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## Article

# Addition of Biochar and Fertiliser Drives Changes in Soil Organic Matter and Humic Substance Content in Haplic Luvisol

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**Abstract:** Humic substances (HSs) constitute a primary component of soil organic matter (SOM) and play a crucial role in soil formation and fertility. However, comprehensive information regarding quantitative and qualitative changes in HS following biochar's application to soil still needs to be improved. This study reports on the impact of biochar application at rates of 0, 10, and 20 t ha<sup>-1</sup> (B0, B10, B20), both with and without nitrogen fertilisation at varying levels (N0, N1, N2), on SOM and HS contents throughout the cropping seasons between 2014 and 2019. The findings reveal changes in SOM and HS contents due to biochar addition and fertilisation. Notably, the most substantial increase in soil organic carbon content was observed in the B20N1 and B10N1 treatments, in stark contrast with the reference B0N0 treatment. A decrease in humification of SOM was noted across all treatments involving biochar, whether alone or combined with different N fertilisation levels. An interesting positive change in HS contents was observed in B10N2, where an increase in humic acids and a decrease in fulvic acids enhanced HS stability and improved HS quality. These findings shed light on the intricate dynamics of SOM and HSs in response to biochar application and nitrogen fertilisation over multiple vegetation seasons of crops on loamy Haplic Luvisols in Central Europe.

**Keywords:** soil organic carbon; humic substances; biochar; Luvisols



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## 1. Introduction

The increase in food production and progress toward the elimination of malnutrition since the 1950s has been driven by the mechanisation of ploughing and other farm operations, the introduction of input-responsive varieties, the use of chemical fertilisers along with herbicides and pesticides, the increase in supplemental irrigation, and reliance on information and communications technology [1]. However, the reverse side of this coin is increased soil degradation, which threatens the long-term sustainability of food production and is intricately tied to the application of many agricultural practices. Addressing these challenges requires concerted efforts and innovation to adapt agricultural practices to safeguard current and future food safety and uphold the principles of environmental sustainability [2,3].

Over recent decades, numerous papers have reported on the existence and properties of Terra Preta Anthrosols, renowned for their high fertility [4]. These soils most likely originate from the low-fertility soils of the Amazon Basin region, improved by purposeful human activity in the distant past [5]. The Terra Preta soils are mainly Oxisols, Ultisols, and Inceptisols, with an anthropic A horizon. Terra Preta soils result from a substantial addition of organic waste materials, including kitchen leftovers, excrements, biomass waste, and

charred residues resembling biochar. Soil organisms and fauna partially decompose this organic material, while a significant part remains in the soil for centuries, enhancing its carbon content [6,7]. Terra Preta soils are not the only examples of agricultural soils where humans have increased, rather than degraded, their organic matter content; C-enriched soils with a history of biochar application are found worldwide [8–10].

With the established potential for enhancing the fertility of soils by prolonged supply of charred organic materials, ongoing research aims to derive soil amendments from a diverse range of waste biomass [11]. Driven by the increasing effort to divert human waste away from landfills and energy recovery facilities [12], the supply of organic waste will likely increase in future. Biogenic waste represents a promising feed source for charring and subsequent deposition to soils [11]. Biochar, undergoing testing globally under diverse soil-climatic conditions, is now a promising soil additive. Originating from the pyrolysis process—thermal degradation of organic material without oxygen—biochar holds potential for agronomic and environmental applications [13]. Applying biochar to soil has consistently demonstrated benefits for crop growth [14,15] by enhancing fundamental soil properties relevant to plant growth.

Biochar application directly increases the stable organic carbon content in the soil [16,17]. However, any additional C source added to the soil affects the mineralisation of organic matter that is already present. Known as the priming effect, biochar's addition can enhance or slow down the existing mineralisation rate [18], with varied impacts on the soil environment. Due to its alkaline pH, biochar effectively increases the soil pH, positively influencing its nutrient storage capacity and fostering plants' growth and development [14,19]. Biochar has emerged as a valuable tool for agroecosystem management, offering a plausible means to effectively elevate soil pH in acidic soils [20]. Furthermore, biochar positively affects soil sorption parameters [21], enhancing soil's water-holding capacity, influencing the air exchange between the soil and the atmosphere, and elevating its cation-exchange capacity [22]. The positive impacts extend to supporting microbial biomass and composition, with reciprocal alterations as microbes interact with the biochar [23]. Key studies highlight biochar's role in reducing emissions of CO<sub>2</sub> and N<sub>2</sub>O from agricultural soils to the atmosphere [24–26].

