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Biochar-induced changes in soil microbial communities: a comparison of two feedstocks and pyrolysis temperatures

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Abstract

Background The application of a biochar in agronomical soil offers a dual benefit of improving soil quality and sustainable waste recycling. However, utilizing new organic waste sources requires exploring the biochar's production conditions and application parameters. Woodchips (W) and bone-meat residues (BM) after mechanical deboning from a poultry slaughterhouse were subjected to pyrolysis at 300 °C and 500 °C and applied to cambisol and luvisol soils at ratios of 2% and 5% (w/w).

Results Initially, the impact of these biochar amendments on soil prokaryotes was studied over the course of one year. The influence of biochar variants was further studied on prokaryotes and fungi living in the soil, rhizosphere, and roots of *Triticum aestivum* L., as well as on soil enzymatic activity. Feedstock type, pyrolysis temperature, application dose, and soil type all played significant roles in shaping both soil and endophytic microbial communities. BM treated at a lower pyrolysis temperature of 300 °C increased the relative abundance of Pseudomonadota while causing a substantial decrease in soil microbial diversity. Conversely, BM prepared at 500 °C favored the growth of microbes known for their involvement in various nutrient cycles. The W biochar, especially when pyrolysed at 500 °C, notably affected microbial communities, particularly in acidic cambisol compared to luvisol. In cambisol, biochar treatments had a significant impact on prokaryotic root endophytes of *T. aestivum* L. Additionally, variations in prokaryotic community structure of the rhizosphere depended on the increasing distance from the root system (2, 4, and 6 mm). The BM biochar enhanced the activity of acid phosphatase, whereas the W biochar increased the activity of enzymes involved in the carbon cycle (β-glucosidase, β-xylosidase, and β-N-acetylglucosaminidase).

Conclusions These results collectively suggest, that under appropriate production conditions, biochar can exert a positive influence on soil microorganisms, with their response closely tied to the biochar feedstock composition. Such insights are crucial for optimizing biochar application in agricultural practices to enhance soil health.

Keywords Biochar, Soil quality, Organic waste recycling, Microbial composition, Enzymatic activity

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Introduction

Biochars, valuable soil amendments produced through a process called pyrolysis, hold great promise for improving soil quality and enhancing microbial activity in agricultural soils. When applied, a biochar enriches soil nutrient levels, adjusts pH, improves moisture retention, enhances fertility, and mitigates soil contamination [23]. Their porous structure, high surface area, and capacity to absorb soluble nutrients and organic matter provide an ideal habitat for various microorganisms, including bacteria, ectomycorrhizal fungi, ericoid mycorrhizal fungi, and arbuscular mycorrhizal fungi (AMF) [96]. These pores serve as a protective shield for microbes against natural predators such as mites and nematodes [56], fostering an increase in total microbial biomass, microbial activity, the abundance of actinomycetes, and ratios of fungi/bacteria and G+/G- bacteria in treated soil [105].

However, a biochar can exhibit various chemical and physical properties that are linked to its production conditions, specifically feedstock type, pyrolysis temperature, and retention time. For instance, different feedstocks have various elemental and structural compositions, resulting in the production of biochars with diverse characteristics [94]. Typically, feedstock containing lignin or cellulose, such as wood or crop biomass, have lower cation exchange capacity (CEC) but higher surface area and carbon content than a biochar originating from animal manure or solid waste sources [88]. A straw-derived biochar can exhibit a higher pH and potassium level than a biochar produced from wood chips [92]. Pyrolyzed sewage sludge lacks P and K; hence, it has to be enriched with these nutrients to ensure its effectiveness as a fertilizer [15]. Along with the type of feedstock, pyrolysis temperature and retention time determine various biochar characteristics, including volatile matter content, ash content, specific surface area, pH, and pore volume [88, 107]. Higher pyrolysis temperatures increase biochar porosity, ash and carbon content, while reducing CEC and the content of volatile matter [88].

As a consequence, the biochar's impact on soil properties and soil microbial community can vary significantly. For instance, a biochar with high lignin content benefits the growth of gram-negative bacteria, while a biochar made of organic waste mainly impacts the soil enzymatic activity [44]. Smaller biochar particles (<1 mm) tend to be more conducive for bacterial growth, whereas larger particles (>2 mm) are preferred by fungi [58]. The nutrient profile, its availability, and soil pH have been identified as key factors shaping the structure of both fungal and bacterial communities in soil [60, 73, 110]. Additionally, pyrolysis temperature has been observed to impact microbial carbon metabolism, using ^{13}C isotope analysis and the incorporation of ^{13}C from biochars prepared at the different temperatures into a specific group of bacteria [64]. The influence of pyrolysis temperature and type of feedstock on soil microbial biomass was also linked together by Li et al. [58], which analyzed 999 paired data points from 194 studies. These alterations in the soil microbial community can affect plant-associated niches, such as the plant endosphere [32, 48], potentially leading to either positive or negative impacts on plant development, stress tolerance, and crop yield [34, 66, 98]. Therefore, understanding how biochar production conditions influence soil and plant-associated microbial communities is essential, especially when introducing new biowaste types into the pyrolysis process. This knowledge will aid in harnessing the full potential of biochars as sustainable soil amendments.

This study investigated the intricate relationship between biochar production conditions, microbial communities in soil and plant-associated niches, enzymatic activity, and plant growth. In the initial phase, we explored the effects of different biochar production and application procedures on prokaryotic communities in bulk soil over one year. The primary objective was to pinpoint the optimal pyrolysis temperature for biochar production, focusing on its long-term benefits for soil microbial communities. The feedstock materials under scrutiny include beech woodchips (W) and waste from mechanical meat separation (BM). Subsequently, the second phase of our experiment assessed the influence of these biochars on prokaryotic and fungal communities in the soil, rhizosphere, and root endosphere using specialized rhizoboxes [97]. In tandem, we studied plant growth and the chemical properties of treated soil to obtain a comprehensive understanding of the biochar's impact on the soil ecosystem. The primary objective of this study was to assess the long-term impact of biochars produced from two distinct feedstocks (beech woodchips and bone-meat residues) and at different pyrolysis temperatures (300 °C and 500 °C) on soil microbial communities in cambisol and luvisol soils. We hypothesized that biochar composition and pyrolysis temperature would lead to significant differences in microbial diversity and community structure. Accordingly, we expected the biochar derived from beech woodchips to enhance enzymatic activities related to carbon cycling, while the bone-meat residue biochar, with its higher phosphorus content, would increase phosphatase activity and induce shifts in microbial communities. We also anticipated that the magnitude of any observed changes in microbial structure, diversity, or enzymatic activity would increase with higher biochar application doses. Furthermore, we hypothesized that these changes in microbial community structure and/or diversity in bulk soil would be mirrored in the rhizosphere and plant root microbiome, reflecting the biochar's influence on both soil and plant-associated microbial environments.

Materials and methods

Phase 1: One-year incubation experiment

In the first phase of the experiment, biochars were produced using two feedstock materials: beech woodchips and bone-meat residues after mechanical deboning from a poultry slaughterhouse. More information about the composition of BM is included elsewhere [82]. The pyrolysis process was conducted in an electrically heated quartz tube at two different temperatures, 300 °C and 500 °C, with a duration of 30 min in a nitrogen atmosphere. The detailed morphological and physiological characteristics of the biochars, as well as the pyrolysis conditions, have been described by Szakova et al. [84]. Two soil types, cambisol and luvisol, were utilized in this phase of the study. Cambisol was collected in Humpolec (East Bohemia, Czech Republic, GPS 49°33'15"N, 15°21'02"E) and is characterized by a sandy loam texture, CEC of 160 $mmol_{(+)}$/kg, oxidizable carbon (C_{ox}) content of 1.24%, and pH 5.1. Luvisol was collected from Hněvčeves (East Bohemia, Czech Republic, GPS 50°18'46"N, 15°43'3"E) and has a loam texture, CEC of 180 $mmol_{(+)}$/kg, C_{ox} content of 1.8%, and pH 6.5. Both soil types were sieved through a 2-mm diameter mesh and mixed together with the biochars at ratios of 2% and 5% (w/w). An illustrational diagram of the experimental design is shown in Fig. 1.

The soil samples, each mixed with or without a biochar (serving as the control treatment), were placed in separate 100-ml pots. These pots were then incubated for up to one year at room temperature with a gravimetrically controlled water-holding capacity set at 60%. The effect of biochar type and dose was monitored at several time points to track changes over time. Samples were collected 3 days, 2 weeks, 1 month, 6 months, and 1 year after the initial biochar-soil mixing. To ensure the robustness of our results, each treatment (soil type, biochar type, biochar dose and time point) was conducted in biological triplicate. The exception was the control soil, for which six biological replicates were set up for each time point. A total of 270 pots were analysed in Phase 1 of the experiment. At each time point, the soil from each individual pot was thoroughly mixed, homogenised and a sample was taken for microbial community analysis.

