0^{bcde}	0.99 ± 0.10^{cd}	0.16 ± 0.01^{b}	13.8 ± 0.7^{ab}	9.55 ± 0.34^{abcd}	92 ± 3^{cd}	112 ± 2^{fg}	90 ± 0^{b}	3466 ± 58^{abcd}
BC30CP70	Fert−	7.50 ± 0.04	1.5 ± 0.0^{abc}	0.96 ± 0.12^{cd}	0.25 ± 0.02^{ab}	14.9 ± 1.5^{a}	9.79 ± 1.03^{abc}	79 ± 1^{e}	120 ± 4^{efg}	120 ± 4^{a}	3446 ± 94^{abcd}
	Fert+	7.50 ± 0.06	1.5 ± 0.0^{abcd}	0.75 ± 0.08^{d}	0.24 ± 0.01^{ab}	13.2 ± 0.6^{abc}	8.90 ± 0.17^{abcd}	83 ± 2^{de}	108 ± 6^{g}	108 ± 2^{a}	3368 ± 20^{bcd}
CP100	Fert−	7.58 ± 0.02	1.7 ± 0.0^{a}	1.78 ± 0.19^{a}	0.24 ± 0.03^{ab}	14.8 ± 0.5^{a}	8.63 ± 0.17^{bcd}	69 ± 1^{f}	156 ± 4^{a}	156 ± 4^{c}	3584 ± 121^{abc}
	Fert+	7.54 ± 0.03	1.6 ± 0.0^{ab}	1.66 ± 0.13^{ab}	0.28 ± 0.03^{a}	14.4 ± 0.6^{a}	8.90 ± 0.17^{abcd}	65 ± 2^{f}	136 ± 6^{bcd}	136 ± 6^{d}	3410 ± 106^{abcd}

CT = non-amended soil, BC100 = soil amended with biochar, BC70CP30 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); BC50CP50 = soil amended with a biochar:compost mixture in the ratio 50:50 (on a dry weight basis); BC30CP70 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); CP100 = soil amended with compost. All amendments were added at a dose of 10 t.ha-1 in total. Fert− = no application of a nitrogen fertilization; Fert+ = application of a nitrogen fertilization. Letters indicate a significant difference between treatments ($p < 0.05$) (n = 5)

