ORIGINAL RESEARCH







Biochar application regulating soil inorganic nitrogen and organic carbon content in cropland in the Central Europe: a seven-year field study

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Abstract

Biochar incorporation into soil has shown potential, in enhancing nitrogen fertilizer (N-fertilizer) efficacy and soil organic carbon content (SOC). This study addresses a critical gap in the literature by investigating the effects of biochar addition over a seven-year period (2014–2020) on inorganic N, SOC, and pH in Haplic Luvisol. The research involved a rain-fed field experiment, with a crop rotation comprising spring barley, maize, spring wheat, and pea. Biochar, applied at the rates of 0, 10, and 20 t ha⁻¹ in 2014, was reapplied to specific plots in 2018. Biochar was also combined with N-fertilizer at three level (N0, N1, and N2). Results showed a significant interactive influence of biochar and N-fertilizer combination on NO₃⁻ and NH₄⁺ contents. Intriguingly, the addition of 10 t biochar ha⁻¹ consistently decreased soil inorganic N levels across most of the examined months. Increasing biochar application rates led to a significant rise in pH, establishing a clear, negative correlation between soil pH and inorganic N content. Biochar significantly increased SOC compared to the control, particularly after the reapplication in 2018. However, this effect showed a diminishing trend over time. The study suggests that incorporating biochar treatments may enhance N-fertilizer effectiveness. However, the long-term implications of biochar application with N-fertilizer on N mineralization are specific to individual soil and biochar combinations. Except the application of 20 t ha^{-1} biochar at N2 in 2019, biochar did not affect the crop yields. Studied soil properties, including those influenced by biochar had nuanced impact on different aspects of crop yield.

Article Highlights

- Biochar obtained from mixed paper fiber sludge and the grain husks resulted in a significant increase of SOC over 7 years.
- Biochar aging resulted in a decrease in pH one year after its application in 2015 and 2019.
- The combination of N-fertilizer with biochar caused an improvement in soil inorganic N content in 2014 and reapplication in 2018.

Keywords Field experiment, Biochar, Inorganic nitrogen, Soil organic carbon, Soil pH

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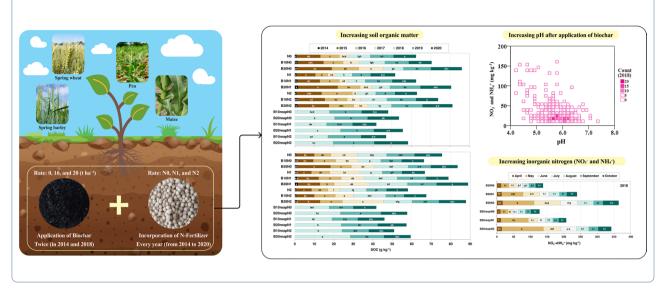
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Graphical Abstract



1 Introduction

The means and intensity of human pressure on land have led to increased CO_2 emissions and have let to a decline in carbon (C) content of soils in recent years (McMillan et al. 2016; Pawlak and Kołodziejczak 2020). The literature review indicated that the application of organic matter would cause an increase in C sequestration (Roß et al. 2022). Moreover, as C fixation increases, there is a corresponding increase in the capture of nitrogen (N) flux in organic matter. This enhanced capture of N in organic matter leads to its turnover, ultimately contributing to plant-available N and promoting greater plant productivity. Therefore, soil organic carbon (SOC) and N are key components of soil fertility, managing their reserves increases soil capability to support plants and ensure food security (Sutton et al. 2022).

Organic and inorganic fertilization is a soil management practice that improves soil and crop productivity (Mahmood et al. 2017). Usually, the application of individual inorganic fertilizer leads to an increase in the soil organic matter in long term, but in some studies no effect was observed (Shen et al. 2004; Dong et al. 2012). However, in some specific cases, an individual N-fertilizer application can cause a decrease of the SOC content which then results in low fertilizer use efficiency contributing to the decline of soil fertility. For instance, Su et al. (2006) observed a significant decrease in SOC content by 18% after inorganic fertilizer application. Biochar is a carbon-rich material obtained by the pyrolysis of different biomass in the partial or complete absence of oxygen (Elnour et al. 2019). The advantages of biochar incorporation have been studied for soil chemical properties such as electrical conductivity (EC), soil pH and cation exchange capacity (CEC) (Yu et al. 2017; Hossain et al. 2020; Sun et al. 2022). Biochar incorporation has been found to further improve N-fertilizer efficacy and SOC content (Neff et al. 2002; Liu et al. 2017; Rehman et al. 2018; Arif et al. 2018; Sial et al. 2019; Dong et al. 2022). The application of biochar together with mineral N fertilizers, has the potential to enhance nitrogen use efficiency (NUE) by decreasing N₂O emissions and NO₃⁻ leaching. This combined approach also improves N uptake by plants (Marcińczyk and Oleszczuk 2022), leading to an increased crop yield compared to the application of synthetic fertilizer alone (Bai et al. 2022; Melo et al. 2022).

Depending on its feedstock and production conditions, biochar usually contains only a certain amount of nutrients. Therefore, for the purposes of instant plant production (just after biochar application), several authors recommended to combine the biochar amendment with application of fertilizer (organic or inorganic). In the long time, high porosity of biochar should lead to the improvement of soil nutrient retention and reduction of nutrient leaching (Blanco-Canqui 2021). Applying biochar with N-fertilizers has been suggested as an effective method for increasing N availability and SOC in soils (Ullah et al. 2020; Peng et al. 2021). The importance of soil pH lies in its ability to significantly impact SOC by regulating the availability of soil nutrients, the turnover of organic matter, and a variety of other soil processes (Kemmitt et al. 2006; Robson et al. 1989). Zhou et al. (2019) observed the strong negative relationship between soil pH and C and N content in the soil. It is essential to understand the mechanisms that may cause change in N-fertilizer

efficacy after biochar application to the soil. For example, inhibited volatilization of N_2O and NH_3^+ by suppressing the enzyme activities of urease, nitrate reductase and nitrite reductase and the microbial activity of denitrifying bacteria (Borchard et al. 2019) is another mechanism by which biochar promotes soil N retention capacity.

So far, several studies showing the changes in the basic chemical soil properties following especially the short-term application of biochar have been published (Toková et al. 2020; Matin et al. 2020; Frimpong et al. 2021). Nevertheless, information on the effect of biochar application with N-fertilizer on NO_3^- and NH_4^+ content, pH and SOC in the field conditions is still limited, particularly in the long-term studies of biochar incorporation into soils (Hernandez-Soriano et al. 2016; Hardy et al. 2019; Kalu et al. 2021). Moreover, the aging effect of biochar on pH and contents of N and SOC in the soils is much less explored in field experiments when compared to short-term application of biochar (Lee et al. 2022; Liu and Chen 2022).