Phase 2: Rhizobox experiment

The same feedstock types, W and BM, were used for biochar production in the second phase of the experiment. The pyrolysis temperature was chosen based on the results of the incubation experiment. The biochar was mixed with the same soil types, cambisol and luvisol, as in the previous experiment, at a rate of 5% (w/w). The amended soil, along with soil without a biochar (control treatment), was transferred into rhizoboxes (Figure SI-2), a detailed description of their compartments is given in the study by Wenzel et al. [97]. *Triticum aestivum* was then planted and cultivated for a duration of 90 days.

To maintain consistency, soil moisture was adjusted to 60% of the maximum water holding capacity (MWHC) using deionized water, and maintained at this level throughout the experiment. Soil sterilization was not performed to preserve the natural microbial community. Specially designed rhizoboxes [97] were used which allow the sampling of the soil rhizosphere's vertical profile. Spring wheat (*Triticum aestivum* L.) was cultivated in

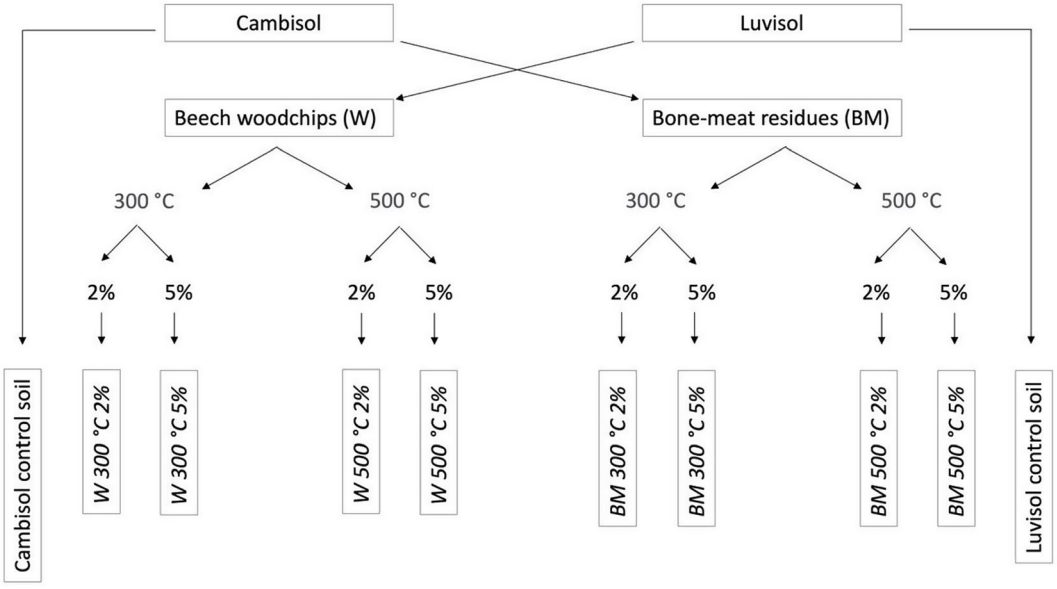

Fig. 1 Biochar Production and Experimental Setup. Description of various feedstock types (W and BM), pyrolysis temperature (300 °C and 500 °C), and application doses (2%, 5%) used for mixing with cambisol and luvisol soil types

the soil–plant compartment, with 10 plants per rhizobox (Figure SI-2).

The experiment was carried out in a greenhouse under controlled conditions at 23/18°C day/night. Each treatment was performed in three biological replicates. At the end of the experiment, the soil was sectioned without freezing into root-parallel segments based on the distance from the plant roots using a specially designed slicing device [30]. Sections included 0–2 mm, 2–4 mm, and 3–6 mm, along with bulk soil sections, which were all separated and homogenized. Additionally, root samples were also collected. Soil pH in the individual sections and bulk soil was determined in a 0.01 mol/L CaCl2 extract (1:10 w/v).

Bulk soil and rhizosphere samples were divided into two parts; one part was for immediate measurement of enzymatic activity, and the other part was stored at -20 °C until the DNA isolation for microbial analysis. Roots were washed under running tap water and cleaned of adhered soil particles. The root surface was then sterilized by submerging into 70% (v/v) ethanol for 30 s, 3% (v/v) hypochlorite (NaOCl) for 6 min, and then washed three times with sterilized nuclease-free water for 5 min per wash cycle [10, 20, 52]. 100 µl of the last wash solution was spread on Luria-Bertrani (LB) agar plates and incubated at 28 °C for one week to verify sterility. Surface-sterilized roots were then stored at -80 °C prior to grinding. Grinding was performed under liquid nitrogen in a ceramic mortar and pestle under aseptic conditions. For the negative control, four 2-ml samples of molecular water (Sigma-Aldrich, St Louis, MO, USA) underwent the same treatment as the surface-sterilized root samples. Hence, they were also subjected to the same sample manipulation, following the exact same procedures of grinding, storage, and DNA isolation for microbiome analysis. This approach enabled us to verify the absence of contamination during these processes and identify any potential sources of contamination.

Table 1 Fluorescent substrates used for enzyme assays

Substrate	Enzyme	Dissolvent
4-Methylumbelliferyl-β-D-glucopyranoside	β-glucosidase	water
4-Methylumbelliferyl N-acetyl-β-D-glucosaminide	β-N-acetyl-hexosaminidase	water
Fluorescein diacetate	Total microbial activity	acetone
4-Methylumbelliferyl phosphate	Acid phosphatase	water
4-Methylumbelliferyl sulfate potassium salt	Sulphatase	water
4-Methylumbelliferyl-β-D-xylopyranoside	β-xylosidase	water

Enzymatic assays

The activity of β-glucosidase, β-xylosidase, β-N-acetylglucosaminidase (NAG), sulphatase, acid phosphatase, and total microbial activity were measured fluorometrically using fluorescent substrates (Table 1, Sigma-Aldrich, USA). The procedure for measuring the enzymatic activity was previously published [53]. In summary, 2 g of soil was mixed with 50 ml of acetate buffer (50 mM, pH 5) and shaken in the dark for 2 h at 28 °C (180 rpm). After the incubation, 200 µl of soil-buffer slurry were transferred into a microtiter plate and mixed with one of the following: (i) 50 µl of substrate of 1,500 µM (to measure fluorescence in the sample), (ii) 50 µl of substrate of 10 µM (to determine the quenching coefficient), (iii) 50 µl of sterile distilled water (as a negative control of the sample). Additionally, acetate buffer mixed with 50 µl of the substrate of 1,500 µM was also used as a negative control of the buffer.

The measurement of enzymatic activity in all bulk soil and rhizosphere samples was performed in three technical replicates. The microtiter plate was then shaken horizontally for another 2 h. After incubation, 10 µl of NaOH (1 M) was added to stop the reaction, and the mixture was incubated for 20 min. Fluorescence was determined using Fluoroskan Ascent (Thermo Fisher Scientific, USA) with 360 nm excitation and 450 nm emission filters. The procedure was the same for all fluorescent substrates, except for the FDA substrate, where phosphate buffer (100 mM, pH 5.8) was used instead of the acetate buffer, and 485 nm excitation and 510 nm emission filters were used. Enzymatic activity (nmol/h·g) was calculated according to the equation [22], in which the weight of dried soil was used to enable meaningful comparisons of enzymatic activity across different soil treatments. For that purpose, 2 g of soil was oven-dried at 105 °C for 24 h.

DNA Isolation and purification

Metagenomic DNA was isolated from 500 mg of bulk soil and rhizosphere samples using a FastDNA Spin Kit for Soil (MP Biomedicals, USA). For root samples, the same procedure was employed, except for a prolonged homogenization period during DNA isolation, lasting 15 min to enhance DNA yield. Isolated DNA was then purified with a Genomic DNA Clean and Concentrator kit (ZYMO Research, USA) according to the manufacturer's protocol, and DNA concentration was measured in a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). DNA concentration was normalized to 10 ng/µl per sample prior to the amplicon generation.

16S rRNA gene and ITS2 region amplicon generation and sequencing

To assess the prokaryotic and fungal community structure and diversity, amplicons of the 16S rRNA gene and ITS2

region were generated from the samples of bulk soil, rhizosphere, and roots. The V4-V5 hypervariable region of the 16S rRNA gene was amplified using the 515 forward (5′-GTGYCAGCMGCNGCGG-3′, Sigma-Aldrich, USA) and 926 reverse (5′-CCGYCAATTYMTTTRAGTTT-3′, Sigma-Aldrich, USA) primers [53]. The ITS2 region was amplified using 5.8 S Fun forward (5′-AACTTTYRRCAAYGGATCWCT-3′, Sigma-Aldrich, USA) and ITS4 Fun reverse (5′-AGCCTCCGCTTATTGATATGCTTAART-3′, Sigma-Aldrich, USA) primers [86].

