agronomy

MDPI

Article

The Effect of Long-Term Farmyard Manure and Mineral Fertilizer Application on the Increase in Soil Organic Matter Quality of Cambisols

Jiří Balík [1,*], Pavel Suran [1], Ondřej Sedlář [1], Jindřich Černý [1], Martin Kulhánek [1], Simona Procházková [1], Dinkayehu Alamnie Asrade [1] and Michaela Smatanová [2]

[1] Department of Agro—Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 16500 Prague, Czech Republic; suranp@af.czu.cz (P.S.); sedlar@af.czu.cz (O.S.); cernyj@af.czu.cz (J.Č.); kulhanek@af.czu.cz (M.K.); prochazkovas@af.czu.cz (S.P.); asrade@af.czu.cz (D.A.A.)

[2] Department of Plant Nutrition, Central Institute for Supervising and Testing in Agriculture, 60300 Brno, Czech Republic; michaela.smatanova@ukzuz.cz

* Correspondence: balik@af.czu.cz

Abstract: Soil organic matter (SOM) quantity and quality are important factors that significantly influence soil fertility. SOM quality indicators change throughout time. In this study, long—term field experiments (22–50 years in duration) on a Cambisol at four sites in the Czech Republic were selected. Seven crops were successively rotated in the sequence: clover, winter wheat, early potato, winter wheat, spring barley, potato, and spring barley with interseeded clover. Five treatments were investigated, including an unfertilized treatment, farmyard manure, and various combinations of farmyard manure and mineral fertilization. A total of 40 t ha^{-1} of farmyard manure was applied to the early potato and potato crops. Combining organic and mineral fertilizers increased soil organic matter quality and quantity over unfertilized or organic only treatment. The highest intensity of mineral fertilizers in our trials elevated the mean of carbon sequestration efficiency to 45.6% in comparison to pure manure treatment which reached only 22.9% efficiency. A strong correlation was established between the total glomalin content and soil organic matter carbon, fulvic acid, humic acid, carbon hot water extraction, potential wettability index (PWI), and aromaticity index. The PWI was also strongly correlated with these indicators. The E4/E6 ratio indicator was shown to be a much less sensitive method for reflecting the change in soil organic matter quality.

Keywords: glomalin; E4/E6 ratio; humic substances; aromaticity index; potential wettability index

check for updates

Citation: Balík, J.; Suran, P.; Sedlář, O.; Černý, J.; Kulhánek, M.; Procházková, S.; Asrade, D.A.; Smatanová, M. The Effect of Long-Term Farmyard Manure and Mineral Fertilizer Application on the Increase in Soil Organic Matter Quality of Cambisols. *Agronomy* **2023**, *13*, 2960. https://doi.org/10.3390/agronomy13122960

Academic Editors: Othmane Merah, Purushothaman Chirakkuzhyil Abhilash, Magdi T. Abdelhamid, Hailin Zhang and Bachar Zebib

Received: 26 October 2023
Revised: 21 November 2023
Accepted: 28 November 2023
Published: 30 November 2023

1. Introduction

The quantity and quality of soil organic matter (SOM) constitute fundamental factors for soil fertility. A great deal of attention has been paid to soil organic matter and its stabilization, particularly concerning its role in mitigating climate change. Soil carbon sequestration can be a part of the solution in tempering climate change processes.

The pivotal role of soil organic matter in various soil characteristics, such as sorption properties and soil structural stability, is widely acknowledged. This, in turn, influences hydrological and filtering characteristics, as well as resistance to water erosion. SOM quantity is a very important factor, but even more important are SOM quality indicators—these significantly influence soil fertility. SOM quality indicators change throughout time as a result of soil organic matter complexities and the evolution of analytical procedures.

Prior notions of humic substances (C_{HS}), fulvic acids (C_{FA}), and humic acids (C_{HA}) characterized as stable, robust macromolecules with substantial molecular weights [1] have been supplanted by novel concepts. Humic substances were better described as supramolecular associations rather than macromolecular polymers [2]. Supramolecular associations can be defined as assemblies of relatively small, chemically diverse organic molecules within the humic structure, linked together by hydrophobic interactions and hydrogen bonds. The accumulation of humus and the creation of microaggregates, characterized by increased hydrophobic traits, result from the concentration of hydrophobic humic molecules in the soil. This, in turn, enhances the stability of soil aggregates. This concept became the base for describing natural humic molecules through sequential fractionation without disrupting the C–C bonds [3]. Infrared spectroscopy in the mid—infrared spectrum can identify SOM composition without physical or chemical extraction or separation from soil. Although it has its limits due to the complex nature of the soil, this method can detect some functional groups within soil organic matter and offer insights into relative differences in SOM composition across distinct soil horizons or soil types [4].

Additionally, the potential wettability index (PWI) defines SOM by evaluating the ratio of aliphatic (C–H) to carboxylic (C–O) bonds. Elevated PWI values point towards reduced aggregate wettability [5]. A correlation between the PWI and C_{SOM} content has also been noted [6]. A robust correlation was observed between the the PWI and the total glomalin content (TG) in a Luvisol [7] but was not confirmed in Chernozem soil type [8].

To further investigate the soil characteristics, the aromaticity index (iAR) can be determined based on the reflectance values of aliphatic and aromatic bands [9]. Increased values of the iAR can be the result of the increased formation of aliphatic compounds within aggregates due to soil organic matter mineralization [10].

Conventional methodsfor determining SOM quality indicators are based on extraction principles. The soil organic matter can be categorized into labile and stable fractions. The stable fraction is represented by humic acids (C_{HA}), fulvic acids (C_{FA}), and humins [1,11]. The labile forms are represented by potentially mineralizable carbon (C_{HWC}) [12] or dissolved organic carbon (C_{DOC}) [7,8].

The amount glomalin (GRSP) in the soil can be regarded as a reliable indicator of soil fertility [13,14] due to the positive correlation between glomalin and C_{SOM} content [15]. In our previous study [7], we identified a positive correlation between GRSP content and humic acid content (C_{HA}), as well as a positive correlation between GRSP content and the ratio of the humic acid to fulvic acid (C_{HA}/C_{FA}) in the context of long-term maize monoculture production on a Luvisol In addition, a positive correlation of glomalin content with the potential wettability index (PWI) was found. Glomalin content in the soil can be increased by through the long—term application of manure [16,17].

The E4/E6 ratio exhibits an inverse correlation with the degree of condensation of the aromatic network in humic substances. A diminished E4/E6 ratio suggests high degree of condensation among aromatic constituents, whereas a heightened ratio signifies smaller degree of aromatic condensation and the prevalence of comparatively large proportions of aliphatic structures [18]. Carboxylic groups contribute to a higher amount of acidity than phenolic groups in fulvic and humic acids using the E4/E6 ratio measurements of these fractions [19]. The E4/E6 ratio has been recorded as higher in fulvic acids than in humic acids. No substantial relationship between (C_{SOM}) and the E4/E6 ratio was discerned in long-term experiments involving maize monoculture [8].