In this study, it was hypothesized that the continuous interaction of paper fibre sludge and grain husks (1:1 ratio) biochar and N-fertilizer will improve inorganic N and carbon content in the soil leading to a permanent ameliorating effect in the chemical properties and soil fertility. This study is a unique investigation about changes of inorganic N, SOC, and pH during 7 years in the rain-fed field experiment on the agricultural land in Central Europe. The novelty of this study is the trend observation of the basic chemical soil properties across the studied period and the evaluation of the need to reapply biochar in the field study along with the continuous N-fertilizer application at rates according to the plant demand to support carbon content and N-fertilizer efficacy. Therefore, this study aims to (i) evaluate the effect of biochar aging and N-fertilizer application on changes in NO₃⁻ and NH₄⁺ contents in the soil for 7 years, (ii) determine the changes of soil pH over 7 years with combined application of biochar and N-fertilizer, and (iii) evaluate the changes of SOC content after the application of biochar and N-fertilizer in the soils over 7 years.

2 Materials and methods

2.1 Site description

The field study was established at the experimental station in the Slovak University of Agriculture located in Dolná Malanta, Slovakia (48° 19′ 23.41″ N, 18° 09′ 0.7″ E). The detailed location of the studied site is presented in Fig. 1 and in previously published literature (Igaz et al. 2018; Horák et al. 2020). The study area is characterized by a warm lowland climate, featuring warm, dry, and long summers, as well as short, dry winters with a very brief duration of snow cover (14–30 days) (Aydin et al. 2020b). The average annual temperature in this area is 9.8 °C and

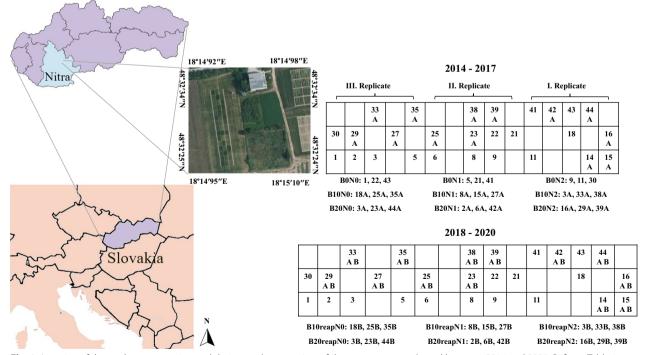


Fig. 1 Location of the study area, experimental design, and an overview of the treatments conducted between 2014 and 2020. Refer to Table 1 for detailed explanations of the abbreviations of the treatments

the total rainfall in this area is 539 mm. The studied soil was identified as Haplic Luvisol based on the Soil Taxonomy (IUSS WRB 2014). The soil texture was a typical silt loam with 15.2% sand, 59.9% silt, and 24.9% clay and it was determined using the pipette method. Before starting the field experiment, the initial soil properties were analyzed from the disturbed soil samples (in March 2014). The initial SOC was 0.91% on average. The initial pH of soil was 5.6 and the total K and P were 300 and 75 mg kg⁻¹, respectively (Horák et al. 2020; Aydin et al. 2020b).

2.2 Experimental design

The experiment began in 2014. The study site was divided into 27 experimental plots, each measuring 6 m×4 m. These plots were separated by 0.5 m and 1.2 m wide protection rows along the length and width of each plot, respectively. The experimental area was subjected to a crop rotation schedule, with spring barley (Hordeum vulgare L. var. Malz) planted in 2014 and 2018, maize (Zea mays L.) planted in 2015 (var. Amanita), 2017 and 2019 (var. LG 30.315), spring wheat (Triticum aestivum L. var. Sensas) planted in 2016, and pea (Pisum sativum L. var. Eso) planted in 2020. In this study, 3 different rates of biochar (0, 10 and 20 t ha^{-1}) were incorporated to the topsoil in March 2014 as part of the initial biochar application event. The respective amounts of biochar were piled on the plots and then manually incorporated into the topsoil using rakes, reaching a depth of 10 cm. In April 2018, for the subsequent biochar reapplication, the plots (6 $m \times 4$ m) that received biochar in 2014 were divided into 2 subplots $(3 \text{ m} \times 2 \text{ m})$ labelled as A and B (Fig. 1). During the reapplication event, biochar was applied exclusively to subplots B. Biochar treatments were also combined with the application of calcium ammonium nitrate ("LAD 27", Duslo Šaľa, Slovakia, containing 27% N) as N-fertilizer at 3 application levels (N0, N1, and N2). Treatments at N0 level were not fertilized during the whole experiment. At N1 fertilization level, the rate of applied fertilizer was adjusted to obtain the average yield of the grown crops. In the case of N2 fertilization level, a higher dose than that in N1 was applied. The exact rate of fertilizer at N1 and N2 fertilization levels varied according to the type of grown crops in different years (Fig. 1, Table 1). The treatments were analyzed in 3 replications. The overview of the field operations is provided in Additional file 1: Table S1.

2.3 Biochar properties

Biochar used in this study was commercially purchased from Sonnenerde company, Riedlingsdorf, Austria. It was produced by the pyrolysis of mixed paper fibre sludge and grain husks (in a ratio of 1:1 by mass) at 550 °C for 30 min in a Pyreg reactor (Pyreg GmbH, Dörth, Germany). The seller also provided information on the biochar properties such as specific surface area that was measured according to DIN 66132/ISO 9277 and the contents of cations (Ca, Mg, K, and Na) which were determined according to DIN EN ISO 11885. The biochar contained 53.1% C, 1.4% N (DIN 51732), 38.3% ash (DIN 51719). The C: N ratio of biochar was 37.9 and biochar pH was 8.8. Biochar contained particles with size ranging between 1–5 mm. The specific surface area of biochar particles was 21.7 m² g⁻¹. Further, biochar contained 57 g Ca kg⁻¹, 3.9 g Mg kg⁻¹, 15 g K kg⁻¹ and 0.77 g Na kg⁻¹ (Horák et al. 2020; Aydin et al. 2020b).

2.4 Soil sampling and analysis

The mixed disturbed soil samples were collected monthly from the top 10 cm surface layer at each plot between April and October from 2014 to 2020. Overall, 243 soil samples were taken from 9 treatments (from 2014 to 2017) and then 15 treatments (from 2018 to 2020). The pH was measured using a pH meter (HI 2211, HANNA Instruments) in a 1:2.5 ratio of soil: KCl. Inorganic N (NO₃⁻ and NH₄⁺) was determined using Yuen and Pollard (1954) method in a K₂SO₄ (1%) solution. Calorimetric procedure was used to determine N contents (NO₃⁻ and NH₄⁺) in isolates by a spectrometer (WTW SPECTROFLEX 6100, Weilheim, Germany). The SOC content was measured twice a year (spring and fall measurement) using Tiurin wet oxidation method (Dziadowiec and Gonet 1999).

2.5 Statistical analyses

The study was conducted from 2014 to 2020 and consisted of 3 rates of biochar application (0, 10, and 20 t ha⁻¹). Initially, the experiment was laid out as a randomized complete block design in a factorial arrangement until 2018 and then, after reapplication of biochar, split plot design was considered in a factorial arrangement with 3 replications. The description of the treatments is shown in Table 1 and Fig. 1. Statistical analysis and data normalization were performed using SAS software version 9.4. The normality of the residuals was tested by Kolmogorov–Smirnov test and the homogeneity of variance was tested by Levene test at 5% significance level. A twoway ANOVA was performed to test the effects of biochar and N addition on inorganic N content and SOC and significant differences were observed at a p < 0.05. Binned scatter plots were employed to illustrate the impact of pH on inorganic N content using IBM SPSS. Regression analysis was used to show the relationship between inorganic N content and soil pH over the studied period 2014 to 2020.