The amplicons generated from bulk soil and rhizosphere samples were amplified using a two-step PCR process. In the first PCR, each 15 µl reaction contained: 0.02 U/µl KAPA HiFi HotStart ReadyMix (Kapa Biosystems, USA), 0.3 µM of each primer (Sigma-Aldrich, USA), template DNA (~ 10 ng/µl), and PCR-grade water (Sigma-Aldrich, USA). The temperature cycling conditions were as follows: an initial DNA denaturation for 5 min at 95 °C, followed by 25–28 cycles of 20 s at 98 °C, 15 s at 56 °C (16S rRNA gene) or 50 °C (ITS2 region), 15 s at 72 °C, and final extension for 5 min at 72 °C. In the second, 0.5 µl of the first PCR product was used as a template DNA with the same primers modified with internal barcodes and sequencing adapters [33]. The 25 µl reaction contained: 0.02 U/µl KAPA HiFi HotStart ReadyMix (Kapa Biosystems, USA), 1 µM of each primer (Sigma-Aldrich, USA), template DNA, and water for molecular biology (Sigma-Aldrich, USA). The temperature cycling conditions were the same as for the first PCR, with the annealing temperature set at 50 °C for both types of amplicons (16S rRNA and ITS2 region), and the number of cycles was reduced to 8–10.

While ITS2 amplicons from root samples were prepared according to the same procedure as the soil samples, 16S rRNA amplicons were generated using a 3-step PCR process. During the amplifications, the DNA from plant organelles was blocked with anti-mitochondrial and anti-plastid peptide-nucleic acids (PNAs) from PNABio, USA. The first 15 µl reaction contained: 1 µM of each peptide nucleic acid probe: mPNAs (5′-GGCA AGTGTTCTTCGGA-3′) and pPNAs (5′-GGCTCAA CCCTGGACAG-3′) (PNA Bio, Thousand Oaks, CA), 0.02 U/µl of KAPA HiFi HotStart ReadyMix (Kapa Biosystems, USA), 1 µM of the 515 forward primer, 1 µM of 1068 reverse primer (5′-CTGRCGRCRRCCATGCA-3′, Sigma-Aldrich, USA), template DNA (~ 10 ng/µl), and PCR-grade water (Sigma-Aldrich, USA) [62]. Initial DNA denaturation at 95 °C for 5 min was followed by 30–35 cycles of 20 s at 98 °C, 15 s at 75 °C (annealing of the PNAs), 15 s at 50 °C, 15 s at 72 °C, and final extension at 72 °C for 5 min. All reactions were performed in 6 copies that were pooled together after the first PCR and separated by electrophoresis on 1.5% agarose gel. The buffer used for the electrophoresis was gamma sterilized to reduce the contaminating DNA. The band at 553 bp was excised from the gel and purified using a Zymoclean Gel DNA Recovery Kit (ZYMORESEARCH, USA). 0.5 µl of the purified product was used in the second PCR as a template DNA. The 25 µl reaction contained: 0.02 U/µl KAPA HiFi HotStart ReadyMix (Kapa Biosystems, USA), 1 µM of mPNAs and pPNAs, 1 µM of the 515 forward primer, 1 µM of 926 reverse primer, template DNA, and PCR-grade water (Sigma-Aldrich, USA). The temperature cycling conditions were the same as for the first PCR, but the number of cycles was reduced to 10–15. 0.5 µl of the second PCR product was used as a template DNA in the final third PCR with 515 forward and 926 reverse primers with internal barcodes and sequencing adapters [33]. The reaction and temperature cycling conditions were the same as for the final PCR use in generation amplicons from soil.

The amplicons generated from bulk soil, rhizosphere, and roots samples were then sent on ice packs to the Core Facility for Nucleic Acid Analysis at the University of Alaska Fairbanks for sequencing. There, the amplicons were purified with SPRIselect magnetic beads (Beckman Coulter, USA) and the DNA concentration was normalized to 1–2 ng/µl using a SequalPrep Kit (Thermo Fisher Scientific, USA) prior to the sequencing. The sequencing was performed using paired-end reads of 300 bp on an Illumina MiSeq platform.

Data processing

Raw Illumina sequences were processed using the DADA2 package following the DADA2 1.16 tutorial pipeline [17] in the R environment (v.4.1.0) [75]. High-quality sequences were filtered, chimeric sequences were identified and removed according to the "consensus" method, and primer sequences were trimmed off. After dereplication, amplicon sequence variants (ASVs) were derived from initial unique sequences by sequencing error removal. Additionally, sequences differing in one base were merged, and the most abundant one was taken as the valid sequence. Taxonomy was assigned to ASVs using the silva_nr_v132_train_set.fa.gz database [16] and the UNITE database [67] for the 16S rRNA gene and ITS region, respectively. The sequence dataset was deposited into the NCBI Short Read Archive under the accession number PRJNA769602.

Multivariate statistical analysis

Further analyses of microbial datasets and enzymatic actives were processed in R using the phyloseq [65], vegan [68], and DESeq2 [63] packages. Graphical outputs were generated using the ggplot2 package [99]. All sequences assigned to organelle DNA (24,784 reads, accounting for 8.9% of all ASVs) were removed from the dataset. Alpha-diversity was determined using the

Shannon diversity index [18]. The normal distribution of the dataset was assessed using the Shapiro-Wilk test. For samples from Phase 1, where the data exhibited a normal distribution, statistical differences in microbial diversity between treatments were tested using analysis of variance (ANOVA), followed by Tukey's HSD test for multiple pairwise comparisons. For Phase 2, in which the data did not exhibit a normal distribution, the Kruskal-Wallis test was used to determine significant differences in microbial diversity between treatments. The results were visualized with boxplots, with significant differences between treatments indicated by letters. The remaining ASVs in the datasets were transformed into compositional counts.

To assess the significance of the influence of soil type, biochar, or distance from the rhizosphere on microbial community structure, we employed permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distances [6, 7]. Additionally, pairwise PERMANOVA was conducted to compare the microbial community structures between treatments. The false discovery rate (FDR) was used to correct p-values [11] in multiple testing analyses. PCoA was conducted to visualize the variation in microbial community structure. Bray-Curtis distance matrices were calculated from the transformed data to quantify dissimilarities between samples. The resulting PCoA plots illustrated the community structure across various treatments and time points. For Phase 1 data, the plots were further divided into facets based on sampling times (3 days, 2 weeks, 1 month, 6 months, and 1 year) to present the data clearly and facilitate interpretation.

To analyze microbial succession in soil following the application of a biochar over the course of one year, we employed the Principal Response Curve (PRC) method for treatments with repeated observations [89]. PRC is a special case of Redundancy Analysis (RDA) for experiments with a repeated observation design [90]. This multivariate method shows how the microbial community structure responds to treatments over time by comparing the community in treatments with an external reference (control soil). PRC also identifies taxa with the strongest response to these treatments.

The enzymatic activity values were first standardized within a range, and the significance of enzymatic activity changes across different treatments was analyzed with a non-parametric Kruskal-Wallis test and pairwise Wilcoxon rank sum test. False discovery rate (FDR) correction was applied to the obtained p-values.

Table 2 Permutational Multivariate Analysis of Variance: pairwise PERMANOVA comparison between individual biochar variants versus control soils

	Cambisol		Luvisol	
	R^2	p_{adj}	R^2	p_{adj}
W 300 °C 2%	0.014	0.73368	0.017	1
W 300 °C 5%	0.024	**0.00036**	0.020	1
W 500 °C 2%	0.036	**0.00036**	0.025	**0.0054**
W 500 °C 5%	0.074	**0.00036**	0.035	0.42804
BM 300 °C 2%	0.084	**0.00036**	0.093	**0.00036**
BM 300 °C 5%	0.233	**0.00036**	0.177	**0.00036**
BM 500 °C 2%	0.146	**0.00036**	0.034	**0.00036**
BM 500 °C 5%	0.137	**0.00036**	0.047	**0.00036**

Tested biochar variants (pyrolysis temperature (300 °C and 500 °C), feedstocks (W - beech woodchips, BM - bone-meat residues after mechanical deboning from a poultry slaughterhouse) and application doses (2% and 5%) in luvisol and cambisol soils on prokaryotic community structure were compared to the control soil (not treated with biochar). Values corresponding to treatments that significantly differed from the control ($p_{adj} \leq 0.05$) are underlined and in bold

Results and Discussion

Phase 1: Impact of biochar's feedstock and pyrolysis temperature on soil prokaryotic communities

In this part, an incubation experiment was conducted to analyze the impact of biochar production conditions and application parameters on the soil prokaryotic community. Samples were collected and monitored over the course of a year, enabling us to closely examine the dynamic changes in microbial diversity and community structure in response to varying biochar treatments. In total, 12,186,287 reads were obtained, with a minimum of 372 reads and a maximum of 238,002 reads per sample.