This study aims to: (i) assess the alterations in both the quality and quantity of theSOM in Cambisol under the influence of long-term exposure to organic and mineral fertilizers.; (ii) evaluate the viability of the glomalin soil extraction method as an indicator of SOM quality; (iii) evaluate the suitability of the PWI method as indicator of the SOM quality; iv) establish, whether the E4/E6 ratio is sensitive enough to reflect the changes in theSOM quality and quantity. We hypothesize that: (a) long—term fertilization will have a significant effect on the SOM quality and quantity; (b) the glomalin soil extraction method will be a suitable method for determining the SOM quality; (c) the PWI determination will be a suitable method to evaluate the SOM quality; and (d) theE4/E6 ratio will be sensitive to changes in the SOM quality and quantity.

For the purpose of this study, four long—term (22 to 50 years) field experiment sites on Cambisol soils in the Czech Republic were selected. We attempted to verify the influence of long—term farmyard manure application as a practice often mentioned in relation to carbon sequestration in agricultural soils. In temperate climate zones, Cambisols characterized by high base saturation are recognized as some of the most fertile and productive soils on Earth. This soil type has an area of 15 million km^2 worldwide [20].

2. Materials and Methods

Between 1972 and 2000, the Central Institute for Supervising and Testing in Agriculture established long-term on-farm trials on Cambisols soil. Table 1 outlines the characteristics of these experimental sites. A rotation of seven crops was implemented in the following sequence: clover, winter wheat, early potato, winter wheat, spring barley, potato, and spring barley with interseeded clover.

Table 1. Experimental site characteristics and their founding years.

Site	Vysoká	Horažďovice	Lípa	U. Ostroh
GPS coordinates	N 49° 22′ 52.8198″ E 13° 34′ 46.6782″	N 49° 12′ 9.5472″ E 13° 24′ 56.8578″	N 49° 20′ 4.7142″ E 15° 19′ 19.2642″	N 48° 35′ 34.6734″ E 17° 15′ 4.2618″
Established	2000	1994	1993	1972
Altitude (m.a.s.l)	595	472	505	196
Precipitation (mm) [1]	611	585	594	521
Air temperature (°C) [1]	7.1	7.4	7.5	9.1
Soil group [2]	Modal Cambisol	Modal Cambisol	Gleyic Cambisol	Modal Cambisol
Soil texture [2]	Loam	Sandy loam	Loam	Sandy loam
Clay (%) (<0.002 mm)	9.00	5.30	13.4	18.3
Silt (%) (0.002—0.05 mm)	40.7	28.9	49.5	26.1
Sand (%) (0.05—2 mm)	50.3	65.8	37.1	55.6
Bulk density (g cm^{-1})	1.42	1.35	1.45	1.36
C_{SOM} in 2022 (%)	1.64	1.72	1.43	1.23
pH_{CaCl_2} *	(5.1; 6.0; 5.9; 5.8; 5.7)	(5.5; 6.2; 6.2; 6.3; 6.4)	(5.5; 6.1; 6.1; 6.0; 6.0)	(6.3; 6.5; 6.3; 6.2; 5.9)
C input in F since the experiment establishment (t ha^{-1})	28.2	28.2	30.8	30.8
Plot size (m^2)	60	67.5	67.2	48.6

C_{SOM}— soil organic matter carbon content; [1] long—term (30 years) annual average; [2] according to NRCS USDA [21]; * values in this row list the following treatment order Con, F, F+M1, F+M2, F+M3; Con—control, F—farmyard manure, F+M—farmyard manure with increasing amounts of mineral fertilizer.

The experiment follows a randomized complete block design across all sites, with three replications of blocks. Specifics regarding plot sizes on each site are outlined in Table 1. The study investigates five treatments: (1) an unfertilized control (Con); (2) farmyard manure only treatment (F); (3) farmyard and mineral fertilizers (F+M1, 57, 13, 33 kg N, P, K ha^{-1}, respectively); (4) F+M2 (85, 26, 66 kg N, P, K ha^{-1}, respectively); and (5) F+M3 (114, 53, 133 kg N, P, K ha^{-1}, respectively). Plant residues are left unincorporated into the soil. A mass of 40 t ha^{-1} of farmyard manure is applied to early potato and potato, occurring twice during a crop rotation. Carbon input over the experiment's duration is calculated from a dry matter content of 23.0% and a content of carbon 27.9% in dry matter. Values derived from long-term monitoring on the sites. Mature farmyard cattle manure (at least six months of storage before application) was used. Average N content was 0.48% in fresh matter, C:N ratio was 13.5:1.

Soil Analysis

Soil samples were collected in 2022 after the harvest of spring barley with interseeded clover. The experimental sites were instituted from 1972 to 2000, employing a crop rotation cycle involving seven crops (as detailed earlier). Soil sampling took place post the barley harvest in 2022, marking the conclusion of the crop rotation, utilizing a soil probe at a depth of 30 cm. From every experimental plot fifteen soil samples were collected and combined. Combined samples were subjected to air-drying at 25 °C, followed by homogenization and sieving through a 2 mm sieve. Furthermore, a 0.4 mm soil size fraction was also prepared for the C_{SOM} determination. Soil was analyzed using following procedures:

The Soil organic carbon (C_{SOM}) content was assessed through oxidation using the CNS Analyzer Elementar Vario Macro (Elementar Analysensysteme, Hanau near—Frankfurt am Main, Germany).

The humic substance fractionation (C_{HS}) followed the procedure outlined by Kononova [11] to acquire the pyrophosphate-extractable fraction, encompassing the cumulative carbon in humic acids (C_{HA}) and fulvic acids (C_{FA}).

Humus quality, as indicated by the E4/E6 ratio, was assessed using the spectrophotometric method. Soil samples underwent extraction with sodium pyrophosphate (0.05 M Na4P2O7) and were then measured for an absorbance ratio at 400 and 600 nm [22] (Lambda 25 UV/Vis (Perkin Elmer, Waltham, MA, USA).

Extractable organic carbon was determined using $CaCl_2$ and hot water extraction.

For $CaCl_2$ extraction (C_{DOC}), the extraction was performed according to Houba et al., [23]. The C_{DOC} content was determined in soil samples using segmental flow analysis with infrared detection using a Skalarplus System (Skalar, Breda, The Netherlands).

Hot water extraction (C_{HWC}) was used to assess extractable soil organic carbon [12]. The C_{HWC} was determined using a segmental flow analysis with infrared detection using a Skalarplus System (Skalar, Breda, The Netherlands).

