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The impact of the long-term application of mineral nitrogen and sewage sludge fertilizers on the quality of soil organic matter

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Abstract

Background: Soil fertility is substantially influenced by soil organic matter quality and quantity. Much attention has also been given to glomalin content as one of the qualitative parameters of soil fertility. Glomalin content is considered an indicator of soil fertility due to its positive correlation with soil organic carbon.

Aim: This study aim to (i) determine the influence of different fertilization systems on maize yield and soil organic matter quality parameters, (ii) ensure that changes in total glomalin content (TG) and easily extractable glomalin (EEG) content are sensitive enough to reflect changes in the SOM quality resulting from long-term fertilization, and (iii) determine whether both forms of glomalin must be determined or if the easily extractable glomalin (EEG) content is sufficient, even for a fertile chernozem.

Materials and methods: Long-term field experiments with silage maize monocultures were used to study relationships under different rates of mineral nitrogen and sewage sludge fertilization. The trials comprised 5 treatments: (i) no fertilization control (Con), (ii) and (iii) two different calcium ammonium nitrate rates (N120 and N240 at doses of 120 and 240 kg N ha⁻¹ year⁻¹, respectively), and iv) and v) two different sewage sludge rates (S120 and S240 corresponding to mineral N doses). Topsoil (0–30 cm) analysis was performed 28 years after the onset of the experiment to determine soil organic carbon, the fractions of humic substances, potentially mineralizable carbon and dissolved organic carbon, the potential wettability index, soil aggregate stability, EEG, and total glomalin (TG).

Results: The control treatment, which did not include fertilization, showed significantly lower average yields (9.76 t DM ha⁻¹ year⁻¹) than the fertilization treatments (on average 12.3 DM ha⁻¹ year⁻¹). Significant differences in yields were not found between the fertilization treatments. A positive correlation between glomalin (EEG, TG) content and soil organic matter carbon content was observed. A periodic application of sewage sludge increased the content of glomalin. However, the highest quality of SOM was found in the control treatment (nonfertilized). This treatment had the highest values for the humic/fulvic acid ratio and the highest contents of humic acids and potentially mineralizable carbon. Furthermore, the Con treatment showed the highest soil aggregate stability and potential wettability index. Mineral N fertilization significantly reduced soil aggregate stability values.

Conclusions: The unfertilized control showed the highest SOM quality but the lowest yields. The data on glomalin content can be used to study soil organic matter quality. Because the EEG extraction method is easy to apply, uses

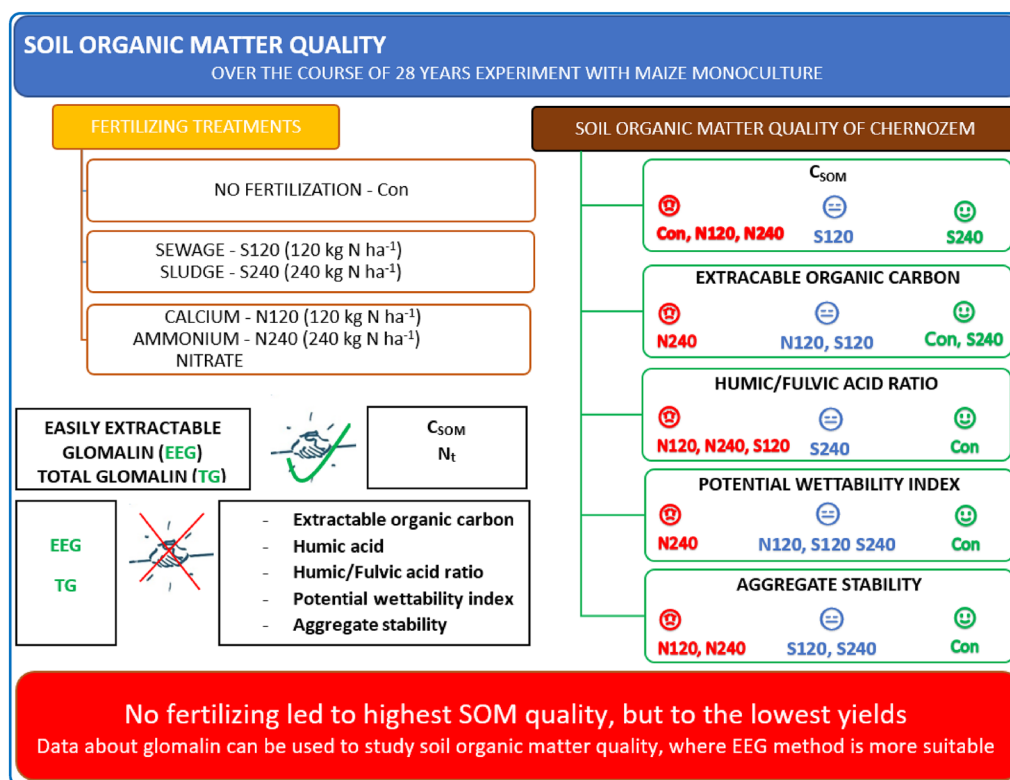
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lower quantities of chemicals and consumes less time, it presents a better option than TG extraction. The sensitivity of the methods for SOM quality determination decreases with increasing SOM content.

Keywords: Maize monoculture, Potential wettability index, Aggregate stability, Glomalin

Graphical Abstract



Introduction

Soil organic matter (SOM) can be divided into stable and labile fractions. The stable fraction is represented by humic acids (C_{HA}), fulvic acids (C_{FA}) and humins (C_{HS}) [1]. The labile forms are represented by potentially mineralizable carbon (C_{HWE}) or dissolved organic carbon (C_{DOC}). The fractionation of SOM into individual forms is important for SOM quality degradability assessment [2–5].