The influence of biochar production conditions (pyrolysis temperature and feedstock type) and application dose on the prokaryotic community structure in both soil types, cambisol and luvisol, was found to be significant ($p_{adj} < 0.05$, PERMANOVA). The explanatory power of these factors varied, as indicated by R^2 values, with feedstock type having the greatest impact (9.2% in cambisol and 4.6% in luvisol), followed by temperature (4% for both soil types) and application dose (1% for both soil types). All variations of the BM biochar resulted in significant shifts in prokaryotic community structure compared to the control soil for both cambisol and luvisol ($p_{adj} < 0.001$, pairwise PERMANOVA) (Table 2). Of all the W biochar variants, only *W 500 °C 2%* caused significant shifts in prokaryotic community structure in both soil types (Table 2). These results suggest that the W biochar significantly alters the prokaryotic communities when produced at higher pyrolysis temperatures (500 °C), whereas the BM biochar can influence the communities even when produced at 300 °C. Differences in prokaryotic community structure are visualized in the PCoA (Figure SI-3). The primary distinction in community structure is driven by soil type, reflected along Axis 1, which explains 51.1% of the variation. Along Axis 2, the most notable difference in community structure is observed between the control soil and *BM 300 °C* treatments across all time points.

The observed changes in prokaryotic community structure are likely associated with the distinct physical and chemical properties of the biochar, which are determined by feedstock type and pyrolysis temperature. For a lignocellulosic feedstock type, such as the W biochar, lower pyrolysis temperatures (300 °C) may not effectively convert lignin into polycyclic aromatic hydrocarbons (PAH), resulting in a biochar with a hydrophobic character [36]. In contrast, higher pyrolysis temperatures can lead to a higher decomposition of organic matter, resulting in higher micropore volume and surface area [88, 108].

For the biochar variants used in this study, the higher pyrolysis temperature (500 °C) transformed the macroporous (>0.08 mm) character of *W 300 °C* biochar into a microporous (<2 nm) structure, while the BM biochar changed from non-porous to mesoporous in character (2–50 nm) [84]. The absence of significant changes in prokaryotic community structure between control soil and the *W 300 °C* biochar suggests that the macroporous nature of this biochar may not strongly influence the living conditions of soil prokaryotes, especially in luvisol soil. Therefore, to induce significant changes in microbial community structure, (W) may need to be subjected to higher pyrolysis temperatures to acquire a microporous character. A biochar's micropores typically enhance soil water retention [14] and promote microbial activity through the sorption of organic matter, which is crucial for sustaining not only a healthy soil microbiome, but also high crop yields [69].

Compared to W biochar variants, the BM biochar caused significant alterations to the prokaryotic community in both cambisol and luvisol soil types, whether it was pyrolyzed at 300–500 °C (Table 2). Furthermore, microbial diversity, described with Shannon's diversity index, significantly dropped in *BM 300 °C 2%* and *BM 300 °C 5%* treatments 3 days after the biochar application (Fig. 2A, Tukey HSD). This reduced diversity persisted even after one year, although the difference compared to the control soil decreased over time (Figure SI-1).

It is well-established that particularly fast-growing (copiotrophic) microorganisms respond rapidly to changes in the ecosystem, due to their shorter generation time compared to oligotrophic bacteria (Nimonkar et al., 2022). The swift response of microorganisms to

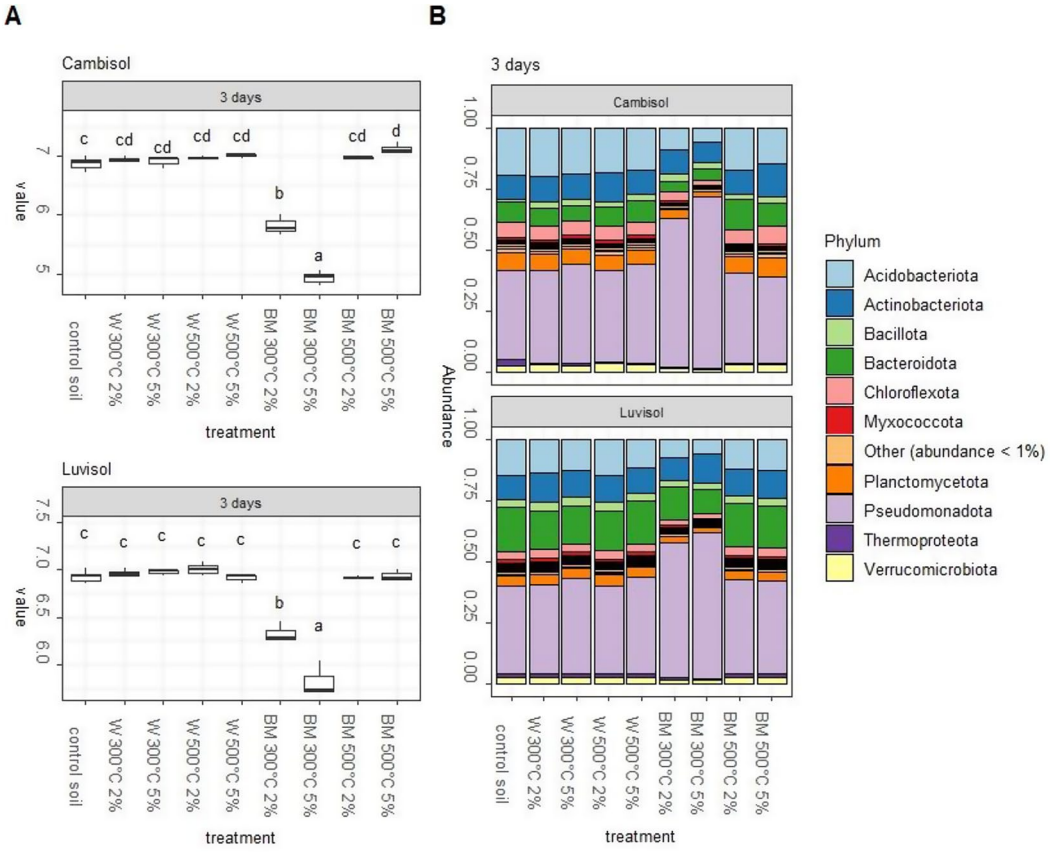

Fig. 2 Shannon diversity index (**A**) calculated from prokaryotic sequence data and Abundance plot (**B**) based on the relative composition of phyla in bulk soil 3 days after biochar application. The biochar variants differed in their pyrolysis temperatures (300 °C and 500 °C), feedstocks (W - Beech woodchips, or BM - Waste after mechanically separated meat), and the application doses at which they were applied to luvisol and cambisol soils (2% and 5%). The control soil was not treated with biochar

environmental changes is also evident in the study by [2], in which litter addition led to a decrease in richness and Shannon diversity by over 50%, primarily due to the proliferation of fast-growing copiotrophs. In line with this pattern, the abrupt decline in diversity in *BM 300 °C 2%* and *BM 300 °C 5%* treatments (Fig. 2B) can be attributed to the increase by almost 50% compared to the control soil of Pseudomonadota, a copiotrophic phylum [29]. The high fat content in BM after mechanical deboning and the apparent oily viscous residues in BM biochar produced at 300 °C [84] probably induced the copiotrophic conditions with access to available carbon sources in soils with treatments *BM 300 °C 2%* and *BM 300 °C 5%*. Concurrently, the relative abundance of other phyla, for example oligotrophic Acidobacteriota, either decreased or remained at similar levels. However, in the case of *BM 500 °C* biochar variants, the higher pyrolysis temperature likely resulted in reduced availability of labile organic compounds and a more inert material structure [84], limiting the rapid proliferation of copiotrophic bacteria such as Pseudomonadota. The mesoporous nature of *BM 500 °C* [84] may also have created fewer immediate carbon and nutrient hotspots, slowing the microbial response compared to the non-porous BM 300 °C biochar. Such findings demonstrate that the BM biochar pyrolyzed at low temperature promoted the growth of Pseudomonadota, known for their copiotrophic tendencies [103], which is likely to be the major factor influencing their relative increase.