The potential wettability index (PWI) and index of aromaticity (iAR) were determined through DRIFT (diffuse reflectance infrared Fourier transform spectroscopy) spectra. DRIFT spectra were captured using the infrared spectrometer (Nicolet IS10, Waltham, MA, USA), covering a range of 2.50 to 25.0 µm (4000 to 400 cm^{-1}). Alkyl C–H groups A (2948–2920 cm^{-1} and 2864–2849 cm^{-1}) bands were considered indicative of hydrophobicity, while C=O groups B (1710 and 1640–1600 cm^{-1}) bands indicated hydrophilicity. The ratio of hydrophobicity to hydrophilicity determined the potential wettability index [24].

$$PWI = A/B$$

The aromaticity index was calculated based on the reflectance values of aliphatic bands ranging from 3000 to 2800 cm^{-1} (AL) and the aromatic band at 1520 cm^{-1} (AR) [24].

$$iAR = AL/(AL + AR)$$

Easily extractable glomalin (EEG) and total glomalin (TG) were performed according to Wright and Upadhyaya [13].

Humification indices were calculated according to Raiesi [25] and Iqbal et al., [26]:

$$\text{Degree of polymerization: } HA = C_{HA}/C_{FA} \quad (1)$$

$$\text{Humification rate: } HR = (C_{FA} + C_{HA})/C_{SOM} \quad (2)$$

$$\text{Humification index: } HI = C_{HA}/C_{SOM} \quad (3)$$

where C_{FA} is the fulvic acid carbon, C_{HA} is the humic acid carbon, and C_{SOM} is the total organic carbon in soil.

The carbon sequestration efficiency (CSE) was calculated as follows:

$$\text{CSE (\%)} = ((C_{SOMtreatment} - C_{SOMunfert.})/\text{TCI}) \times 100 \quad (4)$$

$C_{SOMtreatment}$ represents the quantity of carbon (C) present in the soil under the fertilized treatment. $C_{SOMunfert}$ signifies the amount of C in the soil under the unfertilized Con treatment. TCI denotes the total carbon input (t ha^{-1}) applied through organic fertilizers throughout the course of individual experiments [27].

Statistical analysis

The obtained results underwent assessment through ANOVA analysis and Pearson's correlation coefficient using the Statistica program ver. 12.3 (TIBCO, Palo Alto, CA, USA). One-way ANOVA statistical analysis, complemented by Tukey's test, was employed to evaluate treatment and site effects ($p < 0.05$). Pearson's correlation coefficients were utilized to scrutinize relationships among the studied variables. A significance level of $p < 0.05$ or lower was deemed statistically significant.

3. Results

Statistical evaluation encompassed all the measured values of soil organic matter quality indicators from each treatment, as detailed in Table 2. The impact of site was a major factor in these results and caused statistically significant differences among all observed the SOM quality indicators.

Table 2. Carbon content and qualitative parameters of soil organic matter content at the individual experimental sites.

Site/Indicator	**C_{SOM}**	**C_{HS}**	**C_{FA}**	**C_{HA}**	**E4/E6**	**C_{HWC}**	**C_{DOC}**	**N_t**
	%	**%**	**%**	**%**		**mg kg^{-1}**	**mg kg^{-1}**	**%**
U. Ostroh	1.24 ^{a}	0.232 ^{a}	0.116 ^{a}	0.110 ^{a}	5.48 ^{a}	338 ^{a}	31.2 ^{a}	0.132 ^{a}
Lípa	1.43 ^{b}	0.363 ^{b}	0.169 ^{b}	0.154 ^{b}	8.39 ^{c}	434 ^{b}	60.3 ^{c}	0.137 ^{a}
Horažd'ovice	1.72 ^{c}	0.377 ^{b}	0.196 ^{c}	0.152 ^{b}	5.33 ^{a}	549 ^{c}	50.0 ^{b}	0.160 ^{b}
Vysoká	1.64 ^{c}	0.431 ^{c}	0.210 ^{d}	0.186 ^{c}	6.65 ^{b}	510 ^{bc}	39.3 ^{a}	0.155 ^{b}
Site/Indicator	EEG	TG	DG	PWI	iAR	HA	HR	HI
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}			(C_{HA}/C_{FA})	(C_{FA}+C_{HA}/C_{SOM})	C_{HA}/C_{SOM}
U. Ostroh	712 ^{ab}	1824 ^{a}	1112 ^{a}	0.015 ^{a}	0.034 ^{a}	0.954 ^{b}	0.186 ^{a}	0.090 ^{a}
Lípa	603 ^{a}	2562 ^{b}	1959 ^{b}	0.023 ^{b}	0.044 ^{b}	0.923 ^{ab}	0.228 ^{bc}	0.108 ^{b}
Horažd'ovice	782 ^{bc}	2744 ^{b}	1963 ^{b}	0.026 ^{bc}	0.051 ^{c}	0.781 ^{a}	0.206 ^{ab}	0.089 ^{a}
Vysoká	888 ^{c}	4144 ^{bc}	3256 ^{c}	0.026 ^{c}	0.050 ^{bc}	0.882 ^{ab}	0.243 ^{c}	0.114 ^{b}

Different letters are employed to denote statistically significant differences among sites. Tukey HSD test, $p < 0.05$. n = 15.

To assess the impact of the treatments, the existing variable values were substituted with relative ones. The relative values were computed as follows: $V_{treatment}/V_{site}$—average, where $V_{treatment}$ is represented the value of each treatment, and Vsite—average denoted the average value of a specific site across all the treatments (Table 3). This allowed us to dispose of site influence and focus purely on the influence of individual treatments (Table 3). Along with the relative values, the average values from all four sites are also shown.

The average content of C_{SOM} was 1.51%. The combination of farmyard manure (F) with mineral fertilizers (F+M) led to a significant increase in soil organic carbon content (C_{SOM}) compared to the unfertilized treatment (Con).

Table 3. The impact of fertilization on both soil carbon content and the qualitative parameters of soil organic matter.

