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RESEARCH ARTICLE

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Manure-biochar blends effectively reduce nutrient leaching and increase water retention in a sandy, agricultural soil: Insights from a field experiment

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Abstract

Organic amendments are commonly applied singularly to soils to improve physical, biological and chemical properties, but their combination may be even more advantageous than when applied alone. In this study manure was applied singularly and in combination with biochar (90:10 and 50:50 ratios) to a drought prone agricultural Regosol in a field evaluation. Samples were collected twice a year for 2 years and subjected to testing for moisture retention, nutrient status and microbial activity whilst weed growth was monitored by drone. Substantial seasonal variability in all parameters measured was observed, though all amendments increased actual soil moisture content between 18 and 41% initially; without the addition of biochar (i.e., manure alone) this reverted back to reduced moisture content towards the second year of sampling. None of the tested amendment combinations significantly affected soil-saturated hydraulic conductivity. Cation exchange capacity decreased as a result of manure addition alone, the addition of 10% biochar and 50% biochar increased this significantly (23%–54% increase). Though microbial biomass and enzyme soil health indicators showed no decisive changes as a result of amendment application, and plant biomass was variable by ground sampling, drone imagery proved that plant heights and health were generally increased as a result of biochar addition to manure, compared with manured soil alone. In summary, despite much field seasonal variability limiting the interpretation of the data, this study nonetheless demonstrates a useful maintenance of improved soil moisture achieved by adding biochar together with manure to a drought-prone soil agricultural soil.

KEYWORDS
biochar, field application, soil health, soil retention, UAV mapping

1 | INTRODUCTION

Agricultural intensification, such as repeated tillage and fertilizer applications, has reduced general soil quality, causing soil erosion and loss of organic matter (Liu et al., 2018). Since the formation of healthy soils takes centuries to millennia, but their degradation can occur very rapidly, alternatives to intensive practices such as chemical fertilizer application to enhance nutrient and water retention are increasingly preferable. Much attention has been paid, in recent years, to soil amendment with biochip often based on the hypotheses that the input of a stable form of carbon to soil, which will remain stable for centuries to millennia, will result in prolonged improvements to some soil characteristics. Biochar is the solid product from pyrolysis of waste biomass residues, under anoxic conditions using temperatures ranging from 350°C to 900°C, resulting in material with a very high surface area for minimal mass (Novak et al., 2019). Most studies to date that have utilized biochars as soil amendments have applied them alone and have shown mixed results depending on the soil type and biochar applied. Soils are highly heterogeneous systems, as are biochars, so it is unsurprising that mixed results of their combination have been found. In a meta-analysis by Jeffery et al. (2011), generalized effects of biochars on soil parameters included a 10% increase in crop production, with the most significant improvements (up to 39%) occurring with high biochar application rates, in acidic or neutral pH soils. Other authors have sounded a cautionary note about biochar application to soils more recently; for example, Brtnicky et al. (2021) compiled the results of ~260 studies, finding that biochar application to fine-textured soils may decrease plant available water, contribute to soil salinity and decrease soil fertility through nutrient precipitation caused by the alkaline pH of some biochars. Biochar can also induce a desiccation effect on the soil. Therefore, biochar added alone to soils is no guarantee of improvement to soil properties and scepticism is building about the upscaling of biochar production and usage (Tan & Yu, 2024). Finally, biochar costs usually range between €300–2000 per ton in European markets (depending on its quality), which makes its application in the field expensive, with the common application rates tested in laboratory.

Much longer established materials for amending soils, at least in temperate climates, such as farmyard manure, composts and digestates, are classical on-farm materials with long-proven abilities to alter soil physical, biological and chemical properties (Hairani et al., 2016; Seyedsadr et al., 2022). However, the amorphous nature of these materials means that nutrient leaching is often rapid upon environmental application, and mineralization results in excess greenhouse gas emissions such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Novak et al., 2015). The same effect reduces the longevity of their impacts on crop yields. However, given that the global annual production of livestock manure is expected to reach 0.23 billion tons of nitrogen equivalents by 2030, the increased land application of these materials is inevitable with attendant consequences to nutrient leaching into waters (Lebrun et al., 2022; Wu, Shen, et al., 2017).

In order to gain the greatest benefits from individual soil amendments, the combination of compost and biochar has been tested demonstrating synergistic effects on soil fertility, microorganisms and water retention in agricultural fields (Lebrun et al., 2024; Wu, He, et al., 2017). Banik et al. (2021) demonstrated that the application of biochar combined to manure could stabilize phosphorus and nitrogen released from manure, reducing their leaching, concluding that biochar could act in a regulatory capacity to nutrients from manure. Also, Agbede and Oyewumi (2022) increased concentrations of N, P, K, Ca and Mg in crop leaves using a mixture of biochar and poultry manure more importantly than the sole biochar and sole manure use. This increase might be explained by increased nutrient availability, increased uptake by the crops and reduced nutrient leaching, and increased water retention. Therefore, applying biochar along with other organic materials such as manure may be able to achieve enhanced soil water retention, reduced nutrient leaching and improved fertility and crop yield, in a greater manner than the isolated application of those materials (Lebrun et al., 2022; Seyedsadr et al., 2022), though this remains largely untested in field conditions over extended time periods.

The central aim of this work was to compare the application of manure alone, or in combination with biochar, on key soil parameters under field conditions during two consecutive seasons. Specifically, the objectives were to (1) monitor soil water dynamics, (2) evaluate soil fertility and (3) discuss the findings in the context of the improved utilization of manure in agricultural soils by co-applying biochar.

In this study, we have stated the following four hypotheses:

H1. The incorporation of biochar to manure will improve soil physical properties and thus water retention.

H2. Mixing biochar to manure will stabilize manure nutrients under 2 years real conditions.

H3. Biochar/manure mixtures will increase microbial activity.

H4. Blending biochar to manure will improve plant growth and health.

2 | MATERIALS AND METHODS

2.1 | Site and amendments

The field site in Zvěřínek (Czech Republic, CZE) was chosen for this study because it typifies an increasing cohort of drought prone, predominantly sandy and low organic matter soils of the Czech Republic. Previous studies utilizing soils have identified the soils of this area as Regosols, with an average bulk density of 1.47 g.cm^{-3} and a total porosity of 42.8% (Lebrun et al., 2024, 2022). Particle size distribution indicates sandy soil with mean values of 10% for clay, 13% for silt and 77% for sand fraction (the transition between loamy sand and sandy loam textural class according to USDA classification). The soil is also characterized by a low organic matter content (C_{org} = 9.33 g.kg^{-1}) and is located in a drought-prone region.

Two organic amendments were used for the experiment. The first amendment was a common organic fertilizer, that is, manure. The manure, collected on a farm in Zvěřínek

TABLE 1 Properties of the initial materials. All values are given on a dry-weight basis.

Parameter	Soil	Biochar	Manure
Bulk density (g.cm^{-3})[a]	1.47	0.16	0.67
Total porosity (%)[a]	42.8	74.0	/
Sand (%)[b]	77.4	/	/
Silt (%)[b]	12.9	/	/
Clay (%)[b]	9.69	/	/
pH (–)	4.80	11.2	8.50
EC (μS.cm^{-1})	318	1400	4210
N_{tot} (g.kg^{-1})[c]	0.54	5.80	21.0
C_{tot} (g.kg^{-1})[c]	9.33	868	329
C/N	17.3	150	15.7
S_{tot} (g.kg^{-1})[d]	0.24	0.34	1.88
P_{tot} (g.kg^{-1})[d]	0.41	0.89	7.48
Ca_{tot} (g.kg^{-1})[d]	1.10	16.4	19.1
Mg_{tot} (g.kg^{-1})[d]	0.22	2.85	4.90
K_{tot} (g.kg^{-1})[d]	8.49	3.90	36.0

[a]NF EN 13041.
[b]Hydrometer method (CEN/ISO/TS 17892-42004).
[c]Elemental analyser (TNM-L segment flow analyser).
[d]Aqua-regia digestion.

(CZE), was made from a mixture of cow faeces and bedding straws. The biochar used is a registered additive (Central Institute for Supervising and Testing in Agriculture, CZE) and is made from the gasification of wooden pallets using atmospheric fixed-bed multi-stage gasifier at temperature range of 550–650°C. Details about biochar production can be found in our previous papers (Brynda et al., 2020; Lebrun et al., 2022). From those two materials, two mixtures were prepared, called manured biochars: (i) the first one contained 90% manure and 10% biochar (V/V), while (ii) the second contained 50% manure and 50% biochar (V/V). Such mixtures were made to stabilize manure nutrients, while bringing organic carbon to the soil and lower the primary negative effects that can be seen when applying biochar (high nutrient sorption, desiccation). The two biochar amounts were tested to have two opposite conditions, that is, a low amount of biochar recalcitrant C and a high amount of recalcitrant C, while biochar alone was not tested because of its market price and its primary negative effects on nutrients and soil water (if biochar is not activated). The mixtures were made on a fresh volume basis and left outside in manure heaps to equilibrate for 6 months (from May 2021 to November 2021) under ambient conditions, together with the original manure. This was done to match the farmer's practices.

Soil and original amendments were characterized for their elementary chemical properties, which are shown in Table 1.

2.2 | Experimental design

In November 2021 (after 6-month equilibration), the amendments were applied to the soil at a rate of 40 t.ha^{-1}, a dose commonly used by the farmers, and left over winter time prior to monitoring and sampling. In total, four variants were tested: (i) no amendment, i.e., control (CT), (ii) manure (MA), (iii) the mixture of 90% manure and 10% biochar (MB10 = 'economic manure blend with biochar') and (iv) the mixture of 50% manure and 50% biochar (MB50 = 'experimental manure blend with biochar'). The location of each plot was determined after a characterization of the area, in order to apply the treatments to areas with similar original soil properties, because of the heterogeneity of the site.

2.3 | Field monitoring

The field was monitored over 2 years, with two sampling campaigns per year (in April and August, overall, four campaigns in 2022 and 2023), when the following samplings were done.

2.3.1 | Soil sampling

Two types of soil samples were taken for each variant and sampling campaign: (i) undisturbed soil samples using sampling rings (= no destruction of the soil structure) in seven replicates (overall 4 variants × 7 replicates × 4 campaigns = 112 samples) and (ii) plastic bags in three replicates (overall 4 × 3 × 4 = 48 samples). The undisturbed samples (100 cm^3 in volume, 4 cm in height) were taken at the mean depth of 5 cm for the top of the ring (and 9 cm for the bottom of the ring) and further used to measure bulk density, actual soil moisture in the field, soil water retention curve (SWRC) and related properties such as porosity (estimated at pF 0), field capacity (estimated at pF 2, hereinafter FC), wilting point (pF 4.18, hereinafter WP), available soil water content for plants (the difference between FC and WP, hereinafter AWC) and easily available water content for plants (the difference between FC and pF 3.7, hereinafter EAWC). The SWRC measurements were done in a Sandbox 08.01 (Eijkelkamp, Netherlands) for the suction pressure up to pF 2 (100 cm) using the standard method (Eijkelkamp, 2022); in a Sand/kaolin box 08.02 (Eijkelkamp, Netherlands) for the suction pressure of pF 2.7 using the standard method (Eijkelkamp, 2013); in a 5 Bar Ceramic Plate Extractor 1600 (Soilmoisture, USA) for a suction pressure up to pF 3.7 using the standard method (Soilmoisture, 2008); and finally, a value of WP was measured using the WP4C Dewpoint PotentiaMeter (METER Group, Inc. USA) using the method described by (Campbell, 2023).