Treatment		2014	2015	2016	2017	2018	2019	2020
		Spring barley	Maize	Spring wheat	Maize	Spring barley	Maize	Pea
N0 (Control)	B (t ha ⁻¹)	_	_	-	_	_	_	_
	N (kg ha ⁻¹)	-	-	-	-	_	-	-
N1	B (t ha ⁻¹)	-	-	-	-	-	-	-
	N (kg ha ⁻¹)	40	160	100	160	40	160	30
N2	B (t ha ⁻¹)	-	-	-	-	-	-	-
	N (kg ha ⁻¹)	80	240	150	240	80	240	45
B10N0	B (t ha ⁻¹)	10	-	-	-	-	-	-
	N (kg ha ⁻¹)	-	-	-	-	-	-	-
B10N1	B (t ha ⁻¹)	10	-	-	-	_	-	-
	N (kg ha ⁻¹)	40	160	100	160	40	160	30
B10N2	B (t ha ⁻¹)	10	-	-	-	_	-	-
	N (kg ha ⁻¹)	80	240	150	240	80	240	45
B10reapN0	B (t ha ⁻¹)	10	-	-	-	10	-	-
	N (kg ha ⁻¹)	-	-	-	-	-	-	-
B10reapN1	B (t ha ⁻¹)	10	-	-	-	10	-	-
	N (kg ha ⁻¹)	40	160	100	160	40	160	30
B10reapN2	B (t ha ⁻¹)	10	-	-	-	10	-	-
	N (kg ha ⁻¹)	80	240	150	240	80	240	45
B20N0	B (t ha ⁻¹)	20	-	-	-	_	-	-
	N (kg ha ⁻¹)	-	-	-	-	_	-	-
B20N1	B (t ha ⁻¹)	20	-	-	-	-	-	-
	N (kg ha ⁻¹)	40	160	100	160	40	160	30
B20N2	B (t ha ⁻¹)	20	-	-	-	-	-	-
	N (kg ha ⁻¹)	80	240	150	240	80	240	45
B20reapN0	B (t ha ⁻¹)	20	-	_	-	20	-	-
	N (kg ha ⁻¹)	-	-	_	-	_	-	-
B20reapN1	B (t ha ⁻¹)	20	-	_	-	20	-	-
	N (kg ha ⁻¹)	40	160	100	160	40	160	30
B20reapN2	B (t ha ⁻¹)	20	-	-	-	20	-	-
	N (kg ha ⁻¹)	80	240	150	240	80	240	45

Table 1 Biochar (B) and N-fertilizer (N) application rates for each treatment during the study period from 2014 to 20

"-" indicates that neither biochar nor N-fertilizer were applied

3 Results

3.1 Changes in soil inorganic N

Results on inorganic N are reported in different subsections according to the same crop-specific N-fertilizer rates in the specific years. Figure 2 shows the observed values of $\rm NO_3^-$ and $\rm NH_4^+$ contents in 2015, 2017 and 2019 when the fertilizer application rates at N1 and N2 were based on the maize crop. The accumulation of inorganic N during the studied 7 months of maize vegetation period in N2 treatment was higher when compared to other treatments in three studied years and the maximum amount was observed in 2019 (761.1 mg kg⁻¹). For the same application of N-fertilizer, the concentration of $\rm NO_3^-$ and $\rm NH_4^+$ decreased when 10 t biochar ha⁻¹ was applied in most treatments, while it increased when 20

t biochar ha⁻¹ was added compared to the control treatment. These increases in NO_3^- and NH_4^+ levels were significantly different in most B20-treated soils (soil treated with 20 t biochar ha⁻¹) compared to B10-treated soils (soils treated with 10 t biochar ha⁻¹) and control treatments for most of the months in 2015, 2017, and 2019 (Fig. 2). The application of 20 t biochar ha⁻¹ could store more NO_3^- and NH_4^+ than 10 t biochar ha⁻¹ in most treatments that received N1 and N2, but in the control treatments without biochar, the inorganic N content was higher than in the biochar-treated soils.

Figure 3 compares the NO_3^- and NH_4^+ contents in soil under spring barley cultivation in 2014 and 2018 with the same amount of N-fertilizer applied. Similar to maize in 2015, 2017, and 2019, the highest

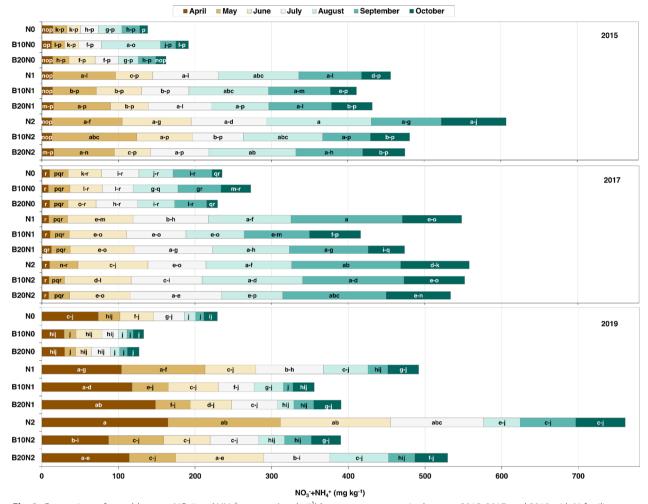


Fig. 2 Comparison of monthly mean NO₃⁻ and NH₄⁺ content (mg kg⁻¹) between treatments in the years 2015, 2017, and 2019 with N-fertilizer application rates based on maize crop. N0: control; B10N0: 10 t biochar ha⁻¹ and without N; B20N0: 20 t biochar ha⁻¹ and without N; N1: without biochar and with 160 kg N ha⁻¹; B10N1: 10 t biochar ha⁻¹ and 160 kg N ha⁻¹; B20N1: 20 t biochar ha⁻¹ and 160 kg N ha⁻¹; N2: without biochar and with 240 kg N ha⁻¹; B10N2: 10 t biochar ha⁻¹ and 240 kg N ha⁻¹; B20N2: 20 t biochar ha⁻¹ and 240 kg N ha⁻¹. Bars followed by at least one same letter do not differ at the confidence level of 5%

cumulative content of NO_3^- and NH_4^+ was observed in the N2 treatments for both 2014 and 2018. Notably, the NO_3^- and NH_4^+ contents for certain treatments with the highest biochar application in 2018 (B20N1, and B20N2) were three to four times higher than the corresponding treatments in 2014. This highlights the significant impact of biochar reapplication on the inorganic N content. Significant differences were noted between treatments for the majority of months in 2018. However, in 2014, significant differences were observed solely in the months of April and June. After the first application of biochar in 2014, the increase in NO_3^- and NH_4^+ content in soil treated with biochar did not fully indicate impact of biochar on soil inorganic N (NO_3^- and NH_4^+) in the first application when compared to control, due to limited effects of biochar in the first application (Hagemann et al. 2017). In 2018, there was a decline in the NO_3^- and NH_4^+ content within B10N0 and B10N1 treatments compared to treatments without biochar. Conversely, these contents increased in the B20N0 and B20N1 treatments. Notably, there was a substantial reduction observed in some months within the N2 treatments as biochar application increased. The cumulative inorganic N content experienced a decrease over a span of 7 months when biochar was applied (specifically in the B10N2 and B20N2 treatments) compared to N2 treatments without biochar addition. During May 2018, noteworthy increases in inorganic N content were evident in N2 and B10N2 treatments compared to other treatments.