Limited knowledge exists regarding the impact of biochar on the properties of humus in the soil [27,28]. The study of humus, a pivotal component of soil, spans over two centuries [29], yet considerable knowledge gaps persist, especially in explaining the humus formation process. Various theories offer differing perspectives, such as Waksman's lignin-protein theory, Trus's theory, microbial synthesis, and carbohydrate-amine condensation theory [29]. However, a recent study by Lehmann and Kleiber [30] challenges the conventional understanding, suggesting that humus exists but is an ephemeral property emerging from soil's interaction with its solution. In this paper, we adhere to the traditional view and consider humus as an integral part of soil organic matter (SOM). Here, humus forms during humification, wherein dead remains transform, lose their original features, and acquire novel, distinct properties [29,31]. Humic substances within humus perform critical functions in soil formation and its physical, nutritional, chemical, biological, and environmental aspects [32–34]. Greenland et al. [35] emphasised that optimal conditions for plant growth arise in soils with organic substance contents exceeding 2%, which are well humified and mature, with high contents of condensed aromatic compounds. The influence of biochar on quantitative and qualitative humus parameters and the humification process in the soil remains a significant and unanswered question. Understanding changes in humus parameters following the application of biochar is crucial. For example, biochar may accelerate soil nitrogen dynamics, with direct implications for plant productivity [36]. Notably, soil management practices, including nitrogen fertilisation, impact the properties of humus. Jagadamma et al. [37] highlighted that nitrogen application increases mineralisation, negatively affecting the stability of organic substances and overall SOM quality, including humic substances.

As previously noted, humus is generally considered to be stable in soil, compared to freshly deposited or undecomposed soil organic matter [38,39]. However, we posit that introducing biochar into the soil may disrupt the humus formation process, ultimately impacting its content in the soil. Upon incorporation into the soil, biochar becomes involved in the formation of humic substances, thereby altering the quality of the humus. This field study aimed to quantify the effects of applying biochar, with and without nitrogen fertilisation, on soil organic matter (SOM) and humus parameters over six years. We hypothesise that (H1) a higher biochar application rate will result in a larger increase in humus content, and (H2) an interaction of biochar and nitrogen fertiliser will impact the humification process. Lastly, we hypothesise (H3) that the effect of biochar's addition on the humification process would diminish over time.

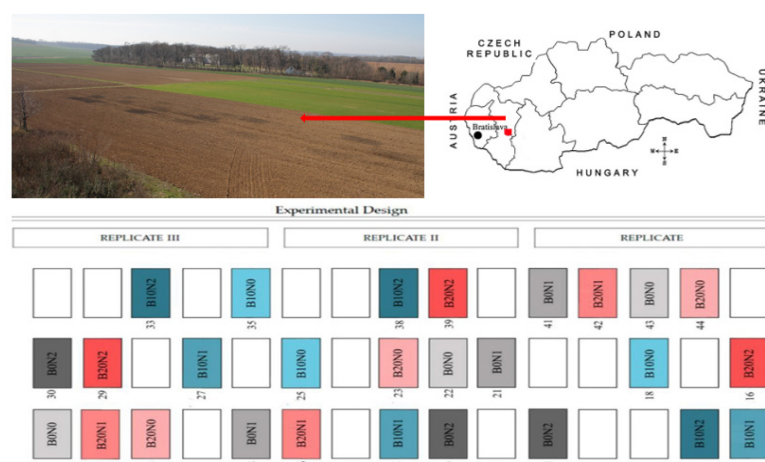
## 2. Materials and Methods

### 2.1. Site Description

The field experiment took place at the Dolná Malanta Experimental Station of the Slovak University of Agriculture in Nitra, Slovakia (latitude 48°19'00"; longitude 18°09'00"). Situated east of Nitra, on the Žitavská upland, the experimental area is flat terrain with a slight southward incline. This location features a warm lowland climate, warm summers, and brief, dry winters. The long-term average air temperature and precipitation (1991–2020) were 10.7 °C and 559 mm, respectively. The geological substratum comprises earlier rocks rich in fine materials. Young Neogene deposits include diverse clays, loams, and sand gravels overlaid with loess in the Pleistocene epoch. The soil in the experimental field is classified as a Haplic Luvisol according to the World Reference Base for Soil Resources, based on the whole soil morphology profile [40]. The properties of the 0–20 cm soil layer before the experiment setup, on average, were as follows: clay—249 g kg<sup>−1</sup>, silt—599 g kg<sup>−1</sup>, sand—152 g kg<sup>−1</sup>, soil organic carbon—9.13 g kg<sup>−1</sup>, CEC—142 mmol kg<sup>−1</sup>, base saturation—85%, and soil pH<sub>KCl</sub>—5.71.

### 2.2. Experimental Design

The field experiment was initiated in March 2014, comprising three replicate plots for each treatment. The 27 plots, each measuring 4 × 6 m (24 m<sup>2</sup>), were randomly distributed across the experimental field (Figure 1). The soil addition treatments were established as follows: application of biochar at rates of 0, 10, and 20 t ha<sup>−1</sup> without N fertilisation (B0N0, B10N0, and B20N0, respectively), biochar at the same rates of 0, 10, and 20 t ha<sup>−1</sup> in combination with the lower level of N fertilisation (B0N1, B10N1, and B20N1, respectively), and finally, biochar at 0, 10, and 20 t ha<sup>−1</sup> with higher N fertilisation (B0N2, B10N2, and B20N2, respectively).