Currently, there are a number of different methods for determining SOM quality parameters. The current aim for agricultural and even scientific practices is to find a “user friendly” method that is characterized by reliable results, a simple procedure, ease of data evaluation, low cost, rapid evaluation time and low chemical consumption. Because of this, much attention has been given to glomalin content as one of the qualitative parameters of soil fertility [6].

Glomalin is produced by arbuscular mycorrhizae and is one of the most important soil proteins. The exact molecular composition of glomalin has not yet been defined, because fractions of glomalin extracted from soils are not sufficiently clean [7, 8]. This is also a reason why the term “glomalin-related soil protein”, abbreviated GRSP, is often used in the literature [9]. Wright and Upadhyaya [10] divided the forms of glomalin into easily extractable (EEG) and total glomalin (TG). Glomalin is hydrophobic and temperature stable. Substantial resistance to degradation in the soil has been described in many studies [9, 11, 12]. The slow change cycle for glomalin in the soil results from its high stability. Rillig [9] claimed that the average time for glomalin degradation in the soil is between 6 and 42 years. Harner et al. [13] estimated a similar time for a change cycle (40 years). Glomalin contains up to 85% polysaccharides that are resistant to microbiological degradation and, therefore, participate in bonding minerals

and organic particles into aggregates for long periods of time [14]. Glomalin also improves C sequestration in the soil [15]. Its influence on the formation of soil aggregates is greater than that on direct plant nutrition [16]. Glomalin acts as a sticky and insoluble biofilm that glues minerals, clays, organic matter and microorganisms together [17]. Organic inputs increase the portion of macroaggregates and the mean weight diameter of aggregates as well as the GRSP fractions [18–20].

Glomalin content can be considered a good indicator of soil fertility [10, 20] due to its positive correlation with the C_{SOM} content [21, 22]. This finding supports the fact that the contribution of glomalin to the total C content in soils is greater than the contribution of microbial biomass [23]. Furthermore, the long-term application of compost and manure increased the glomalin content in a soil [19, 20]. An increase in glomalin content was also found after the application of sewage sludge [22, 24]. There are only a few scientific publications that have focused on glomalin content in soils under long-term maize monoculture, i.e., Galazka et al. [25], and some studies contained in master theses, e.g., Sekgota [26]. However, these studies evaluated other parameters or only short-term monocultures and cannot be directly compared with our results. In our previous study [27], we found a positive correlation between glomalin content and humic acid content (C_{HA}) as well as glomalin content and the humic acid and fulvic acid ratio ($C_{HA/FA}$) under long-term silage maize production on a luvisol. We also found a positive correlation with the potential wettability index (PWI).

“Diffuse reflectance infrared Fourier transform spectrometry” (DRIFTS) can also be used for SOM quality assessment. Usually, it is used to study hydrophobic and hydrophilic functional groups of SOM at wavelengths 3000–2800 cm^{-1} and 1740, 1640–1600 cm^{-1} , respectively [20, 26, 28]. SOM is also characterized by the ratio of aliphatic (C–H) and carboxylic (C–O) bonds, called the potential wettability index (PWI) [29]. Demyan et al. [30] used the DRIFTS method to monitor SOM quality in long-term trials in Bad Lauchstädt (Germany). All of the observations obtained with DRIFTS showed an influence of fertilization.

The abovementioned facts prompted the following objectives: i) determine the influence of different fertilization systems on soil organic matter quality parameters (degradable and stable C fractions including glomalin content), ii) ensure that changes in total glomalin content (TG) and easily extractable glomalin (EEG) content are sensitive enough to reflect changes in the SOM quality resulting from long-term fertilization, and iii) determine whether both forms of glomalin have to be determined or if the easily extractable glomalin content (EEG) is sufficient, even for a fertile chernozem.

Materials and methods

Site description

The study was carried out based on long-term fertilization experiments that were initiated in 1993 at the experimental stations located in Suchdol in the Czech Republic. The basic soil-climatic characteristics are given in Table 1.

Experimental design

This long-term field experiment was initiated in a randomized complete block design (4 replications) with a plot area of 46 m^2 in 1993. In general, the experiment was designed to have five treatments: i) unfertilized control (Con), ii) mineral fertilization in the form of calcium ammonium nitrate (N120), iii) mineral fertilization in the form of calcium ammonium nitrate (N240), iv) sewage sludge (S120) and iv) sewage sludge (S240). A detailed description of the individual treatments is as follows: treatments N120 and N240 received 120 and 240 $kg N ha^{-1} year^{-1}$, respectively, in the form calcium ammonium nitrate, and treatments S120 and S140 received the same dose of N in the form of sewage sludge (calculated based on the sewage sludge analysis). Maize hybrids were planted in each plot at a density of 80 thousand plants ha^{-1} ($= 8 plants m^{-1}$). The maize was sown at the end of April/beginning of May with 70 cm between plant rows. Mineral N fertilizers were applied prior to sowing every year in spring with no additional liming or application of other nutrients. Sewage sludge (S120, S240 corresponding to mineral N doses) was applied every 3 years in autumn (October); therefore, the doses of nitrogen were 360 $kg N ha^{-1} 3 years^{-1}$ and 720 $kg N ha^{-1}$.

