Changes in quantity and quality of organic matter in soil after application of poultry litter and poultry litter biochar—5-year field experiment

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Abstract
Changes of the addition of poultry litter (PL) and poultry litter biochar (PLB) on quantitative and qualitative humus parameters in loamy sand were estimated during the 5-year study period. The following properties were determined in soil: pH, total carbon (Ctotal), total nitrogen (Ntotal), humic and fulvic acids, extracted carbon, and non-hydrolysing carbon. Additionally, light absorbance in the solutions of humic acids was computed at the wavelength of 280, 465, and 665 nm. It was demonstrated that organic matter mineralisation was most intense in soil with the addition of PL, causing significant quantitative and qualitative changes in humus compounds in soil. A slower rate of organic matter mineralisation was observed in soil amended with PLB, especially in a dose of 5.0 t ha⁻¹, which indicated the long-term effect of this material on improving soil properties. Spectrophotometric indexes for the solution of humic acids also showed that PLB had a more favourable effect on the structure durability and lower mobility of humic acid carbon compared with PL. The application of PL and PLB significantly increased the non-hydrolysing carbon content in soil, indicating greater stabilisation of humus compounds and, at the same time, lower CO₂ emissions. It was found that the addition of organic materials to soil significantly increased the soil organic carbon contents. Our study has shown that the identification of changes that may occur in the quantitative and qualitative composition of soil humus after the application of PLB may be helpful in determining the appropriate biochar dose.

Keywords Soil • Humic substances • Biochar • Organic carbon • Carbon stocks

1 Introduction
Soil organic matter (SOM) has a positive effect on soil sorption and buffering properties and biological activity; it stabilises soil structure, consequently limiting its degradation [1]. Due to the multi-directional, beneficial effects of organic matter in soil, solutions that could improve soil richness in SOM have been sought for many years. Application of various organic materials, including waste, is one of the activities potentially increasing OM resources.

Undoubtedly, a significant amount of organic waste is generated on farms and during processing of poultry feedstock. In Poland, the poultry industry is one of the fastest growing segments of the domestic agri-food sector. Poland is a significant poultry producer in Europe and in the world. It is estimated that the production of poultry livestock in Poland in 2018 amounted to over 3.5 million tonnes and was by 4.4% higher than in 2017 [2]. Therefore, the poultry industry development generates more and more organic waste, including significant amounts of poultry litter. Maintaining the current pace of poultry production requires limiting the negative effect of farms on the environment and increasing farmers’ awareness of how to manage faeces and waste from poultry farms [3]. Improper application of poultry litter directly to the soil may pose both chemical and biological hazards to the environment.
Poultry litter is rich in many components that can be dispersed in the environment; for instance, they can be leached into groundwater or cause soil salinity. Due to the possible presence in poultry litter of, among others, pathogenic microorganisms, the material is likely to create health risks. Environmental problems may also result from the storage of poultry litter for several months, releasing a significant load (e.g., of ammonia) into the atmosphere [5]. Considering the above hazards, thermal conversion processes, such as pyrolysis, become a safe alternative for the environmental application of materials alike [1].

According to Shakya and Agarwal [6], thermal processing of poultry litter increases its stability and safety for human health. The physical properties of the converted feedstock, e.g., specific surface area, are also positively affected [7]. The thermal conversion of poultry litter to biochar facilitates the material storage and is a very important direction of environmental resource retardation.

Biochar is carbon-rich material produced from the pyrolysis of biomass feedstock materials under partial or total absence of oxygen [8, 9]. Biochar consists mainly of C (50–90%), volatile substances (0–40%), and mineral substances (0.5–5%). Biochar has a porous carbon structure, containing nano-scale condensed aromatic rings for high specific surface area. Generally, biochar derived from high-temperature pyrolysis is characterised by a large surface area and aromatic carbon content, which may increase the adsorption capacity (a desirable property for bioremediation) as well as the recalcitrant character (for carbon sequestration) [10]. The physical parameters are strongly related to the type of the initial feedstock used and pyrolysis conditions (i.e., temperature, residence time, and pressure). In most cases, as the temperature increases, the specific surface area, pore volume, pH value, and cation exchange capacity increase and, in turn, the contents of volatile elements such as C, N, and S decrease [10–12]. The chemical composition of biochar is stable, and it shows low susceptibility to degradation and microbiological decomposition.