**Figure 1.** Location of the study area and treatments.

In 2014, biochar was applied manually to the soil surface using rakes in all relevant plots and then incorporated into the 0–10 cm soil layer using a combinator. The three rates of N fertilisation (N0, N1, and N2) were applied separately from the biochar, annually added to the soil surface of each fertilised plot, as relevant for the crop rotation. The biochar utilised in the experiment was produced from a mixture of paper fibre sludge and grain husks (1:1 *w/w*) through pyrolysis at 550 °C for 30 min in a Pyreg reactor (Pyreg GmbH, Dörth, Germany). The final biochar, as declared by the company (Sonnenerde, Austria), contained an average of 531 g kg<sup>−1</sup> of total organic C, 14 g kg<sup>−1</sup> of total N, a C:N ratio of 37.9, 57 g kg<sup>−1</sup> of Ca, 3.9 g kg<sup>−1</sup> of Mg, 15 g kg<sup>−1</sup> of K, and 0.77 g kg<sup>−1</sup> of Na. It had a specific surface area of 21.7 m<sup>2</sup> g<sup>−1</sup>, an ash content of 38.3%, a pH of 8.8, and particle sizes ranging from 1 to 5 mm. In N-fertilised treatments, a standard N fertiliser, calc-ammonium nitrate with dolomite (LAD 27), was used at the two N fertilisation levels mentioned above (N1 and N2). LAD 27 is a granular mineral fertiliser containing 27% N (13.5% NO<sub>3</sub><sup>−</sup> and 13.5% NH<sub>4</sub><sup>+</sup>), 7% CaO, and 5% MgO. The pellets had a size range of 2–5 mm. Using the balance method, the N doses for the first level were calculated based on the average crop nutrient demand. The second N level added a further 100%, 50%, 50%, 50%, 100%, and 50% N in 2014, 2015, 2016, 2017, 2018, and 2019, respectively (Table 1). The field experiment followed an annual crop rotation sequence: spring barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), spring wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), spring barley (*Hordeum vulgare* L.), and maize (*Zea mays* L.) in 2014, 2015, 2016, 2017, 2018, and 2019, respectively.

**Table 1.** An overview of the application of biochar and N fertiliser in all combinations of soil amendment treatments across the six years of the experiment.

Year and Sown Crop	2014 Spring Barley		2015 Maize	2016 Spring Wheat	2017 Maize	2018 Spring Barley	2019 Maize
Soil Amendment	Biochar (t ha <sup>−1</sup> )	Fertiliser (kg ha <sup>−1</sup> )	Fertiliser (kg ha <sup>−1</sup> )	Fertiliser (kg ha <sup>−1</sup> )	Fertiliser (kg ha <sup>−1</sup> )	Fertiliser (kg ha <sup>−1</sup> )	Fertiliser (kg ha <sup>−1</sup> )
Treatments							
No fertilisation: N0							
B0N0	0	0	0	0	0	0	0
B1N0	10	0	0	0	0	0	0
B2N0	20	0	0	0	0	0	0
Fertilisation: N1							
B0N1	0	40	160	100	160	40	108
B1N1	10	40	160	100	160	40	108
B2N1	20	40	160	100	160	40	108
Fertilisation: N2							
B0N2	0	80	240	150	240	80	162
B1N2	10	80	240	150	240	80	162
B2N2	20	80	240	150	240	80	162

### 2.3. Soil Sampling and Analysis

Soil samples were collected from depths of 0–20 cm across all treatments. Sampling occurred monthly throughout the growing season of planted crops from 2014 to 2019. Specifically, sampling took place in 2014 at 1, 2, 3, and 4 months after biochar application; in 2015 at 13, 14, 15, 16, 17, and 18 months; in 2016 at 26, 27, 28, and 29 months; in 2017 at 38, 39, 40, 41, 42, and 43 months; in 2018 at 50, 51, and 52 months; and in 2019 at 64, 65, 66, 67, and 68 months. Standard soil analysis methods were employed to measure SOM and humus parameters. Soil organic carbon (C<sub>org</sub>) was determined using the wet combustion method, involving the oxidation of SOM by a mixture of 0.07 mol L<sup>−1</sup> H<sub>2</sub>SO<sub>4</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, followed by titration using Mohr's salt [41]. The group and fraction composition of humic substances (HSs) were determined using a method reported by Belchikova and Kononova, involving extraction with a mixture of 0.01 mol L<sup>−1</sup> Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 10 H<sub>2</sub>O, and 0.1 mol L<sup>−1</sup> NaOH [41]. The light absorbance of HSs and humic acids (HAs) at 465 and 650 nm was

measured with a Jenway 6400 Spectrophotometer to calculate the colour quotients of humic substances ( $Q^{4/6}_{HS}$ ) and humic acids ( $Q^{4/6}_{HA}$ ).