Table 1 Basic description of the Suchdol experimental site at the onset of the experiment

GPS coordinates	50°7'40"N 14°22'33"E
Altitude (m above sea level)	289
Mean annual temperature (°C)	9.1
Mean annual precipitation (mm)	495
Soil type	Haplic Chernozem ^a
Soil texture	Silt loam ^a
Clay (%) (< 0.002 mm)	2.2
Silt (%) (0.002–0.05 mm)	71.8
Sand (%) (0.05–2 mm)	26.0
Bulk density ($g cm^{-3}$)	1.42
C_{SOM} (%)	1.88 ^b
pH (0.01 mol/L $CaCl_2$)	7.5
CEC ($mmol_{(+)} kg^{-1}$)	262

^a Natural resource conservation service—United States Department of Agriculture [31]

^b CNS analyser (see Chapter 2.3., soil analysis)

3 years⁻¹ for S120 and S240, respectively. After application, the fertilizer was immediately incorporated into the soil with ploughing (25 cm depth). The sewage sludge was supplied by the same producer and was produced using the same method over the course of the entire experiment. The average dose of the sewage sludge was 10.17 t of DM ha⁻¹ 3 years⁻¹ and 20.34 t of DM ha⁻¹ 3 years⁻¹ for S120 and S240, respectively. During the experiment, the change in sewage sludge quality was insignificant. The average doses of key nutrients and the C/N ratio are presented in Table 2. The dose of sewage sludge was calculated according to the total nitrogen content determined with Kjeldahl analysis, which includes both organic and inorganic (N–NH₄⁺) forms. However, N–NO₃⁻ and N–NO₂⁻ forms are not determined using this method, because in an anaerobic material, such as sewage sludge, only trace levels of nitrates and nitrites are present.

Plants were protected only against weeds. Two rows of maize aboveground biomass (18 m² per plot) were harvested at silage maturity (roughly 65% biomass moisture content, R4 vegetation stage) and weighed to obtain the aboveground biomass yield (BY). Dry BY was calculated based on the dry mass ratio in the subsamples.

Soil analysis

Topsoil (0–30 cm) analyses were performed with air-dried soil samples (≤ 2 mm), except C_{DOC}, collected in September 2020, after the maize harvest. For aggregate stability (WSA) assessment, the soil aggregates of diameter 2–5 mm were sieved and further analysed.

The soil organic carbon (C_{SOM}) and total nitrogen (N_t) contents of the soils and sewage sludge were determined using oxidation with a CNS analyser Elementar Vario Macro (Elementar Analysensysteme, Germany) after carbonate digestion with HCl and subsequent drying.

Fractionation of humic substances (C_{HS}) was performed according to Kononova [1] to obtain the pyrophosphate extractable fraction, which represents the sum of the carbon in humic acids (C_{HA}) and fulvic acids (C_{FA}). Briefly, C_{HA} and C_{FA} were extracted from a 5 g soil sample with a mixed solution of 0.10 mol L⁻¹ NaOH and 0.10 mol L⁻¹ Na₄P₂O₇ (1:20 w/v). The following fractions of carbon were isolated: C_{FA} was obtained from a solution that was acidified by dilute

H₂SO₄ to a pH of 1.0–1.5 and left undisturbed for 24 h, and C_{HA} was obtained by the dissolution of the previously formed precipitate in a hot 0.05 mol L⁻¹ NaOH solution. Before iodometric titration, the dry matter formed by the vaporisation of each sample was dissolved in a mixture of 0.067 mol L⁻¹ K₂Cr₂O₇ and concentrated H₂SO₄ under an elevated temperature.

For the dissolved organic carbon (C_{DOC}), the extraction agent 0.01 mol L⁻¹ CaCl₂ was used (1:10, w/v) [32]. Analysis was performed on fresh soil samples (≤ 5 mm).

Hot water extraction was used to assess mineralizable soil organic carbon (C_{HWE}). Air dried (≤ 2 mm) soil samples were extracted with water (1:5, w/v). The suspension was boiled for 1 h [33]. The contents of C_{HWE} and C_{DOC} were determined by segmental flow analysis using infrared detection on a SKALAR SAN^{plus} SYSTEM (Skalar, Netherlands).

The potential wettability index (PWI) was determined directly in the soil samples using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). DRIFTS spectra were recorded by an infrared spectrometer (Nicolet IS10, Waltham, USA). The bands of the alkyl C–H groups—A (2948–2920 cm⁻¹ and 2864–2849 cm⁻¹) were assumed to indicate the hydrophobicity, and bands of the C=O groups—B (1710 and 1640–1600 cm⁻¹) indicated hydrophilicity [28].

$$\text{PWI} = A/B \quad (1)$$

Aggregate stability (WSA) was evaluated using the WSA index and was measured using the procedure reported by Nimmo and Perkins [34]. Four grams of air-dried soil aggregates (diameter of 2–5 mm) were sieved for 3 min in distilled water (0.25-mm sieve) with a frequency of 35 cycles per minute and a vertical amplitude of 1.3 cm. The aggregates that remained on the sieve were then sieved (same frequency and amplitude) in a sodium hexametaphosphate solution (2 g L⁻¹) until only the sand particles that remained on the sieve. The aggregates that dissolved by wetting in water or hexametaphosphate solution were subsequently dried at 105 °C and weighed. The WSA index was calculated as follows:

$$\text{WSA} = W_{ds}/(W_{ds} + W_{dw}) \quad (2)$$

where W_{ds} is the weight of aggregates dispersed in a sodium hexametaphosphate solution, and W_{dw} is the weight of aggregates dispersed in distilled water. An increase in the WSA value indicates an increase in soil aggregate stability.