Variable/Treatment	Con	F	F+M1	F+M2	F+M3	Average = 1
C_{SOM} (%)	0.889 ^{a}	0.957 ^{ab}	1.01 ^{bc}	1.06 ^{c}	1.08 ^{c}	1.51%
C_{HS} (%)	0.963 ^{a}	0.903 ^{a}	1.04 ^{a}	1.09 ^{a}	1.00 ^{a}	0.351%
C_{FA} (%)	0.989 ^{a}	0.947 ^{a}	0.973 ^{a}	1.05 ^{a}	1.04 ^{a}	0.173%
C_{HA} (%)	0.850 ^{a}	0.929 ^{ab}	1.02 ^{ab}	1.15 ^{b}	1.04 ^{ab}	0.151%
HA (C_{HA}/C_{FA})	0.859 ^{a}	0.986 ^{a}	1.06 ^{a}	1.10 ^{a}	1.00 ^{a}	0.886
HR (C_{HS}/C_{SOM})	1.04 ^{a}	0.983 ^{a}	0.985 ^{a}	1.03 ^{a}	0.960 ^{a}	0.215
HI (C_{HA}/C_{SOM})	0.956 ^{a}	0.975 ^{a}	1.02 ^{a}	1.09 ^{a}	0.965 ^{a}	0.100
C_{HWC} (%)	0.860 ^{a}	0.885 ^{ab}	1.05 ^{bc}	1.08 ^{c}	1.13 ^{c}	458 mg kg^{-1}
C_{DOC} (%)	0.937 ^{a}	0.915 ^{a}	0.100 ^{a}	1.08 ^{a}	1.07 ^{a}	45.2 mg kg^{-1}
C_{HWC}/C_{SOM} (%)	0.970 ^{a}	0.928 ^{a}	1.04 ^{a}	1.02 ^{a}	1.04 ^{a}	0.030
C_{DOC}/C_{SOM} (%)	1.06 ^{a}	0.960 ^{a}	0.976 ^{a}	1.02 ^{a}	0.980 ^{a}	0.003
N_t (%)	0.889 ^{a}	0.944 ^{a}	1.02 ^{b}	1.06 ^{b}	1.09 ^{b}	0.146%
C_{SOM}/N_t	1.00 ^{a}	1.01 ^{a}	0.995 ^{a}	0.995 ^{a}	0.994 ^{a}	10.3
EEG (%)	0.891 ^{a}	0.880 ^{a}	1.01 ^{b}	1.09 ^{bc}	1.13 ^{c}	746 mg kg^{-1}
TG (%)	0.885 ^{a}	0.954 ^{ab}	0.990 ^{ab}	1.03 ^{bc}	1.14 ^{c}	2819 mg kg^{-1}
DG (%)	0.889 ^{a}	0.983 ^{ab}	0.986 ^{ab}	1.01 ^{ab}	1.13 ^{b}	2072 mg kg^{-1}
EEG/TG (%)	1.01 ^{a}	0.924 ^{a}	1.02 ^{a}	1.05 ^{a}	0.996 ^{a}	0.265
EEG/C_{SOM} (%)	1.01 ^{a}	0.923 ^{a}	0.992 ^{a}	1.03 ^{a}	1.04 ^{a}	0.050
TG/C_{SOM} (%)	1.00 ^{a}	1.00 ^{a}	0.978 ^{a}	0.978 ^{a}	1.04 ^{a}	0.186
E4/E6 (%)	0.958 ^{a}	0.958 ^{a}	1.03 ^{ab}	1.04 ^{b}	1.02 ^{ab}	6.46
PWI (%)	0.876 ^{a}	0.960 ^{ab}	1.00 ^{abc}	1.03 ^{bc}	1.14 ^{c}	0.022
iAR (%)	0.884 ^{a}	0.965 ^{ab}	0.995 ^{abc}	1.03 ^{bc}	1.12 ^{c}	0.045
CSE (%)	-	0.652 ^{a}	0.761 ^{ab}	1.170 ^{b}	1.298 ^{b}	35.1%

Rows represent relative values of monitored variables. Different letters are employed to denote statistically significant differences among treatments. Tukey HSD test, $p < 0.05$. n = 12.

3.1. Carbon Sequestration Efficiency

The carbon sequestration efficiency (CSE) was determined by assessing the difference (C_{SOM}) between the fertilized and unfertilized treatments relative to the total carbon applied in the manure. The application of balanced mineral fertilizer in conjunction with farmyard manure resulted in an increased soil carbon sequestration—specifically, by 22.9% in the farmyard manure (F) treatment and by 45.6% in the F+M3 treatment.

3.2. Carbon Fractions and E4/E6 Ratio

In order to estimate and evaluate the content of C_{HS}, C_{FA}, and C_{HA}, the fractionation of humic substances was performed. The acquired results facilitated the computation of the humification degree (HA), humification rate (HR), and humification index (HI). Notably, there were no significant differences observed among the treatments for the HA, HR, or HI.

Furthermore, the quality C_{SOM} quality was also assessed through hot water extraction (C_{HWC}) and 0.01 M L^{-1} of $CaCl_2$ extraction (C_{DOC}). The application of intensive mineral fertilizer treatments (F+M2 and, F+M3) resulted in higher C_{HWC} values compared to the unfertilized treatment (Con) and other treatments. To obtain better insight into these results, the C_{HWC}/C_{SOM} and C_{DOC}/C_{SOM} ratios were also calculated. The averages for the C_{HWC}/C_{SOM} and C_{DOC}/C_{SOM} ratios were 0.030 and 0.003, respectively. In light of these findings, CHWC and CDOC can be characterized as potentially mineralizable carbon and easily extractable carbon, respectively.

The optical density ratio of the humic acids (E4/E6) serves as an additional qualitative indicator of humic substances, providing insights into the humification process in the soil. The average E4/E6 ratio value was 6.46. A significant increase in the values of this indicator were observed with the F+M2 treatment. To establish and analyze a relationship between the indicators, Pearson's correlation coefficients were used (Table 4). The C_{SOM} was significantly correlated to the C_{HS}, C_{FA}, C_{HA}, and C_{HWC}. There was no correlation between C_{SOM} and E4/E6 ratio. No relationship (Tables 4 and 5) of the E4/E6 ratio with the C_{SOM} was present.

3.3. The Potential Wettability Index

Using the DRIFT spectra, the potential wettability index (PWI) and index of aromaticity (iAR) were determined. Significant differences were recorded between the F + M3 and the Con, the F treatments and their PWIs, and the iAR parameters. A significant relationship between the PWI and the C_{SOM} (Table 4) was established and further confirmed with regression analysis (Table 5). No relationship between the E4/E6 ratio and the PWI or iAR was established. The correlation results indicate a strong relationship of thepotential wettability index and aromaticity index with the C_{HS}, C_{FA}, and $C_{HA,}$ as well as the C_{WHC}.

3.4. The Glomalin-Related Soil Protein (GRSP) Content

The assessment of organic matter quality involved the utilization of indicators such as easily extractable glomalin (EEG) content and total glomalin (TG). content There were significant differences in the EEG and TG among the observed treatments. The average EEG/TG ratio was 0.265. The average values for the EEG/C_{SOM} and TG/C_{SOM} were 0.050 and 0.186, respectively. There were no significant differences among the treatments with respect to these indicators. The EEG and TG exhibited strong correlations with the C_{SOM} content, as well as the C_{FA} and C_{HA} content. Both C_{HA} and GRSP content emerged as crucial contributors to the stability of soil organic matter. Notably, a more robust relationship was identified in the correlation with the G content as opposed to the EEG content. Additionally, a significant and strong relationship was established between the PWI, iAR, and GRSP content, encompassing both EEG and TG contents. Regression analyses further investigated these relationships. A significant influence of the C_{SOM}, C_{HS}, and C_{HA} over the values of the EEG and TG contents was discovered (Table 5).