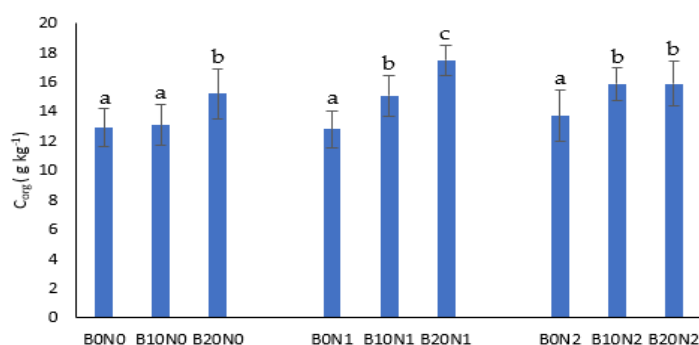
#### 2.4. Statistical Analysis

The data analysis utilised ANOVA tests implemented in the software package Statgraphics Centurion XVI (Statpoint Technologies, Inc., Washington, DC, USA). Comparisons between samples were conducted using Tukey's test at a probability level of  $p = 0.05$ . Single linear regression analyses were employed to assess trends in SOM and humus parameters during the vegetation seasons of planted crops from 2014 to 2019.

### 3. Results and Discussion

#### 3.1. Effect of Biochar on Soil Organic Matter

The local soil type is Haplic Luvisol, which represents about 11% (265.4 thousand ha) of the total agricultural land in Slovakia [42] and approx. 500–600 mil. ha [40] worldwide; this experiment is thus informative for a substantial proportion of arable soils. Biochar stands out as a widely employed soil ameliorant, recognised for its role in elevating soil organic carbon ( $C_{org}$ ) levels [43,44], a trend substantiated by our 6-year study results (Figure 2).



**Figure 2.** Soil organic carbon (average for 2014–2019,  $\pm$  standard deviation). Letters indicate homogeneous groups according to Tukey's procedure at a 0.05 significance level. Different letters between columns indicate that treatment means are significantly different at  $p < 0.05$  according to Tukey's test.

Observations over the six years revealed a significant increase in  $C_{org}$  in non-fertilised treatments, with the B20N0 treatment experiencing a significant increase of  $2.46 \text{ g kg}^{-1}$  compared to its control (B0N0).  $C_{org}$  shows an increasing trend with higher biochar application rates, as reported by numerous studies. The accumulation of  $C_{org}$  in soil is influenced by soil management practices and is generally a gradual process, primarily noticeable in the long term, albeit with variations across experimental sites [45]. About 90% of non-pyrolised carbon entering tilled soil is typically released within a year [46]. However, given that biochar is a source of stabilised organic carbon [43],  $C_{org}$  contents will substantially increase following its addition; this effect is typically observable even after a single application treatment. We saw partial evidence of this process, with our results revealing that the B0N0 and B20N0 treatments significantly increased  $C_{org}$  by 80 and  $222 \text{ mg kg}^{-1} \text{ year}^{-1}$  over the six years of vegetation seasons, respectively (Table 2). Interestingly, changes in  $C_{org}$  can also occur as a result of changes in soil pH, such as after applying alkaline biochar to an acidic soil. As reported Aydin [14] in a paper on the same experiment, the soil was acidic to start with, and after biochar application its pH significantly increased to neutral. The range of pH observed in this experiments, from slightly acidic to neutral, is likely suitable for most soil microorganisms [47], and SOM increases as a result of plant material deposition and microbial activity.