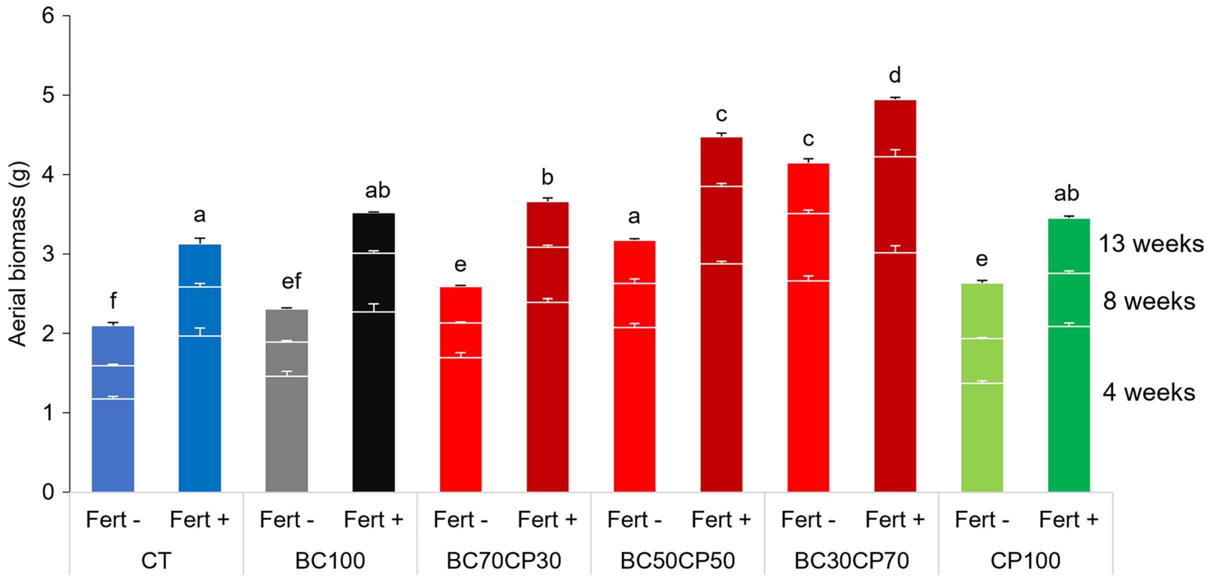


Fig. 1 Plant aerial biomass (g) measured in the aerial tissue of *Lolium multiflorum* after 4 weeks (bottom), 8 weeks (middle), 13 weeks (top) of growth and total aerial biomass produced during the experiment (full bar) on the different substrates. CT = non-amended soil, BC100 = soil amended with biochar, BC70CP30 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); BC50CP50 = soil amended with a biochar:compost mixture in the ratio 50:50 (on a dry weight basis); BC30CP70 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); CP100 = soil amended with compost. All amendments were added at a dose of 10 t ha^{-1} total. Fert− = no application of a nitrogen fertilization; Fert+ = application of a nitrogen fertilization. Letters indicate a significant difference of the total biomass ($p < 0.05$) (n = 5)

Finally, the total aerial biomass collected by the end of the experiment was 2.10 g in the control treatment (CT/Fert−) (Fig. 1). Total aerial biomass production was significantly affected by amendment, fertilisation and their interaction (Table S3). Amendment application increased biomass in all the treatments as well as fertiliser addition. Fertilisation allowed for a higher biomass increase in treatments without compost (+ 48.9% and + 52.5% biomass respectively in CT/Fert+ and BC100/Fert+) and a lower biomass increase in the two treatments with the highest compost contribution (+ 19.2 and + 31.3% biomass respectively in BC30CP70/Fert+ and CP/Fert+). Highest total aerial biomass was recorded for the treatment BC30CP70/Fert+ .

Carbon and macronutrient concentrations (N, P and K) in the plants

At the first harvest, amendment application had a significant effect on the plants' C, N, P and K concentrations, while fertilisation affected N and P concentrations and the interaction amendment*fertilisation affected N and K concentrations (Table S3). In more detail, C values ranged from 395.4 g kg^{-1} (CP100/Fert-) to 442.2 g kg^{-1} (BC50CP50/Fert−) in the amended conditions, although they were not different from the control. The N concentrations were increased by the addition of some mixture up to twofold (BC30CP70/Fert+). The plants' P concentrations were decreased by the biochar amendments and fertilisation up to − 34% (BC30CP70/Fert+). Finally, K concentrations were increased by the amendments up to + 16% (BC30CP70/Fert+).

At the second harvest, amendment application significantly affected all parameters. Fertilisation affected all elements except K concentrations while the interaction amendment*fertilisation only significantly affected N and P concentrations (Table S3). Carbon concentrations increased with amendments and fertilisation up to + 2% (Figure S3) (BC30CP70/Fert+). Nitrogen and P concentrations were both decreased when amendments and fertilisation were