Easily extractable glomalin (EEG) and total glomalin (TG) analysis were performed according to Wright and Upadhyaya [10]. Briefly, to the 1.00 g

Table 2 Doses of applied C, N, P, and K (in kg ha⁻¹ year⁻¹) and the C/N ratio of sewage sludge

Fertilizer	C	N	P	K	C/N
S120	879	120	82.0	15.6	7.32:1
S240	1758	240	164.0	31.2	

S120 sewage sludge (120 kg N ha⁻¹); S240 sewage sludge (240 kg N ha⁻¹)

of air dried soil (<2 mm) was added 8 mL of sodium citrate (20 mmol. L⁻¹ of pH 7.0—EEG, 50 mmol L⁻¹ pf pH 8.0—TG), followed by autoclaving at 121 °C (30 min—EEG, 60 min—TG), cooling and centrifugation at 5000 rpm (10 min—EEG, 15 min—TG). For TG, the centrifugation of the supernatant of the same sample was repeated 5 times until the supernatant no longer showed the red–brown colour that is typical of glomalin. Both forms of glomalin were determined colorimetrically using bovine albumin (BSA) as a standard for quantification and the Bradford protein assay (both from Bio-Rad, California, USA) to achieve the colour change.

Carbon sequestration efficiency (CSE) was calculated according to the following formula:

$$\text{CSE (\%)} = ((C_{\text{Ss}} - C_{\text{S}}) / C_{\text{Sa}}) * 100 \quad (3)$$

where C_{Ss} is the amount of C in the topsoil of the sewage sludge treatments, C_{S} is the amount of C in the topsoil of the control and C_{Sa} is the total amount (for 28 years) of C applied with sewage sludge (all in kg ha⁻¹).

Statistical analysis

The results were evaluated using ANOVA statistical analysis with Tukey's test using the Statistica program (TIBCO, Paolo Alto, California, USA). Principal component analysis (PCA) was performed to evaluate the relationships between the content of glomalin (EEG, TG) and the qualitative parameters of SOM using XLSTAT (Addinsoft, New York, USA). The variables were subjected to

PCA, and eigenvalues > 1, variance (%) and cumulative (%) criteria were used to define the association among variables.

Results

Yield of maize biomass

The silage maize biomass yields for the period 1997–2020 are presented in Fig. 1. The experiments were initiated in 1993, and the fields have been fertilized regularly since. Biomass yields have been monitored accurately since 1997. The average yield in the Con treatment was 9.76 t DM ha⁻¹. The nutrients in the soil at this site could not be fully utilized due to a lack of precipitation. The efficacy of nitrogen fertilization (N120 and N240) was higher than the efficacy of sewage sludge fertilization. The key reason for this difference is that the sewage sludge was applied only once every 3 years, which reduced total nitrogen utilization from sludge.

The dry matter silage maize yields are presented in Fig. 2. Only the average values from 3-year intervals are presented to make orientation easier. The year-to-year variation in the yields at this site was consistently linked mainly to the lack of rainfall during the growing season. Nevertheless, there was a certain decreasing tendency in the yields, especially since 2015.

Approximate C balance

The nitrogen dose and organic matter content in sewage sludge were reflected in the soil organic carbon content (C_{SOM}) (Table 3). The C_{SOM} content was 1.88%

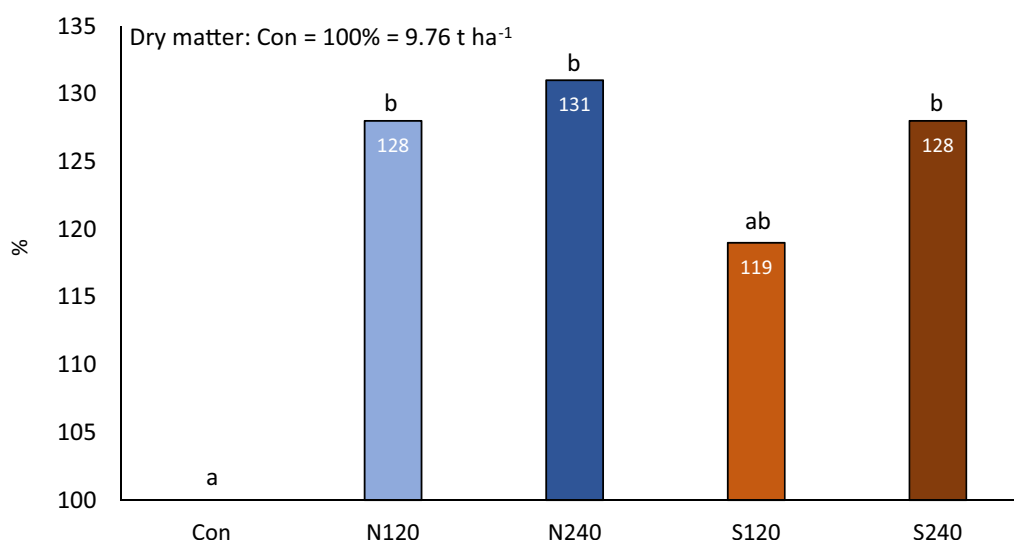


Fig. 1 Relative average silage DM yields (1997–2020). Different letters describe statistically significant differences between treatments. Tukey's HSD test ($p < 0.05$). Con Unfertilized control; N120 Mineral fertilization (120 kg N ha⁻¹); N240 Mineral fertilization (240 kg N ha⁻¹); S120 Sewage sludge (120 kg N ha⁻¹); S240 Sewage sludge (240 kg N ha⁻¹)