**Table 2.** Trends of SOM parameters' distribution in the biochar treatments for the vegetation seasons of 2014–2019.

Treatments	Equations	R <sup>2</sup>	Trend	Probability	Treatments	Equations	R <sup>2</sup>	Trend	Probability
C <sub>org</sub>					HS ration in C <sub>org</sub>				
B0N0	$y = 0.08x + 11.6$	0.2940	increase	**	B0N0	$y = -0.38x + 44.5$	0.4944	decrease	***
B10N0	$y = 0.04x + 12.5$	0.0982	increase	n.s.	B10N0	$y = -0.26x + 41.9$	0.2995	decrease	**
B20N0	$y = 0.22x + 12.5$	0.5375	increase	***	B20N0	$y = -0.54x + 40.0$	0.5178	decrease	***
B0N1	$y = -0.04x + 13.4$	0.0667	decrease	n.s.	B0N1	$y = 0.02x + 39.1$	0.0019	increase	n.s.
B10N1	$y = -0.01x + 15.2$	0.0015	decrease	n.s.	B10N1	$y = -0.02x + 33.1$	0.0020	decrease	n.s.
B20N1	$y = -0.03x + 18.0$	0.0323	decrease	n.s.	B20N1	$y = 0.002x + 28.7$	0.0004	increase	n.s.
B0N2	$y = -0.16x + 16.2$	0.2589	decrease	**	B0N2	$y = 0.29x + 35.3$	0.1592	increase	*
B10N2	$y = -0.08x + 17.1$	0.1062	decrease	n.s.	B10N2	$y = 0.10x + 30.7$	0.0418	increase	n.s.
B20N2	$y = -0.06x + 16.8$	0.1337	decrease	*	B20N2	$y = 0.14x + 28.5$	0.1332	increase	*
Share of HA in C <sub>org</sub>					Share of FA in C <sub>org</sub>				
B0N0	$y = -0.33x + 25.6$	0.6211	decrease	***	B0N0	$y = -0.05x + 19.0$	0.0216	decrease	n.s.
B10N0	$y = -0.28x + 24.6$	0.5315	decrease	***	B10N0	$y = 0.02x + 17.1$	0.0050	increase	n.s.
B20N0	$y = -0.40x + 22.7$	0.6655	decrease	***	B20N0	$y = -0.14x + 17.3$	0.1080	decrease	n.s.
B0N1	$y = 0.05x + 19.6$	0.0206	increase	n.s.	B0N1	$y = -0.03x + 19.7$	0.0030	decrease	n.s.
B10N1	$y = -0.05x + 18.7$	0.0303	decrease	n.s.	B10N1	$y = 0.03x + 14.5$	0.0086	increase	n.s.
B20N1	$y = -0.03x + 15.8$	0.0109	decrease	n.s.	B20N1	$y = 0.03x + 12.8$	0.0166	increase	n.s.
B0N2	$y = 0.15x + 17.8$	0.1178	increase	n.s.	B0N2	$y = 0.14x + 17.4$	0.0987	increase	n.s.
B10N2	$y = 0.06x + 16.4$	0.0291	increase	n.s.	B10N2	$y = 0.04x + 14.3$	0.0114	increase	n.s.
B20N2	$y = 0.08x + 14.6$	0.0865	increase	n.s.	B20N2	$y = 0.06x + 13.9$	0.0456	increase	n.s.

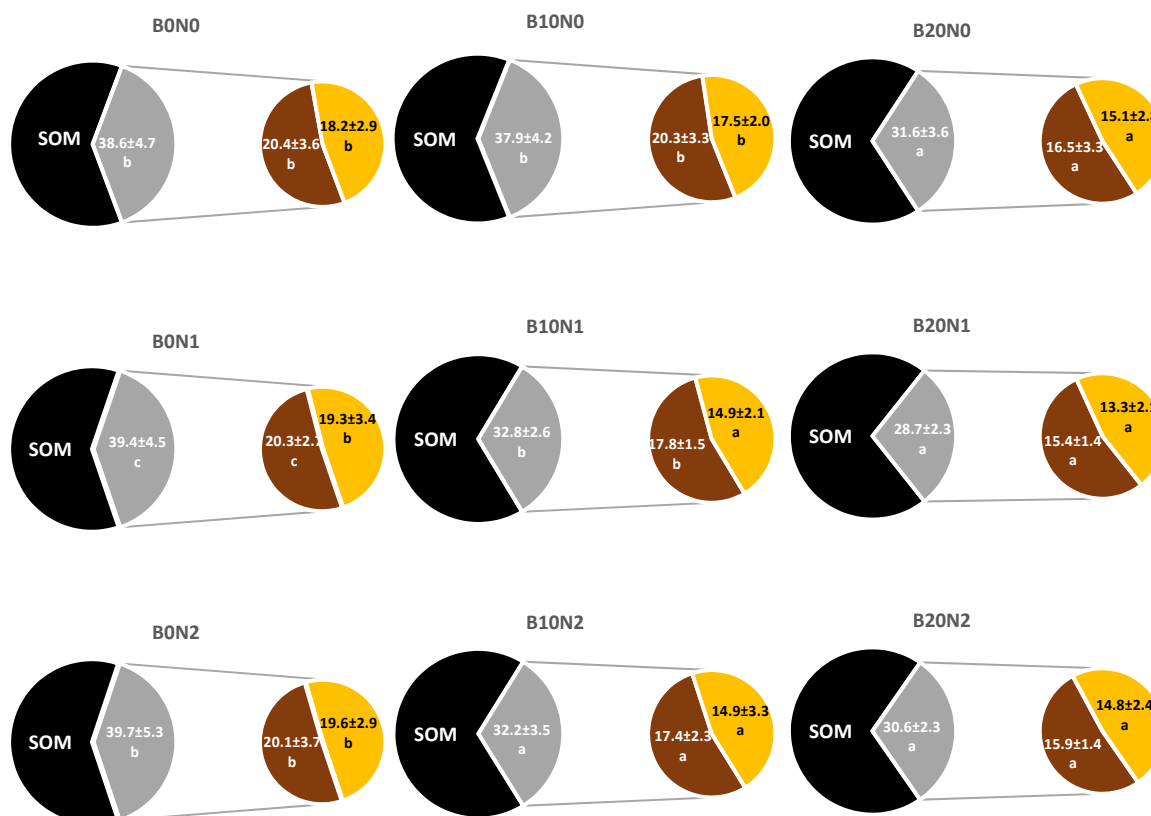
Notes: n.s.—non-significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ;  $y = C_{org}$ , share of HS in C<sub>org</sub>, share of HA in C<sub>org</sub>, and share of FA in C<sub>org</sub> distribution with time  $x =$  months during the vegetation seasons of 2014–2019 ( $n = 30$ ).

