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Biochar Alone Did Not Increase Microbial Activity in Soils from a Temperate Climate That Had Long-Term Acidity Stress

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Abstract: Biochar can increase the microbial activity of a soil and the seed yield of oilseed rape. We performed a field experiment to determine the effect of different doses of biochar and the impact of nutrient additions on microbial activity in soils with low pH (4.69). Different doses of biochar were applied to plots in 2016 (0 t/ha (control), 15 t/ha (B15), 30 t/ha (B30), or 45 t/ha (B45)), and fertilization was applied during 2019 (90 kg P/ha) and 2020 (50 kg N/ha, 80 kg P/ha, and 50 kg K/ha). Maize was grown in 2016, barley in 2017, maize in 2018, wheat in 2019, and winter oilseed rape in 2020. All analyses were carried out in 2020. Higher biochar doses led to reduced seed yield of oilseed rape and inhibition of microbial activity (cumulative soil respiration, dehydrogenase activity (DHA), and leaching of mineral forms of nitrogen). Notably, dehydrogenase activity was more than 60% lower in plots with the highest biochar dose. All doses of biochar increased the soil pH compared to the control (control: 4.51 ± 0.04, B15: 4.69 ± 0.04, B30: 5.06 ± 0.05, B45: 5.05 ± 0.04) but did not increase microbial activity or the seed yield of oilseed rape. Thus, application of biochar alone did not increase the seed yield of oilseed rape or soil microbial activities, although it did increase soil pH.

Keywords: nitrogen; soil enzymes; plot experiment; DHA; mineral nitrogen

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1. Introduction

Biochar is carbonized organic matter that can be used as a soil additive. There is growing interest in the use of biochar in agriculture because it can improve key soil characteristics such as aggregate stability and the retention of water in soil [1]. Biochar can also sequester carbon; hence, its widespread use may assist in slowing climate change [2,3].

Biochar has the potential to increase microbial activity and plant production and to improve the physical and chemical properties of soil. These benefits are especially important for low-quality soils, such as those that are acidic, have low content of organic matter, or are compacted [4]. As demonstrated by Ye et al. [5], the effect of biochar on crop yield is greater in soils from tropical and subtropical climates than in soils from continental and temperate climates (mean increase: 14.8% vs. 1.4%). These authors also concluded that biochar supplements provided less benefit for rice than other cereal crops. Liu et al. [6] concluded that the application of biochar was most suitable for dryland crops. However, both authors also stated that the most important parameter for the successful application of biochar is soil quality. They considered that the most suitable soils for biochar application are sandy soils with a low CEC and a soil pH < 6.5.

However, there is limited commercial availability of biochar. Moreover, the specific climate, type of crop, and/or soil properties at a site may make it unsuitable for biochar application [2].

Biochar is often classified as a liming additive [7,8] because it increases pH after addition. However, it is necessary to consider the variability in properties among different biochars. For example, Singh et al. [9] reported that the pH of biochars can vary from 4 to 12,

although most biochars have pH values greater than 7. Therefore, acid soils are generally considered most suitable for the application of biochar. The microbial communities of these acid soils are often limited by the low pH [10]. The addition of biochar to soils can lead to a more suitable environment for microbes due to direct effects (increasing the content of available nutrients) and indirect effects (sequestering potentially toxic substances) [2]. Moreover, biochar is a porous material exhibiting a wide pore size distribution, and it allows colonization by microbes, it provides microbes refuge from predators, and it adsorbs molecules, such as carbonates and amino acids, that microbes can use to support growth [2,11].

The aim of the present study was to test the effect of supplementing acidic soils with different doses of biochar that was prepared from residual plant biomass on the soil pH, the biomass of rapeseed (*Brassica napus* var. napus), and soil microbial parameters after 4.5 years. Our hypothesis was that biochar would increase the soil pH, biomass yield, and measured soil microbial parameters.

2. Materials and Methods

2.1. Experimental Plots

Experiments were conducted in the spring of 2016 at the Field Forage Research Station in Vatín (49.52° N; 15.97° E) in the Bohemian–Moravian Highlands, which is 7 km south of Žďár nad Sázavou (Czech Republic). The altitude is 540 m.a.s.l., the region has a mild-warm climate, and the average annual precipitation was 736 mm from 1971 to 2000. The soil (Eutric Cambisol) at the experimental site was classified as sandy clay–loam [12] built from clay loam.