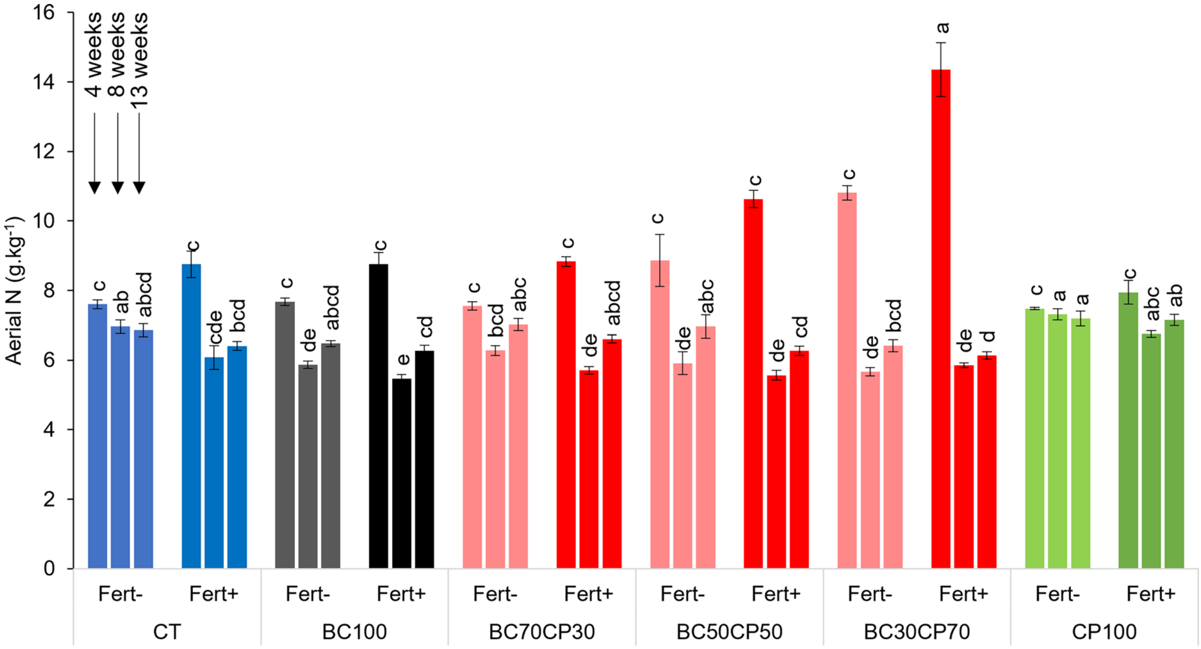


Fig. 2 Nitrogen content (g kg^{-1}) measured after 4 weeks (left), 8 weeks (middle) and 13 weeks (right) of growth on the different substrates in the aerial biomass of *Lolium multiflorum*. CT = non-amended soil, BC100 = soil amended with biochar, BC70CP30 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); BC50CP50 = soil amended with a biochar:compost mixture in the ratio 50:50 (on a dry weight basis); BC30CP70 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); CP100 = soil amended with compost. All amendments were added at a dose of 10 t.ha.$^{-1}$ total. Fert− = no application of a nitrogen fertilization; Fert = application of a nitrogen fertilization. Letters indicate a significant difference for each week ($p < 0.05$) (n = 5)

applied, except for P when CP100 was used (Figs. 2 and 3). More specifically, N concentrations in plants grown on the amended substrates decreased down to − 21% (BC100/Fert+), whereas P concentrations decreased down to − 20% (BC30CP70/Fert−). Finally, K concentrations were only increased in the presence of CP100 up to + 38% (Fert−) and + 58% (Fert+) (Fig. 4).

At the last harvest, the application of the organic amendments significantly affected C, N, P and K concentrations, fertilisation affected only N, P and K concentrations while the interaction amendment*fertilisation had no effect compared to control (Table S3). The carbon concentrations of harvested biomass were increased by the addition of organic amendments up to + 6% (CP100/Fert−) (Figure S3); N concentrations were not significantly changed by amendment addition (Fig. 2). Phosphorus concentrations decreased with the addition of amendment and fertilisation, to reach values down to − 34% (Fig. 3) (BC30CP70/Fert+). Finally, K concentrations were again only increased when CP100 was applied, with values up to + 9% on average (Fig. 4).

Roots were also analysed for their element concentrations, and in general, amendment, fertilisation and amendment*fertilisation had no effect on root element concentrations, except for K (Table S4). Concentrations varied between 319.5 g kg^{-1} (CP100/Fert−) and 365.5 g kg^{-1} (BC100/Fert+) (Table S5) for C, 6.2 g kg^{-1} (CT/Fert+) and 7.0 g kg^{-1} for N (BC30CP70/Fert+ and CP100/Fert+). P concentrations ranged from 698 mg kg^{-1} (CT/Fert−) to 942 mg kg^{-1} (BC100/Fert+) and K concentrations from 3.4 g kg^{-1} (CT/Fert- and BC30CP70/Fert+) to 4.8 g kg^{-1} (BC100/Fert+).