Combining biochar addition with nitrogen fertilisation may be a promising practice for improving the sustainability of agriculture. One of the reasons for this is a decrease in the soil's carbon-to-nitrogen (C:N) ratio observed elsewhere [48]. Accordingly, we explored the effects of varying nitrogen fertilisation intensity in conjunction with biochar addition. C<sub>org</sub> exhibited significant increases in B10N1 ( $15.1 \pm 1.38 \text{ g kg}^{-1}$ ) and B20N1 ( $17.5 \pm 1.02 \text{ g kg}^{-1}$ ) compared to B0N1 ( $12.8 \pm 1.25 \text{ g kg}^{-1}$ ), as well as in B10N2 ( $15.9 \pm 1.11 \text{ g kg}^{-1}$ ) and B20N2 ( $15.9 \pm 1.48 \text{ g kg}^{-1}$ ) compared to B0N2 ( $13.7 \pm 1.75 \text{ g kg}^{-1}$ ) (Figure 2). While the first level of nitrogen fertilisation, both alone and in combination with biochar, showed no significant linear trend in C<sub>org</sub> changes over the six years of vegetation seasons (Table 2), the second level of N fertilisation resulted in a noteworthy linear decline in C<sub>org</sub> in the B0N2 and B20N2 treatments. Our results indicate that the B0N2, B10N2, and B20N2 treatments reduced C<sub>org</sub> by 159, 78, and 61 mg kg<sup>−1</sup> year<sup>−1</sup> over the six years of vegetation seasons, respectively. This means that a high dose of N alone or in combination with a higher dose of biochar contributes to more intensive use of SOM or more labile fractions of the organic matter of the biochar itself by soil microorganisms [49], resulting in SOM transformation. The distinctive impact of nitrogen fertilisation on C<sub>org</sub> changes after biochar's application suggests a dependency on the application rates. Notably, a higher biochar rate combined with the second nitrogen level more effectively suppressed C<sub>org</sub> mineralisation in the soil than lower biochar rates under the same nitrogen level (N2). This is consistent with findings by Yang et al. [50], who concluded that higher nitrogen fertilisation levels, especially when applied as manure, accelerated the decline of soil organic matter compared to lower nitrogen levels. This effect can be attributed to priming effects, where nitrogen fertilisation stimulates microbial activity, allowing the use of the most labile organic fraction of biochar by microbes, temporarily replacing native C<sub>org</sub> as a C source and, eventually, reducing native C<sub>org</sub> mineralisation [51].

Overall, the application of biochar in 2014 at a rate of 20 t ha<sup>−1</sup> without nitrogen fertilisation (B20N0) led to a significant decrease in extractable humic substances (HSs) in soil organic matter (SOM), compared to the B0N0 and B10N0 treatments (Figure 3). The reduction in HSs in the B20N0 treatment was linked with a significant decrease in the amounts of both humic acids (HAs) and fulvic acids (FAs) in SOM within the same treatment. The most substantial decline in extractable HSs over the six years, including HAs and FAs in SOM, was evident in the B20N0 treatment, followed by B0N0 and B10N0 (Table 2). Significant negative linear relationships were observed between C<sub>org</sub> and HSs