The three additional samples, stored in plastic bags, were taken next to the sample rings and used for chemical analysis: (i) pH was measured in 1:20 w/V (Houba et al., 1998); (ii) cation exchange capacity (CEC) was measured as the sum of extractable Ca, Mg, K, Na, Fe, Mn and Al in 0.1 M $BaCl_2$ solution (ISO 11260, 1994); and (iii) available nutrients were determined using the Mehlich III extraction procedure (Mehlich, 1984) followed by ICP-OES (Inductively coupled plasma-optical emission spectrometry) (Agilent 720, Agilent Technologies Inc., USA) measurements.

Finally, field-saturated hydraulic conductivity (Ksat) was determined using the Guelph permeameter (the Model 2800 K1 GP kit, Soilmoisture USA, hereinafter GP) at four replicates for each variant and sampling campaign (overall 4 × 4 × 4 = 64 GP tests) in wells of ca 15 cm depth and 6 cm in diameter. Each GP measurement was made in a separate position with a minimal distance of 1.5 m from other GP tests to avoid a mutual influence. The standard single constant head method (concretely a constant 10 cm depth of flooding and estimated sorptive number 0.12 cm^{-1}) was applied according to Reynolds (2008). This evaluation equation is described, for example, by Jačka et al. (2018).

2.3.2 | Soil microbial properties

As a result of the potential drought stress, which could affect both plant and microorganism survival, soils sampled in the plastic bags in summertime (August 2022 and August 2023) were also analysed for the microbial biomass C and N, using the chloroform fumigation extraction method (Brookes et al., 1985; Gregorich et al., 1990). In addition, diverse enzyme activities involved in C, N, S and P cycles were measured. In the carbon cycle were measured the activities of the β-D-glucosidase (ISO 20130, 2018), the cellobiohydrolase (using 4-Methylumbelliferone (MUF) and p-nitrophenol) (Baldrian, 2009) and the lipase (using MUF and heptonate) (Baldrian, 2009). In the nitrogen cycle were measured the activities of the alanine aminopeptidase (using MUF and L-alanine-7-amide) (Baldrian, 2009), the leucin aminopeptidase (using MUF and L-leucine-7-amide) (Baldrian, 2009) and the chitinase (depolymerisation) (Baldrian, 2009). In the phosphorus cycle the activity of the acid phosphatase was measured (using MUF and p-nitrophenol) (Baldrian, 2009), while in the sulfur cycle the activity of the arylsulfatase was measured (ISO 20130, 2018).

For each element, that is, carbon, nitrogen, phosphorus and sulphur, the sum of all activities was made.

Finally, the geometric mean (GMean) of the enzyme activities was also calculated, based on the formula given in Xu et al. (2021):

$$GMean = \left(\prod_{i}^{n} yi \right)^{1/n},$$

where *yi* is the activity of the enzyme *i* and *n* is the number of enzymes measured.

2.3.3 | Plants sampling

Only in the first year, that is, August 2022, was the natural plant coverage evaluated. The reason for this only one sampling in 2022 was: (i) targeted observation of natural vegetation cover by weed in the first season (= fallow land) representing green fertilizer application and (ii) unexpected cold spring when the corn (*Zea mays*) was sowed during the consecutive second season. First, a relevé of the plant species present and their abundance was recorded inside three squares (1 m^2) for each plot, selected for their representativeness of the entire plot. Inside each of those squares, three samplings were done; each sampling was 500 cm^2 and all the plants of each species present were collected. The plants collected were dried (72 h, 50°C) and weighed. To obtain the biomass produced per m^2, the biomass of the sample was recounted based on the species coverage value. The samples of *Setaria viridis*, a species found in all samplings and at high abundance, were acid-digested to determine P and K concentrations. In addition, inside each variant, five replicates of *Setaria viridis* were sampled fresh and immediately frozen in liquid nitrogen to measure the pigment concentrations, following the protocol of (Kiani et al., 2020).

2.3.4 | Drone monitoring

In order to have a complete picture of the plots (vs. small soil and plant samplings), drone images were acquired. For this, a Trimble R8s GNSS (Global Navigation Satellite System) receiver with a Trimble TSC3 controller was used to obtain precise coordinates at Zvěřínek. The GNSS receiver was connected to the CZEPOS network of permanent stations, and thanks to RTK (Real Time Kinematic) corrections, vertical and horizontal accuracy of up to 4 cm was achieved. In the experimental plots where biochar was applied, the individual devices of the weather station system and the ground control points used in processing UAV (Unmanned Aerial Vehicle) data were measured with precise coordinates. The coordinates were further used to set out the experimental plots during further control measurements. The UAV data was acquired using two different unmanned systems. The first was a DJI Mavic 2 Pro system equipped with an RGB sensor with 20 MPx and the second was a DJI P4 Multispectral system equipped with five monochromatic sensors (blue (B): 450 nm ± 16 nm; green (G): 560 nm ± 16 nm; red (R): 650 nm ± 16 nm; red edge (RE): 730 nm ± 16 nm; near-infrared (NIR): 840 nm ± 26 nm), each with 2 MPx. Primarily, these systems were used to monitor the experimental plots (ca. 3.5 ha); nadir images were taken from 100 m above the terrain, and the unmanned systems flew along programmed trajectories, which allowed for a longitudinal and lateral image overlap of ca. 80%. These images were subsequently processed using various photogrammetric techniques into a high-resolution, seamless orthophoto, positionally refined by RTK GNSS-targeted ground control points. The RGB orthomosaic was used for an overall visual overview of the site, and various vegetation indices, for example, NDVI (Normalized Difference Vegetation Index), can be calculated from the multispectral orthomosaic. Furthermore, a digital elevation model (DEM) was created by photogrammetry. All generated data was then processed in GIS software into map outputs. Secondarily, the DJI Mavic 2 Pro was used to take oblique images of the Zvěřínek site.

One of the outputs was a map of digital elevation models. Since the first imagery was taken before seeding, the first elevation model (04/2022) can be considered a digital terrain model (DTM) representing the elevation of the terrain without vegetation. The result of the subsequent imaging is then represented by a digital surface model (DSM); thus, different vegetation heights can be observed. Calculating the difference between the DSM and the DTM (sometimes referred to as the canopy height model; CHM), the vegetation growth in the observed areas is evident, with an average of 0.5 m. A detailed visual observation of the orthophoto from the first orthophoto shows a variation in soil coloration, which may result in different proportions of brown soil, black soil and sand across the observed area. This was also confirmed by the spatial analysis of the cluster unsupervised classification based on the Maximum Likelihood Classification tool. It outputs a classified raster that distinguishes different soil types. The splines following the dragging of the field are also evident. Further imagery visually shows the amount and asymmetric growth of vegetation.

Another map output showed the state of the NDVI. The NDVI (Normalized Difference Vegetation Index) is a simple and nondestructive graphical indicator that evaluates whether the surveyed surface contains living green vegetation so that changes in vegetation condition can be assessed over time when measurements are repeated. Simply, it indicates how much chlorophyll is present in a plant by measuring its colour (reflectance) in the near-infrared (NIR) part of the spectrum. The NIR spectrum is not visible to the eye; however, the healthier the observed plants are, the more they reflect light in the NIR spectrum. When plants are dehydrated or stressed, they consequently reflect less light in the NIR and still reflect the same amount of light in the visible spectrum (Figure S1). Thus, the map output shows a difference in the observed vegetation, where it takes on values from 0 to 1 (represented by the colour scale from yellow to green) and, conversely, the absence (or degradation) of vegetation takes on negative values (represented by the red colour). The multispectral sensor was imaged three times, and thus, it is possible to evaluate the change in vegetation status.

2.4 | Statistical analysis

Data was analysed on the R software. A repeated measured ANOVA was performed, with the year taken as the repeated factor and treatment as the main factor. This analysis showed non-significant results (Table S1), except for one parameter (available potassium, Figure S2). Based on those results, the repeated factor does not need to be included in the model for the rest of responses and thus we performed simple ANOVA test followed by a Tukey post-hoc. Difference was considered significant at $p < .05$.

3 | RESULTS

3.1 | Initial soil description

The entire area was initially evaluated using drone images and randomized soil samples. From this analysis, we observed an important heterogeneity of the soil (Figure 1). The area is almost flat with a maximum elevation change <11 m within the whole monitored area (240 × 100 m; Figure 1b). The orthophoto mosaic map (Figure 1a) shows the initial situation after the application of the amendments. We can see different colours of the surface, reflecting soil heterogeneity. Sufficient soil sampling/mapping was done to find representative sampling spots within each variant as well as to minimize the heterogeneity of the examined area (and thus be able to compare particular variants with each other).

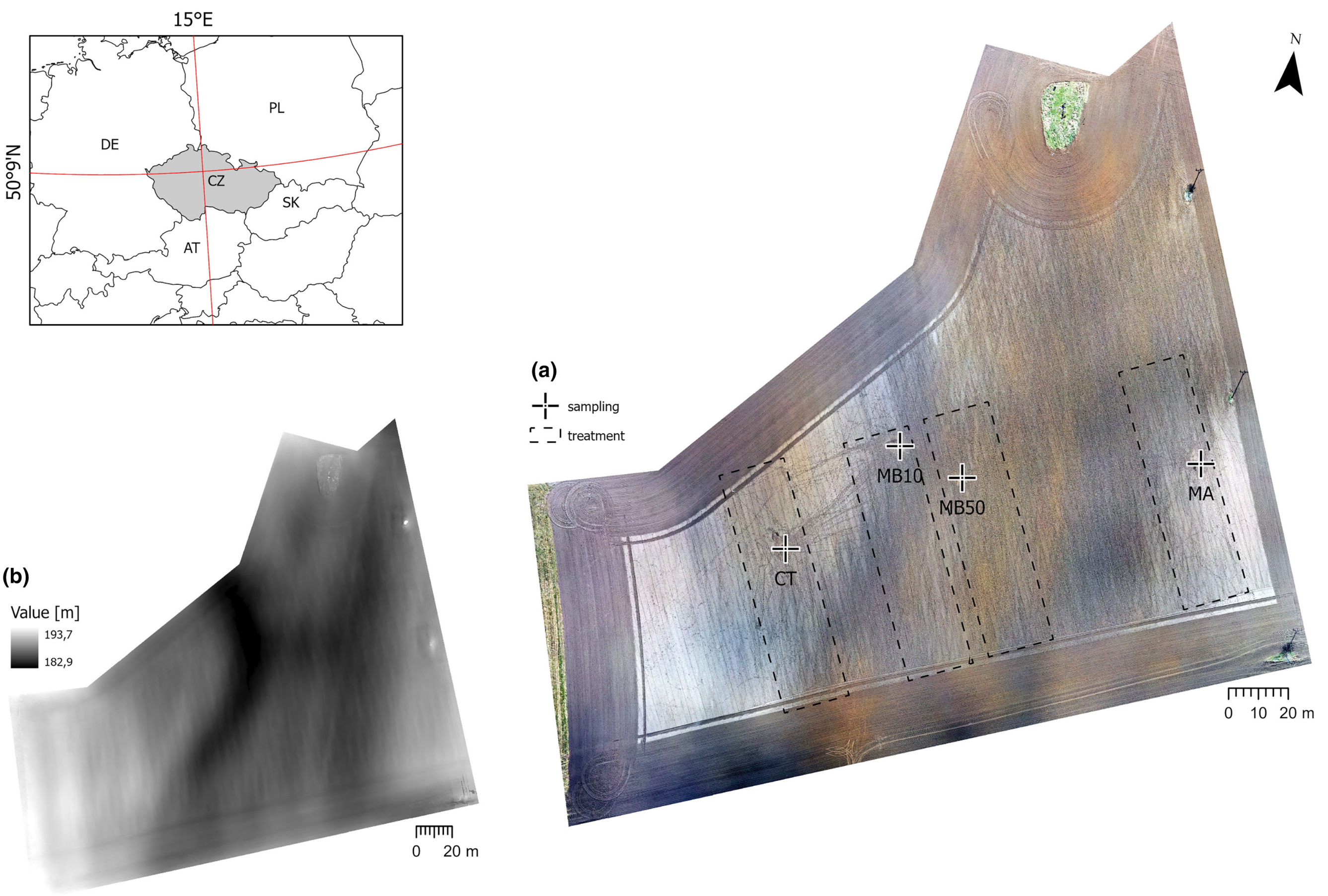

FIGURE 1 Drone image (RGB) of the field surface at the initial state (04/2022). (a) Orthophoto; (b) digital elevation model.