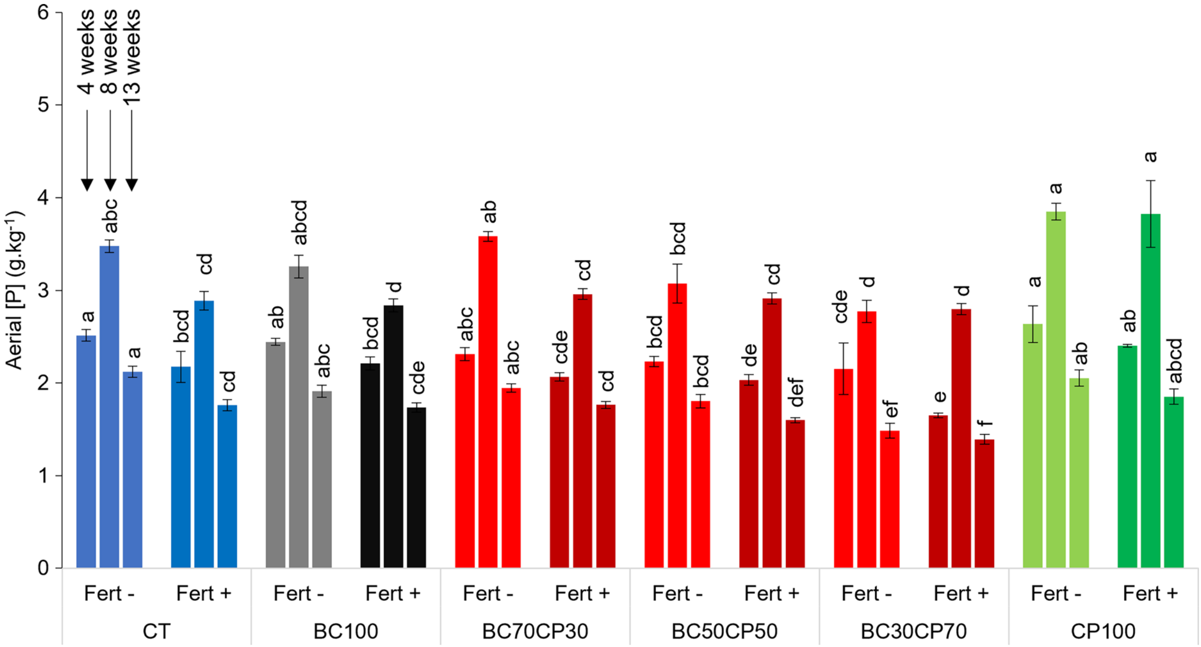


Fig. 3 Phospshorus content (g kg^{-1}) measured after 4 weeks (left), 8 weeks (middle) and 13 weeks (right) of growth on the different substrates in the aerial tissues of *Lolium multiflorum*. CT = non-amended soil, BC100 = soil amended with biochar, BC70CP30 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); BC50CP50 = soil amended with a biochar:compost mixture in the ratio 50:50 (on a dry weight basis); BC30CP70 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); CP100 = soil amended with compost. All amendments were added at a dose of 10 t.ha.$^{-1}$ total. Fert− = no application of a nitrogen fertilization; Fert+ = application of a nitrogen fertilization. Letters indicate a significant difference for each week ($p < 0.05$) (n = 5)

Element stoichiometry of aboveground and belowground plant tissue

For aerial tissues, at the first harvest, the C/N ratio was 51.80 in the control without fertiliser (CT/Fert−) (Fig. 5a) and the N/P ratio was 3.02 (Fig. 5b). Amendment, fertilisation, and amendment*fertilisation significantly affected both ratios (Table S3). The C/N ratio tended to decrease after amendment, with values ranging from 28 (BC30CP70/Fert+) to 53 (BC70CP30/Fert−), while N/P ratio increased and reached values between 2.9 (CP100/Fert−) and 8.7 (BC30CP70/Fert+). Similar trends were observed following fertilisation, *i.e.*, decrease of the C/N ratio and increase of the N/P ratio.

At the second harvest, for the non-fertilized and non-amended control (CT/Fert−), shoot C/N ratio was 57.96 and shoot N/P ratio was 2.00 (Fig. 5a, b). Amendments significantly increased C/N ratio and decreased N/P ratio, with values ranging respectively from 55 (CP100/Fert−) to 74 (BC100/Fert+) and from 1.75 (BC70CP30/Fert−) to 2.1 (BC30CP70/Fert+). Fertilisation and fertilisation*amendment only significantly affected C/N ratio (Table S3).

At the last harvest, the shoot C/N and N/P ratios for CT/Fert- were 55.07 and 3.23, respectively (Fig. 5a, b). Amendment applications tended to significantly increase both ratios and values ranged between 55 (CP100/Fert+) and 64 (BC30CP70/Fert+) for C/N, and between 3.4 (BC100/Fert−) and 4.4 (BC30CP70/Fert+) for N/P.

At the end of the experiment, root C/N ratio was 50.4 on the unfertilised control, and none of the treatments (amendments and fertilisation) affected the root C/N ratios (Table S5). The root N/P ratio was 8.0 for CT/Fert−, and none of the other treatments showed significant difference with the control (Table S5).