The basic characteristics of the soil were determined (Table 1). The soil organic carbon content was determined by oxidation in a chromium–sulfur mixture with a final cyclooxygenase determination using spectrophotometry according to ISO/DIN 14 235: 1998 [13]. The determination of soil pH was performed according to the ISO 10390: 2005 standard [14]. Total nitrogen (Nt) was determined using a LECO analyzer (LECO TruSpec CN, Vancouver, Canada). Particle size distribution was determined according to ISO 11277: 2009 [15], in which a pipetting method was used to determine the sedimentation times of soil particles with different sizes, and evaluation was performed using the USDA Soil Texture Triangle. The nutrients were measured using the Mehlich III [16] soil test (Thermo Scientific iCAP 7400 Duo, Thermo Fisher Scientific, Cambridge, UK). The levels of P (mg/kg), K (mg/kg), Ca (mg/kg), and Mg (mg/kg) were determined using a colorimetric assay with measurement of absorbance at 660 nm.

Table 1. Basic characteristics of the soil (sandy clay loam) in the experimental plots. These characteristics were measured before the experiment was established.

Available P (mg/kg)	Available K (mg/kg)	Available Ca (mg/kg)	Available Mg (mg/kg)	Organic Carbon (%)	pH/KCl	Nt (%)	Texture		
							<0.002 mm (%)	0.05–0.002 mm (%)	2.00–0.25 mm (%)
24.1	164.0	881.5	182.1	1.52	4.69	0.13	33.8	47.5	18.7

2.2. Characteristics of the Experimental Plots and Biochar

There were 4 different biochar treatments, each with 3 replicates (12 plots): (i) no biochar (control), (ii) biochar at 15 t/ha (B15), (iii) biochar at 30 t/ha (B30), and (iv) biochar at 45 t/ha (B45). These variants of biochar doses were distributed randomly on the plot. The biochar was incorporated to a depth of 20 cm, and each plot had an area of 12 m^2.

Biochar was applied in April 2016. Due to the acidic pH of the soil and its low level of phosphorus, superphosphate was applied two months later at a rate of 200 kg/ha. Maize (*Zea mays*) was grown in the plots in 2016, spring barley (*Hordeum vulgare* conv. distichon var. Nici) in 2017, maize in 2018, wheat (*Triticum aestivum*) in 2019, and winter rape (*Brassica napus* var. napus) in 2020. This study focused on the characteristics of the

soils in the experimental plots 4.5 years after the application of biochar; thus, the results reported are from 2020.

The biochar was produced by pyrolysis at 500 °C in the Czech Republic (biouhel.cz, accessed on 20 February 2022). The primary material for biochar production was waste from woody biomass (wood chips). The basic properties of the biochar were determined as described below (Table 2).

Table 2. Basic properties of the biochar.

BET Sorption Analysis	
Specific surface	584 (m^2/g)
Optical emission inductively coupled plasma spectroscopy	
N	0.2 hm%
C	96.3 hm%
H	0.1 hm%
S	0.00 hm%
O	2.2 hm%
Combustion analysis with GC detection	
Ca	59.9 mg/g
K	13.4 mg/g
Na	4.4 mg/g
P	2.0 mg/g
Al	3.4 mg/g
Mg	3.8 mg/g
Mn	3.4 mg/g
Pb	0.0 mg/g
Zn	0.1 mg/g
Cd	0.0 mg/g
Ash	10.7 hm%
Gas chromatography/mass spectrometry	
Naphthalene	0.034 µg/g
Acenaphthylene	0.245 µg/g
Acenaphthene	BLD
Anthracene	0.346 µg/g
Fluorene	0.216 µg/g
Phenanthrene	0.247 µg/g
Benz (a) anthracene	0.636 µg/g
Chrysen	0.259 µg/g
Benzo (b) fluoranthene	0.668 µg/g
Benzo (k) fluoranthene	BLD
Benzo (a) pyrene	BLD
Indene (1,2,3-cd) pyrene	0.807 µg/g
Dibenz(a, h)anthracene	BLD
Benzo(ghi)perylene	0.535 µg/g
Fluoranthene	0.267 µg/g
Pyrene	0.299 µg/g
ISO 10390:2005	
pH	10.2
C/N ratio	520

Abbreviations: hm%: relative content; BLD: below level of detection. Results were obtained by BET sorption analysis, optical emission inductively coupled plasma spectroscopy, and gas chromatography/mass spectrometry. Measurements were made in 2020.