As stated by Jeffery et al. [13] and Yusof et al. [14], application of biochar to soil increases the nutrient content for plants, promotes C sequestration in soil, supports water retention, and increases the microbial activity, soil sorption capacity, and heavy metal ion immobilisation. Biochar is widely recognised as an efficient tool for carbon sequestration and reduces nitrous oxide (N₂O) emission and CH₄ emission [8, 9]. Chemically stable C fractions of biochar contribute to the soil C sequestration and may have an important role for the global C budget. The relative amount of recalcitrant and labile compounds and the degree to which the organic compounds are protected from decomposition determine soil organic matter degradability after biochar addition [7]. In addition, taking into account biochar ageing in soil and the resulting changes in its properties, it is fully justified and necessary to monitor the material effect on soil properties in experiences conducted for many years. There are also data reporting no significant change or even negative effects after biochar application [15]. Also, it should be emphasised that the number of studies on the effect of biochar on the humus compound transformation is very small. Considering the constantly increasing interest in biochar in the context of improving soil fertility and productivity, it is very important to assess the effect of this material on the quantitative and qualitative transformations of humus compounds in soil. This study hypothesises that the application of biochar from poultry litter together with mineral fertilisation stabilises the quantity and quality of humus compounds in soil, promoting the long-term C storage in it.

2 Materials and methods

2.1 Experiment location

The experimental field was located in southern Poland (50° 08.404′ N; 19° 85.362′ E), and the experiment was carried out in the years 2014–2018 (five growing periods). The microplot area was 1 m². The soil in the experiment location was qualified as typical Eutric Cambisols with the granulometric composition of loamy sand (FAO World Reference Base for Soil Resources 1998). The field experiment was established in the spring of 2014 at the Experimental Station of the Agricultural University in Krakow. Laboratory analyses of the study material were carried out at the Department of Agricultural and Environmental Chemistry at the University of Agriculture in Krakow.

2.2 Climate conditions

The total annual precipitation in 2014 was 639 mm. The highest precipitation was recorded in May (110 mm), July (100 mm), and August (97 mm), while the lowest in 2014 occurred in February (17 mm) and December (23 mm). In 2018, the total annual precipitation was 672 mm. The highest precipitation was recorded in January (95 mm) and July (93 mm), while the lowest in February (10 mm), April (13 mm), and March (16 mm). The average temperatures in 2014 ranged from −0.9°C in January to 17.5°C in July, while in 2018, the lowest average monthly temperature of −3°C was recorded in February, and the highest of 20.6°C in August. The air temperatures and precipitations are shown in Fig. 1. Meteorological conditions were recorded using an automatic meteorological station located in Krakow Mydlniki.

2.3 Pyrolysis process and properties of soil, poultry litter, and poultry litter biochar

The pyrolysis process was carried out at a station designed for biomass conversion, under a limited supply of air (1–2%). Temperature in the combustion chamber was 300 ± 10°C, and...
exposure time was 15 min. The process parameters have been configured so that Ctotal losses are as low as possible. The quality of poultry litter biochar depends on the peak pyrolysis temperature. To obtain biochar from poultry litter for agricultural purposes, pyrolysis at 300 °C is recommended, since the resulting biochars feature, among other things, higher cation exchange capacity and increased content of carbon compounds [16]. Additionally, biochars produced at a temperature above 300 °C contain a much smaller quantity of aliphatic carbon compounds and functional groups, which may significantly reduce the effectiveness of these materials in improving the soil quality [17]. Increasing the temperature above 500 °C reduces the obtained amount of biochar and also leads to a significant loss of Ctotal and Ntotal contents [18].