In general, the other factor influencing microbial diversity is the composition of the biochar. Unlike the W biochar, BM is rich in P, which could attracts P-solubilizing bacteria [84, 85]. Many of these bacteria belong to Pseudomonadota, including genera such as *Azobacter*, *Rhizobium*, *Pseudomonas*, and *Enterobacter* [51]. These bacteria are known for facilitateting P release from hydroxyapatite, an inorganic component of bone tissue, by producing organic acids or hydrolytic enzymes, thereby increasing the availability of P for plants [112]. Even though a related study [84] also documented a higher availability of P in BM-treated soil, and showed that P continued to be solubilized from the BM biochar throughout the entire year, further analysis would be required to directly confirm that the genera responsible for P-solubilization in our case study belong to the phylum Pseudomonadota.

This underscores the long-term impact of the BM biochar on soil nutrient dynamics, and its effectiveness on P availability can be further enhanced. For example, this improvement can be achieved by: (i) combination with AMF inoculation [112]; (ii) sulfur bone char modification [45]; or (iii) ensuring that the pyrolysis of bone char occurs between 300 °C and 500 °C, which was also done in our study. At higher pyrolysis temperatures, the crystallinity of hydroxyapatite increases, limiting its plant availability [37]. This reduced plant availability likely also influenced microbial dynamics, as the more crystalline structure of hydroxyapatite in BM 500 °C could have slowed down the release of key nutrients like phosphorus. With fewer nutrients immediately available for microbial use, particularly for fast-growing copiotrophs such as Pseudomonadota, BM 500 °C did not trigger the rapid shifts in microbial diversity seen with the BM 300 °C variant [84].

However, it is essential to consider that microbial diversity is generally recognized as a fundamental determinant of soil stability, dynamics, and overall functionality [40, 43]. Higher diversity is often associated with improved soil quality and disease suppression due to a broader spectrum of potential metabolic pathways being employed [50]. Therefore, the low microbial diversity observed in the *BM 300 °C 2%* and *BM 300 °C 5%* treatments, despite the potential increase in plant-available P, raises concerns about soil health. The significant drop in microbial diversity, which was further intensified with a higher application dose of *BM 300 °C* (Fig. 2A), may be associated with the nonporous structure of *BM 300 °C* biochar variants and the oily viscous residue originating from fats in the feedstock material [84]. Therefore, it is advisable to subject BM to higher pyrolysis temperatures to avoid a drop in microbial diversity, and thereby enhance potential soil health and quality.

In contrast to the drop in soil microbial diversity caused by *BM 300 °C* amendments, W-derived biochar did not significantly stimulate diversity at earlier time points but did show a notable increase in both luvisol and cambisol soils one year after the biochar application, particularly in the case of W 500 °C 2% (Figure SI-1). This increase, however, was observed one year after the biochar application on soil, specifically for *W 500 °C 2%*. Furthermore, *BM 500 °C* treatments only increased the diversity in cambisol 6 months after the biochar application, and this significant difference dissipated over time. While these changes in microbial diversity were particularly noticeable over extended periods or in specific soil types, they remain crucial findings. Soil microbial diversity serves as a vital indicator of the overall environmental health of the soil ecosystem [78]. It reflects how external factors, such as biochar amendments, can profoundly influence the intricate web of microorganisms that play pivotal roles in nutrient cycling, soil structure maintenance, and plant health [49, 72]. A high level of microbial diversity enhances the soil food web, creating a favorable environment for symbiotic microbe populations to thrive. This, in turn, contributes to the enrichment of the soil with essential nutrients crucial for optimal plant growth and productivity [93]. Therefore, even the delayed or soil-specific effects observed in this study underscore the significance of the *BM 500 °C* and *W 500 °C* biochars'

impact on soil microbial diversity, highlighting their relevance for sustainable soil management and agricultural practices.

As previously demonstrated, the impact of biochar variants on soil microbial community structure can become evident as early as 3 days after application. The Principle Response Curve (PRC) diagram (Fig. 3) provides additional insights into the effects of biochar variants on microbial community structure in both cambisol (Fig. 3A) and luvisol (Fig. 3B) soils at multiple time points: 3 days, 14 days, 1 month, 6 months, and 1 year after application. PRC is an extension of redundancy analysis (RDA), and is particularly useful for visualizing changes in species composition over time due to biochar treatments compared to a control.

In the PRC plots (left panels of Fig. 3), the y-axis, labeled "Effect," represents the deviation of the microbial community structure of each treatment from the control soil (baseline set at zero). A higher effect value indicates a greater alteration to the community structure relative to the control. The curves for each treatment show how the community response evolves over time.

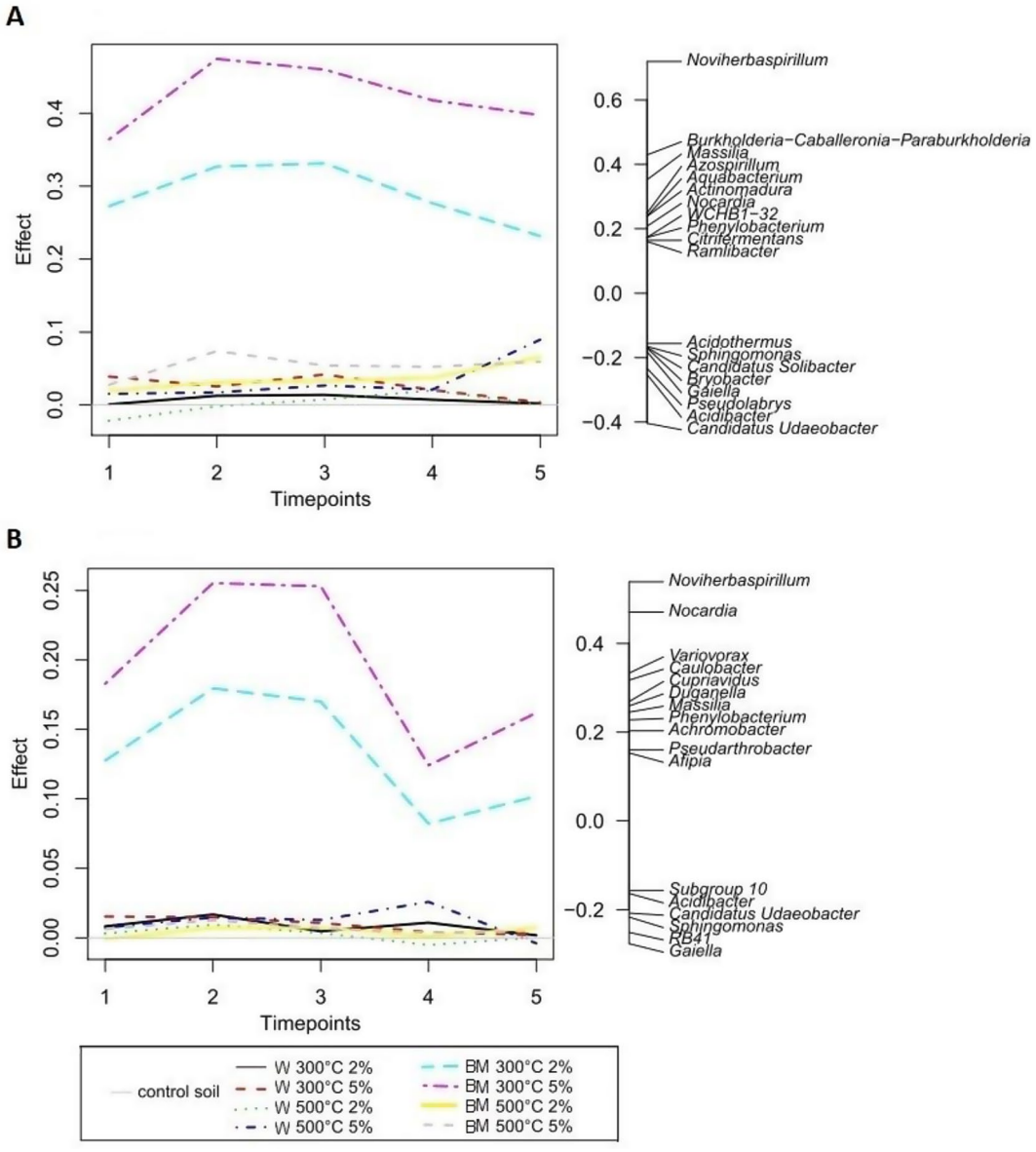

Fig. 3 Principal response curves (PRC) showing trends of community response to biochar variants over time compared to control soil (baseline) in (**A**) cambisol and (**B**) luvisol. Time points represent samples that were collected (1) 3 days, (2) 2 weeks, (3) 1 month, (4) 6 months, and (5) 1 year after adding the biochar. The biochar variants differed in pyrolysis temperature (300 °C and 500 °C), feedstocks (W - Beech woodchips, or BM - Waste after mechanically separated meat), and application doses at which they were applied to luvisol and cambisol soils (2% and 5%). The control soil was not treated with a biochar

The right panels display the species weights, which reflect the contributions of individual taxa to the observed changes. Positive species weights indicate taxa that are more abundant in treatments that deviate positively from the control, while negative weights represent taxa that are more abundant in the control or less affected treatments. Notably, the range of the y-axis differs between the treatment effect plots and the species weights plots, because the former shows the overall impact on community composition, while the latter highlights the relative importance of individual taxa.