The results of the element stoichiometry of plant tissue identify the ratio BC30CP70 as optimal for a reducing N limitations, as we observed a decrease

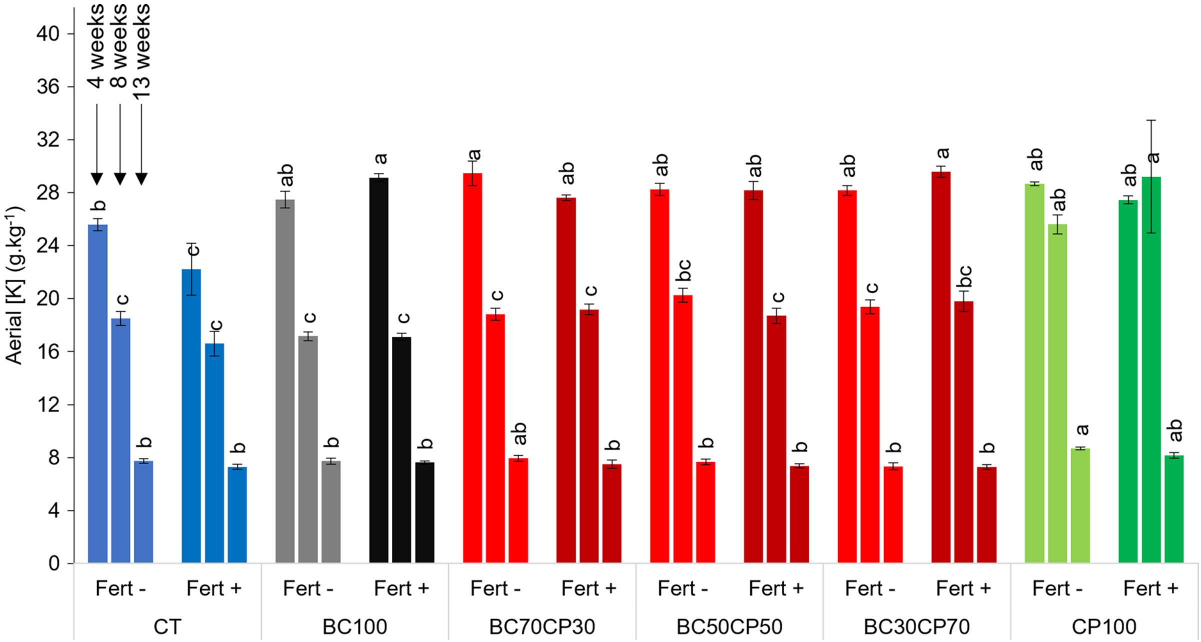


Fig. 4 Potassium content (g kg^{-1}) measured after 4 weeks (left), 8 weeks (middle) and 13 weeks (right) of growth on the different substrates in the aerial tissues of *Lolium multiflorum*. CT = non-amended soil, BC100 = soil amended with biochar, BC70CP30 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); BC50CP50 = soil amended with a biochar:compost mixture in the ratio 50:50 (on a dry weight basis); BC30CP70 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); CP100 = soil amended with compost. All amendments were added at a dose of 10 t ha^{-1} total. Fert− = no application of a nitrogen fertilization; Fert+ = application of a nitrogen fertilization. Letters indicate a significant difference for each week ($p < 0.05$) (n = 5)

of C/N and increase of N/P ratios of plant tissues compared to the control treatment.

Discussion

Although the combination of biochar and compost has been often studied to improve the fertility of agricultural soils, the importance of the ratio between these two organic amendments still lacks proper evaluation. The objectives of our study were to assess the influence of biochar/compost mixing ratios on soil and plant parameters to determine the optimal ratio. We have demonstrated an additive effect of biochar and compost combination on the soil fertility (in particular nutrient content and availability). In contrast, combining biochar and compost induced a synergistic effect on plant growth and nutrition. Finally, we showed that the intensity of this synergistic effect was highly influenced by the ratio between both components of the mixture, with the treatment containing 30% biochar and 70% compost having the best effects.