2.3. Sampling and Analysis of Soil Properties and Measurements

In 2020, three months before soil sampling, ion exchange disks were incorporated into the soil to measure the leaching of mineral forms of nitrogen at a depth of 30 cm [17]. The disks were applied 59 days after nitrogen fertilization (40 kg N/ha in the form of LAV 27% = 180 g LAV per parcel 12 m^2). Soil was randomly sampled from each plot at a depth of 0 to 10 cm during autumn of 2020. The samples were then placed in a refrigerator at 4 °C and analyzed after 1 week (Mendel University, Brno). Immediately before soil sampling, biomass (only seeds) was harvested (August) from the experimental plots by hand and placed in paper bags. The biomass was subsequently dried at 105 °C to a constant weight us-

ing measurements from an analytical scale (model AEJ 100-4NM, KERN, Berlin, Germany). Qualitative analysis of the biomass composition was not performed.

Six basic soil properties were determined:

Dehydrogenase Activity (DHA) was determined as described by Casida et al. [18] and Šimek et al. [19] using an incubator (model FC/FC 222, BMT Medical Technology, Brno-Zábrdovice, Czech Republic) and a spectrophotometer (model DR 3900, HACH Company, Düsseldorf, Germany).

The Nitrogen Availability Index was measured using a short-term incubation procedure as described by Keeney [20] This method characterizes the available nitrogen as the fraction of soil nitrogen that can be obtained microbially or by root activity in a relatively short time. The distillation apparatus used was a Behr S3 Steam Distillation Unit (Behr Labor-Technik, Düsseldorf, Germany).

The Leaching of Mineral Forms of Nitrogen was measured using on-site ion exchangers as described by Binkley and Matson [17]. This method measured the loss of nitrogen continuously throughout the cultivation experiment. The distillation apparatus was the same as above.

Cumulative soil respiration was measured as described by Keith and Wrong [21] using alkaline absorbent natrocalcite (soda lime) once per week for 48 h. Before the measurement, the granules (50 g of undried granules per 0.08 m^2) were dried at 105 °C to a constant weight, which was then recorded. During the measurement, the soil was incubated with natrocalcite granules for 48 h.

The formation of carbonates was accompanied by a weight gain, which was measured after incubation and drying of the calcite. The difference in weight before and after incubation was then used to determine CO_2 production (g of C per m^2 in 24 h) as follows:

The measurement was repeated in 4 weeks using mature rape seeds. These results were multiplied by 3.5 to calculate respiration per week, and each result therefore indicated "cumulative respiration". The analytical scale was the same as described above.

$$CO_2 \text{ production} = \left\{ \frac{\{(sample\ weight\ (g) - mean\ blank\ weight\ gain\ (g) \times 1.69)\}}{chamber\ area\ (m^2)} \right\} \times \frac{48\ (h)}{duration\ of\ exposure\ (h)} \times \frac{12}{44} \quad (1)$$

Mineral Forms of Nitrogen (nitrate) were measured as described by Binkley and Matson [17] using the same distillation apparatus as described above.

The Soil Exchange Reaction was determined according to ISO 10390: 2005 [14] using a pH meter (model MS 22, Laboratory Instruments, Prague, Czech Republic).

Polycarbonates were extracted by toluene using a Soxtec system as follows: 2 mg of biochar was extracted by toluene for 2 h at 180 °C, then filtered, evaporated in vacuum, and dried under a nitrogen atmosphere. The rest of the toluene was dissolved in 1 mL of isooctane, passed through a nylon filter (0.45 µm), and analyzed by GC/MS–TOF using a Pegasus IVD instrument as previously described [22].