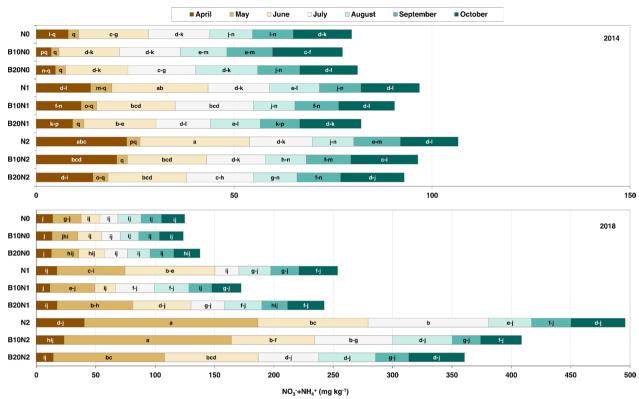


Fig. 3 Comparison of monthly mean NO_3^- and NH_4^+ content (mg kg⁻¹) between treatments in the years 2014 and 2018 with N-fertilizer application rates based on spring barley crop. N0: control; B10N0: 10 t biochar ha⁻¹ and without N; B20N0: 20 t biochar ha⁻¹ and without N; N1: without biochar and with 40 kg N ha⁻¹; B10N1: 10 t biochar ha⁻¹ and 40 kg N ha⁻¹; B20N1: 20 t biochar ha⁻¹ and 40 kg N ha⁻¹; N2: without biochar and with 80 kg N ha⁻¹; B10N2: 10 t biochar ha⁻¹ and 80 kg N ha⁻¹; B20N2: 20 t biochar ha⁻¹ and 80 kg N ha⁻¹; B20N2: 20 t biochar ha⁻¹ and 80 kg N ha⁻¹; B20N2: 10 t biochar ha⁻¹ and 80 kg N ha⁻¹; B20N2: 20 t biochar ha⁻

Similarly, B20N2 also exhibited significant distinctions from most treatments during May. In August and October 2018, there was an increasing trend in inorganic N as biochar increased in most of the treatments (Fig. 3). The lowest levels of NO_3^- and NH_4^+ contents were observed in May 2014, ranging from 0.8 to 7.8 mg kg^{-1} . Conversely, the highest inorganic N content was recorded in the N2 treatments without biochar application across most months, with the exception of August, during which B10N2 displayed a higher inorganic N content compared to N2. The comparison of NO_3^- and NH_4^+ (mg kg⁻¹) content when 3 different rates of N-fertilizer were applied to spring wheat in biochar-treated soil in 2016 is presented in Table 2. For the same N-fertilizer application, the content of NO₃⁻ and NH_4^+ generally increased after biochar application, but these increases varied across different months. For instance, in August 2016, a higher increase in the NO₃⁻ and NH₄⁺ content was observed at the application of 10 t biochar ha⁻¹ compared to 20 t biochar ha⁻¹ application in some treatments. In May 2016, the inorganic N content decreased with biochar application but in

April and October, most treatments showed an increase after biochar addition. In 2016, the maximum content of $\mathrm{NO_3}^-$ and $\mathrm{NH_4}^+$ was observed in the N2 treatment (without biochar addition) across most months, except of June (99.9 mg kg⁻¹) and August (43.0 mg kg⁻¹), when the highest significantly different amount of inorganic N was measured in B10N2 treatment when compared to other treatments.

The effect of N-fertilizer and biochar application on NO_3^- and NH_4^+ (mg kg⁻¹) content is shown in Table 2 when pea was planted in 2020. Across most months, there was an increase in inorganic N content with an increasing biochar rate. We recorded significant differences between treatments that received biochar when compared to control. But there were exceptions such as a decrease in August 2020, when the NO_3^- and NH_4^+ (mg kg⁻¹) content decreased with biochar application while it increased in April and October, like in 2016 for spring wheat. The highest content of NO_3^- and NH_4^+ was observed in different treatments depending on the month, with significant differences between treatments

Table 2 Monthly comparison of NO_3^- and NH_4^+ content (mg kg⁻¹) (mean ± standard deviation) between control and treatments with biochar and N-fertilizer addition in 2016 and 2020 when spring wheat and pea crops were grown, respectively

Treatments	2016*								
	April	Мау	June	July	August	September	October		
N0	18.1±0.9 f	15.7±2.6 f	11.1±1.6 f	17.7±1.0 e	17.9±0.1 e	13.3±3.0 e	13.6±0.8 d		
B10N0	18.4±2.3 f	15.1±3.3 f	10.9±1.4 f	16.2±1.6 e	22.4±4.1 de	17.6±3.2 de	13.3±1.1 d		
B20N0	18.9±1.0 f	14.8±3.4 f	9.6±0.7 f	17.4±3.2 e	20.9±3.4 de	17.6±4.0 de	15.0±4.6 d		
N1	47.3±2.3 e	62.5±4.8 b	50.5±4.9 c	67.1±4.2 b	25.5±2.9 d	24.2±2.0 c	21.8±2.6 c		
B10N1	54.1 ± 1.4 d	34.2±1.4 e	38.1 ± 3.9 d	26.3±4.3 d	32.4±3.4 c	17.7±3.5 de	20.4±1.1 c		
B20N1	46.6±4.6 e	40.4±2.7 d	32.7±2.7 e	39.8±2.8 c	31.9±3.4 c	20.5 ± 2.1 cd	34.6±3.8 b		
N2	129.5±4.2 a	75.0±2.7 a	75.7±3.7 b	87.3±3.4 a	35.6±2.7 bc	42.3±4.6 a	48.0±4.0 a		
B10N2	116.2±4.0 b	63.1±3.8 b	99.9±2.3 a	83.5±1.8 a	43.0±2.7 a	37.3±4.3 ab	42.9±3.0 a		
B20N2	84.5±3.1 c	47.3±2.2 c	79.2±3.0 b	63.7±3.0 b	40.4±3.5 ab	32.2±2.2 b	33.7±2.9 b		
Treatments	2020**								
	April	May	June	July	August	September	October		
NO	12.5±1.1 de	18.9±4.7 e	12.0±1.8 bc	17.8±2.7 cde	20.2 ± 2.0 d	29.7±3.0 de	15.2±1.5 a		
B10N0	11.2±0.8 e	19.2±3.1 e	11.3±3.0 c	17.1±1.6 e	21.9±1.9 cd	24.8±1.1 e	13.2±1.4 a		
B20N0	11.8±1.1 de	18.4±4.5 e	13.5±2.7 abc	19.3±2.7 cde	21.8±2.8 cd	27.6±3.8 de	14.2±2.0 a		
N1	13.5±1.4 cde	60.7±2.5 b	12.0±1.3 bc	23.2±3.1 cd	25.6±2.2 bc	34.5±2.2 abc	14.3±1.1 a		
B10N1	14.3±0.8 cde	42.6±2.1 d	12.3 ± 2.2 bc	19.9±2.2 cde	25.7±1.9 bc	30.7±3.2 bcd	14.7±2.0 a		
B20N1	21.6±4.3 a	52.1±4.4 c	14.7±3.9 abc	21.9±3.9 cde	20.0±1.3 d	35.0±3.3 ab	14.0±0.6 a		
N2	16.3±4.4 bcd	70.4±3.6 a	18.8±2.0 a	28.6±1.1 b	32.2±2.8 a	36.0±2.3 a	14.2±0.8 a		
B10N2	17.3±2.6 abc	66.5±2.7 ab	17.4±4.0 ab	38.2±1.5 a	34.2±3.1 a	35.1±2.1 ab	15.2±1.2 a		
B20N2	19.1±2.5 ab	38.8±2.9 d	12.9±3.0 bc	26.5±5.2 bc	26.7±3.2 b	30.1 ± 2.4 bcd	15.4±2.1 a		