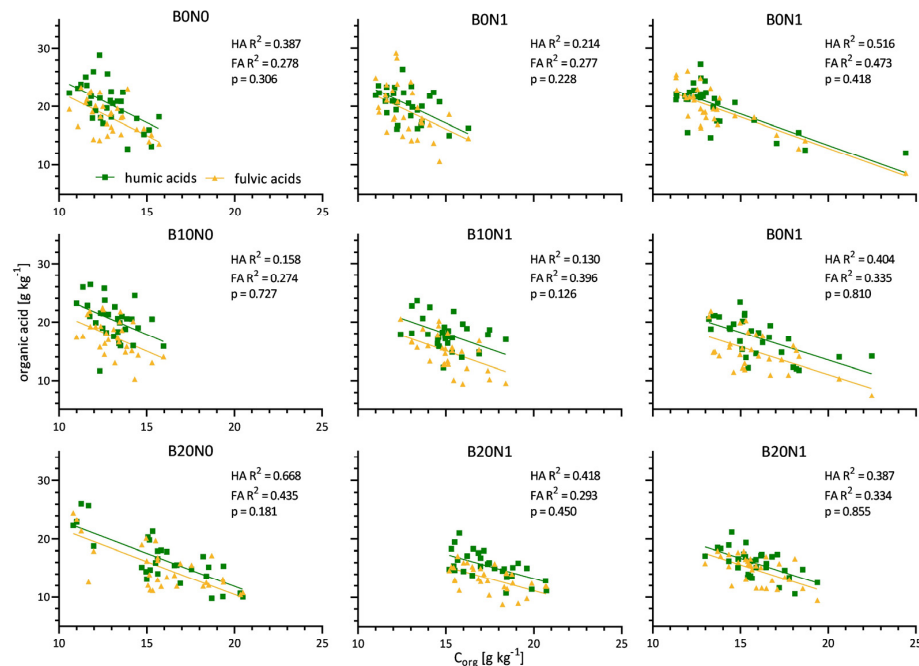


in SOM (Figure 4), and the strength of the mutual relationship decreased in the following order: B20N0 > B0N0 > B10N0. These results predominantly indicate mineralisation of SOM and elimination of humification between  $C_{org}$  and  $C_{HS}$ , but no significant linear trend was determined. These findings imply that a higher dose of biochar, rather than a lower dose, decelerates the humification of SOM after biochar is applied to the soil. This is consistent with the findings of Aydin et al. [14], who observed a similar decreasing trend in the extraction of HSs in SOM after applying  $20 \text{ t ha}^{-1}$  of biochar without nitrogen fertiliser.



**Figure 3.** Soil organic matter parameters (average for 2014–2019,  $\pm$ standard deviation). The letters indicate homogeneous groups according to Tukey's procedure at a 0.05 significance level. Different letters between slices in graphs of the same colour in rows indicate that treatment means are significantly different at  $p < 0.05$ .

Combining a higher rate of biochar with both the first and second levels of nitrogen (B20N1 and B20N2, respectively) resulted in a significant decrease in the extraction of HSs, including HAs and FAs (Figure 3). Higher  $C_{org}$  contents corresponded to lower extraction of HSs, HAs, and FAs in SOM in biochar treatments combined with both levels of nitrogen fertilisation (Figure 4). The mineralisation of soil organic carbon can also be inhibited through the sorption of labile carbon onto the biochar surface, subsequently forming relatively stable organic substances [52]. Overall, the extraction of HAs in SOM across all nine treatments did not exceed 40% and ranged from 16.5 to 20.4%. A level of 40% indicates a very high degree of SOM humification, while the range of 10–20% suggests a low level of humification [53]. In our soils, even after applying biochar, organic matter primarily prevailed over humus, meaning that most of the SOM mineralised at the expense of humus formation. Even in unfertilised soil (control), it was constantly replenished through root exudates, plant and root residues, the biomass of microorganisms, etc. [39].



**Figure 4.** The contents of humic and fulvic acids in soil samples modified by adding biochar and fertiliser, as a function of total organic carbon content (C<sub>org</sub>); *p* values represent differences between the slopes of linear fits.

### 3.2. Effects of Biochar on Humic Substances

Applying organic matter to arable soils, including biochar, has great potential to increase, stabilise, and improve the amount and quality of humic substances in the soil. Predicting the effects of biochar's addition on HSs is challenging due to its wide-ranging properties [54] and its application to diverse soil types under different climatic conditions. For this reason, little is known about its effects on the speed of humification and the creation of humic substances or their stabilisation in the soils. Several authors have stated that biochar application holds significant potential for forming and stabilising soil humic substances [27,28,55–60]—a conclusion supported by our research, but only in the case of both biochar doses combined with the second level of nitrogen fertilisation (Table 3).