2.4. Statistical Analysis

The statistical significance of different values was determined using analysis of variance (ANOVA). The assumption of homoscedasticity was tested using the Levene test; in cases of non-homoscedasticity (Levene test: $p \leq 0.05$), the Kruskal–Wallis test was used. Mean differences between the different groups were determined using Tukey's test or the Games–Howell test. In all analyses, a p value of 0.05 or less was considered significant. Correlation was determined by calculation of Pearson's r. All statistical analyses were performed using STATISTICA version 12 for Windows.

3. Results

We first determined the effect of biochar dose on the seed yield of oilseed rape, pH, and mineral nitrogen (Table 3) and used ANOVA to compare the different groups (Table 4). The dose of biochar had a statistically significant effect on oilseed rape seed yield (Table 4, $p = 7.9 \times 10^{-5}$; $\alpha = 0.05$), in that an increased biochar dose led to reduced oilseed rape seed

yield. The oilseed rape seed yield in the control group (1.02 g ± 0.03) was 59.4% greater than in the B45 group (0.64 g ± 0.07).

Table 3. Effect of biochar on the seed yield of oilseed rape, soil pH, and nitrate content.

Treatment	Replicates	Mean	Median	Standard Deviation
Oilseed Rape Seed Yield (t/ha)				
C	3	1.02	1.02	0.03
B15	3	0.95	0.97	0.02
B30	3	0.88	0.90	0.03
B45	3	0.64	0.68	0.07
pH (KCl)				
C	3	4.51	4.51	0.04
B15	3	4.67	4.68	0.04
B30	3	5.05	5.05	0.05
B45	3	5.06	5.05	0.04
Mineral Nitrogen, NO_3^- (mg/kg)				
C	3	3.86	3.83	0.04
B15	3	3.84	3.88	0.04
B30	3	4.00	3.88	0.05
B45	3	3.91	3.65	0.04

Table 4. ANOVA for the effect of biochar on the seed yield of oilseed rape, soil pH, and soil mineral nitrogen.

Soil Parameter	*p*-Value (α = 0.05)
Oilseed rape seed yield	7.9×10^{-5}
pH	4.0×10^{-7}
Mineral nitrogen (NO_3^-)	0.9

Biochar also significantly affected soil pH (Table 4, $p = 4.0 \times 10^{-7}$; $\alpha = 0.05$), with the pH increasing with increasing biochar dose. The control group had the lowest pH (4.51 ± 0.04), and the B45 group had the highest pH (5.06 ± 0.04).

However, biochar had no significant effect on the mineral nitrogen content (Table 4, $p = 0.9$, $\alpha = 0.05$). The level of mineral nitrogen only varied by about 4%, from 3.84 mg/kg in the B15 group to 4.00 mg/kg in the B30 group (Table 3).

We also measured the effect of biochar dose on DHA, leaching of mineral forms of nitrogen, and cumulative respiration (Table 5). The level of DHA declined significantly with increasing biochar dose (Table 6, $p = 7.4 \times 10^{-5}$, $\alpha = 0.05$), and the level was 59.5% lower in the B45 group than in the control group.

Biochar also had a statistically significant effect on leaching of mineral forms of nitrogen (Table 6, $p = 3.1 \times 10^{-7}$, $\alpha = 0.05$). The level was greatest in the control group (0.40 mg/mm^2 ± 0.02), lowest in the B15 group (0.17 mg/mm^2 ± 0.02), and intermediate in the B30 group (0.26 mg/mm^2 ± 0.01) and B45 group (0.37 mg/mm^2 ± 0.02).

Cumulative respiration decreased significantly as the biochar dose increased (Table 6, $p = 7.0 \times 10^{-16}$, $\alpha = 0.05$). In particular, cumulative respiration in the control group (49.08 g CO_2/g soil/24 h ± 0.03) was 38.8% greater than in the B45 group (35.36 g CO_2/g soil/24 h ± 0.11).

Table 5. Effect of biochar dose on DHA, leaching of mineral forms of nitrogen, and cumulative soil respiration.