The PRC analysis reveals that approximately 35% (cambisol) and 36% (luvisol) of the variation in genera composition is attributed to within-year variation, while another 50% (cambisol) and 44% (luvisol) can be attributed to variation between treatments (including the interaction with time). This underscores the dynamic and persistent nature of the microbial community responses to biochar treatments.

The largest shifts in prokaryotic community structure were observed in *BM 300 °C 2%* and *BM 300 °C 5%* treatments, as shown by the high effect values consistently maintained throughout the time series in both cambisol and luvisol soils. These treatments, indicated by the cyan and purple dashed lines respectively, show the most significant and persistent deviation from the control soil, suggesting that the BM biochar produced at 300 °C results in a prolonged impact on microbial communities. This effect was then intensified with increasing application dose. In contrast, the separation of curves representing the W-treated soils gradually became less distinct over time. The prokaryotic community structures in the W-treated soils (except for *W* 500 °C *5%* in cambisol) tended to resemble that of the control soil one year after biochar application. Therefore, it can be argued that the W biochar only caused temporary shifts in the prokaryotic community structure.

As observed in Fig. 3, the abundance of genera with a positive species weight is expected to increase (Fig. 3) relative to the control soil in treatments with curves above the control soil (baseline). Conversely, genera with a negative value are expected to decrease in such treatments. The greater the separation of a biochar variant's curve from the control soil, the more significant the observed changes in the abundance of the shown genera. Specifically, *Noviherbaspirillum*, *Nocardia*, and *Massilia* exhibited positive species weights in both cambisol and luvisol soils, indicating their increased relative abundance with BM 300 °C 2% and BM 300 °C 5% treatments. In contrast, *Sphingomonas* and *Gaiella* exhibited negative species weights, suggesting a reduction in their relative abundance with these treatments compared to the control soil.

Notably, *Noviherbaspirillum* (Pseudomonadota) had the highest species weight in both soil types. This denitrifying bacterium utilizes simple organic acids as a carbon source [42], and its stimulated growth after the addition of pyrogenic organic matter was previously observed in various soil types [101]. Genera such as *Nocardia*, *Actinomadura* (both Actinomyceota), *Massilia*, *Burkholderia/Caballeronia/Paraburkholderia*, *Azospirillum*, *Aquabacterium*, *Variovorax*, *Cupriavidus*, and *Duganella* (all Pseudomonadota) exhibited positive species weight in at least in one of the soil types treated with *BM 300 °C 2%* and *BM 300 °C 5%*. These genera have previously been associated with phosphate-solubilizing activity [12, 71, 76, 87, 111], which aligns with the observed higher phosphate availability in these treatments [84]. Similarly, Azeem et al. [8] found that bone-derived biochar improved the phosphorus availability in contaminated soils, further supporting the role of bone biochar in nutrient mobilization. Additionally, the presence of genera belonging to the Pseudomonadota phylum corresponds to its higher relative abundance, as presented in Fig. 2B.

In summary of phase one, our study highlights the importance of pyrolysis temperature in biochar production, with distinct effects observed for different feedstock types. For bone-meat residues, pyrolysis at 300 °C significantly influenced the community structure and promoted the growth of phosphate-solubilizing bacteria, making it a potential alternative to chemical P fertilizers. However, this treatment also resulted in a significant drop in soil prokaryotic diversity. In contrast, pyrolysis at 500 °C for the BM biochar maintained higher prokaryotic diversity while still enhancing phosphate mobility [84]. For beech woodchips (higher pyrolysis temperatures (500 °C) were necessary to induce substantial changes in the soil microbial community microbial structure, likely due to the shift from macroporous to microporous biochar characteristics [84]. Therefore, for phase two of our study, a pyrolysis temperature of 500 °C was selected for both feedstock types, aiming to strike an optimal balance between microbial community enrichment and diversity preservation.

Phase 2: Impact of biochar variants on enzymatic activity and prokaryotic and fungal communities in soil, rhizosphere and roots

In this phase of the study, we conducted a rhizobox experiment (Figure SI-2) in which the rhizoboxes were filled with cambisol or luvisol and enriched with W biochar or BM biochar, both of which were pyrolyzed at 500 °C (5% w/w). *Triticum aestivum* L. was planted in the soil-plant compartment for 90 days. The prokaryotic and fungal communities were analyzed in plant roots and in the rhizosphere at distances of 2, 4, and 6 mm from the root system. This analysis was followed by an assessment of enzymatic activity in the bulk soil. A total of 2,525,117

Table 3 Influence of biochar application (W, BM, or control soil) and distance (various sections from the root system (0–2, 2–4, 4–6 mm) of *T. aestivum* L.) on the structure of prokaryotic and fungal communities in soil (PERMANOVA)

	Prokaryotes				Fungi			
	Cambisol		Luvisol		Cambisol		Luvisol	
	R^2	P	R^2	P	R^2	P	R^2	P
Biochar treatment	23%	**0.0009**	14%	**0.0400**	11%	0.1	12%	**0.03**
Distance	7%	**0.0070**	5%	0.2000	7%	0.4	6%	0.50
Biochar x Distance	12%	**0.0200**	10%	0.5000	13%	0.5	12%	0.74

Significant ($p \leq 0.05$) p-values are underlined and shown in bold

16S rRNA ASVs were obtained, with read counts ranging from a minimum of 2,561 to a maximum of 58,852 per sample. For ITS ASVs, there were 2,734,826 reads in total, with a minimum of 135 reads and a maximum of 81,548 reads per sample.

The diversity of prokaryotes, both in the rhizosphere and roots, was not significantly influenced by soil type, biochar treatment, or the distance from the root system ($p > 0.1$, Kruskal-Wallis test). Conversely, the fungal diversity significantly differed between the soil types ($p < 0.05$). Specifically, luvisol exhibited higher fungal diversity than cambisol (Figure SI-4). Neither the application of biochar nor the distance from the root system exerted a significant influence on fungal diversity ($p > 0.1$). These findings align with the general trends observed in the phase one experiment, where, for the most part, we did not detect significant alterations in soil prokaryotic diversity less than a year after the application of either the W biochar pyrolyzed at 500 °C (5% w/w) or the BM biochar pyrolyzed at 500 °C (5% w/w). The response of microbial diversity in rhizosphere to biochar treatment was previously linked to the nutrient richness of such treatments [55], where nutrient-rich biochar maintained bacterial species richness, while low-nutrient biochar led to reduced diversity. This suggests that the biochar treatments in our study provided the necessary nutrients to sustain diversity compared to the control soil.

While significant changes were not observed in microbial diversity, the microbial community structure exhibited noticeable changes due to the biochar application. The biochars had a significant influence on the community structure of prokaryotes in both soil types, whereas fungi were only significantly affected in luvisol (Table 3). In cambisol, a significant difference in the prokaryotic community structure was found between all the biochar treatments (W, BM, and control), distance from the root system and their combination (pairwise PERMANOVA, $p_{adj} < 0.05$). In contrast, in luvisol, the prokaryotic community differed only between the control soil and BM, while the fungal community differed between the control soil and both BM and W treatments (pairwise PERMANOVA, $p_{adj} < 0.05$).

