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The Effect of Anaerobic Digestate on the Soil Organic Carbon and Humified Carbon Fractions in Different Land-Use Systems in Lithuania

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Abstract: The most important component of agricultural system are soils as the basis for the growth of plants, accumulation of water, plant nutrients and organic matter. The main task of our research was to ascertain changes in soil organic carbon (SOC) and mobile humified carbon fractions in digestate-treated soils. We have performed three field experiments using the same design on two soil types in 2019–2020. We studied the fertilization effects of different phases of digestate on Retisol and Fluvisol. Fertilization treatments: control; separated liquid digestate 85 kg ha^{-1} N; and 170 kg ha^{-1} 170 N; separated solid digestate 85 kg ha^{-1} N; and 170 kg ha^{-1} N. We have found a greater positive effect on the increase in SOC because of the use of the maximum recommended fertilization rate of the solid digestate. The content of mobile humic substances (MHS) tended to increase in grassland and crop rotation field in digestate-treated soil. In our experiment, maximum concentration of SOC was found in 0–10 cm soil layer, while in the deeper layers the amount of SOC, MHS and mobile humic acids proportionally decreased. We concluded, that long-term factors as soil type and land use strongly affected the humification level expressed as HD (%) in the soil and the highest HD was determined in the grassland soil in Fluvisol.

Keywords: carbon fractions; mobile humic substances; humic acids; soil; solid digestate fraction; liquid digestate fraction; Fluvisol; Retisol

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Citation: Slepetiene, A.; Kochiieru, M.; Jurgutis, L.; Mankeviciene, A.; Skersiene, A.; Belova, O. The Effect of Anaerobic Digestate on the Soil Organic Carbon and Humified Carbon Fractions in Different Land-Use Systems in Lithuania. *Land* **2022**, *11*, 133. https://doi.org/10.3390/land11010133

Academic Editors: Evgeny Lodygin and Cezary Kabala

Received: 29 November 2021
Accepted: 12 January 2022
Published: 15 January 2022

1. Introduction

As the world is facing climate changes issues today, there is a strong demand for the alternative ways to manage our ecosystems. The long lived standard of the linear economy is becoming less and less widely used, as the concept of the circular economy is being promoted politically, economically and socially [1]. There is a strong effort to reduce agriculture systems dependency on pesticides and antimicrobials, reduce excess fertilization, increase organic farming, improve animal welfare, and reverse biodiversity loss [2]. Soils form a landscape, accumulate water, plant nutrients and organic matter, captures pollutants and exert climate control functions [3]. The soil and the multitude of organisms that live in it provide us with food, biomass and fibers, raw materials, regulate the water, carbon and nutrient cycles and make life on land possible [4]. Soil use, different cultivation and fertilization have an impact on its chemical properties. The improper use of agricultural land leads to the deterioration of the soil agricultural properties and the decrease of its fertility [5]. Furthermore, the excessive agricultural activity causes the destruction of soil organic matter (SOM) due to the depletion of the organic carbon (C_{org}) compounds. Erosion of soils can lead to degradation of soil organic carbon (SOC), which can be released as carbon dioxide or methane.

The intensity of agricultural crops' cultivation supports the process of mineralization and decreases organic matter in the soil, which often causes the deterioration of chemical and other properties of the soil [6,7]. Recently, the soil has been identified as a potential tool to reduce the greenhouse gas emissions [8]. There are different approaches for the carbon sequestration in order to return the organic carbon into the soil as, for example, by using compost or digestate streams. Therefore, it is important to evaluate the changes of SOM and its constituent valuable compounds in soils with different properties and their different land uses.

Although the content of SOM, the amount and quality of humic substances (HS) in the soil depend on soil genesis, the changes in farming methods also have influence on the SOM, and SOC [9]. The main component of SOM is SOC [10]. However, SOC is included in the soil in organic compounds of different stability. HS are the main source of stable carbon in the soil. One of them is the fraction of humic acids (HA) in the soil, which increases the soil's C storage capacity. HS are characterized to be the compounds soluble in alkaline solution. Extractable by 0.1 N NaOH, soil mobile humic substances (MHS) can be further subdivided into the mobile humic acids (MHA) and the mobile fulvic acids (MFA) and both are considered as already affected by humification process. Soil MHS are formed in the initial stages of the humification process. Therefore, they are relatively "young" HS. HS were thought to be large, irregular, aromatic organic molecules resistant to the microbial decomposition [11].