The soil fertility improvement is driven by the compost proportion, in an additive interaction between biochar and compost

Addition of biochar and compost to the soil increased its organic C concentrations directly after the amendment application and until the end of the experiment, between 25 and 60%. This was expected as the addition of organic amendment, such as biochar and compost, is often shown to raise SOC rapidly after their application (Agegnehu et al. 2015, 2016; Chan et al. 2007; Fischer and Glaser 2012). An interesting result regarding SOC was that soil MBC measured in soils at the end of the experiment was not impacted by amendment application, which is inconsistent with results obtained by other authors (Irfan et al. 2019; Li et al. 2020;

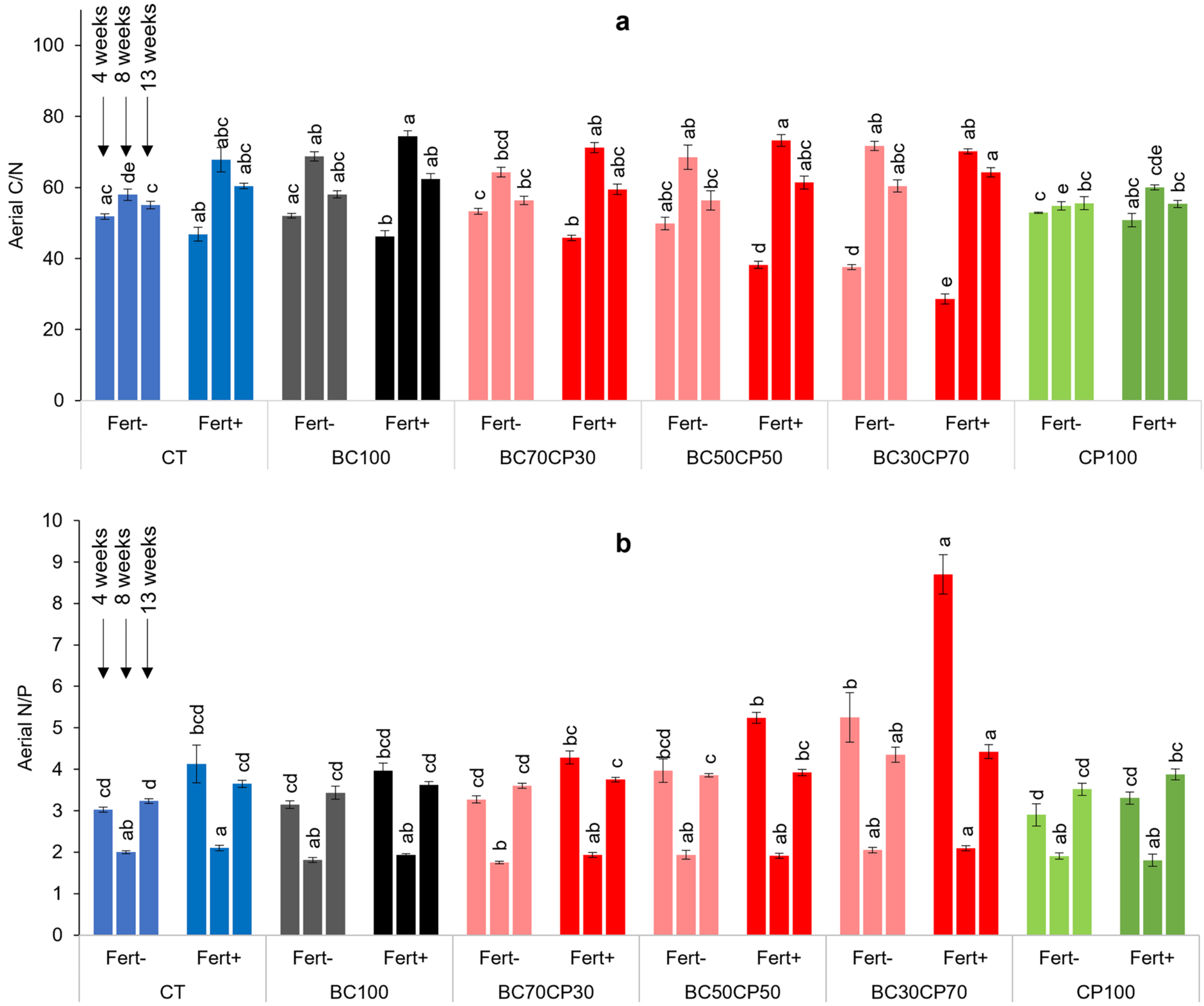


Fig. 5 C/N ratio (**a**) and N/P ratio (**b**) measured after 4 weeks (left), 8 weeks (middle) and 13 weeks (right) of growth on the different substrates in the aerial tissues of *Lolium multiflorum*. CT = non-amended soil, BC100 = soil amended with biochar, BC70CP30 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); BC50CP50 = soil amended with a biochar:compost mixture in the ratio 50:50 (on a dry weight basis); BC30CP70 = soil amended with a biochar:compost mixture in the ratio 70:30 (on a dry weight basis); CP100 = soil amended with compost. All amendments were added at a dose of 10 t ha^{-1} total. Fert− = no application of a nitrogen fertilization; Fert + = application of a nitrogen fertilization. Letters indicate a significant difference for each week ($p < 0.05$) (n = 5)

Liu et al. 2021). Indeed, after soil application, compost usually stimulates soil microbial communities, which in turn impacts biogeochemical cycling and plant growth (Abbott et al. 2018). Similarly, biochar has often beneficial effects on soil microorganisms by providing a microhabitat (Lehmann et al. 2011), protecting them from predation and allowing a good water retention in its microporosity protecting them from desiccation (Abbott et al. 2018). MBC is generally increased after biochar application particularly at low pH soil (< 6.5) (Pokharel et al. 2020). Nevertheless, in our experiment where soil pH was 7.9, MBC was unchanged by the amendment applications after 13 weeks, suggesting that the growth conditions for microbial soil communities were similar in all treatments at the end of the experiment. The absence of effects could be related to the fact that water was provided at optimal rate, and thus microorganisms did not particularly need protection from desiccation (Griffin 1981;

Young et al. 2008). As well, MBC was measured only once, 13 weeks after the amendment application, and thus may not be sufficient to ensure that the added substrates had no effect on microorganisms´ development. Otherwise, measurements of soil gas emissions during such experimentation could have brought more insights on possible microbial activities.