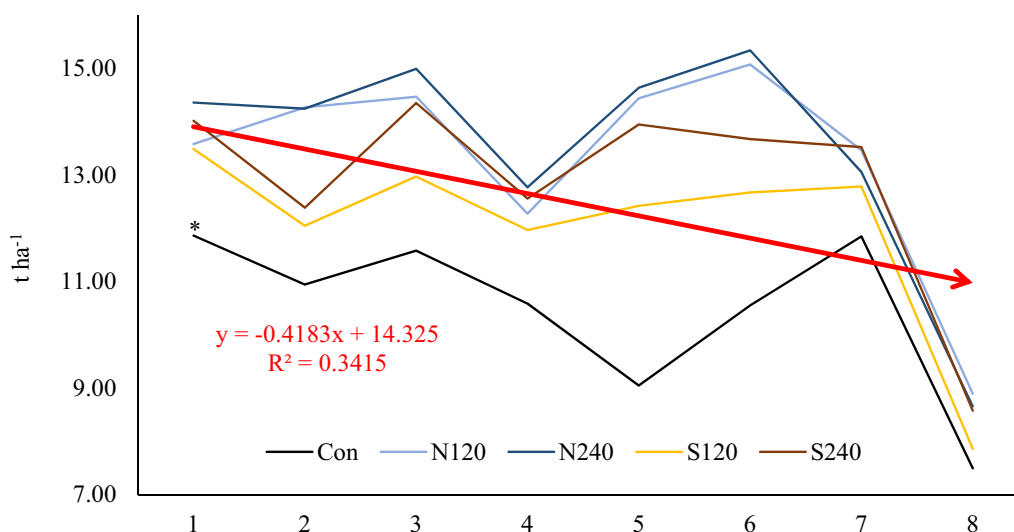


Fig. 2 Trends in maize monoculture silage dry mass yields during the experiment. The red trend line is an average of all treatments. The horizontal axis represents 3-year periods starting with 1997–1999 (1) and ending with 2018–2020 (8). *Con* Unfertilized control; *N120* Mineral fertilization (120 kg N ha⁻¹); *N240* Mineral fertilization (240 kg N ha⁻¹); *S120* Sewage sludge (120 kg N ha⁻¹); *S240* Sewage sludge (240 kg N ha⁻¹) *only the unfertilized control treatment showed significantly low yields (Tukey HSD test; $p < 0.05$) during all periods, and differences among the fertilization treatments were not significant

Table 3 Qualitative and quantitative soil organic matter (SOM) parameters

Parameter	Con	N120	N240	S120	S240
C _{SOM} (%)	1.62 ^a	1.61 ^a	1.61 ^a	1.75 ^a	1.84 ^a
C _{HWE} (mg kg ⁻¹)	196 ^{cd}	165 ^{ab}	148 ^a	172 ^{bc}	189 ^c
C _{DOC} (mg kg ⁻¹)	26.4 ^c	21.1 ^b	15.4 ^a	22.4 ^b	29.7 ^d
C _{HA} (%)	0.244 ^c	0.162 ^a	0.185 ^{ab}	0.182 ^{ab}	0.222 ^{bc}
C _{FA} (%)	0.182 ^a	0.173 ^a	0.195 ^a	0.165 ^a	0.189 ^a
C _{HS} (%)	0.487 ^a	0.486 ^a	0.529 ^a	0.450 ^a	0.544 ^a
C _{HA/FA}	1.352 ^b	0.950 ^a	0.948 ^a	1.102 ^a	1.215 ^{ab}
N _t (%)	0.144 ^a	0.138 ^a	0.148 ^a	0.155 ^a	0.162 ^a
C _{SOM} /N _t	11.2 ^a	11.6 ^a	10.9 ^a	11.3 ^a	11.4 ^a
PWI	0.029 ^b	0.021 ^{ab}	0.020 ^a	0.021 ^{ab}	0.023 ^{ab}
WSA	0.509 ^b	0.397 ^a	0.387 ^a	0.426 ^{ab}	0.418 ^{ab}
EEG (mg kg ⁻¹)	983 ^{ab}	952 ^a	999 ^{ab}	971 ^{ab}	1052 ^b
TG (mg kg ⁻¹)	2091 ^a	2027 ^a	2013 ^a	2126 ^a	2145 ^a

Different superscript letters describe statistically significant differences between treatments. Tukey HSD test; $p < 0.05$

Con Unfertilized control; *N120* Mineral fertilization (120 kg N ha⁻¹); *N240* Mineral fertilization (240 kg N ha⁻¹); *S120* Sewage sludge (120 kg N ha⁻¹); *S240* Sewage sludge (240 kg N ha⁻¹). C_{SOM} Soil organic matter carbon; C_{HWE} Mineralizable soil organic carbon; C_{DOC} Dissolved organic carbon; C_{HA} Carbon of humic acids; C_{FA} Carbon of fulvic acids; C_{HS} Carbon of humic substances; C_{HA/FA} Carbon in humic and fulvic acid ratio; N_t Total nitrogen content; C_{SOM}/N_t Soil organic matter carbon/total nitrogen ratio; PWI Potential wettability index; WSA Aggregate stability index; EEG Easily extractable glomalin; TG Total glomalin

at the beginning of the trial in 1993, and after 28 years, the content changed to 1.62% in the *Con* treatment—a

reduction of 0.26%. Relatively speaking, the decrease was 13.8%. This means that there was a loss of carbon from the soil. The loss of carbon amounted to 11310 kg C ha⁻¹ in the topsoil (0–30 cm). On average, the carbon loss in the *Con* treatment was 404 kg C ha⁻¹ year⁻¹. A similar decrease was observed in the *N120* and *N240* treatments. The C_{SOM} content in the *S120* treatment was 1.75% in 2020. This is a decrease of 0.13% in comparison with 1993. The difference between the organic carbon content (2020 minus 1993) for the *S120* treatment represented losses of 5655 kg C ha⁻¹. To the total balance, we added the carbon applied with sewage sludge, i.e., 24612 kg C ha⁻¹ 28 years⁻¹. The carbon losses from soil combined with sewage sludge were, therefore, 30267 kg C ha⁻¹ 28 years⁻¹. Using the same calculation principle for the *S240* treatment, the total carbon loss was 50964 kg C ha⁻¹ 28 years⁻¹.

Soil organic matter quality parameters

Although there were no significant differences, the *S120* and *S240* treatments showed an increasing tendency in the C_{SOM} content related to *Con*. This trend was more evident in *S240*. The total nitrogen (N_t) content was not significantly influenced by fertilization, despite the increasing tendency for *S240* (Table 3).