Physical and chemical properties of the soil before the experiment, poultry litter (PL), and poultry litter biochar (PLB) are presented in Table 1. The attached (see graphic abstract) as graphical abstract. In order to obtain biochar, the pretreated material (dried at 70 °C, ground in a laboratory mill, and 4 mm sieved) was thermally converted at 300 °C in a combustion chamber for 15 min. A detailed methodology of analyses performed in organic materials and soil before the experiment was presented in the studies of Mierzwa-Hersztek et al. [19, 20].

2.4 Experiment design

The following treatments were carried out: C: control soil without fertilisation; MF: NPK fertilisers; PL+MF: MF + poultry litter in a dose of 5 t ha⁻¹; PLB 2.25+MF: MF + biochar in a dose of 2.25 t ha⁻¹; and PLB 5.0+MF: MF + biochar in a dose of 5 t ha⁻¹. The experiment included 3 replicates for each variant. Due to the need to create comparable conditions in the experiment, the same mineral fertilisation was used in all treatments, except control (total component doses): 100 kg N ha⁻¹, 40 kg P ha⁻¹, and 120 kg K ha⁻¹. A dose of phosphorus was used once in the form of enriched triple phosphate, before harvesting the first swath. Potassium was used in the form of potassium salt, and nitrogen in the form of ammonium nitrate. The doses of potassium and nitrogen were divided into three equal parts. Organic materials (PL, PLB) and mineral fertilisers were used once in the first year of the experiment (spring 2014) by mixing them with a 0–10-cm layer of soil, and then, the seeds of a grass pasture mixture of Phleum pratense ‘Erecta’ 15%, Festuca pratensis ‘Ardeinia’ 10%, Festuca arundinacea ‘Alix’ 10%, Lolium perenne ‘Victorian’ 20%, Lolium perenne ‘Solen’ 20%, Lolium multiflorum ‘Gaza’ 10%, Trifolium pratense ‘Dajana’ 5%, and Lolium westervoldicum ‘Movester’ 10% in the amount of 60 kg ha⁻¹ were sown, and a rolling operation was carried out. The increased sowing of the mixture was aimed at better covering the experiment surface.

2.5 Soil sampling and analysis

Soil samples were collected from each plot (1 m²), from the 0–10-cm soil layer, after the 1st year (8 months after the application of organic materials) and after the 5th year (54 months after the application of organic materials), after growing was finished. For chemical and physicochemical analyses, soil samples were dried and 1 mm sieved. The pH values were determined electrochemically (CP-505 pH meter), while the EC values conductometrically (CPC-502 conductometer), maintaining the material:water ratio of 1:2.5. The total carbon (Ctotal) and nitrogen (Ntotal) in soil were determined using the CNS analyser (Vario EL Cube, Elemental). Humus compound content was extracted from soil by a mixture of 0.1 mol dm⁻³ Na₂P₂O₇ solution + 0.1 mol dm⁻³ NaOH. Humic acid carbon (Cha) was isolated in the extract of 0.1 mol dm⁻³ Na₄P₂O₇ solution + 0.1 mol dm⁻³ NaOH. In turn, fulvic acid carbon (Cfa) was calculated from the difference between the amount of carbon in the extract (Cext) and the amount of humic acid carbon (Cha) in the extract [21]. The extraction residue—non-hydrolysing carbon (Cnh)—was computed from the difference between the Ctotal and the amount of carbon in the extract [22]. Light absorbance was then measured in the obtained solutions of humic acids at the

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**Fig. 1** Climatic conditions in the experiment location from 2014 to 2018
wavelength of 280, 465, and 665 nm, and the colour ratio (A2/6, A2/4, and A4/6) was computed. It has been assumed that the value of A464 determines the absorbance of substances at the initial stage of humification, and A664 determines the absorbance of substances with a high degree of humification [23, 24].