*Nitrogen application rates in 2016: treatments N0, B10N0 and B20N0: 0 kg N ha⁻¹; N1, B10N1 and B20N1: 100 kg N ha⁻¹; N2, B10N2 and B20N2: 150 kg N ha⁻¹

^{**} Nitrogen application rates in 2020: treatments N0, B10N0 and B20N0: 0 kg N ha⁻¹; N1, B10N1 and B20N1: 30 kg N ha⁻¹; N2, B10N2 and B20N2: 45 kg N ha⁻¹. Different letters within the same column in a specific year indicate significant differences between treatments, as determined by two-way ANOVA with *p* < 0.05

that received biochar when compared to the control. For example, in 2020, the maximum content of NO_3^{-1} and NH₄⁺ was observed in April and July for B20N1 and B10N2, respectively. These contents were significantly different from other treatments across all months in 2020 except for October (Table 2). Figure 4 demonstrates the comparison between treatments with single biochar application in 2014 and treatments with biochar reapplication in 2018. The content of NO_3^- and NH_4^+ significantly increased in most N1 application treatments when compared to N0 and N2 in 2019 and 2020 in both types of biochar amendment intervals. There were no significant differences between most treatments with biochar reapplication when compared to treatments with single biochar application in 2014. However, in 2019, significant differences were observed between reapplied biochar treatments (10 t biochar ha^{-1}) and treatments with single application of biochar (Fig. 4) because the application of coupled biochar and N-fertilizer directly influenced nitrification rate.

3.2 Changes in soil pH

A comprehensive comparison of soil pH across all studied months and years in soil treated with biochar and N-fertilizer is provided in Additional file 1: Tables S2 and S3. Additionally, Additional file 1: Fig. S1 showcases the Pearson correlation coefficients between soil pH and NO_3^{-} and NH_4^{+} content for the entire 7-year study period in our designated area. The pH values ranged between 4.0 and 6.3 throughout all the studied years. A consistent negative and significant relationship existed between these two parameters, apart from the years 2014 and 2019. The highest correlation coefficient between these two parameters (r = 0.44; p < 0.01) was observed in 2018 after the reapplication of biochar. Moreover, this outcome indicates that the reapplication of biochar statistically enhanced pH in both 10 and 20 t biochar ha⁻¹ application rates.

In April 2014, just after the first biochar application, the pH in treatments ranged from 5.0 (B0N1) to 6.1 (B20N2) and it generally increased after biochar application. In the following years, the increases were lower than

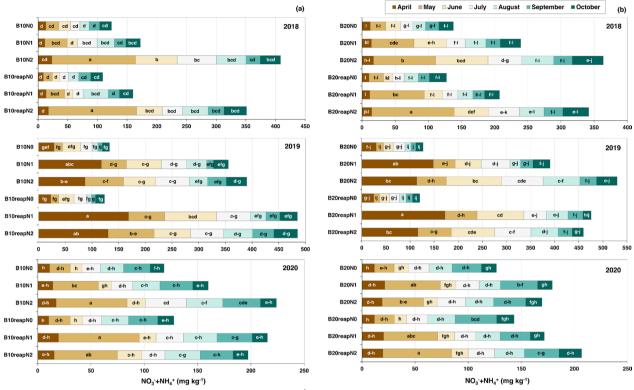


Fig. 4 Comparison of monthly mean NO_3^- and NH_4^+ content (mg kg⁻¹) between biochar and reapplied-biochar treatments with different N-fertilizer rates applied in 2018, 2019 and 2020. Treatments are combination of biochar application and reapplication at (**a**) 10 t ha⁻¹ and at (**b**) 20 t ha⁻¹ with different N-fertilizer rates: N0: control with no soil amendments; N1 and N2: 40 kg N ha⁻¹ and 80 kg N ha⁻¹ in 2018; 160 kg N ha⁻¹ and 45 kg N ha⁻¹ in 2020, respectively. Bars within the same month followed by at least one same letter do not differ at the confidence level of 5%

in the first year, which might be caused by the buffering capacity of soils (Xu et al. 2012). In October 2017, the pH of treatments without biochar ranged from 4.4 up to 5.1 and treatments with 10 t biochar ha⁻¹ application had pH of around 5.0 (4.8 in B10N2 and 5.1 in B10N0). Then, the pH rose by 0.4 to 0.9 units in the treatments that received the second biochar application (in 2018) compared to treatments with single biochar addition (in 2014). This increase continued in most treatments up to 2 years after biochar reapplication. However, the differences were not statistically significant for some treatments (Table S3). Then, the increase in pH dropped in 2020 (2 years after reapplication of biochar). No significant differences in pH were observed in July, June, and August 2017, which showed that the effect of biochar on pH decreased with aging.

Binned scatter data were used to group pH and N inorganic content into specific bins, and an aggregate statistic was employed to summarize the data within each bin (Fig. 5). The highest counts of NO_3^- and NH_4^+ contents were observed in 2016 (20) and 2017 (7) at pH=5.5 and pH=5.1, respectively. These results indicate that the pH range of 5 to 6, which is lower than the initial soil pH before the experiment began, exhibited the highest concentrations of NO_3^- and NH_4^+ .