Intensive mineral nitrogen fertilization (*N240*) increased the mineralization rate of stable organic matter, which in turn caused a substantial decreasing tendency in the C_{SOM}/N_t ratio (Table 3). Soil organic matter

quality was also monitored by hot water extraction and $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$ extraction (Table 3). The lowest values were consistently present in the N240 treatments. In the previous time period, easily degradable carbon fractions were mineralized in the N240 treatment. The lowest content of extractable carbon was observed during the most recent soil analysis (from 2020). Significant increases in the C_{HWE} and C_{DOC} contents were recorded in the S240 treatment, although the last sludge application occurred in the autumn of 2017, 3 years prior to this analysis. The C_{HWE} and C_{DOC} contents in the S240 treatment were comparable to those in the Con treatment, which demonstrated the relative stability of SOM in the Con treatment. The highest humic and fulvic acid ratios were found in the Con treatment, which was even higher than that in S240 (Table 3). None of the observed treatments produced significant differences in either humic substances content (C_{HS}) or fulvic acid content (C_{FA}).

The SOM quality was further monitored using the DRIFTS method. The potential wettability index (PWI) determines the hydrophobic and hydrophilic group ratio (Table 3). Significant differences were discovered between the Con (highest value) and N240 (lowest value) treatments. To express soil aggregate stability, the WSA index was used. The N120 and N240 treatments showed the lowest stability, with values significantly lower than those of the other treatments. This WSA method confirmed the results from the C_{HWE} and C_{DOC} extraction methods—intensive nitrogen fertilization degrades stable organic matter and, in turn, degrades soil structure. In contrast, the Con treatment had the highest soil aggregate stability, with values surpassing that of sludge application in the S240 treatment.

One of the goals of our experiment was to evaluate how all the changes influenced the glomalin (EEG and TG) content (Table 3). A significant increase in EEG content was observed only in the S240 treatment. The treatments produced no significant differences in terms of TG content. The relationships between glomalin

content and other parameters related to SOM quality are presented in Table 4. Glomalin content was significantly related only to C_{SOM} and N_t content. The correlations between C_{SOM} and other parameters (C_{SOM}/N_t ; PWI; C_{HWE} ; C_{DOC} ; C_{HA} ; $C_{\text{HA/FA}}$; WSA) were not significant (Table 4).

Principal component analysis (PCA) was applied to the variables mentioned in Table 5. The variable associations and number of significant components were systematically selected based on three basic criteria, i.e., eigenvalues > 1.1 , loading factors ≥ 0.59 and percentage of variability $> 8\%$.

The biplot position of the components in Fig. 3 explained 78% of the cumulative variance, where the first factor described 52% and the second described 26%. PC1, which explained 52% of the total cumulative variance, and 6.7 of the eigenvalue were highly dominated by positively and strongly associated variables, such as TG, C_{SOM} , PWI, C_{HWE} , C_{DOC} , $C_{\text{HA/FA}}$, WSA and C_{HA} . Approximately 30–32% of the variable associations in PC1 was contributed by the S240 and N120 treatments. The second PC2 explained approximately 26% of the total cumulative variance and 3.4 of the eigenvalue and was dominated by the positively and strongly associated variables EE, C_{HS} and C_{FA} , and these variables were positively influenced by the S₂₄₀ treatment, whereas the third PC accounted for 14% of the total cumulative variance and 1.8 of the eigenvalue and was dominated by the weakly associated variables C_{SOM}/N_t and N_t . PC4 also explained 8% of the total cumulative variance and 1.1 of the eigenvalue, and no variables dominated this component.

Table 4 Relationship between glomalin and other soil parameters

	C_{SOM}	N_t	C_{FA}
EEG	0.791 ^b	0.773 ^b	0.458 ^a
TG	0.818 ^b	0.732 ^b	0.357
C_{SOM}	—	0.909 ^b	0.391

Pearson's correlation coefficient. Correlations with other parameters (C_{SOM}/N_t ; PWI; C_{HWE} ; C_{DOC} ; C_{HA} ; $C_{\text{HA/FA}}$; WSA) were not significant

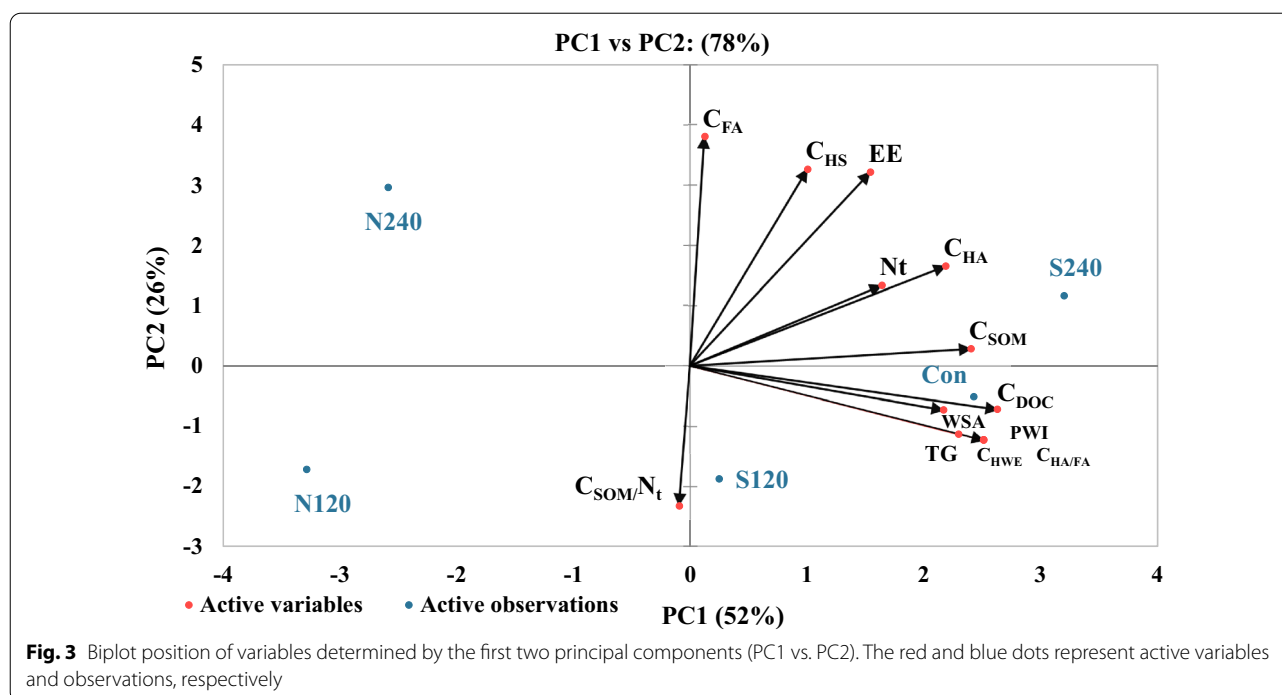
C_{SOM} Soil organic matter carbon, N_t Total nitrogen content, C_{FA} Carbon of fulvic acids, EEG Easily extractable glomalin, TG Total glomalin