On the basis of Ctotal content (in %) and volumetric density of soil ($\rho_{\text{cinM g}^{-3}}$), organic carbon stocks ($ZC$) were calculated after the first and after the fifth year of the experiment at the 0–10-cm soil layer [25]:

$$ZC = \frac{C_{\text{total}}}{\rho_{\text{cinM g}^{-3}}}$$

(1)

where $ZC$ is the soil carbon stock (in Mg C ha$^{-1}$), $C_{\text{total}}$ is the carbon content (mg C g soil$^{-1}$), $\rho_{\text{cinM g}^{-3}}$ is the soil density (bulk density in Mg m$^{-3}$), with depth of the soil layer in cm.

### 2.6 Statistical analysis

The differences between each treatment and the control, as well as between individual treatments, were evaluated using one-way analysis of variance (ANOVA, Duncan’s test, $\alpha \leq 0.05$). Variation within treatments was determined by calculating the standard deviation (±SD) values. All statistical analyses were performed using Statistica PL 13 software (StatSoft Inc.).

### 3 Results and discussion

#### 3.1 Effect of biochar on soil pH and electrical conductivity

After the first year of the experiment, the pH H$_2$O values (Table 2) determined in soil with the addition of MF, PLB 2.25+MF, and PLB 5.0+MF were higher compared with the values determined in the C and MF treatments. Regardless of the treatment, soil pH H$_2$O values determined after the fifth year of the experiment were smaller compared with the values determined after the first year. Comparison of contents obtained after the first and fifth years of the experiment showed that the pH value decreased the most in the soil of PLB 2.25+MF (by 13.4%) and the least in the soil of the C treatment (by 1.8%). A similar trend was found after analysing the pH values determined in KCl.

The determined values of electrical conductivity (EC) are shown in Table 2. The results obtained allowed to state the increase in the EC values in all treatments after the fifth year of the experiment, regardless of fertilisation applied, compared with the values obtained after the first year. In the C, MF, and PL+MF treatments, the EC values increased by over 300%, while in the PLB 2.25+MF and PLB 5.0+MF treatments, the
increase was 109% and 190%, respectively. These EC values showed that the addition of both doses of biochar caused a much smaller increase in the soil electrical conductivity over a five-year period compared with treatments without biochar. However, it should be noted that the determined EC values did not limit plant growth and development.

Agbna et al. [26] discovered a reduced pH (by 27%) after applying different doses of wheat straw biochar. These authors also showed that the EC values in soil decreased with increased biochar doses. On the other hand, in their pot experiment, Chan et al. [27] determined increased pH, 6 weeks after biochar application. As stated by the authors, these changes resulted from introducing with biochar a significant load of basic cations. The deacidifying effect of poultry litter biochar and increased soil EC were demonstrated by Sikder and Joardar [28].

3.2 Effect of biochar on Ntotal and Ctotal content

According to Lehmann [10], the addition of biochar to soil can affect Ntotal retention in soil and thus limit its leaching to groundwater. The analysis of Ntotal content in soil (Table 3) obtained after the first and fifth years of experiment allows observing an increase in Ntotal content in all treatments. However, the largest increase in Ntotal content (by 12%) was found in PLB 2.25+MF. Ntotal content obtained in the PLB 2.25+MF soil was 4% higher after the first year and 13% higher after the fifth year than that in the C treatment. Ntotal contents obtained in the PLB 5.0+MF treatments were slightly smaller than those in the control, while the smallest contents after both the first and fifth years of experiment were determined in MF treatment. However, it should be emphasised that the experiment involved supplementary N fertilisation in the amount of 100 kg N ha\(^{-1}\) for each treatment, except for C treatment. Moreover, the perennial grass mixture yield (N uptake), which was collected from each treatment, played a large role in the N cycle. The study of Zhan et al. [29] revealed increased total carbon content by 27.6% and Ntotal content by 75.6% in soil amended with rice straw biochar after the fourth year of the experiment. The mechanism of increasing the C and N contents concerns mainly the difficult to degrade compounds of both elements that are present in BC and, therefore, the long residence of BC in the soil. In the case of nitrogen, there may be a risk of its protonisation; i.e., microorganisms may build up in the protein structures of the microorganisms, so that N is not taken up or leached from the soil.