The scientific literature states that HS usually are formed in the soil [12]. However, they can also form in other environments. There is little scientific evidence of humification occurring during anaerobic digestion [13]. During digestion process, organic matter of the digestate is changed and modified to the more stable compounds. Characterizations of the C_{org} compounds of digestates by FTIR spectra indicated that the anaerobic stabilization of organic matter is mainly due to the buildup of more stable compounds in the dry matter rather than humification processes [14,15]. According to Wang et al., HA, a byproduct formed during the biological conversion of organic matter into biogas in the anaerobic digestion (AD) process, contains complex structures and redox functions [16]. However, the evolution mechanism of HA and their interaction with CH_4 production during the AD process have not been fully explored, particularly with respect to various substrates and temperature conditions. The aforementioned researcher investigated the evolutionary dynamics of the structure and function of HA that naturally formed in the AD processes of chicken manure and corn stover under mesophilic (37 °C) and thermophilic (55 °C) conditions. There was reported, that the HA performed positive and negative effects on CH_4 production in the fast and slow CH_4 production stages, respectively. The adequacy of digestate as a tool for the soil amendment is based on its modified OM content. Most OM is converted into biogas, while the biological stability of remaining OM was increased during AD with the increase of more recalcitrant molecules such as lignin, cutin, HA, steroids, complex proteins. These aliphatic and aromatic molecules are possible humus precursors with high biological stability [17]. It was determined that during sludge anaerobic digestion, 16.3% of HA and 27.0% of FA were degraded, but the degradation rate was relatively low compared with that of other organic substances in sludge. Besides the mineralization of sludge HS, humification processes also took place [18]. The HS extracted from the digested sludge have more oxygen functional groups, more aromatic structures and larger molecular sizes compared with the HS extracted from the raw sludge. However, the degree of humification was low, and mineralization was still the main process that occurred during sludge anaerobic digestion. According to Li et al. this information on HS degradation is helpful in understanding anaerobic digestion and also provides guidance on treatment or utilization of digested sludge [18].

Alternatively, it was concluded, that anaerobic digestion has only a minor influence on the total amounts of highly recalcitrant compounds in the organic manures, which basically influences long-term SOM contents and long-term soil fertility [19]. However, there is still no scientific consensus on the extent to which the anaerobic digestion process is important for the conversion of organic compounds into more stable forms that could be useful for the C storage in the soil. It is thought that anaerobic digestate has a negligible effect on SOM in the long term.

The benefit of anaerobic digestate as a fertilizer or amendment depends on the composition and quality of feedstocks [20]. As has already been mentioned before, the organic fraction of anaerobic digestate is more recalcitrant than the input feedstock due to the mineralization processes during anaerobic digestion, although not the total easily degradable organic matter is degraded [21–23]. During the anaerobic digestion process along with the biogas production the HS precursors or humic-like substances, or naturally formed HS, are already formed in the fermentation media and the digestate. Once the digestate is inserted into the soil, the residual organic matter is transformed again by the microorganisms present in the soil and mineralization and humification processes occur. Nevertheless, the digestate behavior and transformation process depend on the soil characteristics, plants grown, the type and rate of fertilizer applied, and other conditions.

It is important to assess the potential benefits of solid and liquid phases of the digestates not only as the biofertilizers, but also as the potential improvers of the soil properties. There is a lack of scientific knowledge on the changes in HS, MHS and MHA in digestate-treated soils. Therefore, the present research would fill in this currently under-filled research niche.

The aim of this study was to demonstrate changes in SOC and mobile humified carbon fractions in digestate-treated low fertility and eroded soils in different land-use systems.