	Replicates	Mean	Median	Standard Deviation
DHA (g TPF/g soil/h)				
C	3	1.73	1.81	0.14
B15	3	1.68	1.64	0.10
B30	3	1.36	1.32	0.07
B45	3	1.03	1.02	0.06
Leaching of mineral forms of nitrogen (mg/mm^2)				
C	3	0.40	0.40	0.02
B15	3	0.17	0.17	0.02
B30	3	0.26	0.26	0.01
B45	3	0.37	0.37	0.02
Cumulative respiration (mg CO_2/g soil/24 h)				
C	3	49.08	49.09	0.03
B15	3	42.21	42.22	0.10
B30	3	37.78	37.79	0.02
B45	3	35.36	35.36	0.11

Table 6. ANOVA for the effect of biochar on DHA, leaching of mineral forms of nitrogen, and cumulative respiration.

Soil Parameter	*p*-Value (α = 0.05)
DHA	7.4×10^{-5}
Leaching of mineral forms of nitrogen	3.1×10^{-7}
Cumulative respiration	7.0×10^{-16}

4. Discussion

4.1. Effect of Biochar on Soil pH, Soil Nitrate Content, and Seed Yield of Oilseed Rape

The current understanding is that biochar influences crops by increasing the availability of nutrients due to its increase of soil pH [23,24]. However, some research also found that biochar had positive effects in alkaline soils [25]. Thus, biochar can increase production in acidic soils because it increases pH, but it also provides benefits to plants in more alkaline soils [26].

Our study confirmed that the application of biochar led to a significant dose-related decrease in seed yield from oilseed rape. We did not consider the possibility of an initial increase in biomass due to the increased nutrient content of biochar, because biomass measurements were recorded 4.5 years after biochar application, a time when these nutrients were already used by the soil [27]. Among the available nutrients, we only measured nitrate–nitrogen (Table 3).

Our results indicated that soil pH increased with biochar dose (control: 4.51 $\pm$ 0.04, B15: 4.67 $\pm$ 0.04, B30: 5.06 $\pm$ 0.05, B45: 5.05 $\pm$ 0.04; Table 3). Although our experiments were conducted in a temperate zone, they were in a region with relatively acidic soil (pH 4.69). Our results are thus similar to those of Chintala et al. [28] who found that the pH of acidic soils increased 165 days after the addition of biochar (control: 4.78 $\pm$ 0.04; 52 t/ha: 5.51 $\pm$ 0.02).

However, the increase of soil pH in our study was not accompanied by an increase in seed yield of oilseed rape (Table 3). In particular, the yield was similar in the B15 group (0.95 t/ha $\pm$ 0.02) and the control group (1.02 t/ha $\pm$ 0.03), and higher doses of biochar reduced the yield, which was more than 10% lower in the B45 group. It is possible that biochar did not increase the yield (even though it did increase soil pH) because it decreased the level of soil microorganisms, as indicated by our measurements of soil respiration (Table 5). The present results thus differ from those of Hale et al. [29]. These previous

authors stated that biochar did not achieve as strong a liming effect as conventional liming, but it had a greater effect on reducing the availability of exchangeable Al^{3+}, which binds to the surface of biochar. Thus, they found that biochar provided a greater benefit to crop yields.

Jin et al. [30] also measured the effect of biochar on seed yield from rapeseed over 5 years. They reported a positive response, probably due to the increased pH. However, the magnitude of this effect decreased after the second year.

Previous studies reported that biochar increased the seed yield of oilseed rape by 13% [31], 10% [32], and 11% [33],. However, these field studies did not consider the effect of geographical location. Jeffery et al. [6] analyzed 1125 results from 109 independent studies that assessed the effects of biochar additions on plant production. They concluded that most publications that reported positive effects were from tropical areas (i.e., areas with acidic soils). They also found that biochar did not provide this benefit in temperate zones, probably because there was no liming effect. Soils in tropical regions are usually limed to maintain a pH more suitable for plant growth, but this practice is not common in temperate regions. Jeffery et al. [31] also found that the relationship between soil pH and dose of biochar only occurred in tropical soils.

Another possible reason that biochar decreased the yield in the present work may be that toxic substances in the biochar itself inhibited plant growth, as well as microbial activity. Thus, we compared the levels of polycyclic aromatic hydrocarbons (PAHs) in our plots at 4 years after biochar addition with the levels in plots in previous studies that were recorded soon after biochar addition (Table 7). Notably, Rombola et al. [31] reported that 4.5 years after the addition of biochar (16 t/ha biochar from orchard pruning biomass), the levels of PAHs gradually declined to near the level of untreated controls.