Table 4. Pearson's correlation coefficients among variables.

	C_{SOM}	C_{HS}	C_{FA}	C_{HA}	HA	HR	HI	E4/E6	C_{HWC}	C_{DOC}	N_t	C_{SOM}/N_t	EEG	TG	PWI
C_{HS}	0.610 ***														
C_{FA}	0.664 ***	0.897 ***													
C_{HA}	0.630 ***	0.810 ***	0.660 ***												
HA	0.001	−0.039	−0.353 **	0.462 ***											
HR	−0.083	0.659 ***	0.584 ***	0.568 ***	0.023										
HI	−0.022	0.523 ***	0.285 *	0.752 ***	0.598 ***	0.807 ***									
E4/E6	−0.024	0.336 **	0.193	0.323 *	0.172	0.442 ***	0.450 ***								
C_{HWC}	0.844 ***	0.601 ***	0.682 ***	0.574 ***	−0.090	0.060	0.033	0.045							
C_{DOC}	0.343 **	0.350 **	0.300 *	0.265 *	0.005	0.127	0.104	0.538 ***	0.386 **						
N_t	0.948 ***	0.498 ***	0.540 ***	0.575 ***	0.074	−0.178	−0.048	−0.104	0.817 ***	0.249					
C_{SOM}/N_t	0.681 ***	0.639 ***	0.698 ***	0.497 ***	−0.187	0.219	0.071	0.171	0.556 ***	0.371 **	0.427 ***				
EEG	0.552 ***	0.379 **	0.439 ***	0.431 ***	0.003	0.043	0.078	−0.246	0.562 ***	−0.050	0.658 ***	0.047			
TG	0.635 ***	0.748 ***	0.763 ***	0.698 ***	−0.031	0.420 ***	0.350 **	0.187	0.589 ***	0.162	0.580 ***	0.497 ***	0.618 ***		
PWI	0.842 ***	0.749 ***	0.783 ***	0.685 ***	−0.058	0.230	0.183	0.215	0.741 ***	0.411 **	0.746 ***	0.725 ***	0.445 ***	0.720 ***	
iAR	0.835 ***	0.665 ***	0.704 ***	0.619 ***	−0.045	0.124	0.106	0.148	0.725 ***	0.382 **	0.758 ***	0.683 ***	0.429 ***	0.636 ***	0.982 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ n = 60

Table 5. Linear regression equation of C_{SOM} and selected indicators of soil organic matter quality.

Indicator	C_{SOM}	R^2
E4/E6	y = −0.1161x + 6.6391	0.001
EEG	y = 339.13x + 235.2	0.304 ***
TG	y = 2291.7x − 634.75	0.403 ***
PWI	y = 0.018x − 0.0047	0.708 ***
	C_{HS}	R^2
E4/E6	y = 5.1262x + 4.6657	0.113 **
EEG	y = 742.49x + 485.74	0.144 **
TG	y = 8594.3x − 196.53	0.559 ***
PWI	y = 0.0512x + 0.0045	0.561 ***
	C_{HA}	R^2
E4/E6	y = 10.558x + 4.8709	0.104 *
EEG	y = 1810.2x + 473.07	0.186 ***
TG	y = 17218x + 220.32	0.488 ***
PWI	y = 0.1005x + 0.0073	0.470 ***

* significant at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ n = 60; $C_{SOM;}$ $C_{HS;}$ C_{HA} independent variables; n = 60.

4. Discussion

The focus of this study was the Cambisol soil type of the Czech Republic.The combination of the location of the Czech Republic incentral Europe and the fact, that Cambisol soil covers a vast area of the world [20] may provide useful insight for similar conditions. Older sources in the literature describe Cambisols as a soil type with a low proportion of humin acids with aromatic compounds and high fulvic acid content; in addition, these soils have been described as containing more aliphatic compounds [1,28].

Cambisols typically have a lower organic matter quality index and decomposition index in comparison to Chernozems and Luvisols [4]. However, the stability of soil organic matter is influenced not only by the composition of organic matter, its aromaticity, and nitrogen content but also by other factors. The presence of iron oxide is an important factor, with polysaccharides and proteins selectively preserved in organo-metallic complexes [29,30]. Other studies showed that microbiologically originated biomolecules that are rich in nitrogen, such as amino acids, are also selectively bound to mineral surfaces [31]. In Cambisols, the stabilization of soil organic matter (SOM) occurs not solely through the formation of complexes with the mineral fraction but also through the preservation of stable aggregates, aided by the positive influence of various forms of iron on aggregate stability [32].

4.1. Carbon Sequestration Efficiency

As per Wang et al., [27] carbon sequestration efficiency is predominantly associated with soil fertility. On average, 22.9% and 45.6% of the carbon input in the farmyard manure in our experiment was transformed to the soil organic carbon content in the F and F+M3 treatments, respectively. These results are close to the ones reported by Sedlář et al., [33], where an increase in the carbon sequestration in the soil was recorded following mineral fertilizer treatments. The F+M2 treatment in our experiment showed the highest content of C_{HA} and the highest C_{HA}/C_{FA} ratio. In addition, the F+M2 treatment produced the highest humification index (HI). An elevated humification index fosters more favorable conditions for carbon sequestration [34,35].

4.2. Carbon Fractions and the E4/E6 Ratio

Based on the results of the E4/E6 ratio (Table 2), the Cambisols of the highest quality were at the U. Ostroh and Horažd'ovice sites. The relationship between the E4/E6 ratio and the C_{HA}/C_{FA} has been found to be site—specific [36,37]. There were no statistically significant differences in the C_{HA}/C_{FA} ratios between the treatments. On the other hand, significant differences were established between the F+M2 and unfertilized

Con treatments for the E4/E6 ratio. Similar results of increasing E4/E6 ratios in farmyard manure treatments were also published by Song et al., [38] and Galantitni and Rossel [39], although they reported increased contents of fulvic acids resulting fron farmyard manure treatments. Higher aliphatic and phenolic –OH group contents are generally found post organic fertilizer application [39]. The impact of fertilization on carbon fractions (C_{FA}, C_{HS}, HI, HR, HA) was smaller compared to impact on the E4/E6 ratio. Similarly, Gerzabek et al., [40] and Oktaba et al., [41] reported significant changes in theE4/E6 ratio caused by fertilization, while there was no effect on the C_{HA}/C_{FA} ratio. The mineral fertilizer treatments exhibited a higher E4/E6 ratio compared to the Con or F treatments. Notably, the F+M3 treatment displayed a significantly distinct E4/E6 ratio in comparison to the Con (refer to Table 3). A parallel finding was observed in another study, wherein treatments involving mineral + organic fertilizer yielded a higher E4/E6 ratio than the control or pure organic fertilizers [42]. The elevated E4/E6 ratio observed in the F+M2 treatment in our experiment is indicative of a low aromaticity of humic substances and the prevalence of substantial quantities of aliphatic structures. No significant relationship and no significant regression were present between the C_{SOM} and E4/E6 ratio (Tables 4 and 5).