In our study, Ctotal (Table 3) after the first year of the experiment ranged from 8.67 (MF) to 9.90 g kg\(^{-1}\) DM (PLB 2.25+MF). After the fifth year of the experiment, regardless of the fertilisation applied, the Ctotal contents increased significantly compared with those obtained after the first year. The highest (13.62 g kg\(^{-1}\) DM) contents after 5 years were determined in PL+MF, and the Ctotal increase over these 5 years was 53.2%. The lowest Ctotal content (10.95 g kg\(^{-1}\) DM) was found in the treatment with only MF treatment applied. The study showed that the Ctotal contents in the soil of PL+MF, PLB 2.25+MF, and PLB 5.0+MF after the fifth year of the experiment were significantly higher than those in the C and MF treatments. In the results presented by Agbna et al. [26], the Ctotal content in treatments with different doses of biochar was 1.2 to 1.7% higher compared with that in the C treatment. Abbasi and Anwar [30] observed an increase in carbon content by 82% after applying 30 t ha\(^{-1}\) of PLB. On the other hand, the study of Bhattarai et al. [31] revealed a 35% increase in Ctotal content after applying 10 t ha\(^{-1}\) of PLB. The results presented by Sikder and Joardar [28] indicate an increase in Ctotal content by 55% after the application of 5 t ha\(^{-1}\) of PL, by 47% after applying 2 t ha\(^{-1}\) of PLB, and by 100% when 4 t ha\(^{-1}\) dose of PLB was used. In our study, the highest Ctotal content after the fifth year of the experiment was determined in the treatment fertilised with PL. In the study of juriga et al. [32], the Ctotal content in soil increased significantly after the addition of biochar in a dose of 20 t ha\(^{-1}\), which is several times higher than doses used in our study.

The C:N ratio is a value that reflects the organic matter humification level. Bednarek et al. [33] showed that the organic matter decomposition was faster when the Ctotal to Ntotal ratio value was lower. It resulted from the higher content of N which is a building component of microbial biomass. After the first year of the experiment, the C:N ratio values
The extracted carbon contents (Cext) after the first year of the experiment ranged from 5.70 to 6.37 g kg\(^{-1}\) DM and, after the fifth year, from 3.52 to 5.85 g kg\(^{-1}\) DM (Table 4). Comparing the results obtained after the first year and the fifth year of the experiment, it was found that the Cext content significantly increased in treatments fertilised with PL+MF (by 4%) and PLB 5.0+MF (by 19%). However, it should be noted that this fraction is represented by simpler organic compounds with low molecular weight, which are most labile in soil and susceptible to mineralisation.

### 3.3 Effect of biochar on C extracted with Na\(_4\)P\(_2\)O\(_7\)+NaOH, C humic acid, C fulvic acid, and C non-hydrolysing content

The extracted carbon contents (Cext) after the first year of the experiment ranged from 5.70 to 6.37 g kg\(^{-1}\) DM and, after the fifth year, from 3.52 to 7.82 g kg\(^{-1}\) DM (Table 4). Comparing the results obtained after the first year and the fifth year of the experiment, it was found that the Cext content significantly increased in treatments fertilised with PL+MF (by 4%) and PLB 5.0+MF (by 19%). However, it should be noted that this fraction is represented by simpler organic compounds with low molecular weight, which are most labile in soil and susceptible to mineralisation.