3.3 Changes in SOC

The changes and comparison of SOC means in biochar and reapplied biochar treatments from 2014 to 2020 are shown in Fig. 6. These contents were measured twice in a year (spring (Fig. 6a) and fall (Fig. 6b)). The results revealed fluctuations in SOC over the course of 7-year period. Notably, for the treatments with biochar reapplication from 2018 to 2020, the extent of these changes demonstrated a decreasing trend. The results of the study also showed that the SOC content ranged from 0.95 up to 1.8 (g kg⁻¹) during the spring of 2014. Following the initial biochar application, no significant changes were observed during the subsequent fall sampling of the same year. However, when biochar was applied at high rate of 20 t ha^{-1} , the SOC content in spring was higher than in fall sampling. This increase in SOC with 20 t ha⁻¹ biochar when compared to 10 t ha⁻¹ biochar application was confirmed by Šimanský and Šrank (2021) who also

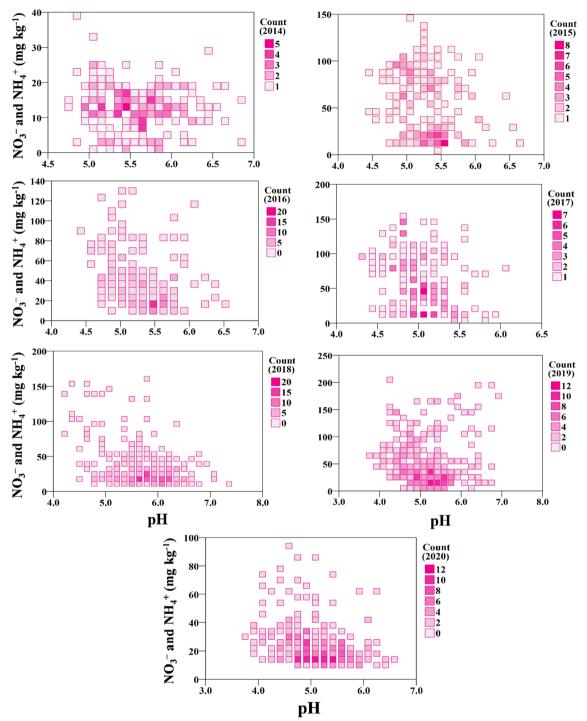


Fig. 5 Changes in pH and NO_3^- and NH_4^+ contents (mg kg⁻¹) across various treatments during the period from 2014 to 2020 depicted through binned scatter plots. Distinct colors represent the frequency of data points within each bin. Increasing color saturation refers to increasing number of bins

reported an increase in SOC content after reapplication of biochar in 2018 and two years after that. The content of SOC in reapplied biochar treatments increased in half of the treatments (0.4 to 115%) when compared to treatments with the first application of biochar. For example, the highest increase was observed in the spring of 2018

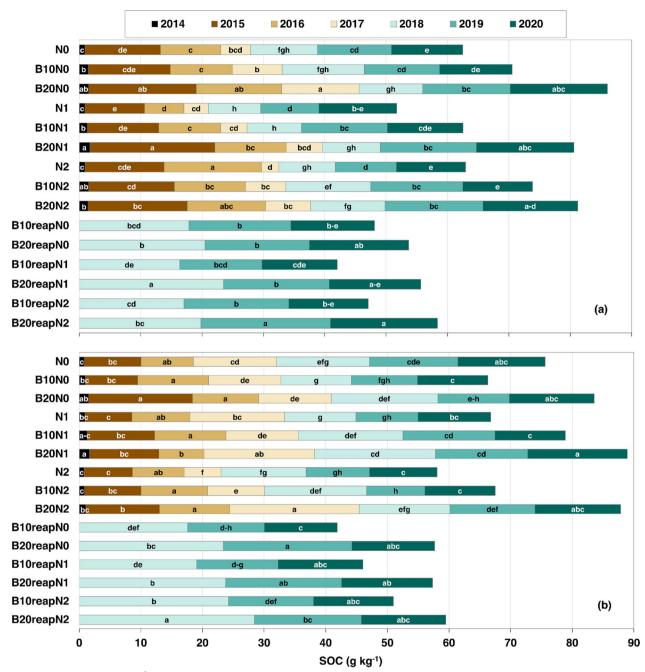


Fig. 6 The SOC content (g kg⁻¹) of treatments with single biochar application and biochar reapplication from 2014 to 2020 measured biannually in (**a**) spring and (**b**) fall. B10 and B20: 10 and 20 t biochar ha⁻¹; B10reap and B20reap: 10 and 20 t reapplied-biochar ha⁻¹ with different rates of N-fertilizer: N0: control; N1 and N2: 40 kg N ha⁻¹ and 80 kg N ha⁻¹ in 2014 and 2018; 160 kg N ha⁻¹ and 240 kg N ha⁻¹ in 2015, 2017, and 2019; 100 kg N ha⁻¹ and 150 kg N ha⁻¹ in 2016; 30 kg N ha⁻¹ and 45 kg N ha⁻¹ in 2020, respectively. Bars within the same sampling period (spring/fall) and year followed by different letter indicate significant differences between treatments, as determined by two-way ANOVA with p < 0.05

for B20reapN1 and B20reapN2 treatments. The largest increase in SOC for 2019 was found in the B10reapN0 treatment. In 2020, the content of SOC increased in four reapplied treatments (B10reapN0, B10reapN1, B10reapN2, and B20reapN2) when compared with the

first biochar application. The increase in SOC due to the reapplication of biochar was also observed during the fall measurement, in most treatments in 2018 and half of the treatments in 2019. In our study, biochar application had both direct and indirect impacts on soil fertility resulting in increased in N and SOC contents, as well as pH. The highest SOC content in 2018 was recorded in B20reapN1 (23.5 g kg⁻¹) (Fig. 6a) and B20reapN2 (28.4 g kg⁻¹) during spring and fall measurements (Fig. 6b), respectively. Biochar-amended treatments maintained a significantly higher SOC content than that of B0 treatments, especially after the reapplication of biochar.