^a $p < 0.05$;

^b $p < 0.001$

Table 5 Principal components (PCs) or factors and their loading factor values, eigenvalues, and variabilities (%)

Parameters	PC1	PC2	PC3	PC4
EE	0.55	0.82	0.17	0.00
TG	0.82	− 0.29	0.48	− 0.06
C_{SOM}	0.86	0.07	0.40	− 0.31
C_{SOM}/N_t	− 0.03	− 0.59	0.60	0.54
N_t	0.59	0.34	0.59	− 0.43
PWI	0.90	− 0.31	− 0.25	0.17
C_{HWE}	0.90	− 0.31	− 0.25	0.17
C_{DOC}	0.94	− 0.18	0.18	0.22
C_{HS}	0.36	0.83	0.14	0.41
C_{FA}	0.04	0.97	− 0.09	0.23
C_{HA}	0.78	0.42	− 0.45	0.08
$C_{\text{HA/FA}}$	0.90	− 0.31	− 0.25	0.17
WSA	0.78	− 0.19	− 0.45	− 0.40
Eigenvalue	6.7	3.4	1.8	1.1
Variability (%)	52	26	14	8



Discussion

The benefits of silage maize monoculture experiments belong to a small set of variable factors that could influence changes in the quality and quantity of soil organic matter. It is generally accepted that maize belongs to a group of crops that is substantially dependent on arbuscular mycorrhizae and, at the same time, promotes their development [35]. Another variable was the difference in the quality of the applied fertilizers (mineral N/organic). Sewage sludge was included as a treatment, because its production is consistently increasing with the increasing world population, and 37% is currently being utilized on farming lands in EU countries [36].

Aboveground biomass yields are closely correlated with the quantity of roots and stubble, which influences the soil organic matter content. The number of roots in the topsoil was not monitored in the current experiment. However, in our previous study on a luvisol [37], the yearly production of root biomass carbon was 394 kg ha⁻¹, while the fertilized treatments had 1.9 × more, approximately 749 kg ha⁻¹. The carbon content in stubble was 208 kg C ha⁻¹ year⁻¹ in the control treatment and 1.5 × higher in the fertilized treatment (312 kg C ha⁻¹ year⁻¹). The content in stubble + root biomass was 1061 kg C ha⁻¹ (312 + 749). If we base the following calculation on results from this study, we can assume the chernozem reached approximately 10% higher values. In the fertilized treatments, stubble + root biomass (topsoil only) supplied 1100–1200 kg

C ha⁻¹ year⁻¹. The quantity of organic matter supplied to the soil by sewage sludge in the S120 and S240 treatments was 879 and 1758 kg C ha⁻¹ year⁻¹, respectively (Table 2). The annual addition of C was lower than that with stubble + root biomass in the S120 treatment. This may be the reason for the lack of a significant increase in C_{SOM} content in the S treatments in comparison with the Con treatments. The other reason is the fact that organic matter quality in sewage sludge is not high in comparison with farmyard manure, and it does not contribute much to the SOM content. The relatively low C/N ratio (7.32:1) could also promote fast mineralization of the organic matter in sewage sludge. The carbon sequestration efficiency from both sewage sludge treatments was calculated to estimate their effect on carbon accumulation. The S240 treatment showed lower CSE (19.0%) than S120 (22.5%). The mentioned balances and data on CSE clearly show that the organic matter present in sewage sludge is not stable. Intensive nitrogen fertilization (N240) caused an increase in aboveground biomass, which caused an increase in root biomass. Root biomass has a lower C/N ratio than Con and is mineralized faster [37]. In addition, the mineralization rate of soil organic matter is greater due to the “priming effect”.

The fulvic acid content was influenced by neither the intensity nor the dose of fertilizer. The humic acid content was highest in the Con treatment. The humic/fulvic acid ratio is an important parameter for the determination of soil organic matter quality [1]. The highest quality

in terms of the $C_{HA/FA}$ ratio was present in the Con treatment. The changes in soil organic matter quality (in terms of C_{HA} , C_{FA} , C_{HS} , and $C_{HA/FA}$) were determined to be relatively small.