4.3. The Potential Wettability Index

The potential wettability index (PWI) can be used to describe the quality of the soil organic matter. Lower aggregate wettability is indicated by high PWI values [5]. Significant differences in the PWI values between F+M2 and other treatments were observed. In our previous work, a significant influence of farmyard manure fertilization was reported [7]. In line with this, Demyan et al., [6] reported a similar increase in the PWI following farmyard manure application. The application of manure led to an augmentation of the hydrophobic particles and played a role in the development of larger soil aggregates in these treatments. Secondary metabolites generated during the organic matter decay process can possess hydrophobic characteristics [43]. Additionally, mineral fertilization has been shown to contributed to heightened root biomass production, an increase in root exudate production, and the formation of stable aggregates [5].

A significant correlation between the PWI and the C_{SOM}, C_{HS}, C_{FA}, C_{HA}, C_{HWC} and C_{DOC} was observed (Table 4). The relationship between PWI and C_{SOM} was further investigatedusing regression analysis and confirmed (Table 5). Literature also confirms this robust correlation [44]. In our experiment, The TG and PWI exhibited a strong correlation, as depicted in Table 4. This correlation was also identified in our prior study involving a maize monoculture on a Luvisol [7]. Given that Glomalin-Related Soil Protein (GRSP) is a temperature-stable, adhesive, hydrophobic glycoprotein [13], the rise in glomalin content resulted in a higher ratio of hydrophobic particles, leading to an observable increase in the PWI. The calculation of the aromaticity index (iAR) was based on the reflectance of aliphatic and aromatic bands [9]. Notably, significant differences in the iAR were observed among the treatments, as detailed in Table 3. Furthermore, a robust correlation was identified between the iAR and the C_{SOM}, along with its quality indicators (C_{HS}, C_{FA}, C_{HA}, and C_{HWC}).

4.4. Relationship of GRSP and Organic Matter Quality Indicators

Glomalin estimation is a very specific extraction method. Glomalin—related soil proteins (GRSPs) are a mixture of humic substances and heat—stable glycoproteins [45]. A strong correlation was established between the glomalin content (EEG and TG contents) and the C_{SOM}, C_{HA}, C_{FA}, and C_{HWC} contents (Table 4). Regression analysis further confirmed these relationships (Table 5). A positive correlation was also observed between the GRSP content and the C_{SOM}, C_{HA}, and C_{HA}/C_{FA} ratios in our previous work on long—term experiments with a maize monoculture on a Luvisol [7]. Similarly, other studies in the literature [46] established a correlation between humic and fulvic acid contents, C_{OX}, glomalin content, and the C/N ratio. Correlation coefficient values were greater for theTG content than for the EEG content. The repeated extraction used for the TG estimation could be the cause for a higher correlation for the TG content. In addition to GRSP, there is an additional release of humic substances. It's worth noting that the Bradford assay can exhibit a cross-reaction with humic acids, polyphenolic compounds, sugars, and lipids [47], and this can interfere with glomalin determination. Likewise, Li et al., [15] highlighted that the total glomalin content in soil exhibits strong linkages with the overall content of the soil organic matter. In our study, a significant increase in the glomalin content after treatment with farmyard manure was observed. Similarly, Bertagnoli et al., [48] and Zhang et al., [16] reported a heightened the TG content following the application of farmyard manure. The synergistic use of organic and mineral fertilizers led to an enhanced TG content, particularly evident in the F+M3 treatment. The contents of TG and EEG in the F+M3 treatment were 25% and 24% higher than in the Con treatment, respectively. The F+M3 treatment yielded a high biomass, closely linked to elevated post-harvest residues (stubble) and root biomass production. This likely resulted in an increased exudate production, manifesting as a rise in soil organic carbon (C_{SOM}) content. Therefore, higher arbuscular mycorrhiza activity does not have to be the primary contributor to the increase in the TG content. More clarity into this issue can be provided by a different study with an 80—year fallow [49]. The authors conclude that recycling of the SOM continually produces soil protein. 28.3% of TG content was founded by the EEG content. Interestingly, a very similar proportion (28.8%) was estimated on a Luvisol at the Červený Újezd experimental site [7]. The ratio of TG in C_{SOM} was found to be 0.186, on average. This is a significant proportion, and it is obvious that glomalin has a role in soil carbon sequestration. Glomalin plays an important role in promoting the stability of soil organic carbon, in terms of the slow decay of glomalin (and a low soil turnover rate) [50].

5. Conclusions

Drawing from the outcomes of long-term field experiments conducted on Cambisols at four sites in the Czech Republic, it can be asserted that:

(i) The amalgamation of organic and mineral fertilizers enhances both the quantity and quality of soil organic matter when compared with unfertilized conditions or treatments involving solely organic fertilizer. The highest intensity of mineral fertilizers (F+M3) increased the average value of carbon sequestration efficiency (CSE) to 45.6% in comparison with pure manure treatment (F) which reached only 22.9%.

(ii) We established a robust correlation between total glomalin (TG) content and indicators of both the quality as well as quantity of soil organic matter. (C_{SOM}, C_{FA}, C_{HA}, C_{HWC}, and C_{SOM}/N_t). On the other hand, the correlation between easily extractable glomalin (EEG) and the same indicators of soil organic matter quality and quantity mentioned above was weaker.

(iii) We demonstrated a strong correlation between the PWI, and soil organic matter quality as well as quantity indicators (C_{SOM}, C_{FA}, C_{HA}, C_{HWC}, $C_{DOC,}$ and C_{SOM}/N_t) TG content and EEG content. The data regarding the (PWI) can be employed for the investigation of organic matter quality. The E4/E6 ratio indicatorwas found to be a considerably less sensitive method for reflecting alterations in both the quality and quantity of soil organic matter.

Author Contributions: Conceptualization, J.B.; Data curation, J.Č. and M.K.; Methodology, M.S. and J.B.; Validation, D.A.A., S.P. and O.S.; Writing—original draft, P.S. and J.B. All authors have read and agreed to the published version of the manuscript.

Funding: Funds for the creative process of this manuscript were provided by: the Ministry of Agriculture of the Czech Republic, grant numbers QK21010124 and QK23020056.