The humic acid carbon (Cha) contents determined in soil after the first year of the experiment ranged from 1.85 (C) to 2.74 g kg\(^{-1}\) DM (PLB 2.25+MF) (Table 4). After the fifth year of the experiment, a significant increase in the Cha content was also found in the PL treatment (by 17%). In turn, the Cha contents in C, PLB 2.25+MF, and PLB 5.0+MF after the fifth year were lower than those determined after the first year, by 32%, 45%, and 28%, respectively. The reduced Cha content may indicate the transformation of this carbon fraction into more complex compounds with lower mobility [21]. On the other hand, the reduced Cha content may be due to the use of this carbon fraction by microorganisms or its displacement to deeper layers of the soil profile. Our study revealed a significant effect of the used poultry litter on increasing the Cha content compared with the treatment in which only mineral fertilisation was used.

The Cfa contents determined after the first year of the experiment ranged from 3.26 to 4.03 g kg\(^{-1}\) DM and, after the fifth year, from 3.52 to 5.85 g kg\(^{-1}\) DM (Table 4). Comparing the Cfa contents obtained after the first and fifth years of the experiment, their significant increase was observed in treatments with the addition of biochar: in PLB 2.25+MF by 15% and in PLB 5.0+MF by 34%. However, in the MF and PL treatments, the Cfa contents slightly decreased. The Cfa contents in all treatments were higher than the Cha contents, both after the first and fifth years of the experiment. Cfa content are easily soluble in water, and this determines their mobility and ability to move deeper into the soil profile. Greater Cfa contents may lead, among others, to faster leaching of heavy metals and alkaline cations from soil (distribution of complex metal connections with humic substances) [7].

The Cnh contents after the first year of the experiment ranged from 2.59 (PL+MF) to 3.91 g kg\(^{-1}\) DM (PLB 2.25+MF and PLB 5.0+MF) (Table 4). In our study, after the fifth year of the experiment, the Cnh content significantly increased in each of the treatments and regardless of the fertilisation applied. The increase in Cnh content after 5 years in individual treatments was 27% for C, 49% for MF, 63% for PL+MF, 35% for PLB 2.25+MF, and 13% for PLB 5.0+MF.

| Table 3 | The content of Ntotal and Ctotal in soil and C:N ratio after the 1st and 5th years of the experiment |
|---------|----------------------------------|
| Treatment | After the 1st year | After the 5th year | After the 1st year | After the 5th year | After the 1st year | After the 5th year |
|          | Ntotal (g kg\(^{-1}\) DM) | Ctotal (g kg\(^{-1}\) DM) | Ntotal (g kg\(^{-1}\) DM) | Ctotal (g kg\(^{-1}\) DM) | Ntotal (g kg\(^{-1}\) DM) | Ctotal (g kg\(^{-1}\) DM) |
| C        | 1.09b ± 0.04 | 1.12bc ± 0.06 | 9.61b ± 0.12 | 10.77c ± 0.40 | 8.82a ± 0.16 | 9.62cd ± 0.47 |
| MF       | 0.96a ± 0.05 | 1.06b ± 0.03 | 8.67a ± 0.13 | 10.95c ± 0.20 | 9.03ab ± 0.31 | 10.33cd ± 0.35 |
| PL+MF    | 1.11bc ± 0.04 | 1.20cd ± 0.08 | 8.89a ± 0.11 | 13.62e ± 0.46 | 8.01a ± 0.26 | 11.35d ± 0.74 |
| PLB 2.25+MF | 1.13bc ± 0.06 | 1.26d ± 0.05 | 9.90b ± 0.14 | 11.72d ± 0.15 | 8.76a ± 0.07 | 9.30bc ± 0.48 |
| PLB 5.0+MF | 1.08b ± 0.03 | 1.10b ± 0.02 | 9.59b ± 0.17 | 11.54d ± 0.12 | 8.88a ± 0.33 | 10.49bc ± 0.25 |

± standard deviation, n = 3; means marked with the same letters do not differ significantly according to Duncan’s test at α < 0.05; factor: fertilisation × year
Based on the results obtained, it can be stated that fertilisation with PL and PLB in a dose of 2.25 t ha\(^{-1}\) in conjunction with MF had the greatest effect on the C\(\text{nh}\) contents.