All the prior and representative soil analyses are presented (together with both pristine amendments) in Table 1. Analyses showed that soil had an acidic pH and low nutrient contents, while both pristine amendments were alkaline (biochar > manure) and rich in nutrients (manure > biochar). In addition, the soil had a low field capacity (ca. 22%), in relation to the big size of its pores. In addition, Ksat showed a high value ($10^{-5}\,m\,s^{-1}$) and thus that soil is highly subject to drought (Figure S3).

3.2 | Soil hydrophysical and hydropedological properties

3.2.1 | Bulk density

The average bulk density of the control plot over the 2 years of monitoring was $1.45\,g.cm^{-3}$. The only significant effect was observed in the first sampling date (April 2022) (ANOVA results: p-value = 8.85 e^{-6}, $F = 15.32$), with a significant increase in BD in the MA treatment (+20%) and, in a lower extent, MB10 (+10%), and in the last sampling (ANOVA results: p-value = .00829, $F = 4.853$), with a significant increase in bulk density with the manure (+9%) (Figure S3a).

3.2.2 | Field capacity

Over the 2 years, FC of the control plot was on average $0.23\,cm^3.cm^{-3}$. The FC was affected by the amendments. In April 2022 (ANOVA results: p-value = 6.87 e^{-7}, $F = 20.94$), manured biochar application significantly increased FC compared with control, with a higher effect of MB10 (+27%) than MB50 (+14%). In the second sampling (ANOVA results: p-value = 1.49 e^{-9}, $F = 51.02$), MA treatment showed a significantly lower FC than control (−28%), while MB10 had a higher FC (+12%) and MB50 had no effect. In the second year, field capacity was affected by the amendments in April (ANOVA results: p-value = 1.07 e^{-10}, $F = 59.21$) and August (ANOVA results: p-value = 2 e^{-16}, $F = 217.7$) in a similar way: the application of manure decreased FC (−20% and −14%) while MB10 and MB50 increased it to similar levels (+12% and +25%) (Figure 2).

3.2.3 | Actual Soil Moisture

Actual soil moisture was $0.20\,cm^3.cm^{-3}$ on average on the control plot during the 2 years of monitoring. At the first sampling (April 2022) (ANOVA results: p-value = .00036, $F = 8.988$), all amendments increased ASM (between 18% and 41%). In the following samplings (ANOVA results for August 2022: p-value = 7.38e^{-8}, $F = 36.47$; ANOVA results for April 2023: p-value = 5.55e^{-14}, $F = 63.13$; ANOVA results for August 2023: p-value = 1e^{-13}, $F = 77.66$), a reduction in ASM was observed with manure alone (−25% to −29%), and an increase with MB10 and MB50. No difference between MB10 and MB50 was observed, except at the last sampling (MB50 > MB10) (Figure S3b).

3.2.4 | Saturated hydraulic conductivity

On average, Ksat was 20 e^{-6} $m.s^{-1}$ on average. Overall, amendment application did not significantly affect soil Ksat (ANOVA results for April 2022: p-value = .593, $F = 0.659$; ANOVA results for August 2022: p-value = .962, $F = 0.093$; ANOVA results for April 2023: p-value = .614, $F = 0.621$; ANOVA results for August 2023: p-value = .704, $F = 0.477$) (Figure S3c).

3.3 | Soil chemical properties

3.3.1 | Cation Exchange Capacity

Control CEC was on average $99\,mmol.kg^{-1}$ and was affected by amendment application over the 2 years of monitoring (ANOVA results for April 2022: p-value = 3.68e^{-5}, $F = 16.55$; ANOVA results for August 2022: p-value = 1.46e^{-5}, $F = 51.05$; ANOVA results for April 2023: p-value = 1.43e^{-11}, $F = 104.3$; ANOVA results for August 2023: p-value = 4.81e^{-8}, $F = 222.6$). In more detail, MA induced a decrease in CEC in the four sampling dates (15%–31% decrease). On the contrary, manured biochar treatments increased CEC, with MB10 (33%–54% increase) inducing a significantly higher effect than MB50 (23%–44% increase) only at the last sampling (August 2023) (Figure 3a).

3.3.2 | pH

The control plot was acidic, with an average pH value of 5.78 over the 2 years of monitoring. In the first sampling (April 2022) (Results of ANOVA: p-value = .03, $F = 3$), pH only significantly increased with the application of MB10 (+0.2 unit), while in the two summer samplings (August 2022 (Results of ANOVA: p-value = .000623, $F = 18.19$) and August 2023 (Results of ANOVA: p-value = .02692, $F = 9.186$)), both MA (+0.6 unit and +0.4

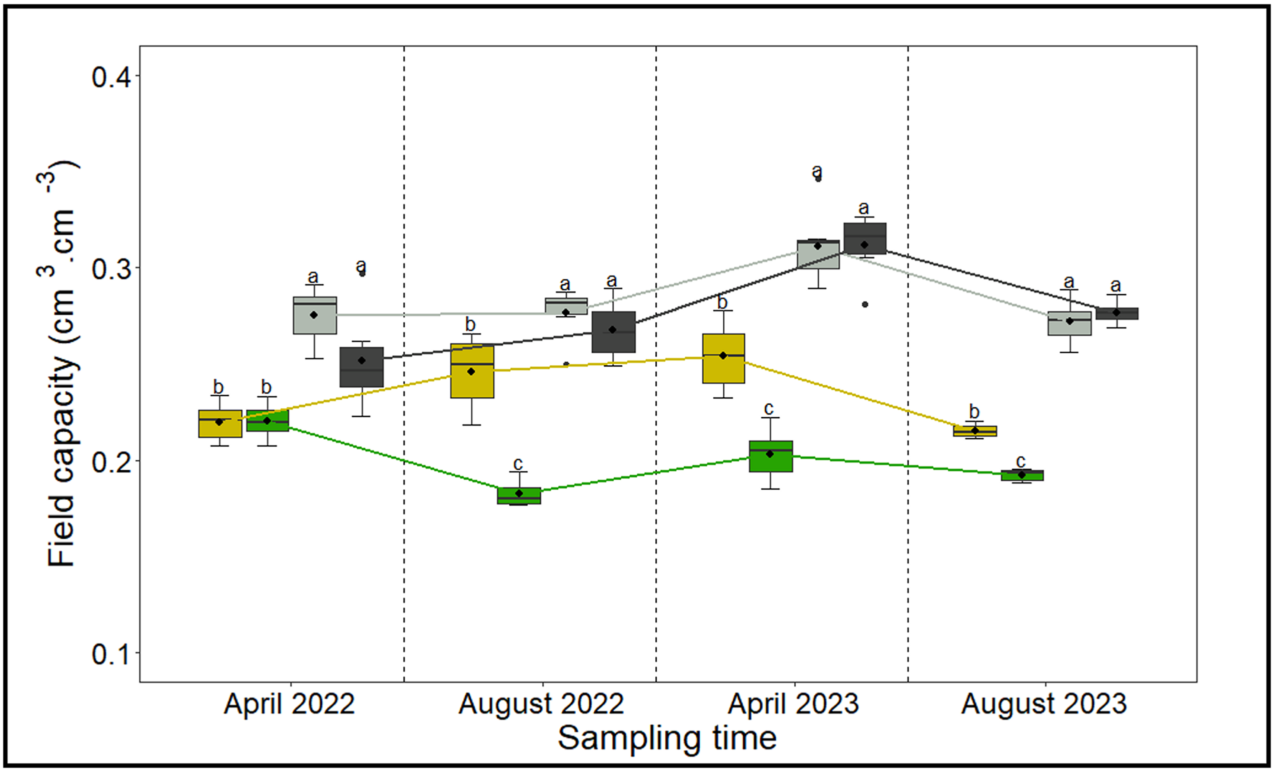

FIGURE 2 Soil field capacity measured on the field in the four variants (yellow = control, green = manure, grey = MB10, black = MB50) over the 2 years of monitoring. Letters indicate significant difference between variant within each sampling time ($p < .05$).

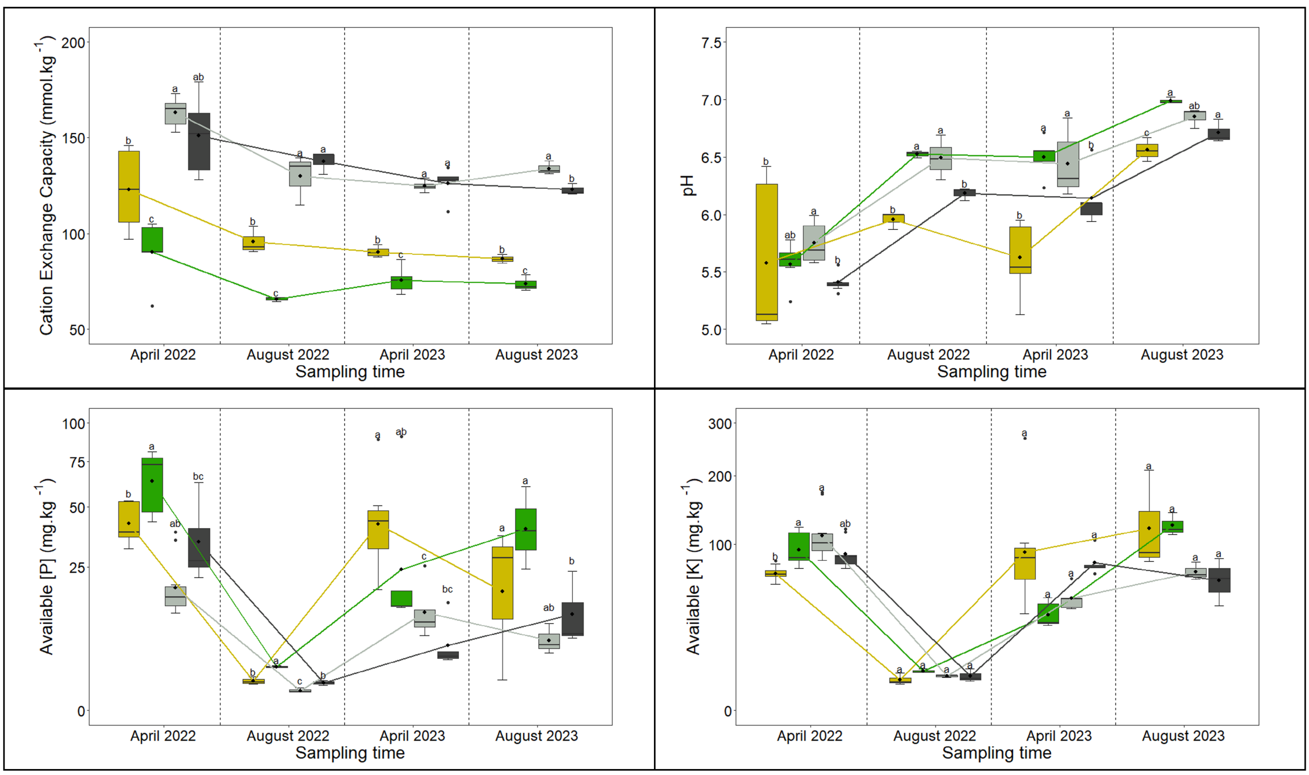

FIGURE 3 Soil chemical properties (cation exchange capacity, pH, available P and K concentrations) measured on the field in the four variants (yellow = control, green = manure, grey = MB10, black = MB50) over the 2 years of monitoring. Letters indicate significant difference between variant within each sampling time ($p < .05$).

unit) and MB10 (+0.5 unit and +0.3 unit) increased soil pH, and MB50 had no effect. In April 2023 (Results of ANOVA: *p*-value = 4.91e^{-5}, *F* = 14.43), all amendments increased soil pH to similar levels (+0.5 to 0.9 unit) (Figure 3b).