Zhang et al. [34] reported that the soil nitrate content may continue to increase for many years after the application of biochar because biochar reduces leaching losses and not because it increases nitrogen immobilization. This decline in losses from leaching occurs immediately after the application of biochar. Kammann et al. [35] also reported an increased concentration of nitrate–nitrogen after the application of biochar and attributed this to reactions with functional groups on the surface of biochar, reactions with the organo-mineral complex, and non-conventional H-bonding. However, our measurements of mineral nitrogen leaching (Table 5) and nitrate content (Table 3) suggested that these mechanisms did not explain why the addition of biochar reduced nitrogen losses.

Table 7. Comparison of the value of selected PAHs in several biochars (mg/kg).

Anthracene	Acenaphthylene	Fluorene	Benzo(ghi)perylene	Authors	Type of Biochar	Temperature
0.13	0.03	0.07	0.15	Fabbri et al. [36]	Hard wood	Not specified
0.14	0.77	0.24	BLD	Quilliam et al. [37]	Soft wood	450 °C
0.10	0.01	-	-	Wang et al. [38]	Reed	450 °C
0.05	0.01	0.11	0.03	Paneque et al. [39]	Paper sludge	620 °C
0.04	0.05	0.07	0.01		Wood	
0.34	0.01	0.04	0.02		Sewage sludge	
0.58	0.04	0.23	0.02		Grape wine wood	
0.44	0.09	0.84	0.26	Bucheli et al. [40]	Wood	500 °C
0.34	0.24	0.22	0.53	This study		500 °C

Notably, even after 4 years, our biochar-treated soil had higher levels of selected PAHs than the other studies, with the exception of anthracene and fluorene in the study of Bucheli et al. [40] and acenaphthylene in the study of Quilliam et al. [37]. It should be noted that the European Biochar Certificate (EBC) and the U.S. EPA [2] have guidelines for the permissible level of the total of 16 PAHs in biochar (12 mg/kg for "ordinary biochar"). Our biochar was well below this level (4.55 mg/kg). However, the EPA also has a classification of "premium

biochar" (which contains more than 60% organic carbon) with a total PAH level below 4 mg/kg, and our biochar exceeded this level.

The previously reported LD50 values for anthracene (430 mg/kg in mice), acenaphthylene (1700 mg/kg in rats), fluorene (2 mg/kg in mice), and benzo(ghi)perylene (636 mg/kg in rats) confirm high rates of rodent mortality at very low doses of PAH [2]. This supports the hypothesis that the high levels of PAHs in our biochar had a negative impact on soil biota. Moreover, we measured microbial activity after the application of biochar and found that it significantly decreased DHA, cumulative soil respiration, and the leaching of mineral forms of nitrogen (all $p < 0.05$).

4.2. Effect of Biochar on DHA, Leaching of Mineral Nitrogen, and Soil Respiration

Dehydrogenases are among the most important enzymes in the soil environment because they catalyze intracellular hydrogen transfer from organic substrates to inorganic acceptors due to the activity of microbial redox systems. DHA is therefore a good measure of microbial oxidation activities in soils and of the biological oxidation of soil organic matter [40]. Importantly, this enzyme is rapidly degraded after cell death and does not accumulate in the soil [39–41]. We found that DHA decreased with the dose of biochar (Table 5). Chinata et al. [28] and Ameloot et al. [42] reported similar results, and they attributed this decrease to decreased mineralization of C and N. In contrast, Park et al. [43] and Paz-Ferreiro et al. [44] reported significant increases in DHA after the addition of biochar from chicken manure and sewage sludge, but they used container experiments. These authors attributed the increased DHA to improvements in the soil environment, but our experiments reported no improvements in any of the measured soil indicators of biological activity (Table 5).

Ameloot et al. [42] concluded that biochar produced at high temperatures had high porosity and a large active surface, and this led to reduced DHA because it degraded the soil. Lehman et al. [2], Zhou et al. [44], and Mierzwa-Hersztek et al. [45] also recommended against the use of biochar that was produced at temperatures above 400 °C to maintain DHA. Our biochar was produced at 500 °C, which may explain why it reduced the activity of soil microbes. In particular, our DHA was more than 40% lower in the B45 group than in the control group (Table 5).