The addition of the biochar-compost mixtures increased the total content and availability of the macronutrients nitrogen (from 11 to 36%), phosphorus (from 10 to 84%) and potassium (from 11 to 65%). Similar amelioration of soil organic matter and nutrient contents has been previously observed following the application of compost and biochar, and related to the amendment properties (high organic matter and available nutrient contents) (Plaza et al. 2016; Radin et al. 2018; Ravanbakhsh et al. 2019). However, an immobilisation of N following the addition of amendments with high C/N ratio has also been observed, especially in the case of biochar (Bong Cassendra Phun Chien et al. 2021; Jien et al. 2018). One of the reasons for such immobilisation is sorption of N on the biochar surface (Garbowski et al. 2023; Jien et al. 2018) and, more importantly, its incorporation into the microbial biomass (Irfan et al. 2019). At the end of our study, the application of biochar and compost did not show any microbial immobilisation of N, as shown by the lack of effects of organic amendment application on MBN after 13 weeks, which is likely related to the lack of change in MBC. When biochar and/or compost are applied to the soil, the mineralisation of the labile C by microorganisms may, in the short term, induce a microbial immobilisation of the available soil N, resulting in higher MBN (Abbas et al. 2020; Chen et al. 2021; Schofield et al. 2019). Nitrogen incorporated into the microbial biomass is not available for plants, which could consequently reduce their growth. For these two reasons, it has been recommended to apply, together with an organic C-rich amendment such as biochar or compost, a mineral N fertiliser to compensate for the N immobilisation. Indeed, N provided by mineral fertilisation is directly available for plants although rapidly depleted, while the one from organic amendments will require more time to become available (Dey and Mavi 2021; Gao et al. 2019). By contrast, adding biochar and/or compost increased total N in the soil (from 11 to 36%) and, more importantly, the concentration of nitrate-N at initial time (between 1.8 and 5.6 times), which might be due to the direct release of nitrate-N by amendments and to higher nitrification (Clough et al. 2013; Zhang et al. 2021). We hypothesised that adding biochar and/or compost improved the availability of N, and thus could ameliorate plant growth, as nitrate is the form of N preferentially taken up by plants. Our results confirmed this hypothesis, as the ryegrass biomass increased in the amended conditions, under both N fertilisation regimes, although the increase diminished after repeated harvesting. Previous studies have also shown that following compost/biochar amendments, the improvement of soil fertility was followed by an increase in biomass production of maize (Abbas et al. 2020; Manolikaki and Diamadopoulos 2019; Zahra et al. 2021), *Salix purpurea* (Seehausen et al. 2017), lettuce (Trupiano et al. 2017), and *Phragmites karka* (Abideen et al. 2020). The importance of N in this growth amelioration was confirmed by the decrease in C/N ratio (T1) and an increase in the N/P ratio of the plants, demonstrating a reduction of N limitation under amended conditions (Cao and Chen 2017), and a change of the system from a N limitation towards a more equilibrated system, in terms of plant nutrition.