4 Discussion

4.1 Effect of biochar and N-fertilizer application on inorganic N content

The higher content of inorganic N in control treatment is probably related to the surface area and the ability of biochar to sorb NO_3^- and NH_4^+ , which can make them unextractable for a short time (Peng et al. 2021). Badu et al. (2019) confirmed that the application of 10 t biochar ha⁻¹ was more efficient in N uptake and grain yield than the amendment of 20 t biochar ha⁻¹. In addition, Khan et al. (2022) reported improved N-uptake by rice and rapeseed with higher nitrate reductase, glutamate synthase and glutamine synthetase at seedling and flowering stages in soils treated with biochar at 30 t ha⁻¹ compared to a high level of biochar application (60 t ha^{-1}). Active sites and functional groups rapidly increased with the application of biochar, which could improve available forms and sorption of NO₃⁻ and NH₄⁺ by plants from the soil solution and cause a decrease in $\mathrm{NO_3}^-$ and $\mathrm{NH_4^+}$ contents in the soil. In our study, the highest contents of NO3⁻ and NH4⁺ were detected in N2 treatments and these contents were significantly different from the most treatments with biochar application across all studied years (Fig. 2). In April, significant differences were evident between the identical treatments in 2019 and those of other years (2017 and 2015). This divergence is likely attributed to the reapplication of biochar in 2018. When utilizing the highest rate of N-fertilizer and biochar (B20N2), a significant increase was observed in comparison to B20N1 during specific months (August in 2015; October, July, and September in 2017; May, June, July, and August in 2019). However, this significant increase was not observed in the remaining months. A study similar to ours, wherein maize was grown in soil treated with biochar (6 t ha⁻¹) and chemical fertilizers containing N, phosphorus and potassium, revealed a noteworthy increase exceeding 290% in the δ 15N peak within the topsoil after a span of 3 years. This increase facilitated N uptake, marking a significant contrast to the control treatment (Peng et al. 2021). Likewise, Ullah et al. (2020) reported analogous findings. In their investigation conducted on paddy soils, the application of N-fertilizers in conjunction with biochar resulted in significantly higher levels of NO_3^- and NH_4^+ content compared to treatments without such amendments. This approach aimed at enhancing C and N content of the soil through the utilization of N-fertilizer in tandem with biochar. It was repeatedly concluded that biochar plays an important role in enhancing N content due to its high CEC, which has great nutrient retention capability and is generally enhanced with aging (Ippolito et al. 2012; Cayuela et al. 2014; Li-li et al. 2017; Oni et al. 2019). However, the exact mechanisms for NH_4^+ retention are not yet understood. Fuertes-Mendizábal et al. (2019) hypothesized that NH₄⁺ is physically captured in the biochar pore structures, which may be responsible for NH_4^+ retention in soils treated with biochar. Bista et al. (2019) observed that soil NO₃⁻ decreased with an increase in biochar rates when N-fertilizer was applied with biochar because of the wheat shoot and root biomass improvement and higher NO₃⁻ uptake than at individual N-fertilizer application. Similarly, studies on wheat, maize, soybean, and barley have also revealed the beneficial effects of biochar on improving N fertilizer utilization and crop productivity (Agegnehu et al. 2016; Nagappan et al. 2020; Xiu et al. 2021; Dong et al. 2022). The study by Berihun et al. (2017) also showed an increase in total N (0.17%) with application of 12 t ha⁻¹ of biochar for peas compared to the highest rate of biochar application (18 t ha^{-1}). These findings are consistent with the results of the present study as across most of studied months the inorganic N content was lower in treatments with 20 t ha⁻¹ biochar application when compared to treatments with10 t ha⁻¹ (Table 2).

Tian et al. (2017b) and Liao et al. (2020) reported a significant increase in nitrification rates in biochar-treated soils when N-fertilizers were applied during the growing season. However, in contrast to these findings, Li et al. (2021) observed a decreased nitrification rate when urea and biochar were applied together. They suggested that the high CEC and exchange sites for NH_4^+ absorption on biochar might have contributed to this effect. A microbial mechanism inhibited nitrification in the presence of biochar by activating heterotrophs for NH_4^+ consumption and attributing reduced nitrification rates (Martin et al. 2015). Nonetheless, Clark and Tang (2015) reported that biochar might provide NO_3^- and NH_4^+ or enough available N to increase N-mineralization and nitrification by microorganisms during the growing season.

The concentration of inorganic N in the soil, which influences N transportation and assimilation, is a crucial factor to consider during biochar application. Biochar demonstrates the potential to alleviate N deficiency by decomposing organic N or mitigating N toxicity through adsorption (Khan et al. 2023). This action optimizes the N balance in the soil. To regulate and offset the low or high levels of inorganic N in the soil and enhance N uptake and utilization by plants, it is recommended to apply biochar at appropriate levels (Li et al. 2019). Adopting such environmentally friendly fertilization strategies becomes essential to reduce N inputs, enhance NUE, ensure high yields, and promote sustainable agriculture in the future.

4.2 Effect of biochar and N-fertilizer application on soil pH The results have validated our expectations, indicating that incorporating biochar led to an elevation in soil pH compared to the control treatment in the first year. The biochar's alkalinity may be linked to its surface organic functional groups, including phenolic, hydroxyl, and carboxyl groups. These functional groups possess the capacity to absorb protons and swiftly mitigate soil acidity (Geng et al. 2022; Murtaza et al. 2024). In this study area, Juriga and Šimanský (2019) also reported a significant increase (by more than 0.4 units) in pH (measured in H₂O as well as KCl) at the highest rates of biochar application. Results obtained by Oladele et al. (2019) showed that soil pH dropped with an increase in N-fertilizer rates, while it increased with enhancing biochar application. Biochar has the capacity to improve soil acidity due to its alkaline nature and high pH buffering capacity (Dai et al. 2017). Also, soil pH significantly decreased in the presence of individual N-fertilizer during harvest stages in studied soils as reported by Liao et al. (2020). This reduction could be ascribed to the high level of N-fertilizer application because roots uptake NH_4^+ and exchange H^+ to alleviate charge balance. The application of biochar generally enhanced soil pH, and changes were strongly affected by different properties like soil type, feedstock material and the liming amount of biochar (DeLuca et al. 2015; Tian et al. 2017a). According to the study reported by Sarfraz et al. (2017), the combined application of biochar and N-fertilizer significantly decreased soil pH, which could be due to increased N mineralization by N-fertilizer transformation to the NH4⁺. Additionally, Liao et al. (2020) reported that soil pH significantly decreased during the harvest stages in the studied soils in the presence of individual N-fertilizer, which could be attributed to the high level of N-fertilizer application. This process, along with the slow oxidization and decomposition of biochar, can enhance hydrogen (H⁺) concentration in soil solution, causing acidification and a decrease in soil pH by production of acidic functional groups such as carboxyl (Zavalloni et al. 2011; Liu et al. 2014).

4.3 Effect of biochar and N-fertilizer application on SOC content

Our findings are consistent with outcomes from other long-term experiments where the incorporation of plant residue has demonstrated a propensity to elevate SOC and sequester C from the atmosphere (Lal 2004; Lugato et al. 2006). Overall, our study showed that the SOC in reapplied biochar treatments decreased with aging in both sampling events across the years (Fig. 6). Horák and Šimanský (2016) suggested that further biochar application in this area after 2014 could lead to an increase in SOC and positive effects on soil fertility and nutrient retention, which supports the findings of this study. However, further investigation is needed to determine the potential of replacing chemical fertilizers with biochar for plant nutrition support. Berihun et al. (2017) also observed an increase in SOC from 3.35% to 5.05% and 4.89% when 12 t ha⁻¹ or maize cob and lantana biochar, respectively were applied with 2 kg of nitrogen, phosphorus, and sulfur (NPS) fertilizer to soil compared to the control treatments.

The impact of incorporating organic matter (OM) and N-fertilizer on SOC and N content was investigated in various studies (Tian et al. 2017b; Ali et al. 2020). Tian et al. (2017b) explored different mechanisms to explain diverse responses of SOC to OM and N-fertilizers incorporation. Ullah et al. (2020) found a significantly positive correlation between SOC and N in paddy soils after applying N-fertilizers with biochar. Cameron et al. (2013) and Khan et al. (2022) observed an increase in SOC content with the application of biochar and N-fertilizers compared to non-biochartreated soils. Biochar with stable interior structures can improve SOC and reduce the mineralization rate of SOC, enhancing its stability against surface oxidation and microbial degradation. This effect is attributed to the enhanced adsorption and polymerization of the organic molecules to form stable OM through surface catalytic activity (Zhang et al. 2019). Furthermore, Ali et al. (2020) found that the application of biochar and N-fertilizers increased the amount of N metabolism enzymes, including $\mathrm{NO_3}^-$ reductase, glutamine synthetase and glutamine 2-oxoglutarate aminotransferase playing critical roles in the absorption and translocation of soil N.