In our previous work, in which we used the same levels of fertilization on a luvisol, much larger differences in the C_{SOM} qualitative parameters were observed among treatments [27], which was caused by significantly lower organic matter content and quality (for example, in the control treatment: C_{SOM} —0.98%; C_{HA} —0.078%; $C_{HA/FA}$ —0.565; C_{SOM}/N_t —10.2). For comparison, the following values were measured for the current work on the chernozem: C_{SOM} —1.62%; C_{HA} —0.244%; $C_{HA/FA}$ —1.352; C_{SOM}/N_t —11.2. This is the most likely reason for their nonsignificant relationships with glomalin (both EEG and TG) content in this experiment. Another factor could be the stability of glomalin in soils; it can persist for up to several decades [9], and therefore, the influence of even long-term fertilization can appear very slowly.

A significant increase in EEG content was observed in the S240 treatment after the application of an extremely high dose of sewage sludge. Sandeep et al. [24] and Balík et al. [22] published similar results resulting from sewage sludge application.

The significant increase in EEG content in the S240 treatment was probably caused by increased root biomass and, therefore, increased glomalin production. Sewage sludge could not be confirmed to be a direct source of glomalin. The method used by Wright and Uppadhyaya [10] was designed to extract glomalin from soil and not from organic fertilizers. The presence of arbuscular mycorrhizae and glomalin was not expected in the sewage sludge, because it was obtained directly from the producer without intermediate storage.

Cross-reactivity of the Bradford assay with humic acids, polyphenolic compounds, sugars, and lipids can interfere with GRSP determination [39]. The aforementioned organic compounds can lead to misestimations of GRSP contents [21]. In particular, the treatments fertilized with sewage sludge probably increased the production of organic molecules in the soil (lipids, carbohydrates and others), which may have led to interferences during glomalin analysis.

In our experiments, mineral nitrogen fertilization did not influence glomalin content, contrary to the conclusions of other studies that reported a positive influence of N fertilization on glomalin content [22, 39]. A probable reason is that intensive mineral N fertilization leads to the degradation of stable organic matter and lowers the stability of soil aggregates. This results in soil structure deterioration, which could later decrease the abundance of arbuscular mycorrhizae and subsequently glomalin production.

Increased degradation of SOM was present during the experiment due to intensive mineral nitrogen fertilization (N120, S120). This is why the N240 treatments produced the lowest contents of C_{HWE} and C_{DOC} . This fact was not reflected in the EEG and TG contents and their correlation with C_{HWE} and C_{DOC} . These results indirectly confirmed that glomalin is relatively stable during mineralization. Glomalin persists for longer periods of time than the original organic matter present in bulk soil [40].

In this study, only a significantly positive correlation of glomalin with the C_{SOM} and N_t parameters was confirmed. Relationships between glomalin content and other soil organic matter quality parameters (C_{HA} , $C_{HA/FA}$, PWI) were not found, in contrast to our previous study with a luvisol [27]. A difference between the sensitivity of both the EEG and TG methods in relation to C_{SOM} content and other qualitative parameters of SOM was not found. Therefore, the higher usability of the EEG method was supported by its ease of implementation, as mentioned in the introduction section.

The presented results seem to suggest that soil glomalin content determination can be used to estimate the quality of soil organic matter, but with limited indicative value, especially for soils with naturally high fertility within a relatively short time horizon (for example, 10 years). This method is sensitive for estimating site characteristics. The influence of soil type can be demonstrated by comparing the glomalin content of the chernozem and luvisol: the EEG content in the Con treatment in this work (chernozem) was 983 mg kg^{-1} , while the content of EEG in the Con treatment of the luvisol was 578 mg kg^{-1} . Furthermore, the EEG/TG ratio was 0.47 in the chernozem, while in our previous study with a luvisol, it was only 0.29 [27].

The potential wettability index (PWI), which includes the ratio of aliphatic (C–H) and carboxyl (C–O) bonds, can be used to describe soil organic matter quality [29]. The Con treatment showed the highest PWI values; this was in accordance with the fact that this treatment also showed the highest $C_{HA/FA}$ ratio, which represented the highest SOM quality. The higher PWI values in the Con treatment also indicated higher stability of the soil aggregates. Statistically, the lowest values of PWI were present in N240, which also corresponded with the lowest soil aggregate stability (WSA). The S240 treatment showed a slight increase in the PWI value. Adani and Tambone [41] also reported a higher content of the aliphatic carbon fractions in humic acids, even with a lower sewage sludge dose of $1 \text{ t ha}^{-1} \text{ year}^{-1}$ over a duration of 10 years. The correlations between EEG and PWI as well as the correlations between C_{SOM} and PWI presented in our earlier study [27] were not confirmed in this study.

The Con treatment showed the highest soil aggregate stability. The sewage sludge treatments led to an increasing trend in stability as well, which was also confirmed in several other studies [42]. The values of PWI and WSA in our experiment showed a nonsignificant correlation with the C_{SOM} content. This might be caused by the aforementioned fact that the current experiment was conducted on a fertile chernozem.

Conclusions

In general, the highest quality of SOM was found in the unfertilized control treatment. However, this treatment showed significantly lower yields. Because of this, other fertilizing strategies should be tested to obtain higher yields together with higher SOM sustainability.

The data on glomalin content can be used to study soil organic matter quality. EEG extraction is more advantageous than TG extraction, because the procedure and data evaluation are easier and it has lower costs, requires less time and consumes fewer chemicals relative to TG analysis.

The results suggest that with increasing SOM quantity, there was a decrease in sensitivity for all the methods used for SOM quality determination. Nonetheless, further studies focused on different crops, soil and climate conditions are necessary to prove this.

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Author contributions

JB and MK—conceptualization; JC and OS—formal analysis and methodology; JB—writing original draft; PS, SP and ADA—laboratory and statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated and presented in this study as well as source data are available at the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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