When considering the effect of biochar on the mineralization of soil organic matter, it is important to consider the "priming effect" (i.e., changes in the dynamics of decomposition of organic matter caused by the addition of other fresh organic matter). We did not consider this phenomenon, because it is generally not important when easily available resources from fresh organic matter are depleted. In the case of biochar, the most easily absorbable sources of organic matter are carbonized, and the priming effect may be masked by the secondary availability of easily mineralizable substances from the dead cells of the previous acidophilic microbial community [2]. This is in line with the results of biochar experiments by Azeem et al. [46], who measured CO_2 production every month for 2 years and attributed the rapid declines during the second year to the depletion of readily available carbon sources and the stabilization of soil organic carbon due to the formation of organo-mineral interactions with biochar. We measured cumulative soil respiration 4.5 years after the application of biochar and found that the highest level was in the control group and the lowest was in the B45 group (Table 5).

We also measured soil respiration (another indicator of microbial activity) directly at the experimental sites for 4 weeks, and the results indicated that respiration decreased as biochar dose increased (Table 5). Lu et al. [47], Lu et al. [48], and Zhang et al. [49], who measured CO_2 production in field experiments where biochar was applied 2 to 5 years previously, reported similar results. These previous studies proposed that most of the biochar became part of the metabolic cascades that led to the stabilization of organic matter in the soil and was not subject to mineralization. Wardle et al. [50] verified this interpretation.

The leaching of mineral nitrogen from soils is very undesirable, and many studies have reported that biochar has the potential to reduce this effect. Our B15 group had less leaching

of mineral nitrogen than the control group, but higher doses of biochar led to increased leaching (Table 5). These results may be because biochar affected multiple interactions between soil organisms and plants, as indicated by our measurements of microbial activity and oilseed rape grain yield, which would otherwise consume available nitrogen (Table 3).

In a study of biochar additions to soil, Zhao et al. [51] concluded that nitrification decreased as acidity increased. Although we found that biochar increased soil pH, it also increased mineral nitrogen leaching. Thus, it is possible that the slight increase of pH in our experiments was insufficient to increase the seed yield of oilseed rape (which would lead to decreased leaching of nitrogen) but was too dramatic to maintain or increase the activity of existing microbes (Tables 3 and 5).

Our results are similar to those of Yang et al. [52]. These authors concluded that the leaching of mineral nitrogen is affected by the physical characteristics (entrapment in biochar pores) and chemical nature (functional groups on the surface of biochar) of biochar and is also strongly influenced by biological processes (i.e., mineralization and subsequent nitrification). Therefore, nitrogen leaching should increase as DHA and cumulative respiration decrease. We found declines in all microbiological indicators relative to the control group. In particular, relative to the control group, the B45 group had an increase of 7.8% in mineral nitrogen leaching, a decrease of 41% in DHA, and a decrease of 18% in cumulative soil respiration.

5. Conclusions

The aim of the present study was to determine the effects of adding different doses of biochar to acid soils in a temperate region at four years after application. In particular, the aim was to determine whether the addition of biochar increased soil pH, oilseed rape seed yield, and microbial activity of the soil. We found that the application of biochar slightly increased the soil pH but did not increase the seed yield of oilseed rape. Addition of biochar also did not increase soil DHA or soil respiration. The leaching of nitrogen was lowest in the B15 group (indicating some benefit of biochar on this parameter), but it increased with higher doses of biochar, probably due to the decreased nitrogen utilization by the crops. Our results indicated that the application of raw biochar to an acidic soil did not lead to increased microbial activity at 4 years after application.

Our study showed that biochar significantly increased soil pH and the leaching of mineral forms of nitrogen; significantly decreased plant biomass, DHA, and cumulative respiration; and had no significant effect on soil mineral nitrogen content. Thus, biochar alone did not increase the microbial activity in soils that had long-term acidity stress. Further research should focus on tropical and subtropical soils, which appear to benefit more from biochar. We also acknowledge certain limitations of our work. We are aware that the results of our work are limited by the fact that the analyzes were not performed in the previous 4 years.

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