Except for the available P, nutrient availability was mainly dependent on the compost content, as higher values were found in the compost only treatment and diminished with the proportion of compost, which could be explained by the fact that compost contains more available nutrients. On the contrary, P availability was more important in mixtures with higher proportions of biochar. Wood biochar seems to have the potential to raise P availability through a direct addition, through a pH raise of soil or by inducing a competition with organic anions brought by biochar on soil fixation site (Houben et al. 2017). However, our results reject the first explanation as pH changes were not significant. Moreover, compost had a slightly higher P content than biochar, and thus a competition with other anions seems more likely. Altogether, soil parameters show that compost and biochar have no synergistic effect on soil but rather an additional effect.

The optimal ratio for inducing a synergistic interaction between biochar and compost

Interestingly, the soil analysis revealed that N availability was dependent on the ratio of compost and biochar, and the highest availability of N (ammonium-N + nitrate-N) was found for the ratio BC50CP50, while N chemical fertilisation further increased N availability. We hypothesised that the highest growth and uptake of N would occur in this treatment. However, the best growth was measured for the ratio BC30CP70, which showed even higher biomass production than the N fertilisation treatment (CT/Fert+). In addition, compared to the other treatments, including mineral N fertiliser, this biochar/compost ratio still increased plant growth at the successive harvests. It also induced an increase in the N/P ratio of plants, and thus a reduction in N limitation, after 13 weeks, indicating a longer lasting effect. Thus, this study demonstrated that a ratio of 30% biochar and 70% compost allowed for synergistic interactions between those two amendments to increase nutrient uptake by plants, although the synergism was not observed in terms of nutrient availability in the soil. This could be related to the high amount of carbon and nutrients added through compost and their stabilisation via a small dose of biochar, and a modification of plant physiological response to the changes in soil conditions (Kidd et al. 2009). This specific biochar/compost ratio also improved growth under N fertilisation more than the other treatments. Finally, as the ratio BC30CP70 showed the best results, higher than the fertilised control, we can conclude that adding a mixture of biochar and compost at a specific ratio could reduce mineral N fertilisation or improve its effect. Biochar and compost used together as soil amendment are extensively studied but few works address the ratio differences of such mixtures and their potentialities in terms of soil functioning and plant development. Such practice needs, however, to be evaluated for other biochar/compost mixtures and in terms of cost/benefits for the farmer. Our results agree with our previous study showing that this specific ratio (BC30CP70) was able to substitute for P and K fertilisation for the same soil, under field conditions (Nobile et al. 2022). Taken together, we suggest that biochar-compost mixtures with specific ratios (30/70% in our case) have potential to reduce the regular N, P and K fertilisation, although more testing is needed, using different types of biochar and compost and NPK fertilisers under contrasting pedoclimatic conditions and cropping systems.

Conclusion

A pot experiment was performed to evaluate the influence of the biochar/compost ratio in amendment mixtures with regards to their effects on soil fertility and plant growth. This study confirmed our two hypotheses: (i) biochar and compost association resulted in better plant growth and nutrient availability than their single applications, and (ii) the effect of biochar/compost mixtures depends on their ratio and nitrogen fertilisation. Altogether, the results showed that associating biochar and compost can have synergistic effects and may be able to alleviate mineral N fertilisation if used in an appropriate ratio. We therefore suggest that adding a small amount of biochar to compost before field application could be useful to reduce mineral fertiliser input. This may lead to better plant growth and carbon storage than compost application alone. Such synergism between different biochar and compost types remains to be elucidated.

Author contributions Conceptualization: ML, CV, CR, DH; Methodology: ML, CV; Formal analysis and investigation: ML, CV; Writing-original draft preparation: ML, CV; Writing-review and editing: NH, CR, DH; Funding acquisition: CR, DH.

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Data availability The data will be made available by the corresponding authors on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Consent for publication Not applicable.

Ethical approval Not applicable.

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