The beneficial impact of biochar on nitrogen efficacy can be attributed to 2 important mechanisms. Firstly, biochar itself contains N nutrients that undergo gradual release into the soil solution. This phenomenon could contribute to the observed increases in NH_4^+ and $NO_3^$ levels, which were particularly notable in certain treatments of our study. This effect became more pronounced as biochar application rates increased, even when maintained at similar N-fertilizer rates. Secondly, biochar promotes soil N retention, increases SOC, and prevents N loss by improving electrostatic adsorption and retention of NH_4^+ due to biochar's high CEC and large surface area. It can also increase N immobilization by soil microorganisms by providing labile carbon and changing carbon content in the soil (Jeffery et al. 2017; Mehmood et al. 2020; Phares et al. 2022; Li et al. 2023).

4.4 Effect of biochar and N-fertilizer on crop production

Since the establishment of the field experiment in Dolná Malanta in 2014, the application of soil amendments (single biochar, N-fertilizer, and their combination), did not exhibit a significant influence on observed crop yields, except for one notable exception in treatment B20N2 in 2019 (Aydin et al. 2020a). This specific treatment demonstrated a significant increase in crop yield by +71%, compared to the fertilized control treatment N2, and +50% compared to the control without any amendment. Intriguingly, this effect manifested six years after the initial biochar application in 2014.

Šimanský et al. (2019) studied nutrient uptake and the effect of biochar application on soil properties in Dolná Malanta in years 2015 and 2017 and they found that biochar significantly increased soil pH and specific nutrients, especially in treatment with 20 t ha⁻¹ biochar (B20N0). However, variations in macro- and micronutrient content in corn organs indicated complex relationships. Negative correlations occurred between P in corn seeds and total/ available P, while positive correlations linked total K in soil to K in leaves and corn seeds. Higher total Mn in soil correlated with increased Mn in stalks and corn seeds, and positive correlations emerged between total Zn in soil and Zn in corn roots. The authors suggested that the apparent disconnection between soil nutrient availability and plant nutrient uptake may be attributed to the intricate interactions occurring in the rhizosphere. Their study emphasizes the need to consider various factors influencing nutrient cycling, including microbial activity, soil pH, and specific crop demands. Negative correlations with crop yield elements suggest that the impact of biochar on nutrient availability may not uniformly enhance plant growth. Šimanský et al. (2019) reported synergistic and antagonistic effects, highlighting the necessity for a nuanced understanding of how biochar influences nutrient dynamics in diverse soils and cropping systems.

Aydin et al. (2020b) investigated soil properties and yield in Dolná Malanta during the maize and spring barley seasons in 2017 and 2018, respectively. The study revealed positive correlations between grains per ear and SOC. Conversely, average grain weight showed negative correlations with both SOC and soil pH, emphasizing the varied impact of soil properties, including those influenced by biochar, on crop yield. This confirmed the fact that the effect of biochar application on crop yields is generally not as pronounced as that on C sequestration and improvement of soil properties (Blanco-Canqui 2021). The results of this study are consistent with a field experiment conducted by Karer et al. (2013), under

relatively similar climatic (temperate climate zone) but different soil conditions (Cambisols and Chernozems). In the first year after biochar application, Karer et al. (2013) found that despite significant increases in pH and SOC, plant nutrient uptake did not increase significantly and the improvement in soil properties had no effect on the yields achieved for maize and spring barley.

An in-depth analysis of rainfall and average air temperature highlighted suboptimal weather conditions during the 2014–2019 seasons (Aydin et al. 2020b; Aydin 2021). Variability, including warmer periods and uneven rainfall distribution, likely adversely affected crop yields. Water stress, influenced by temperature and rainfall patterns, could impact nutrient availability and susceptibility to pests, contributing to fluctuations in crop yields.

In summary, while biochar application has shown positive effects on soil nutrient content, its impact on nutrient uptake by plants is multifaceted and influenced by a range of factors. In the context of soil amendments, the primary concern for producers is the impact on crop production and resulting profitability. However, it should be noted that biochar does not necessarily act as a universal solution for all ecosystem services, and its effects vary across different parameters evaluated (Blanco-Canqui 2021). Insights from different studies contribute to a more holistic understanding, emphasizing the importance of context-specific assessments to harness the full potential of biochar as a soil amendment for optimizing nutrient availability and enhancing crop productivity.

5 Conclusions

Investigating the effects of biochar as a soil amendment is crucial in predicting the dynamics of N and C and other chemical properties in treated soils with this organic material. Results from a 7-year field experiment showed that adding 10 t biochar ha $^{-1}$ along with N-fertilizer, led to a significant decrease in NO3- and NH4+ content across most months when compared to the 20 t of biochar ha⁻¹ and control treatments. This indicates that optimal biochar application can improve the efficacy of N-fertilizer by increasing the availability of N for plant uptake. Increasing biochar rates also resulted in a rise of soil pH and SOC in soils treated with biochar. The surface and porous structures of biochar can be coated in organic compounds, leading to an increase in SOC. However, this effect diminished over time as the compounds decomposed, highlighting the need for biochar reapplication after a few years. The study also found that biochar had a more significant impact on inorganic N concentration in soils with low organic matter due to the high content of C embedded in the soil matrix. The results suggest that the first application of biochar had a significant influence on the levels of nitrates, ammonium, and organic carbon in the soil. However, the efficacy of field-aged biochar was limited due to microbial and chemical composition, emphasizing the importance of biochar reapplication. Further research could help determine the optimal timing for biochar reapplication in long-term field studies and clarify whether biochar merely increases available N to plant uptake. Climatic conditions during the growing season, rather than biochar type or rates, appeared to have a more significant influence on yield values.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-024-00307-4.

Additional file 1: Table S1. Field management operations in the period 2014–2020 at the Dolná Malanta experimental site. Figure S1. Pearson's correlation coefficients between NO_3^- and NH_4^+ contents (mg kg⁻¹) and soil pH from 2014 to 2020. Table S2. The effect of regular N-fertilizer and single biochar application (in 2014) on soil pH (mean \pm standard deviation) during an observation period from 2014 to 2017. Table S3. The effect of regular N-fertilizer, single biochar application (in 2018) on soil pH (mean \pm standard deviation) during an observation period from 2014 to 2017. Table S3. The effect of regular N-fertilizer, single biochar application (in 2014) and its reapplication (in 2018) on soil pH (mean \pm standard deviation) during an observation period from 2018 to 2020.

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Author contributions

Material preparation and data collection were performed by DI, EA, and JH. Data analyses were performed by NHM. The first draft of the manuscript was written by NHM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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