### 3.4 Biochar effect on the value of humic to fulvic acid ratio and the optical properties of humic acid

The Cha:Cfa ratio is one of the basic indicators to assess the quality of soil organic matter and, above all, its stability. It is generally accepted that higher Cha:Cfa ratio values are typical for more fertile soils [37]. Changes in the carbon content in humic and fulvic acids after fertilisation consequently altered the Cha:Cfa ratio value (Table 5). The results obtained after the first year prove that soil in treatments with organic materials had significantly higher Cha:Cfa ratios than the soil from the C treatment and MF treatment. The results also indicate that the fertilisation applied increased the Cha:Cfa ratio in most treatments (MF, PL+MF, and PLB 2.25+MF) after the fifth year of the experiment and, in others, did not affect this ratio at all. The analysis of changes in the Cha:Cfa ratio in the experiment revealed that the most beneficial effect on improving the organic matter quality after 5 years had treatment with the addition of poultry litter, where a significant increase in Cha:Chf (by 23% compared with 2014) was noted. Although the Cha:Cfa values obtained did not exceed 1 in any of the fertiliser variants, the highest rate of organic matter humification in the soil was determined in the PL treatment in conjunction with MF, confirming earlier study results.

Kononowa [21] argued that the A\(4/6\) quotient values greater than 5 indicate a simpler structure of humic acids and their greater mobility. The obtained A\(4/6\) quotient values after the first year and after the fifth year of the experiment were comparable and ranged from 4.83 to 5.37 (Table 5). The applied fertilisation had no significant effect on this value after 1 year. On the other hand, statistical analysis of the A\(4/6\) quotient value after the fifth year of the experiment showed that fertilisation with organic materials significantly increased this value compared with the control.

| Treatment          | After the 1st year | After the 5th year |
|--------------------|--------------------|--------------------|
| C                  | 0.48b ± 0.03       | 0.35a ± 0.01       |
| MF                 | 0.50b ± 0.02       | 0.56b ± 0.07       |
| PL+MF              | 0.67c ± 0.00       | 0.87d ± 0.11       |
| PLB 2.25+MF        | 0.89d ± 0.04       | 0.50b ± 0.05       |
| PLB 5.0+MF         | 0.70c ± 0.06       | 0.34a ± 0.02       |
| C                  | 0.70a ± 0.02       | 1.53bc ± 0.02      |
| MF                 | 0.87a ± 0.09       | 1.59c ± 0.07       |
| PL+MF              | 0.78a ± 0.03       | 1.35b ± 0.14       |
| PLB 2.25+MF        | 0.77a ± 0.03       | 1.49bc ± 0.23      |
| PLB 5.0+MF         | 0.76a ± 0.04       | 1.67c ± 0.12       |
| A\(2/4\)           | 0.79a ± 0.03       | 1.53bc ± 0.02      |
| A\(2/6\)           | 0.97a ± 0.17       | 6.52b ± 0.33       |
| MF                 | 4.23a ± 0.61       | 7.69c ± 0.66       |
| PL+MF              | 3.91a ± 0.17       | 6.66b ± 0.09       |
| PLB 2.25+MF        | 3.96a ± 0.22       | 7.42c ± 0.32       |
| PLB 5.0+MF         | 3.67a ± 0.01       | 8.91d ± 0.54       |

\(\pm\) standard deviation, \(n = 3\); means marked with the same letters do not differ significantly according to Duncan’s test at \(\alpha < 0.05\); factor: fertilisation × year.
The A2/4 quotient presents the ratio of humification-resistant substances to substances at the initial decomposition stage, and a significant increase in its value was observed in all treatments after 5 years of the experiment. After 1 year, the highest A2/4 quotient (0.87) was found in MF, while the lowest in PLB 5.0+MF (0.76). After 5 years, the highest A2/4 quotient was found in PLB 5.0+MF (1.67), while the lowest in PL+MF (1.35).