The main driver of such changes could be a significant alteration of soil pH caused by biochar treatment in both

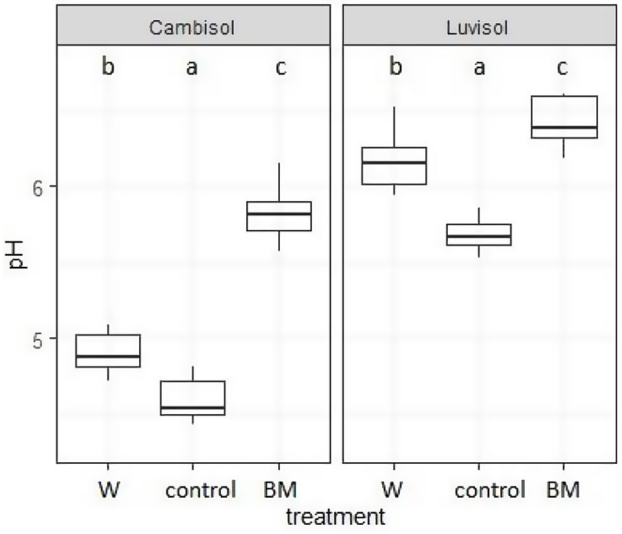

Fig. 4 Soil pH in control soil, W (beech woodchips) and BM (bone-meat residues after mechanical deboning from a poultry slaughterhouse) treatments. The control soil was not treated with a biochar. Different letters indicate significant differences between treatments ($p_{adj} \leq 0.05$), and were assigned according to conducted pairwise Wilcoxon tests

cambisol and luvisol soils (Fig. 4). Both the W and BM treatments led to a significant increase in soil pH levels; notably BM caused a considerably larger increase compared to W. The difference in soil pH between biochar treatments was even larger in cambisol, which is typically more acidic than luvisol. This might also be the reason why biochar treatment explained more variability of prokaryotic community structure in cambisol than in luvisol (Table 3) since biochar has a larger effect on prokaryotic communities, mainly in acidic soils [106]. Microorganisms, especially prokaryotes, are known to be highly responsive to pH fluctuations. Therefore, a shift in soil pH can have a substantial influence on the structure of the microbial community [21].

Differential abundance analysis using DESeq2 revealed that, compared to the control soil in both cambisol and luvisol, the relative abundance of 18 genera was significantly higher in BM-treated soils, while the relative abundance of 10 prokaryotic genera was significantly higher in W-treated soil (Table 4). Of these genera, the relative abundance of *Sphingopyxis* stood out, having the highest

relative abundance in both treatment groups. *Sphingopyxis* is known for its remarkable metabolic versatility and adaptability to a wide range of environmental conditions [80], suggesting that the microbial communities in BM- and W-treated soils may be better equipped to respond to environmental changes.

The relative abundance of certain fungal genera was also significantly higher in soils with the BM and W biochar (Table 4). Specifically, *Selinia* and *Leohumicola* were found to have significantly higher relative abundance with both W and BM treatments compared to control soils of cambisol and luvisol. Notably all genera, whether prokaryotic or fungal, that had significantly higher relative abundance in W-treated soils compared to the control soils were also among those significantly enriched in BM-treated soils compared to the control soils. This outcome suggests that there were no genera with a significantly different relative abundance that was specific to the W treatment.

In contrast, BM treatment alone produced significantly higher relative abundance, compared to the cambisol and luvisol control soil, of genera such as *Lacibacter*, *Desulfocapsa*, *Geobacter*, *Herbaspirillum*, *Nitrosospira*, *Flavisolibacter*, *Noviherbaspirillum*, *Oxalicibacterium*, *Methylorosula*, *Candidatus Alysiosphaera*, and *Nitrospira*. Several of these genera are known for their involvement in essential soil processes, including sulfate reduction (*Desulfocapsa*) [59], Fe(III) reduction (*Geobacter*) [57], phosphate solubilization (*Herbaspirillum*) [27], or their roles in the nitrogen cycle (*Herbaspirillum*, *Noviherbaspirillum*, *Nitrosospira*, and *Nitrospira*) [19, 24, 81, 102]. These activities exert a profound and lasting influence on the soil environment, suggesting that the BM biochar has the potential to enhance soil quality.

Biochar amendments also had a significant impact on the community of prokaryotic endophytes within the roots of *Triticum aestivum* L., particularly when planted in cambisol soil (Table 5). In this specific case, the biochar treatments accounted for 53% of the variability in the prokaryotic community structure. Conversely, neither the community of prokaryotic endophytes in luvisol nor the fungal endophytes in either soil type showed a significant influence due to the biochar treatments.

The observed changes in the community structure of prokaryotes in the root endosphere are likely linked to alterations in the prokaryotic community structure in the rhizosphere in cambisol (Table 3). Endophytes can be horizontally acquired from the rhizosphere, where they are selected through interactions with root exudates [32]. Root exudates play a pivotal role in plant-microbe interactions, and shape the selection of potential endophytes based on their composition [9]. Given that changes in the prokaryotic community structure were observed across different rhizosphere zones (2, 4, and 6 mm), it is plausible to argue that the microorganisms in these zones experienced selection pressures, which subsequently led to changes in the endophytic community structure.

The only prokaryotic genus that exhibited a significantly different relative abundance between biochar-treated plants and the control groups (as determined by DeSeq, $p_{adj} < 0.05$) was *Duganella*. A significantly higher relative abundance of it was observed in both W and BM treatments when compared to the control. Interestingly, *Duganella's* relative abundance did not show a significant increase in W 500 °C or BM 500 °C treated soils (Table 4), suggesting that biochar treatments directly influenced its presence within the endosphere rather than in the treated soil.

The presence of *Duganella* in the roots could be indicative of its beneficial role in enhancing soil health and

Table 4 Results of Differential abundance analysis (DeSeq) showing prokaryotic or fungal genera with significantly ($p_{adj} < 0.05$) higher abundance in biochar-treated soil compared to control groups of both soil types (cambisol and luvisol)

Treatment	Total	Genera
BM	18 prokaryotes	*Sphingopyxis*, WCHB1-32 (Bacteroidetes), *Lacibacter*, *Desulfocapsa*, *Flaviaesturariibacter*, *Geobacter*, OM27 clade (Proteobacteria), *Herbaspirillum*, *Nitrosospira*, *Ramlibacter*, *Flavisolibacter*, *Xylophilus*, *Noviherbaspirillum*, *Oxalicibacterium*, *Methylorosula*, *Candidatus Alysiosphaera*, *Nitrospira*, Unclassified (from Bacteroidetes)
	2 fungi	*Selinia*, *Leohumicola*
W	10 prokaryotes	*Sphingopyxis*, *Flaviaesturariibacter*, OM27 clade (Proteobacteria), *Xylophilus*, *Ramlibacter*, Unclassified (from Bacteroidetes, Acidobacteria, and 3x Proteobacteria)
	2 fungi	*Selinia*, *Leohumicola*

The biochar treatments were BM (bone-meat residues after mechanical deboning from a poultry slaughterhouse) and W (beech woodchips). The control group was soil without any biochar treatment

Table 5 Influence of biochar application (W, BM, or control soil) on the structure of endophytic prokaryotic and fungal communities in the root system of *T. aestivum* L. (PERMANOVA)

	Endophytic prokaryotes				Endophytic fungi			
	Cambisol		Luvisol		Cambisol		Luvisol	
	R^2	P	R^2	P	R^2	P	R^2	P
Biochar treatment	53%	**0.007**	29%	0.144	23%	0.525	30%	0.088

Significant ($p \leq 0.05$) p-values are underlined and shown in bold

plant growth. This genus is known for its diverse metabolic capabilities, including activities such as phosphate solubilization [104] and nitrogen fixation [28], which can contribute to improved nutrient availability for plants. Moreover, *Duganella* has been indentified as a member of the core endophytic microbiome of *T. aestivum* [54] and has been associated with enhancing plant tolerance to cold [74], along with its known anti-fungal activities [39]. These findings underscore the potentially beneficial role of *Duganella* in bolstering plant health and resilience, emphasizing the significance of biochar amendment in the context of sustainable agriculture and soil management practices.

The activity of several key enzymes, including β-glucosidase, β-xylosidase, β-N-acetylglucosaminidase (NAG), sulphatase, acid phosphatase, and total microbial activity (fluorescein diacetate method), was assessed in soil samples collected from both bulk and rhizosphere compartments of the rhizoboxes (2, 4 and, 6 mm). The enzymatic activity was found to be significantly associated with soil type and biochar application (PERMANOVA, $p_{adj} < 0.001$), but not with the distance from the root system ($p_{adj} = 0.588$). Additionally, the interaction between soil type and biochar application also had a significant impact on enzymatic activity ($p_{adj} < 0.001$), leading to further statistical analysis being conducted separately for each soil type.

β-glucosidase, β-xylosidase, and NAG exhibited a significant increase in activity relative to the control soil after the application of the W biochar in at least one of the soil types (Fig. 5). β-glucosidase and β-xylosidase play vital roles in the carbon cycle, catalyzing the breakdown of polysaccharide bonds into simple sugars [3, 13]. NAG is involved in the cycle of both carbon and nitrogen by facilitating the hydrolysis of chitin into amino sugars [25]. Of particular note β-glucosidase, known for its sensitivity as an indicator of soil quality [83], suggests an enhancement in the quality of both cambisol and luvisol soils with the addition of the W biochar.