3.3.3 | Available P concentration

Available P concentration presented an important variation in time, with higher values in both April samplings and August 2023 (CT = 22–44 mg.kg^{-1}) and much lower values in August 2022 (CT = 1.1 mg.kg^{-1}), when plants developed more importantly. The main effects were observed during the first year (Results of ANOVA for April 2022: *p*-value = 5.86e^{-8}, *F* = 21; Results of ANOVA for August 2022: *p*-value = 1.14e^{-5}, *F* = 54.59), with an increase in available P following MA application (51%–117% increase), while MB10 decreased P availability (56%–53% decrease) and MB50 had no effect. In the second year of monitoring, the only significant effect was found in April (Results of ANOVA: *p*-value = .0248, *F* = 3.965), with an 86% decrease in available *P* concentration in the treatment MB50 compared with control (Figure 3c).

3.3.4 | Available K concentration

Similarly to *P*, available *K* concentrations (RM ANOVA results: *p*-value = .01, *F* = 5.56) showed variations with time, with higher values in April 2022 and April–August 2023 (CT = 68–127 mg.kg^{-1}), while in August 2022, values were much lower (CT = 3.55 mg.kg^{-1}). The only significant effect was observed at the first sampling (April 2022) (Results of ANOVA: *p*-value = .000629, *F* = 7.304), with an increase in available K concentration with MA (+40%) and MB10 (+65%) application (Figure 3d).

3.3.5 | Mobile C and N contents

Mobile C and N contents were only assessed in summer samplings (August 2022 and August 2023). No significant effect was observed, for both mobile C (Results of ANOVA for August 2022: *p*-value = .0104, *F* = 7.482; Results of ANOVA for August 2023: *p*-value = .0192, *F* = 5.997) and N (Results of ANOVA for August 2022: *p*-value = .296, *F* = 1.463; Results of ANOVA for August 2023: *p*-value = .241, *F* = 1.714), in both years. However, in the first year, mobile C was higher in MB50 compared with MA and MB10 (Figure 4).

3.3.6 | Total C, H, N

An elemental analysis of soil sampled in August 2022 and in August 2023 was done. Results of the first year showed an increase in total C and N contents only with MB50 application (+35% for C and +36% for N). Total H and the C/N and C/H ratios were not affected by amendment applications. In the second year, total C content was not different in the amended plots compared with the control, although it was higher in MB50 compared with MA. Content in H increased significantly in MB50 (+63%) compared with the control, while nitrogen content was only significantly decreased in the MA treatment (−40%). The C/N ratio was not affected by the amendments, while the C/H ratio decreased in the manured biochar plots (−30%) (Table 2).

3.4 | Microorganisms

3.4.1 | Microbial biomasses

Microbial biomass C was 12 and 216 mg.kg^{-1} while microbial biomass N was 3.6 and 6.9 mg.kg^{-1}, in the control plot, in August 2022 and August 2023, respectively. Neither of those two parameters was significantly affected by the

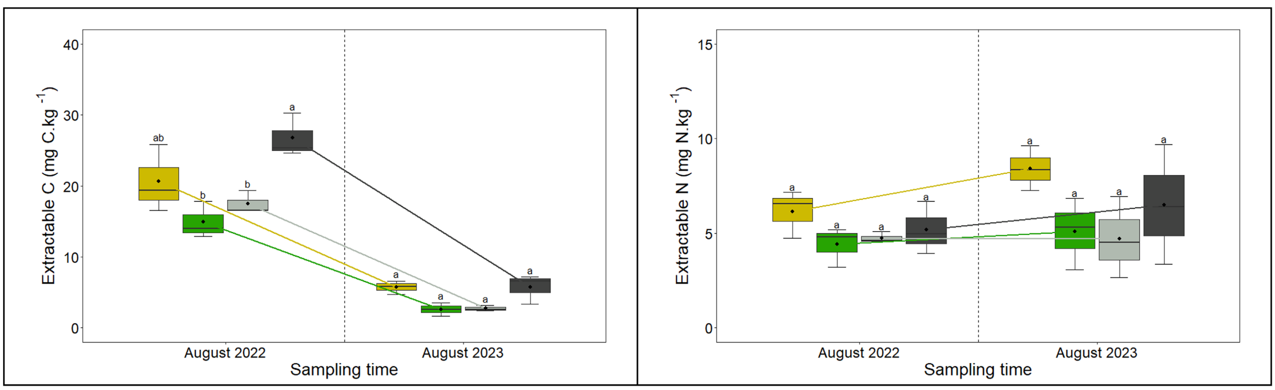

FIGURE 4 Soil mobile C and N measured on the field in the four variants (yellow = control, green = manure, grey = MB10, black = MB50) over the 2 years of monitoring. Letters indicate significant difference between variant within each sampling time ($p < .05$).

TABLE 2 Total C, H, N contents and C/N and C/H ratios measured in August 2022 and August 2023, in the treatments, CT = non-amended soil, MA = manure, MB10 = manured biochars (10:90), MB50 = manured biochars (50:50).

		Total C (%)	Total H (%)	Total N (%)	C/N	C/H
August 2022	CT	1.41 ± 0.05[b]	0.27 ± 0.01[b]	0.14 ± 0.01[b]	10.3 ± 0.3[a]	5.2 ± 0.1[a]
	MA	1.13 ± 1.17[b]	0.29 ± 0.27[ab]	0.11 ± 0.12[b]	6.7 ± 5.8[a]	2.8 ± 2.4[a]
	MB10	1.58 ± 0.18[ab]	0.48 ± 0.06[a]	0.16 ± 0.02[ab]	9.8 ± 0.2[a]	3.3 ± 0.1[a]
	MB50	1.91 ± 0.03[a]	0.47 ± 0.02[ab]	0.19 ± 0.00[a]	9.9 ± 0.2[a]	4.1 ± 0.1[a]
August 2023	CT	1.47 ± 0.13[ab]	0.24 ± 0.03[bc]	0.15 ± 0.02[a]	9.9 ± 0.6[a]	6.2 ± 0.4[a]
	MA	1.03 ± 0.36[b]	0.14 ± 0.02[c]	0.09 ± 0.02[b]	10.2 ± 1.0[a]	6.7 ± 0.6[a]
	MB10	1.56 ± 0.12[ab]	0.41 ± 0.10[ab]	0.17 ± 0.01[a]	9.3 ± 0.4[a]	3.9 ± 0.6[b]
	MB50	1.91 ± 0.03[a]	0.39 ± 0.01[a]	0.19 ± 0.02[a]	9.6 ± 0.2[a]	4.7 ± 0.4[b]

Note: Letters indicate significant different ($p < .05$).

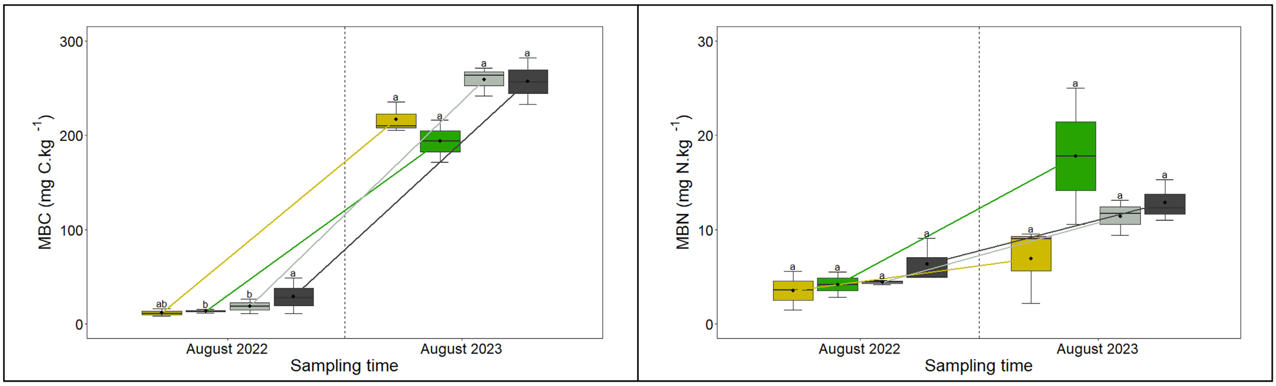

FIGURE 5 Soil microbial biomass C and N measured on the field in the four variants (yellow = control, green = manure, grey = MB10, black = MB50) in August over the 2 years of monitoring. Letters indicate significant differences between variant within each sampling time ($p < .05$).

amendment application, in both sampling times (ANOVA results for MBC in 2022: p-value = .398, F = 1.163 and 2023: p-value = .010, F = 7.575; ANOVA results for MBN in 2022: p-value = .436, F = 1.052 and 2023: p-value = .175, F = 2.207). However, a higher MBC was measured in the manured biochar treatments compared with MA treatment in 2023 (Figure 5).

3.4.2 | Enzyme activities

Enzyme activity values were used to calculate cumulative enzyme activities in relation to the elements (C, N, P and S). In August 2022, cumulative C-enzyme activity was 11 nmol MUF.g^{-1}.min^{-1} and it highly increased in the MA amended plot (3-fold increase), while the MA had little effect. Cumulative N activity was 1.1 nmol MUF.g^{-1}.min^{-1} on control, and, as for C-enzymes, it highly increased with MA amendment (twofold increase), while MB10 induced a small increase (45%) and MB50 had no effect. Enzymes related to P and S were very low in the CT plot (0.03 nmol MUF.g^{-1}.min^{-1} and 0.05 nmol.g^{-1}.min^{-1}, respectively) and those values increased in the amended plots. For P enzyme, it increased in the order MB50 (9-fold) < MB10 (28-fold) < MA (34-fold); for the S enzyme, the order was MA (2.6-fold) < MB10 (3.2-fold) (Figure 6a).

In August 2023, cumulative C and N enzyme values were 18 and 0.8 nmol MUF.g^{-1}.min^{-1}. Both increased in MA treatment (6% for C and 2.3-fold for N). The application of MB10 decreased cumulative C enzymes (−50%) while MB50 increased cumulative N enzymes (88%).

Finally, using these data, GMean was calculated (Figure 6b). Values in control plot were 0.8 in August 2022 and 0.17 in August 2023. No significant effect of amendment application was observed (ANOVA results: p-value = .138, F = 2.453). Although values tended to be higher in manure and, to a lesser extent, manured biochar plots.