Data Availability Statement: All data are available from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Stevenson, F.J. *Humus Chemistry, Genesis, Composition, Reactions*, 2nd ed.; John Wiley and Sons, Inc.: New York, NY, USA, 1994; p. 512.
2. Piccolo, A. The supramolecular structure of humic substances: A novel understanding of humus chemistry and implications in soil science. *Adv. Agron.* **2002**, *75*, 57–134. [CrossRef]
3. Nebbioso, A.; Piccolo, A. Advances in humeomics: Enhanced structural identification of humic molecules after size fractionation of a soil humic acid. *Anal. Chim. Acta.* **2012**, *720*, 77–90. [CrossRef]
4. Pavlů, L.; Zádorová, T.; Pavlů, J.; Tejnecký, V.; Drábek, O.; Reyes Rojas, J.; Thai, S.; Penížek, V. Prediction of the distribution of soil properties in deep Colluvisols in different pedogeographic regions (Czech Republic) using diffuse reflectance infrared spectroscopy. *Soil Tillage Res.* **2023**, *234*, 105844. [CrossRef]
5. Haas, C.; Gerker, H.H.; Ellerbrock, R.H.; Hallet, P.D.; Horn, R. Relating soil organic matter composition to soil water repellency for soil biopore surfaces different in history from two Bt horizons of a Haplic Luvisol. *Ecohydrology* **2018**, *11*, e1949. [CrossRef]
6. Demyan, M.S.; Rasche, F.; Schulz, E.; Breulmann, M.; Muller, T.; Cadisch, G. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. *Eur. J. Soil Sci.* **2012**, *63*, 189–199. [CrossRef]
7. Balík, J.; Kulhánek, M.; Černý, J.; Sedlář, O.; Suran, P.; Asrade, D.A. The influence of organic and mineral fertilizers on the quality of soil organic matter and glomalin content. *Agronomy* **2022**, *12*, 1375. [CrossRef]
8. Balík, J.; Kulhánek, M.; Černý, J.; Sedlář, O.; Suran, P.; Procházková, S.; Asrade, D.A. The impact of the long-term application of mineral nitrogen and sewage sludge fertilizers on the quality of soil organic matter. *Chem. Biol. Technol. Agric.* **2022**, *9*, 86. [CrossRef]
9. Cunha, T.J.F.; Novotny, E.H.; Madari, B.E.; Martin-Neto, L.; Re-zende, M.O.; Canelas, L.P.; Benitesm, V.M. Spectroscopy Characterization of Humic Acids Isolated from Amazonian Dark Earth Soils (Terra Preta De Índio). In *Amazonian Dark Earths: Wim Sombroek's Vision*, 1st ed.; Woods, W.I., Teixeira, W.G., Lehmann, J., Steiner, C., Eds.; Springer: Dordrecht, The Netherlands, 2009; pp. 363–372. ISBN 978-1-4020-9031-8.
10. Jakab, G.; Filep, T.; Kir, C. Differences in mineral phase associated soil organic matter composition due to varying tillage intensity. *Agronomy.* **2019**, *9*, 700. [CrossRef]
11. Kononova, M.M. *Soil Organic Matter: Nature, Properties and Methods of Study*; Pergamon Press Ltd.: Oxford, UK, 1966.
12. Körschens, M.; Albert, E.; Armbruster, M.; Barkusky, D.; Baumecker, M.; Behle-Schalk, L.; Bischoff, R.; Čergan, Z.; Ellmer, F.; Herbst, F. Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: Results from 20 European long-term field experiments of the twenty-first century. *Arch. Agron. Soil Sci.* **2013**, *59*, 1017–1040. [CrossRef]
13. Wright, S.F.; Upadhyaya, A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil.* **1998**, *198*, 97–107. [CrossRef]
14. Dai, J.; Hu, J.L.; Lin, X.G.; Yang, A.; Wang, R.; Zhang, J.B.; Wong, M.H. Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. *J. Soils Sediments* **2013**, *13*, 1–11. [CrossRef]
15. Li, X.; Han, S.; Luo, X.S.; Chen, W.L.; Huang, Q.Y. Arbuscular mycorrhizal-like fungi and glomalin-related soil protein drive the distributions of carbon and nitrogen in a large scale. *J. Soil Sediments* **2020**, *20*, 963–972. [CrossRef]
16. Zhang, X.; Wu, X.; Zhang, S.; Xing, Y.; Wang, R.; Liang, W. Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. *Catena* **2014**, *123*, 188–194. [CrossRef]
17. Turgay, O.C.; Buchan, D.; Moeskops, B.; De Gusseme, B.; Ortas, I.; De Neve, S. Changes in soil ergosterol content, glomalin-related soil protein, and phospholipid fatty acid profile as affected by long-term organic and chemical fertilization practices in Mediterranean Turkey. *Arid Land Res. Manag.* **2015**, *29*, 180–198. [CrossRef]
18. Quatmane, A.; Orazio, V.D.; Hafidi, H.; Senesi, N. Chemical and physico chemical characterization of humic acid like materials from compost. *Compost Sci. Util.* **2002**, *10*, 39–46. [CrossRef]
19. Kale, S.P.; Bhakare, B.D.; Kausadikar, H.K. Characterisation of soil Humic and Fulvic acids from different land use patterns. *Pharma Innov.* **2023**, *12*, 301–308.
20. Food and Agriculture Organization of the United Nations. World Reference Base for Soil Resources 2014. In *International Soil Classification System for Naming and Creating Legends for Soil Maps*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2015; Available online: http://www.fao.org/3/i3794en/I3794en.pdf (accessed on 1 September 2020).
21. United States Department of Agriculture–Natural Resources Conservation Service. Soil Taxonomy. Available online: https://www.nrcs.usda.gov/sites/default/files/2022-06/Soil%2520Taxonomy.pdf (accessed on 4 October 2023).
22. Sparks, D.L. *Methods of Soil Analysis. Part 3—Chemical Methods*; John Wiley & Sons: Hoboken, NJ, USA, 1996; p. 1390. ISBN 9780891188254.
23. Houba, V.J.G.; Temminghoff, E.J.M.; Gaikhorst, G.A.; van Vark, W. Soil analysis procedures using 0.01 *M* calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* **2008**, *31*, 1299–1396. [CrossRef]
24. Ellerbrock, R.H.; Gerke, H.; Bachman, J.; Goebel, M. Composition of organic matter fractions for explaining Wettability of three forest soils. *Soil Sci. Soc. Am. J.* **2005**, *69*, 57–66. [CrossRef]