The observed increase in β-glucosidase, β-xylosidase, and NAG activity following the application of the W biochar to agronomical soil aligns with findings from other studies [4, 5, 95]. This increase in enzymatic activity was correlated with the higher carbon content of the biochar [31], which was 83.1% for the W biochar, in contrast to the 22.8% carbon content of the BM biochar [84]. The biochar effectively enriches the soil's organic matter content, thereby increasing the availability of carbon for microbial utilization [109]. It is also the microporous nature of the W biochar that creates favorable conditions for microbial growth by absorbing essential nutrients and moisture from the soil environment [70]. Simultaneously, it acts as a protective shield for microbes against their predators [56]. The biochar also absorbs extracellular enzymes and/or their substrates, leading to stabilization of their catalytic reactions [26], which inherently contributes to the observed increase in enzymatic activity. This multifaceted effect underscores the numerous advantages

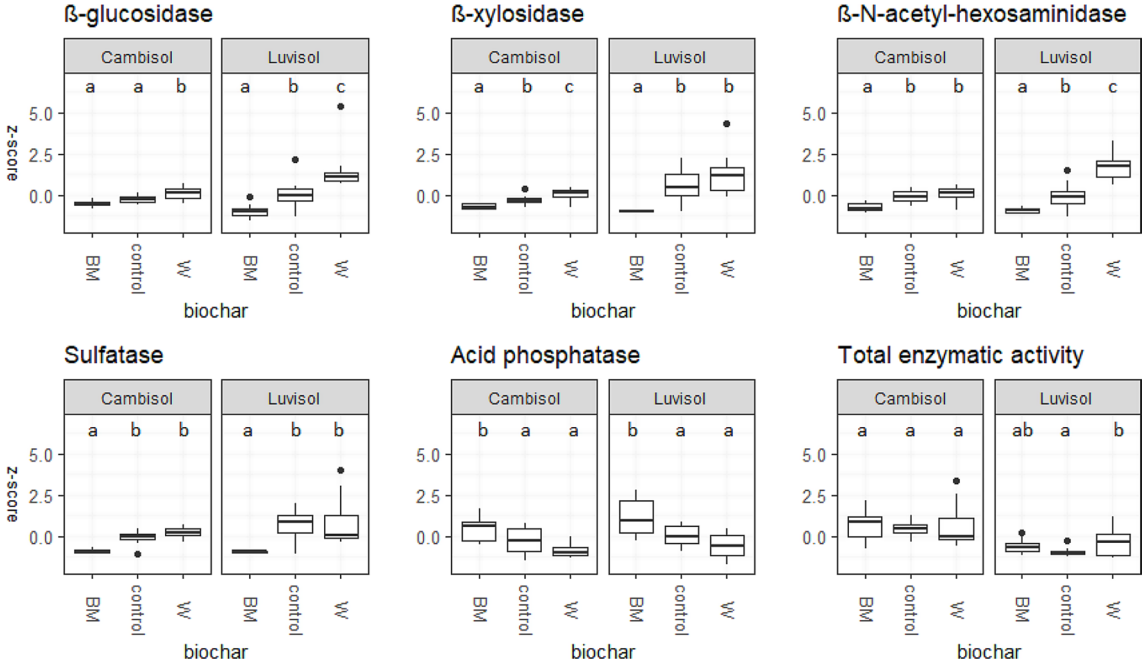

Fig. 5 Enzymatic activity in cambisol and luvisol soil types mixed (5% w/w) with W (beech woodchips) or BM (bone-meat residues after mechanical deboning from a poultry slaughterhouse) biochar prepared at 500 °C. The control soil was not treated with a biochar. Different letters indicate significant differences between treatments ($p_{adj} \leq 0.05$), and were assigned according to conducted pairwise Wilcoxon tests

of applying the W biochar to agricultural soils, ranging from carbon enrichment to the creation of a more conducive environment for soil microbes, ultimately leading to an enhancement of soil quality.

Additionally, we observed significantly higher total microbial activity in W-treated luvisol (Fig. 5), which is consistent with previous research [61]. Total microbial activity is measured by the hydrolysis of FDA, a substrate that can be acted upon by various enzymes, including lipases, esterases, and proteases [46]. Many decomposers possess these enzymes, making FDA hydrolysis and its response to external factors a reliable indicator of total microbial activity in the soil [1]. Interestingly, the activity of proteases, one of the enzymes responsible for FDA hydrolysis, has been linked to the activity of NAG, which was also elevated in W-treated luvisol. Both proteases and NAG are inducible by microbial-derived sources such as chitin and proteins [35]. In this context, it may be hypothesized that it is not only biochar amendments that contribute to increased enzymatic activity, but the stimulated turnover of microbial communities over an extended period could drive this activity surge.

In contrast to the W biochar, the BM biochar led to a significant decrease in the activity of β-xylosidase, NAG, and sulfatase in both soil types, as well as β-glucosidase in luvisol (Fig. 5). This decline in β-xylosidase, NAG, and β-glucosidase activity can be attributed to the feedstock origin and composition of the resulting BM biochar [84]. Furthermore, the mesoporous structure of the BM biochar may not offer as protective an environment for soil microbes and extracellular enzymes as the W biochar can. Additionally, there is a decreased activity of sulfatase; sulfatase is responsible for the hydrolysis of organosulfur compounds, releasing sulfate forms that are made available for plant uptake [100]. It has been reported that the activity of sulfatase is suppressed in the presence of sulfate [77], which was significantly increased in BM-treated soils [84].

In contrast to the trends observed with these enzymes, a different pattern emerged for acid phosphatase. Acid phosphatase was the sole enzyme whose activity significantly increased in BM-treated soils, while no such increase was noted with the W biochar (Fig. 5). This increase in activity aligns with the findings of other incubation experiments focused on the biochar's impact on P availability [38]. The addition of the BM biochar enhances the bioavailability of P in treated soils, likely by promoting the growth of phosphate-solubilizing bacteria [8, 41]. Such bacteria play a pivotal role in converting insoluble forms of phosphorus into soluble ones, accomplished through producing organic acids or phosphatases [79]. In this particular case, BM 500 °C treatment resulted in an approximately 19-fold increase in the mobile phosphorus content in the unplanted cambisol, and a roughly 14-fold increase in the luvisol [84]. As phosphorus is an essential and often limiting nutrient for plant growth, the potential increase in its bioavailable form in agronomical soil holds significant value.

Unlike chemical fertilizers, which elevate the risk of phosphorus leaching and are a primary contributor to water eutrophication, a biochar derived from animal waste, such as a BM biochar, elevates phosphorus levels without imposing such ecological burdens [47, 91]. Hence, a BM biochar can be considered an environmentally friendly phosphorus fertilizer alternative.

Conclusion

Incorporating new organic wastes into biochar production demands a thorough assessment of their impact on agricultural soil. This study underlines the critical influence of feedstock type, pyrolysis temperature, application dosage, and even soil type on soil and endophytic microbial communities. Remarkably, the utilization of both feedstock types — BM (bone-meat residues after mechanical deboning from a poultry slaughterhouse) and W (beech woodchips) — both processed at 300 °C, either led to a substantial decrease in soil microbial diversity, as observed with the BM treatment, or failed to elicit significant alterations in microbial community structure or microbial diversity, as seen with the W treatment. In contrast, the application of a biochar produced at a higher pyrolysis temperature of 500 °C, using both organic waste sources, was found to induce changes in the soil microbial community that correlated with improved soil quality. These biochar treatments led to substantial shifts in the community structures of prokaryotes and fungi. The BM biochar exhibited a tendency to support the growth of Pseudomonadota relative to the other phyla, while the W biochar significantly stimulated carbon cycling processes. These changes were particularly pronounced in cambisol, a soil type typically characterized by higher acidity compared to the tested luvisol soil. Enzymatic activity in soil was also impacted, with the BM biochar increasing the activity of acid phosphatase while decreasing the activity of β-glucosidase, β-xylosidase, NAG, and sulfatase. In contrast, the W biochar enhanced the activity of enzymes involved in the carbon cycle, including β-glucosidase, β-xylosidase, and NAG. Importantly, the distinct composition of the two biochar variants was the main driver behind the observed shifts in microbial community structure within the soil, rhizosphere, and root endosphere, as well as the significant changes in soil enzymatic activity. The next study should include a field experiment with biochar pyrolysed at the higher temperature to assess the complexity of all biotic and abiotic interactions.

Supplementary Information

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Supplementary Material 1

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Author contributions
All authors contributed to the study's conception and design. Biochar preparation, ensuring one-year incubation and rhizobox experiments were performed by FM, JS and PT. Enzymatic assays, amplicon preparation and data analysis were performed by MKF, EA, AK and HS. The first draft of the manuscript was written by MKF, and HS and KD commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

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Data availability
The sequence dataset was deposited into the NCBI Short Read Archive under the accession number PRJNA769602.

Declarations

Ethics approval
Not applicable.

Consent to publish
All authors agree with the submission and publication in this journal.

Competing interests
The authors declare no competing interests.

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