3.5 | Plants

Plants were assessed in August 2022. On average, total biomass production on control plot was 86 g.m^{-2} (Figure 7a), no significant effect of the amendment was found (ANOVA results: p-value = .11, F = 2.178), although biomass was higher in amended plots, in the order

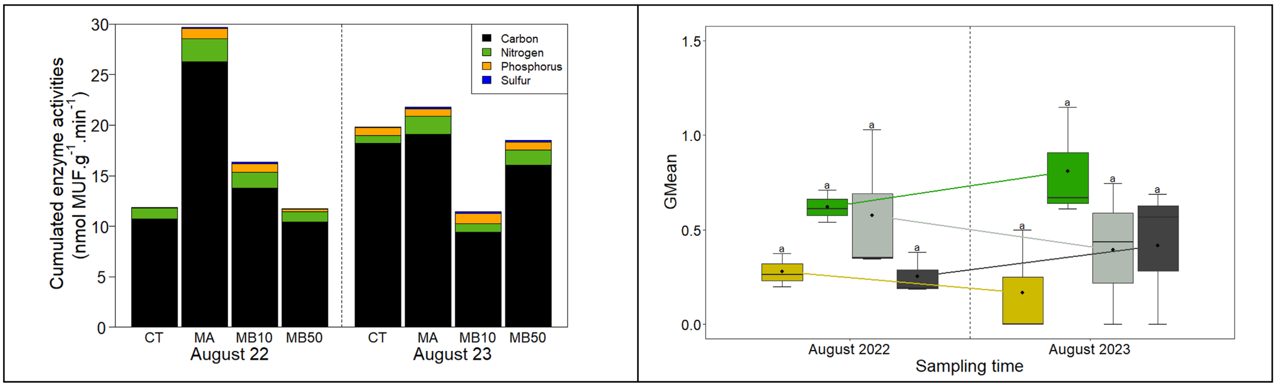

FIGURE 6 Soil-cumulated enzyme activities and Gmean measured on the field in the four variants (yellow = control, green = manure, grey = MB10, black = MB50) over the 2 years of monitoring. Letters indicate significant difference between variant within each sampling time ($p < .05$).

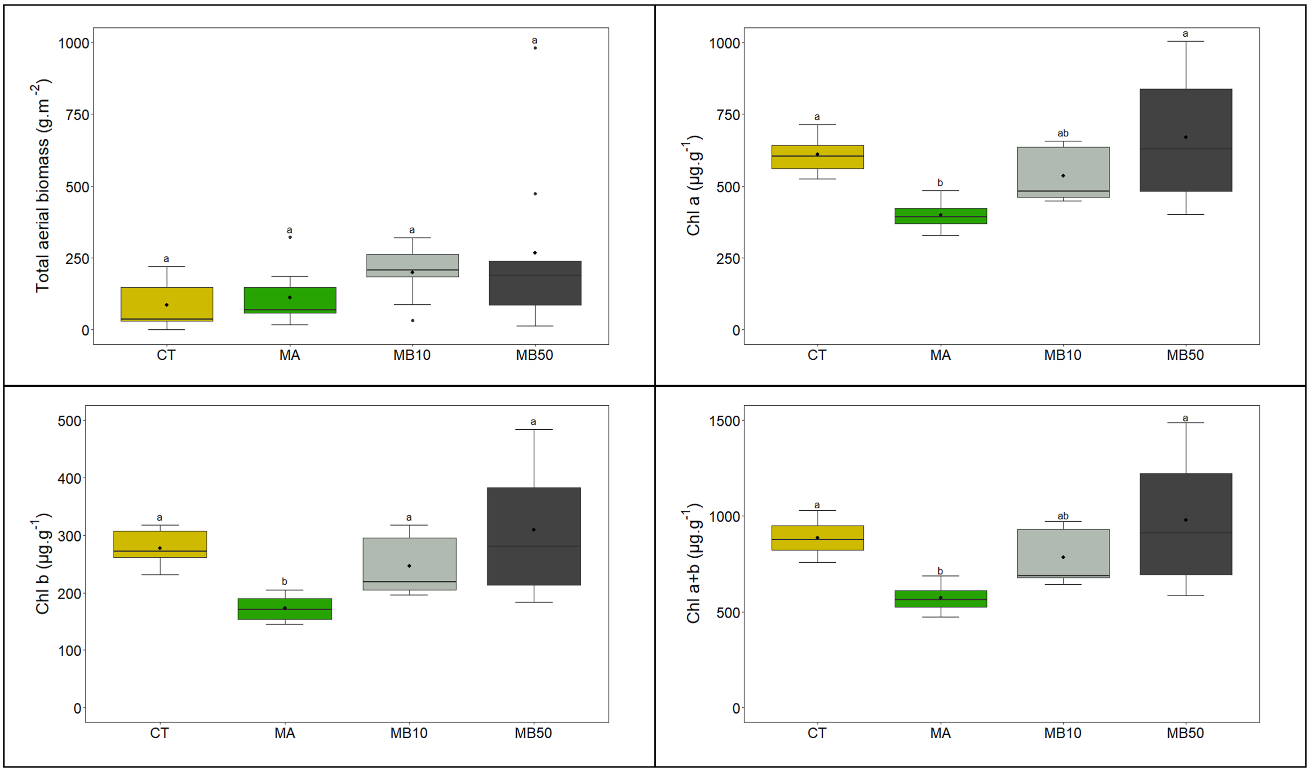

FIGURE 7 Total plant biomass and *Setaria viridis* leaf chlorophyll contents measured on the field in the four variants (yellow = control, green = manure, grey = MB10, black = MB50) on August 2022. Letters indicate significant difference between variants ($p < .05$).

MA < MB10 < MB50. One species, encountered in all the plots, was selected for pigment analysis, which showed that under MA treatment, chlorophyll a, chlorophyll b and chlorophyll A + B contents decreased (34%–38%) (Figure 7b–d). Moreover, the root to shoot ratio was on average 0.22 on the control plot and increased in the order: MB10 (0.27) < MA (0.33) < MB50 (0.44) (Figure S4).

The images provided by UAV confirm those results (Figure 8), that is, the height of the plants is seen higher in the area where MBs were applied, compared with control and manure (Figure 8b), while the health of the plants looks similar which was confirmed by measured NDVI index (Figure 8c).

4 | DISCUSSION

4.1 | Amelioration of water retention with biochar

The blending of biochar to manure lowered the increase in bulk density induced by manure. This demonstrates the

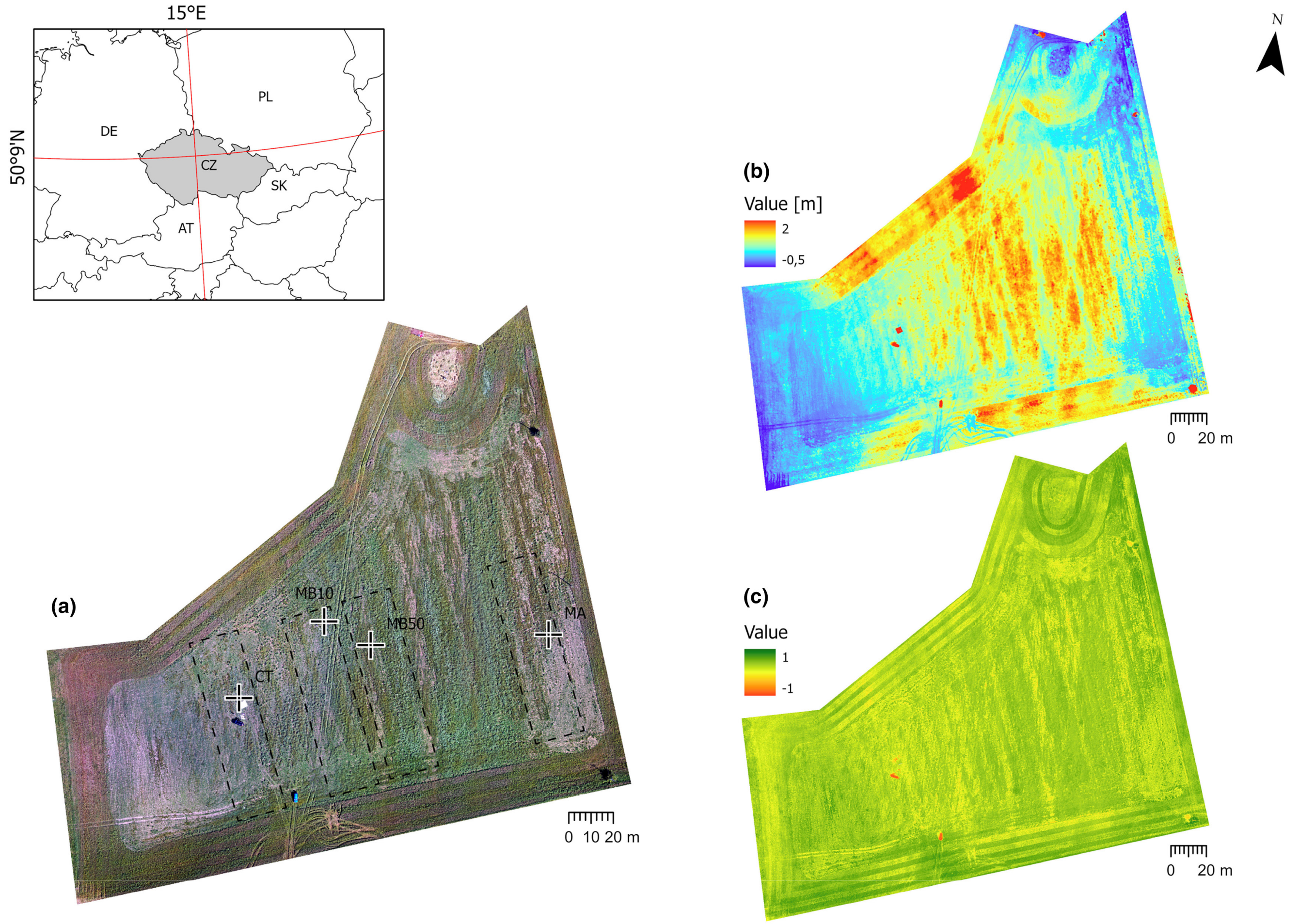

FIGURE 8 Drone image (RGB) of the field surface during vegetation season (08/2022). (a) orthophoto; (b) Canopy height model; (c) Normalized difference vegetation index (NDVI).

benefits of biochar towards soil bulk density, which can be attributed to the porosity of the biochar and the improvement of soil aggregation (Védère et al., 2022) as well as can indirectly confirm better retention of water in the manure (usually bulk density of manure is lower than water).

Blending manure and biochar also increased the field capacity of the soil. Organic amendments are known to improve soil water retention (Ajayi, Holthusen, & Horn, 2016; Głąb et al., 2020; Zhang et al., 2019), via direct and indirect mechanisms. Biochar contains many macro- and mainly meso-pores, in which water can infiltrate (Abrol et al., 2016), and be then available to plants (Seyedsadr et al., 2022). Biochar also contains carboxyl functional groups on its surface, which can form hydrogen bonds with water (Jačka et al., 2018), and cations with which water can interact (Kutilek & Nielsen, 1994). Amendment application reduced bulk density, which increases porosity and thus water infiltration (Védère et al., 2022). The effect of presented biochar to increase field capacity (= water retention) could be explained via not only the direct interaction of water molecules with biochar surface (i.e., via the presence of functional groups, hydrogen bonds and pí binding sites) but also indirectly through: (1) the improved persistence of soil pores (increasing aggregate stability); (2) the reduction of soil pore sizes (filled by small 'sticked' biochar particles) and (3) the presence of inner biochar pores (Seyedsadr et al., 2022). Finally, the organic matter in the soil has a crucial impact on water dynamic in soil, with a positive correlation between organic matter content and water content (Védère et al., 2022).

Overall, our study confirmed our first expectation (H1), as blending biochar to manure improved soil structure and thus water content. The underlying mechanisms of such observation are (i) a reduction of soil bulk density, (ii) an amelioration of the aggregation and (iii) an increase in porosity, which allows water to be stored more efficiently in the soil.

However, the effects were mainly significant during the first sampling and faded with time. Other studies have observed amelioration of water content with manure and/or biochar on the medium-term (Table 3b), our results were less strong. Even tough amendment effects are superior on sandy soil (Seyedsadr et al., 2022; Védère et al., 2022),

TABLE 3 Comparison of our findings to other published works on the effect of biochar/manure on soil properties.