**Table 3.** Humus parameters (average for 2014–2019, ±standard deviation).

Treatments	C <sub>HS</sub>	C <sub>HA</sub>	C <sub>FA</sub>	C <sub>HA</sub> :C <sub>FA</sub> Ratio	Q <sub>HS</sub> <sup>4/6</sup>	Q <sub>HA</sub> <sup>4/6</sup>
	g kg <sup>-1</sup>					
B0N0	4.96 ± 0.34 a	2.62 ± 0.37 a	2.38 ± 0.37 a	1.15 ± 0.29 a	4.63 ± 0.46 a	3.88 ± 0.35 a
B10N0	4.99 ± 0.31 a	2.67 ± 0.35 a	2.29 ± 0.35 a	1.21 ± 0.35 a	4.54 ± 0.44 a	3.85 ± 0.29 a
B20N0	4.85 ± 0.37 a	2.51 ± 0.39 a	2.33 ± 0.42 a	1.17 ± 0.44 a	4.61 ± 0.41 a	3.90 ± 0.31 a
<i>p</i> -value	0.2702	0.2742	0.7033	0.8181	0.6685	0.8597
B0N1	5.01 ± 0.35 a	2.58 ± 0.33 a	2.44 ± 0.48 a	1.12 ± 0.33 a	4.76 ± 0.53 a	3.95 ± 0.38 a
B10N1	4.95 ± 0.25 a	2.70 ± 0.35 a	2.25 ± 0.37 a	1.26 ± 0.38 a	4.61 ± 0.41 a	3.93 ± 0.33 a
B20N1	4.95 ± 0.35 a	2.62 ± 0.33 a	2.42 ± 0.66 a	1.19 ± 0.28 a	4.67 ± 0.45 a	3.96 ± 0.40 a
<i>p</i> -value	0.7221	0.3888	0.3026	0.2529	0.4305	0.9628
B0N2	5.28 ± 0.28 c	2.68 ± 0.34 ab	2.60 ± 0.12 b	1.05 ± 0.23 a	4.88 ± 0.48 b	4.07 ± 0.38 a
B10N2	5.07 ± 0.30 b	2.75 ± 0.43 b	2.33 ± 0.43 a	1.23 ± 0.27 b	4.63 ± 0.28 a	3.98 ± 0.39 a
B20N2	4.82 ± 0.29 a	2.50 ± 0.30 a	2.32 ± 0.15 a	1.11 ± 0.26 ab	4.66 ± 0.21 a	3.94 ± 0.36 a
<i>p</i> -value	0.0000	0.0235	0.0034	0.0534	0.0327	0.4408

Notes: C<sub>HS</sub>—carbon of humic substances, C<sub>HA</sub>—carbon of humic acids, C<sub>FA</sub>—carbon of fulvic acids, Q<sub>HS</sub><sup>4/6</sup>—colour quotient of humic substances, Q<sub>HA</sub><sup>4/6</sup>—colour quotient of humic acids. The letters indicate homogeneous groups according to Tukey's procedure at a 0.05 significance level. Different letters between rows indicate that treatment means are significantly different at *p* < 0.05.

However, if it is applied at a dose of 10 or 20 t ha<sup>−1</sup> alone, or in combination with the lower level of N fertilisation, to Haplic Luvisols in a mild climate, it has no significant effect on the humic substances in the soil. On the other hand, the B10N2 and B20N2 treatments resulted in significantly lower contents of humic substances ( $5.07 \pm 0.30$  and  $4.82 \pm 0.29$  g kg<sup>−1</sup>, respectively) and fulvic acids ( $2.33 \pm 0.43$  and  $2.32 \pm 0.15$  g kg<sup>−1</sup>, respectively), compared to the reference B0N2 treatment ( $C_{HS}$ :  $5.28 \pm 0.28$  g kg<sup>−1</sup>,  $C_{FA}$   $2.60 \pm 0.12$  g kg<sup>−1</sup>). The B10N2 treatment contributed to a higher content of humic acids than the B20N2 treatment. In B10N2 and B20N2, the  $C_{FA}$  content was significantly reduced by 10 and 9%, respectively, compared to B0N2, which is positive because higher fulvic acid contents may contribute to faster leaching of heavy metals and alkaline cations from soil, compared to situations when the share of  $C_{HA}$  predominates. Fulvic acids are very easily soluble, with high levels of migration in soil profiles; they dissociate in solutions much more strongly than humic acids and show a strongly acidic pH [29,31,61]. This suggests that biochar, in conjunction with the higher nitrogen level, decreased the contents of humic substances. However, nitrogen fertilisation at the second level was a stabilising element for humic substances in the soil. By stabilising humic substances after applying both doses of biochar with the higher nitrogen level, the quality of humus in the soil was increased, more strongly in the B10N2 than the B20N2 treatment (Table 3). In all treatments, the colour quotient values indicated more humified and mature organic matter in the soil, with high contents of condensed aromatic compounds and a low representation of aliphatic compounds [31,62]. The molecular weight and degree of condensation of humic substances increased after applying biochar, but this was only statistically significant in the cases of B10N2 and B20N2 compared to B0N2.

#### 4. Conclusions

Our research highlights the potential of biochar to enhance soil organic carbon contents, representing a significant step towards sustainable agriculture. The investigation emphasised that biochar, especially at higher application rates, influences the composition of humic substances, contributing to the complex formation mechanisms of stable soil organic substances. Moreover, this study demonstrates the intricate relationship between biochar, nitrogen fertilisation, and the soil's ability to form humic substances, albeit potentially impacted by the specific soil and climate conditions. The biochar treatment at the rate of 10 t ha<sup>−1</sup> with an increased level of N fertilisation was the optimal solution for reducing mineralisation and supporting humification, thus increasing the quality of humic substances and their stabilisation in Luvisols. In the future, it will be essential to observe the evolution of the biochar's effects over the years after its application to the soil, as the extent of the effects of biochar and its combination with N fertilisation on SOM and humus may change over time.

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