The ratio of the content of substances difficult to humidify to the content of substances characterised by an advanced degree of humification is presented as the A2/6 ratio (Table 5). In the analysed soil, the quotient values after the first year of the experiment ranged from 3.67 in PLB 5.0+MF to 4.23 in MF. After the fifth year of the experiment, a significant increase in the A2/6 ratio was found in all treatments. The highest increase of 59% was observed in PLB 5.0+MF, and the smallest of 39% in the control. Dębska [37] stated that the organic matter in the soil, where natural fertilisation was applied in the form of slurry, was characterised by a higher Cha:Cfa ratio. Additionally, humic acids had higher values of absorbance coefficients than organic matter of soil with only mineral fertilisation applied.

The results showed that the rate of transformation and humification of organic matter decreased to the greatest extent in treatments amended with PLB 5.0+MF after 5 years of the experiment. The mineralisation of organic matter was the fastest in the PL+MF treatment. As reported by Wang et al. [38], apart from providing a carbon-resistant fraction, biochar indirectly affects C sequestration by reducing the intensity of transformations leading to SOM degradation, at least for several years after its application.

3.5 Carbon stocks in soil

Organic carbon stocks (SCS) in soil are conditioned by many factors, such as climate, soil properties, cultivation type, fertilisation type, irrigation, or the type and frequency of agro-technical operations performed [39]. Our results indicated an increase in SCS values after 5 years of the experiment (Fig. 2). The most beneficial effect on the SCS increase was observed in the PL+MF treatment where the parameter value increased by 34.7% after the fifth year. It should also be emphasised that the SCS values in PL+MF, PLB 2.25+MF, and PLB 5.0+MF after the fifth year of the experiment were significantly higher than in C and MF treatments.

When considering the issue of the SOC increase, not only the type of fertilisation applied but also environmental factors should be taken into account. Given that the organic materials were applied only once (in 2014) in the experiment, it is difficult to clearly conclude on their individual/exclusive impact on the SOC content increase after 5 years. According to Zeraatpishe and Khormali [40], climate and, above all, soil moisture conditions are the most important environmental factors affecting SOC content. In addition, these authors also indicate the mineral composition and soil structure as factors stabilising the SOC content. Dignac et al. [41] also emphasised the important role of plants and soil macro- and microorganisms that can significantly affect the organic matter mineralisation rate. According to the cited authors, the type and number of agro-technical operations, including fertilisation, are also significant. Comparing our results with determinants of the increased SOC contents described in the literature, one can state the indirect effect of the organic materials applied on the rate and direction of biochemical processes in the soil, which, in turn, led to an increase in the C content in the soil.

4 Summary

The conducted experiment proved that the fertilisation with PLB+MF reduced the organic matter transformation rate. The process of organic matter mineralisation was the most intensive in the soil with the addition of PL, proving that this material can, in a relatively short time, significantly increase the SOC content. Unfortunately, due to the rapid decomposition
of PL, this effect may be of short duration in contrast to treatments where PLB was applied. Determined A4/6 quotient values showed that PLB had a more favourable effect on the structure durability and lower mobility of Cha compared with poultry litter. It was shown that in both the 1st and 5th years of the experiment, the use of PLB in a lower dose (2.25 t ha\(^{-1}\)) was more beneficial for increasing the pool of Cn, compared with the treatment where PL was used in a higher dose (5.0 t ha\(^{-1}\)). The addition of PL and PLB to the soil significantly increased the SOC contents. Our study showed that the identification of changes that may occur in the quantitative and qualitative composition of soil humus after the application of poultry litter biochar may be helpful in determining the appropriate biochar dose in the future.

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