	Experimental design			
Reference	Soil type	Experiment type & time	Treatments	Findings of the study and comparison with our results (in italic)
Part A. Soil microbial properties				
Sun et al. (2024)	Clay loam texture	Field 4 years	Three different manures	After 4 years, plots having received manure showed higher microbial biomass C content, and higher enzyme activities involved in carbon cycle (sucrase and cellulase), because of the easily mineralizable C contained in the manure *Our results did not show any increase in microbial biomass C, even at the initial time. This may be because of a different C resistance in our manure, although enzyme activities showed higher C-related enzyme activities with manure. It may be possible that microbial biomass C will increase in the next years* Manure application increased soil microbial biomass P and the enzymes related to the P cycle (phosphatase and phytase). The authors explained this by the nutrient-poor characteristics of the site and the introduction of microorganisms through manure *Even though we did not measure microbial biomass P, our results on P enzymes are in accordance with this study*
Wang, Gao, et al. (2023)	Clay texture 2 sites	Field Over 30 years	Manure (with and without NPK)	Manure amendment increased gross N mineralization and NH_4 immobilization while decreasing gross nitrification. Manure plots also had higher microbial biomass N content. The activity of N acquiring enzymes were increased by manure as well • The results showed the capacity of manure to improve soil N supply and retention. *Although we did not measure N mineralization and nitrification, we can hypothesize that manure has increased N mineralization, in relation to the higher enzyme activities related to N* The abundance of the genes related to N mineralization (sub, chiA and exo-chi) increased with manure, while manure decreased AOA abundances, which a gene related to gross nitrification
Ma et al. (2020)	Sandy loam texture	Field 28 years	Farmyard manure: 10 t.ha^{-1}; 25–50 t.ha^{-1}	Long term manure application induced an increase in microbial biomass C and N and soil respiration, higher NO_3 production and consumption but no effect on gross NH_4^+ production and consumption Enzymes related to C and N were also increased by manure Although PLFA contents of bacteria, actinomycetes and anaerobes increased, the authors did not observe a change in microbial community structure *Similarly to this study, we have observed an increase in the enzymes related to C and N cycling. However, our results differed in terms of microbial biomass C and N but this could be due, at least partly, to the high variability we had on our field* *Based on our results, we can hypothesize that manure will continue to increase N mineralization over time*

(Continues)

TABLE 3 (Continued)

Reference	Experimental design: Soil type	Experiment type & time	Treatments	Findings of the study and comparison with our results (in italic)
Holatko et al. (2022)	Silty clay loam texture (mix with sand)	Pot 12 weeks	Fermented manure Manure enriched with biochar	Basal respiration was higher with biochar-enriched manure than with manure alone. Dehydrogenase and urease were increased by both manures at similar levels. Both manures also decreased arylsulfatase activity, but enriching manure with manure led to a lower decrease. N-acetylglutamate synthase and glucosidase activities increased only in the case of the enriched manure, which implies a higher fungal abundance, N mineralization and nitrification in this treatment *Our results concord with this study, with an increase in C and N related enzymes with manure. However, we found that mixing biochar with manure reduced the activities of those enzymes compared with manure alone, while the authors found the reverse. Our different results may come from the fact that we let biochar and manure aged outside for 6 months while the authors did it for 2 months at stable temperature and humidity*
Wang, Wu, et al. (2023)	Silty sandy loam texture	Field 3 years	Biochar: 5 t.ha^{-1}; 10 t.ha^{-1}; 20 t.ha^{-1} Manure: 7.5 t.ha^{-1}; 10 t.ha^{-1}	Both amendments rose microbial biomass C and N, with a more important effect of manure *Our study showed that manure had no effect on microbial biomass C but increased microbial biomass N, only on the second year. We can conclude that half our results are in accordance with this study. We could also hypothesize that with time and the activity of microorganisms, microbial biomass C will increase in manure plots*
Dodor et al. (2018)	Sandy soil		Manure: 13 t.ha^{-1}; 26 t.ha^{-1} Biochar: 20 t.ha^{-1}; 40 t.ha^{-1} Manure/Biochar mixture	Manure and biochar application increase CO_2-C emissions, and their mixtures even led to a higher increase. Alone, biochar and manure induced a positive priming effect, showing a higher mineralization of the organic matter (in relation to more microorganisms), while their combination led to a negative priming effect, because of the immobilization of C on the biochar surface as carbonates, which is then unavailable for microbial oxidation Similar results were observed regarding net N mineralization, with a positive effect with biochar and manure alone and a negative effect with biochar/manure mixtures, showing N immobilization *Our results are in accordance with this work, as we observed higher C-cycle enzyme activities, and related CO_2 emission (shown in our previous work,* Lebrun et al., 2024*), which was reduced when biochar was incorporated to manure. Similarly, even thought it was less visible, N mineralization (approximated by the measure of N related enzymes) was increased by manure and lowered with the addition of biochar to manure*

TABLE 3 (Continued)

Reference	Experimental design: Soil type	Experiment type & time	Treatments	Findings of the study and comparison with our results (in italic)
Gautam et al. (2020)	Silty loam texture	Field 16 years	Manure	Manure application affected several microbial processes in the soil but only at the high application rate: increase in microbial biomass C and N, β-glucosidase, urease, alkaline phosphatase and PLFA biomass. However, it did not affect community diversity, whatever the dose applied *Although we did not look at the microbial diversity, we observed that functionality of the community (assessed by Gmean) was no affected by manure, which could corroborate the results of this study* *We also found higher enzyme activities with manure, but at a lower dose than the one of this study. This shows that our manure had higher effects than their manure. As the authors did not provide detailed information on initial soil and manure properties, it is difficult to try to explain the differences*
Irmak Yilmaz and Ergun (2019)	Clayey texture	Greenhouse 1 crop season	Biochar: 20 t.ha^{-1} Manure: 20 t.ha^{-1} Combination of biochar (5 t.ha^{-1}) with manure (5 t.ha^{-1} or 10 t.ha^{-1}) Combination of biochar (10 t.ha^{-1}) with manure (5 t.ha^{-1} or 10 t.ha^{-1}) Combination of biochar (15 t.ha^{-1}) with manure (5 t.ha^{-1} or 10 t.ha^{-1})	The enzymes related to C cycle (dehydrogenase), N cycle (urease) and S cycle (arylsulfatase) increased while the enzyme related to P cycle (alkaline phosphatase) was not affected. In general, the combination of manure and biochar had a higher effect than their single application on dehydrogenase *Our results are only partly in accordance with this study. Manure increased C, N, P and S related enzymes while the incorporation of biochar to manure decreased them compared with manure alone. The timing between mixing of biochar and manure and their application may have led to different results (on the same day vs. 6 months after mixing), as elements could have been fixed on biochar during the 6 month ageing and thus made unavailable for microorganisms*
Lima et al. (2021)	Sandy clay loam texture	Field 1 cropping season	Biochar: 10 t.ha^{-1}; 20 t.ha^{-1}; 40 t.ha^{-1} Manure: 5 t.ha^{-1} Biochar/Manure mixtures	The content in microbial biomass C was not affected by the amendments, while two enzymes had their activities increased, i.e., acid phosphatase (with 40 t.ha^{-1} biochar, 5 t.ha^{-1} manure and 10 t.ha^{-1} biochar +5 t.ha^{-1} manure) and urease (with 5 t.ha^{-1} manure, 10 t.ha^{-1} biochar +5 t.ha^{-1} manure and 40 t.ha^{-1} biochar +5 t.ha^{-1} manure) *Our results are in accordance with this study*

(Continues)

TABLE 3 (Continued)

Reference	Experimental design: Soil type	Experimental design: Experiment type & time	Experimental design: Treatments	Findings of the study and comparison with our results (in italic)
Brtnicky et al. (2021)	Luvisols	Field 3 years	Manure: 50 t.ha^{-1} Biochar: 15 t.ha^{-1} Manure/Biochar mixture All in combination with NPK fertilizer application	Dehydrogenase in manure/biochar mixture treatment was similar to control, showing no effect on C related bacteria, while PLFA fungi increased in this amendment and PLFA bacteria increased with manure application, after 3 years. The authors also observed a higher microbial biomass C content with the manure/biochar mixture and a higher number of copies of the 16S rDNA AOB with manure and manure/biochar mixture, indicating a higher microbial activity in N mineralization *Even though C cycle enzymes were decreased with the incorporation of biochar to manure compared with manure alone, the C enzymes were slightly higher than control in year 1 but decreased in year 2. This can predict that C mineralization will decrease with time. This contradicts the results of this study as the authors found no effect on dehydrogenase, even with a higher dose of amendment than our study. However, our data on N enzymes match the results of this study, as they indicate N mineralization, which will continue over the long time*
Ye et al. (2021)	Quaternary red clay	Field 18 years	Manure: 9 t.ha^{-1}; 18 t.ha^{-1}; 27 t.ha^{-1}	The authors evaluated the changes in microbial community after 18 years and observed that manure increased α-diversity of the bacteria, and the relative abundance of diazotrophs, nitrifiers and saprotrophs while it decreased the denitrifiers and the plant pathogens and parasites *We did not make such evaluation in our study but as our manure did not increase available nutrients after two years, we can hypothesize that the increase in microbial colonization may not be promoted over the long term*
Bera et al. (2016)	Silt loam texture	Field 3 years	Manure: 168,000 L.ha^{-1} Biochar: 22 t.ha^{-1} Biochar/Manure mixture	Manure alone increased FDA enzyme while only when it was combined with biochar did it increase microbial biomass C, urease activity and alkaline phosphatase, while this mixture decreased acid phosphatase and β-glucosidase *Overall, biochar/manure mixtures fitted the results of this study (increase in N and P enzymes, decrease in C enzymes). In addition, such effect compared with the control increased with time and can predict a continuous influence over the long time*
Xie et al. (2023)	Purple fluvo aquic soil	Pot 7 months	Manure Biochar Biochar/Manure mixture	Manure, alone or combined with biochar, reduced potential nitrification rate, while it increased alkaline phosphatase In terms of microbial community structure, manure and manure/biochar mixture reduced the number of copies of the genes AOA and amoA and the AOB Shannon index *We did not proceed to such analysis but as we measured higher N related enzymes, we can hypothesize that nitrification could also be lower*

TABLE 3 (Continued)

Reference	Experimental design: Soil type	Experiment type & time	Treatments	Findings of the study and comparison with our results (in italic)
Elzobair et al. (2016)	Silt loam texture	4 years	Manure: 42 t.ha^{-1} Biochar: 22.4 t. ha^{-1} Biochar/Manure mixture	After 1 year, manure and biochar had no effect on enzyme activities, while the total microbial biomass increased with manure and biochar/manure mixture After 4 years, there was no effect on enzyme activities and total microbial biomass *We have found that manure and manure/biochar mixture affected enzyme activities after 2 years. However, effects were lower on the second year with manure alone, which could predict that manure will not have an effect over the long time, while the manure/biochar mixtures tended to have higher effects, while contradict this study. Our amendment mixtures could have a higher effect on sandy soils than silt loam soils*
Foster et al. (2016)	Loam texture	Field 1 cropping season	Biochar: 30 t.ha^{-1} Manure: 30 t.ha^{-1}	Manure increased microbial biomass C, while biochar decreased it, and microbial biomass N was not affected. Only biochar had an effect on soil enzymes *Our results did not show any significant effect on microbial biomass C with manure and even a reverse trend with a non-significant decrease*
Biederman et al. (2017)		Field 5 years	Biochar: 2.6%; 5.2% Manure: 4.5 kg. m^{-2} Biochar/Manure mixtures	No effect on microbial biomass C *Our results are in accordance with this study.*
Overall effects From those different studies, we can see that manure and/or biochar amendments generally increase microbial biomass C and N and enzyme activities, especially the ones related to C and N cycling, over a wide range of soil texture and application doses *Our results about the influence of manure on microbial community could thus be generalized to other soil within the same climate region, and trends could be drawn based on results on those studies regarding the long term effect of manure. However, the few studies in which biochar and manure were combined also showed higher enzyme activities, while we found that biochar incorporation reduced those activities compared with manure alone. But as our amendment was singular, with the mixing of biochar and manure in advance and mixture ageing for 6 months before application, it was expected that biochar influence would differ. We need to continue to monitor our field to evaluate long term effect of blending biochar into manure*				
Part B. Soil physical properties				
Sun et al. (2024)	Clay loam texture	Field 4 years	Three different manures	The authors found that all three manures increased the proportion of large macroaggregated while decreasing the proportion of microaggregates *We did not evaluate this physical soil parameter. Macroaggregates are good water holders. However, we did not observe an increase in soil water content with manure, and thus cannot expect that macroaggregate proportion was increased. But we can assume that it was the case for manure/biochar mixtures*