25. Raiesi, F. The quantity and quality of soil organic matter and humic substances following dry-farming and subsequent restoration in an upland pasture. *Catena* **2021**, *202*, 105249:1–105249:11. [CrossRef]
26. Iqbal, M.K.; Shafiq, T.; Hussain, A.; Ahmed, K. Effect of enrichment on chemical properties of MSW compost. *Bioresour. Technol.* **2010**, *101*, 5969–5977. [CrossRef]
27. Wang, S.C.; Zhao, Y.W.; Wang, J.Z.; Zhu, P.; Cui, X.; Han, X.Z.; Xu, M.G.; Lu, C.A. The efficiency of long-term straw return to sequester organic carbon in Northeast China's cropland. *J. Integr. Agric.* **2018**, *17*, 436–448. [CrossRef]
28. Sposito, G. *The Chemistry of Soils*; Oxford University Press, Inc.: New York, NY, USA, 2008; p. 321.
29. Nierop, K.G.J.; Van Bergen, P.F.; Buurman, P.; Van Lagen, B. NaOH and $Na_4P_2O_7$ extractable organic matter in two allophanic volcanic ash soils of the Azores Islands—A pyrolysis GC/MS study. *Geoderma* **2005**, *127*, 36–51. [CrossRef]
30. Tonneijck, F.H.; Jansen, B.; Nierop, K.G.J.; Verstraten, J.M.; Sevink, J.; De Lange, L. Towards understanding of carbon stocks and stabilization in volcanic ash soils in natural Andean ecosystems of northern Ecuador. *Eur. J. Soil Sci.* **2010**, *61*, 392–405. [CrossRef]
31. Kopittke, P.M.; Hernandez-Soriano, M.C.; Dalal, R.C.; Finn, D.; Menzies, N.W.; Hoeschen, C.; Mueller, C.W. Nitrogen-rich microbial products provide new organo-mineral associations for the stabilization of soil organic matter. *Glob. Chang. Biol.* **2018**, *24*, 1762–1770. [CrossRef] [PubMed]
32. Pavlů, L.; Kodešová, R.; Vašát, R.; Fér, M.; Klement, A.; Nikodem, A.; Kapička, A. Estimation of the stability of topsoil aggregates in areas affected by water erosion using selected soil and terrain properties. *Soil Tillage Res.* **2022**, *219*, 105348. [CrossRef]
33. Sedlář, O.; Balík, J.; Černý, J.; Kulhánek, M.; Smatanová, M. Long.term application of organic fertilizers in relation to soil organic matter quality. *Agronomy* **2023**, *13*, 175. [CrossRef]
34. Mockeviciene, I.; Repsiene, R.; Amaleviciute-Volunge, K.; Karcauskiene, D.; Slepetiene, A.; Lepane, V. Effect of long-term application of organic fertilizers on improving organic matter quality in acid soil. *Arch. Agron. Soil Sci.* **2022**, *68*, 1192–1204. [CrossRef]
35. Hao, X.X.; Han, X.Z.; Zou, W.X.; Wang, S.Y.; Kwaw-Mensah, D. Changes in soil organic carbon and its fractions after 13 years of continuous straw return in a soybean-maize cropping system. *Appl. Ecol. Environ. Res.* **2020**, *18*, 8267–8284. [CrossRef]
36. Maroušek, J.; Bartoš, P.; Filip, M.; Kolář, L.; Konvalina, P.; Maroušková, A.; Moudrý, J.; Peterka, J.; Šál, J.; Šoch, M.; et al. Advances in the agrochemical utilization of fermentation residues reduce the cost of purpose-grown phytomass for biogas production. *Energy Sources A Recovery Util. Environ.* **2020**, 1–11. [CrossRef]
37. Kopecký, M.; Kolář, L.; Perná, K.; Vachalová, R.; Mráz, P.; Konvalina, P.; Murindangabo, Y.T.; Ghorbani, M.; Menšík, L.; Dumbrovský, M. Fractionation of Soil Organic Matter into Labile and Stable Fractions. *Agronomy* **2022**, *12*, 73. [CrossRef]
38. Song, X.Y.; Liu, S.T.; Liu, Q.H.; Zhang, W.J.; Hu, C.G. Carbon sequestration in soil humic substances under long-term fertilization in a wheat-maize system from North China. *J. Integr. Agric.* **2014**, *13*, 562–569. [CrossRef]
39. Galantini, J.; Rossel, R. Long-term fertilization effects on soil organic matter quality and dynamics under different production systems in semiarid Pampean soils. *Soil tillage Res.* **2006**, *87*, 72–79. [CrossRef]
40. Gerzabek, M.H.; Pichlmayer, F.; Kirchmann, H.; Haberhauer, G. The response of soil organic matter to manure amendments in a long-term experiment at Ultuna, Sweden. *Eur. J. Soil Sci.* **1997**, *48*, 273–282. [CrossRef]
41. Oktaba, L.; Odrobinska, D.; Uzarowicz, L. The impact of different land uses in urban area on humus quality. *J. Soils Sediments.* **2018**, *18*, 2823–2832. [CrossRef]
42. Kutova, A.; Hetmanenko, V.; Skrylnik, I.; Paramonova, T.; Kuts, A. Effect of irrigation and fertilization on the content and composition of humus of chernozem in the vegetable-fodder crop rotation. *AgroLife Sci. J.* **2020**, *9*, 192–197.
43. Hallett, P.D.; Young, I.M. Changes to water repellence of soil aggregates caused by substrate-induced microbial activity. *Eur. J. Soil Sci.* **1999**, *50*, 35–40. [CrossRef]
44. Leue, M.; Hoffman, C.; Hierold, W.; Sommer, M. In-situ multi-sensor characterization of soil cores along an erosion-deposition gradient. *Catena* **2019**, *182*, 104140. [CrossRef]
45. Rillig, M.C. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can. J. Soil Sci.* **2004**, *80*, 355–363. [CrossRef]
46. Vlček, V.; Pohanka, M. Glomalin-An interesting protein part of the soil organic matter. *Soil Water Res.* **2020**, *15*, 67–74. [CrossRef]
47. Bedini, S.; Avio, L.; Sbrana, C.; Turrini, A.; Migliorini, P.; Vazzana, C.; Giovanneti, M. Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem. *Biol. Fertil. Soils.* **2013**, *49*, 781–790. [CrossRef]
48. Bertagnoli, B.G.P.; Oliveira, J.F.; Barbosa, G.M.C.; Colozzi Filho, A. Poultry Litter and Liquid Swine Slurry Applications Stimulate Glomalin, Extraradicular Mycelium Production, and Aggregation in Soils. *Soil tillage Res.* **2020**, *202*, 8. [CrossRef]
49. Cissé, G.; Essi, M.; Kedi, B.; Mollier, A.; Staunton, S. Contrasting effects of long term phosphorus fertilization on glomalin-related soil protein (GRSP). *Eur. J. Soil Biol.* **2021**, *107*, 103363. [CrossRef]
50. Rillig, M.C.; Caldwell, B.A.; Wösten, H.A.B.; Sollins, P. Role of proteins in soil carbon and nitrogen storage: Controls on persistence. *Biogeochemistry* **2007**, *85*, 25–44. [CrossRef]