(Continues)

TABLE 3 (Continued)

Reference	Experimental design: Soil type	Experimental design: Experiment type & time	Experimental design: Treatments	Findings of the study and comparison with our results (in italic)
Wang, Wu, et al. (2023)	Silty sandy loam texture	Field 3 years	Biochar: 5 t.ha^{-1}; 10 t.ha^{-1}; 20 t.ha^{-1} Manure: 7.5 t. ha^{-1}; 10 t.ha^{-1}	Manure and biochar increase mean soil water soil content, in a concentration-dependent manner *Our study only partly concurs with this work. Manure tended to reduce soil field capacity and soil moisture, while bringing biochar to manure increased both parameters. As soil texture is a crucial parameter in soil retention, our results can be explained by the difference in initial texture*
Lima et al. (2021)	Sandy clay loam texture	Field 1 cropping season	Biochar: 10 t.ha^{-1}; 20 t.ha^{-1}; 40 t.ha^{-1} Manure: 5 t.ha^{-1} Biochar/Manure mixtures	The amendments had no effect on: bulk density, total porosity, field capacity and plant available water *We observed that manure alone decreased field capacity and actual soil moisture while combining biochar and manure increased those properties. The fact that biochar and manure had an effect on our soil can be related to the soil texture, which was more sandy in our case*
Agbede and Oyewumi (2022)	2 soils Sandy texture Sandy loam texture	Field 2 years	Biochar: 10 t.ha^{-1}; 20 t.ha^{-1}; 30 t.ha^{-1} Manure: 5 t.ha^{-1}; 10 t.ha^{-1} Biochar/Manure Mixtures	Organic amendments decreased bulk density and increased porosity and soil moisture *Our results only partly matched this study, as biochar incorporation to manure only decreased bulk density compared with manure and not to control. But the application rate was higher in this study, and the ratio between biochar and manure was reverse. However, this mixture of manure/biochar was able to increase soil moisture*
Are et al. (2017)	Coarse-grained texture	Field 2 years	Solid non-composted poultry manure	Manure application led to a lower bulk density, a higher soil hydraulic conductivity, and a higher soil moisture retention *Our results regarding the influence of manure amendment are opposite to this study*
Overall effects From those different studies, it is clear that adding biochar and/or manure increase water retention in the soil in most soil texture range, over a few years *Our results differ from those studies as manure showed an increase in bulk density, associated to a reduction in soil water content. This shows that our manure may not be the best to improve soil physical properties. However, blending biochar to it helps reaching this goal. Few studies here evaluated biochar/manure mixtures and found similar results, even with contrasted soil texture. We can hypothesize that our unique amendment will increase soil water content of different soil types, at least on a few years time scale*				
Part C. Soil chemical properties				
Sun et al. (2024)	Clay loam texture	Field 4 years	Three different manures	The evaluation of the soil after 4 years revealed that manures increased labile and moderately labile phosphorus content as well as stable phosphorus; such improvement was related to the labile phosphorus content of the added manure *Our results similarly showed that available phosphorus increased with manure. However, this improvement was significant only in the first year. Our results may have differed because our manure contained less phosphorus and the application dose of our manure added less phosphorus to the soil, therefore the phosphorus may have been depleted more quickly in our case*

TABLE 3 (Continued)

Reference	Experimental design: Soil type	Experiment type & time	Treatments	Findings of the study and comparison with our results (in italic)
Wang, Gao, et al. (2023)	Clay texture 2 sites	Field Over 30 years	Manure (with and without NPK)	After 30 years, manure application increased pH, and the contents in organic C, total N and organic N, while decreasing NH_4 content. It induced a lower C/N imbalance *Our results also found an increase in soil pH with manure. This shows the capacity of manure to increase soil pH over different soil texture. However, we did not observe a significant increase in C and N content. It is possible that soil initial properties (higher organic C content and same to higher N content in this study) and manure nutritious content led to such discrepancies*
Ma et al. (2020)	Sandy loam texture	Field 28 years	Farmyard manure: 10 t.ha^{-1}; 25–50 t.ha^{-1}	Long-term application of manure increased OM content, DOC content, total N content and NH_4^+ concentration *Our results did not show the significant increases observed in this study. Since we do not have information about initial soil and manure properties, we cannot hypothesize on the reasons of such discrepancies. However, it is possible that the differences in results are because of the fact that this long-term study was made with regular inputs in manure, potentially building up nutrients in soil, while we applied our amendments only once*
Holatko et al. (2022)	Silty clay loam texture (mix with sand)	Pot 12 weeks	Fermented manure Manure enriched with biochar	On the short term, manure enriched with biochar increased total N to similar levels as manure and total carbon to higher content than manure *Our results showed different results in terms of N content. Although the soil of this study had higher initial N content than our soil, manure application increase N content, but such discrepancy could be because of (i) a higher N content of the enriched manure and (ii) a difference in soil texture* *In terms of carbon, we also found that adding biochar to manure increase C content compared with manure alone. This is in relation to the high C content of biochar and show its potential over different soil texture*

(Continues)

TABLE 3 (Continued)

Reference	Experimental design			Findings of the study and comparison with our results (in italic)
	Soil type	Experiment type & time	Treatments	
Wang, Wu, et al. (2023)	Silty sandy loam texture	Field 3 years	Biochar: 5 t.ha^{-1}; 10 t.ha^{-1}; 20 t.ha^{-1} Manure: 7.5 t. ha^{-1}; 10 t.ha^{-1}	The pH of the soil decreased following the application of biochar and manure. On the contrary, total organic C and total N and its forms (NH_4-N and NO_3-N) were increased by the amendments, with a higher effect of manure compared with biochar *Our study showed contradictory results in terms of pH, since we found an increase in soil pH. But such discrepancies can be easily explained by the difference in initial soil pH, as our soil was acidic and the soil of this study was neutron-basic* *Our results also differed in terms of C and N, with our data showing a temporary reduction in extractable C with manure and no effect on extractable N nor total C and N in the manure alone treatment. Based on initial soil and amendment data, we should have observed an increase in those parameters. However, we can explain this with the fact that the authors added manure on a yearly basis and may have build up C and N content while our single application may not allow that*
Dodor et al. (2018)	Sandy soil		Manure: 13 t.ha^{-1}; 26 t.ha^{-1} Biochar: 20 t.ha^{-1}; 40 t.ha^{-1} Manure/Biochar mixture	The amendments increased water extractable organic carbon and mineral nitrogen
Gautam et al. (2020)	Silty loam texture	Field 16 years	Manure	Manure application increased soil organic matter, organic carbon and nitrogen contents over 16 years *Our results are not in agreement with this study, as we observed no significant effect on extractable C and N with manure* *However, the authors of this study re-applied manure over 15 years and performed the measurements after this re-application. After 1 year, only the medium and high manure dose increased organic matter, which could explain, at least partly, the differences observed (our application corresponds to the medium rate of this study). Soluble C and N were also increased only with the high manure application dose, twice as much as we put, which could explain the differences in results*

TABLE 3 (Continued)

Reference	Experimental design: Soil type	Experimental design: Experiment type & time	Experimental design: Treatments	Findings of the study and comparison with our results (in italic)
Irmak Yilmaz and Ergun (2019)	Clayey texture	Greenhouse 1 crop season	Biochar: 20 t.ha^{-1} Manure: 20 t.ha^{-1} Combination of biochar (5 t.ha^{-1}) with manure (5 t.ha^{-1} or 10 t.ha^{-1}) Combination of biochar (10 t.ha^{-1}) with manure (5 t.ha^{-1} or 10 t.ha^{-1}) Combination of biochar (15 t.ha^{-1}) with manure (5 t.ha^{-1} or 10 t.ha^{-1})	The organic amendments, alone or combined, increased soil organic matter, total N and available P and K after one cropping season *Our results in terms of available P and K differed from this study, especially for P, which increased with manure but decreased with biochar/manure mixtures. In this study, manure and biochar were applied at the same time and measures were made after one cropping season, while we incorporated biochar to manure 6 months before applying to the field and monitoring was made over 2 years. It is thus possible that manure nutrients were not yet sorbed on biochar after one cropping season*
Lima et al. (2021)	Sandy clay loam texture	Field 1 cropping season	Biochar: 10 t.ha^{-1}; 20 t.ha^{-1}; 40 t.ha^{-1} Manure: 5 t.ha^{-1} Biochar/Manure mixtures	Manure and biochar, applied alone or in mixture, had no effect on soil total organic carbon, pH, P and K contents and cation exchange capacity; except for pH which increased with the mixture 40 t.ha-1 biochar +5 t.ha-1 manure and P content, which increased with the mixture 10 t.ha-1 biochar +5 t.ha-1 manure *Our sandy soil showed more response than the sandy clay loam of this soil to biochar and manure amendment applications. Such discrepancies could be because of the texture of the soil, as sandy soils are more responsive to amendment*
Ye et al. (2021)	Quaternary red clay	Field 18 years	Manure: 9 t.ha^{-1}; 18 t.ha^{-1}; 27 t.ha^{-1}	Manure application decreased soil pH while it increased soil organic carbon, dissolved organic carbon, nitrogen and available P and K contents *Our manure has increased soil pH after 2 years, which can be explained by the high pH of the manure, although we cannot confirm it, as the authors did not provide this data. But the decrease could also be because of the high microbial activity that occurred for 18 years to degrade manure organic matter, and thus the released of acidifying substances. We could thus observe similar effects on the long time* *On the contrary, we observed similar results in terms of nutrients, although in our case, it was significant only at the first year, which indicates exhaustion of the manure nutrients rapidly*
Bera et al. (2016)	Silt loam texture	Field 3 years	Manure: 168,000 L.ha^{-1} Biochar: 22 t.ha^{-1} Biochar/Manure mixture	Manure application alone decreased total organic N while the mixture of biochar and manure increased total organic carbon *We measured a slight reduction in extractable N with manure and manure/biochar mixtures, but it was not significant. However, the range of decrease was higher in year 2 than year 1, which could predict a significant reduction in extractable N in the longer time*

(Continues)

TABLE 3 (Continued)

	Experimental design			
Reference	Soil type	Experiment type & time	Treatments	Findings of the study and comparison with our results (in italic)
Xie et al. (2023)	Purple fluvo aquic soil	Pot 7 months	Manure Biochar Biochar/Manure mixture	Manure, alone or combined with biochar, increased soi pH and total N after 7 months *Our results also showed that amendments increased pH but we found no effect on extractable N*
Agbede and Oyewumi (2022)	2 soils Sandy texture Sandy loam texture	Field 2 years	Biochar: 10 $t.ha^{-1}$; 20 $t.ha^{-1}$; 30 $t.ha^{-1}$ Manure: 5 $t.ha^{-1}$; 10 $t.ha^{-1}$ Biochar/Manure Mixtures	The application of the organic amendments increased pH, organic C and the nutrients N, P, K, Ca and Mg *Our results fitted this study in terms of pH, which can be because of the fact that the two soils in the study had a similar texture than our soil. However, we did not observed such increase in nutrients, which can be related to (i) an exhaustion of the manure nutrients, (ii) an immobilization of biochar surface and (iii) the dose of amendment applied*
Elzobair et al. (2016)	Silt loam texture	4 years	Manure: 42 $t.ha^{-1}$ Biochar: 22.4 $t.ha^{-1}$ Biochar/Manure mixture	After 1 year, manure and manure/biochar mixture increased total N, while all amendments increased organic C, extractable phosphorus and NO_3-N contents After 4 years, the effects were not visible anymore
Foster et al. (2016)	Loam texture	Field 1 cropping season	Biochar: 30 $t.ha^{-1}$ Manure: 30 $t.ha^{-1}$	Biochar increased total carbon while manure increase total N and available P
Biederman et al. (2017)		Field 5 years	Biochar: 2.6%; 5.2% Manure: 4.5 $kg.m^{-2}$ Biochar/Manure mixtures	Soil pH, inorganic N and exchangeable P were not affected by the amendments Biochar increased total C content, and in plots receiving manure, decreased available P but increased it in plots without manure

Overall effects

From those different studies, we can conclude that manure and biochar can improve soil fertility, especially nutrient levels (mainly related to manure) and carbon content (mainly related to biochar)

Our study is in accordance with those studies and shows that manure can increase nutrient contents. This shows the ubiquity of manure to increase nutrient content over a wide range of soil texture. However, the effects in our case did not last long and shows that our manure was not stable within time. That is why our particular amendment benefits from biochar to stabilize nutrients on the long-term

when the straightforward effect wasn't observed. This is attributed to the high heterogeneity of the soil properties, as well as the influence of the climate; meta-analyses have demonstrated that organic amendments have higher effects on tropical climate and much less under temperate climate (Biederman & Harpole, 2013; Jeffery et al., 2011; Wortman et al., 2017).

4.2 | Reduction of nutrient loss

The contribution of available P and K to the amended soil from manure was expected given that manure has a much greater total P and K than both soil and biochar (Table 1), which is generally reflected in available concentrations of amended soil (Figure 3c,d). However, the positive effect of manure, which has been found in many studies (Table 3c) was only observed in the first year, and effects were lost in the second year, probably because of the exhaustion of the nutrients brought by manure. Considerable seasonal variability in mean and range of values makes firm conclusions difficult to draw. Biochars themselves, if unmodified, seldom offer much nutrition to soils other than from their ash fraction, which can be considerable depending on source material (e.g., up to 50% for manure-derived, or even 85% for bonemeal-derived, source materials (Amonette & Joseph, 2009)). In the present case, biochar was wood-derived, which will yield a lower ash proportion, though EC values for biochar were still much greater than soil (Table 1). Compared with manure and unamended soil, whose C:N ratio is ~15 (Table 1), biochar has a C:N ratio of 10x that value, hence why it was not applied alone into the soil in this study. Detailed meta-analyses on specific elements have demonstrated biochar dose-dependent increase in total and available P in acidic agricultural soils (pH < 6.5), depending on biochar type (Glaser & Lehr, 2019; Zhang, Yang, Chen, Zhang, & Zhou, 2024). Since the pH at the start of this study was generally <6 but was approaching neutral at the end of the study, then the large seasonal fluctuations in available P (Figure 3c) could have been expected. For potassium (K), the large surface areas of biochars and strongly negative charges generally favour the retention of K in soils in a wider pH range (Jindo et al., 2020). In the present study, this appears to have been the case, since available K mean values regardless of proportion of manure to biochar were similarly reduced compared with manure alone at the final sample period (Figure 3d). The fact that the presence of biochar, regardless of dose, clearly and uniformly resulted in a higher CEC than soil alone or with manure (Figure 3a) supports the notion that a biochar application of 10% was more than sufficient to provide a mitigating effect on manure. This finding clearly indicates a positive effect of presented biochar on conventional soil fertilizer application, confirming our second statement (H2). Biochar blending stabilized the nutrients from manure (reduction in availability), via direct sorption on its surface and/or indirectly via the increase in soil CEC; but those immobilized nutrients were still available for plant uptake as plant growth and health was not negatively affected by the presence of biochar.

4.3 | Improvement of microbial activity

Microorganisms (as a marker of soil healthiness) are crucial for nutrient cycling, and such activity can be modulated depending on soil properties; thus, amendment application can affect microorganism functions. Our results have shown the modification of microorganism activities mainly in relation to carbon and nitrogen, which was also observed in previous studies (Table 3a).

Taken altogether, the measure of soil enzyme activities showed higher total activity in the manure treatment and similar or lower activities in the manured biochar treatments. Manure does not only contain organic matter and nutrients but also microorganisms. Those microorganisms will participate in the general soil microbial activity (Khan et al., 2020). In addition, the high organic matter will be processed by the soil and manure microorganisms (Khan et al., 2020). When biochar is blended to manure, organic matter can be stabilized and protected into biochar pores, and thus not available for microorganisms (Joseph et al., 2021), reducing microbial activity compared with manure.

In addition, GMean, an index of functional diversity, was higher under the manure amendment. Again, manure is a reservoir of microorganisms, with species, and thus functions differently from the ones of the soil. Such an increase will help the microbial community to survive under stress, which generally causes a loss of microorganisms and their associated functions (Siebielec et al., 2020). However, when looking more closely, manure application led to an important increase in C-related enzymes and, to a lesser extent, N-related enzymes. This is in accordance with previous studies (Antonious et al., 2020; Ma, 2020). Manure is an organic amendment highly degradable by microorganisms (Lupwayi et al., 2019). Especially, the organic C present in manure can be easily processed by soil microorganisms (Abagandura et al., 2019), which can explain the important increase in C-related enzymes. Such an increase in enzyme activities related to carbon can indicate a higher mineralization of carbon and thus its potential emission to the atmosphere as CO_2, as demonstrated in our previous study (Lebrun et al., 2024).

The blending of biochar to manure reduced such carbon cycling activities, as we previously observed (Lebrun et al., 2024). As biochar carbon has a different structure than the one of manure, it cannot be consumed/processed by microorganisms (Abagandura et al., 2019; Wu et al., 2021), which can partly explain such decrease. In addition, the carbon from the soil and manure could be sorbed on the biochar surface and thus not be accessible to microorganisms, further lowering the microbial C activities.

In relation to the increase in C microbial consumption in manure, N is mineralized to maintain C/N ratio in microorganisms (Abbas et al., 2020; Schofield et al., 2019), which explains the slight increase in N-related enzymes in the manure treatment. Similarly, in relation to the lower C microbial consumption with biochar blending, the activities of N-related enzymes were lowered when biochar was blended to manure.

Taken together, the application of manure can increase C and N mineralization, and thus emissions in the atmosphere, while blending biochar to manure reduces these potential emissions coming from manure, and thus conserves C and N in soil, as shown by the higher total C and N content in the manured biochar treatments.

Overall, our third statement (H3) was not confirmed, as microbial activities were reduced when biochar was blended to manure. Manure highly increased C and N enzyme activities, through: (i) the addition of readily available carbon and (ii) the consumption of N to counterbalance C consumption. Adding biochar to manure reduced such an increase to the control level. The potential mechanisms behind such a decrease are: (i) the recalcitrance of C coming from biochar, (ii) sorption of elements (including C and N) on the biochar surface and (iii) microlocalization of desiccation at the surface of the biochar, hindering microorganism activities. However, such reduction in C and N enzymes further confirm the second hypothesis, as C and N mineralization, and thus potential loss by emission, seems to be lower by biochar blending.

4.4 | Vegetation cover

The vegetation was only assessed in terms of natural development of weeds in the first year, because of the freezing of planted crops in the second year. Such an evaluation showed that biochar incorporation to manure led to a nonsignificant amelioration of plant development (coverage and biomass). This can be related to the amelioration of soil water content as well as the retention of manure nutrients by biochar (Abbas et al., 2020; Lebrun et al., 2022). In addition, from the pigment analysis, we can assume that manure caused slight stress to the plants (lower pigment contents). Such effect may have been related to less water retention (Lebrun et al., 2022); however, based on the root to shoot ratio, which was not higher in manure, a lack of nutrients seems more likely.

Moreover, plants were healthier in the manured biochar treatments than manure alone (as shown by the NDVI). This shows the benefits of biochar for plant growth and confirm our fourth expectation (H4). Such results are in accordance with our previous observations in pots (Lebrun et al., 2022). Unfortunately, the effects on crop yields and quality were not confirmed because of unexpected cold weather in the second season (2023) of the monitoring.

4.5 | Overall medium-term improvement of soil quality

Blending biochar to manure improved the soil fertility, as shown by the better coverage and biomass production of plants naturally developing on the area. Although manure added nutrients to the soil, plant growth was not improved. Therefore, the amelioration of plant development on the manured biochar treatments can be related not only to the quantity of nutrients added to the soil, but to their retention in the soil with time, as well as an improvement of the water retention (Agbede & Oyewumi, 2022; Sistani et al., 2019). From the general point-of-view, the biochar presence in manure mainly improved soil water retention, represented via actual measured volumetric water content and field capacity, and reflecting a potential higher accessibility of the capillary water to plants, and higher CEC value (representing mainly exchangeable form of K and Ca, therefore key nutrients in soil).

The most important benefits of the biochar presence are as follows. First, its high ability to retain water and nutrients, therefore it is possible to achieve a better quality of the blended manure and reduce its initial GHG emissions as well as the release of nutrients (e.g. NO_3^-) via leachate to groundwater bodies. Second, its potentially long-term soil persistence, which supports the strategy of manure blending (where 10% V/V is sufficient dosage) how to sustainably sequester carbon and to increase the amount of fundamentally missed organic matter, which in form of manure or compost will disappear from the soil after few years (and biochar will remain). Regardless of the aforementioned, pyrolysis could be presented as a C-negative technology and together with the presented soil benefits its implementation to agriculture, it will present a sustainable solution against global changes (i.e. agricultural drought and mitigation of GHGs emissions).

5 | CONCLUSION

The field soil application of manure and biochar was investigated here singularly and in combination, primarily to alleviate drought conditions but also to bolster soil and plant nutrition. In common with field conditions, substantial seasonal variability was observed, though the actual soil moisture content was sustainably increased notably with the combined addition of amendments.

Given that soil chemical parameters (CEC, etc.) were also somewhat improved by the amendment combination, and that microbial biomass and enzyme soil health indicators were not detrimentally affected, then the combination of manure and biochar here can be concluded to have generally improved soil conditions. Confirmation of the beneficial effects of the amendments was also found in drone imagery indicating plant height and health were generally improved, compared with soil alone. In addition, biochar blending could greatly reduce the C emission induced by the manure in the first months of its application.

Since the results of this study are generally positive, it remains for future investigation to prove whether these impacts are seen longer-term (i.e. 3–5 years hence), and to what extent addition amendment may be required to maintain the effects seen in this 2-year study. Indeed, several studies have shown that amendment effects were lost with time. But we are hypothesizing that our specific amendment (blending of biochar to manure before the application to soil) will help maintain effects in the long run. However, as some effects (mainly water retention) were lost after 2 years, such hypothesis will need to be verified in the next consecutive years. Further work on a wider range of soil textures must also be conducted to allow better informed decisions on precisely where to apply the amendment combination to achieve maximum benefit.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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