2. Materials and Methods

2.1. Experimental Conditions

Experimental sites were selected using the LTDK 99 (Classification of the soils of Lithuania) and WRB 2015 (World reference base for soil resources) [24] soil databases (Figure 1).

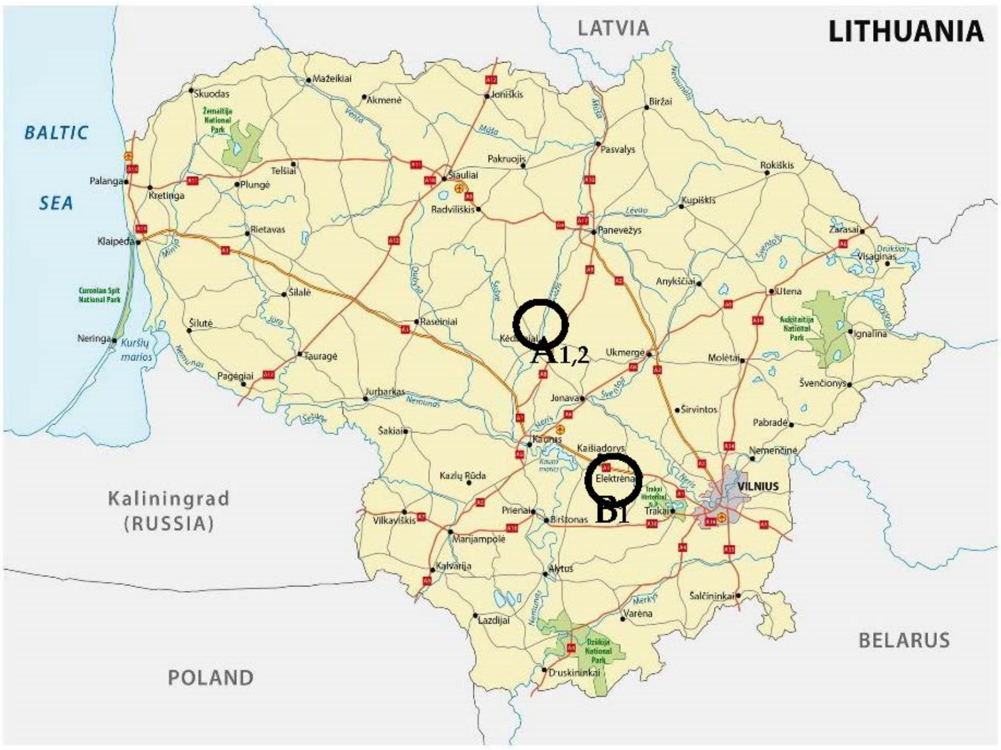

Figure 1. Experimental sites. The three field experiments' locations. $A_{1,2}$—two field experiments in Kėdainiai district, the Fluvisol soil type. A_1—the land-use system of semi-natural grassland, A_2—the field of crop rotation (cereals were grown), B_1—the field experiment in Elektrenai district, the Retisol soil type and semi-natural grassland growth.

Three field experiments were carried out according to the same fertilization design on two types of soil. The effects of digestates on Retisol in Elektrenai district, where the land-use system was semi-natural grassland were studied. In Fluvisol, Kedainiai district, the influence of digestate on the soil in the two land-use systems: semi-natural grassland and a field of crop rotation, where cereals were grown, was studied.

Fertilization treatments: (1) control (without fertilizers); (2) separated liquid digestate 85 kg ha^{-1} N (85N LD); (3) separated liquid digestate 170 kg ha^{-1} 176 N (170N LD); (4) separated solid digestate 85 kg ha^{-1} N (85N SD); (5) separated solid digestate 170 kg ha^{-1} 177 N (170N SD). The application rates of LD and SD were calculated based on the total Kjeldahl nitrogen (TKN) content in the digestate. A randomized experimental design with three field replicates was used, and the plots were $2 \times 3 = 6$ m^2.

2.2. Digestate and Soil Sampling

Fertilizers were applied in early spring of 2019 and 2020. For fertilization, the samples of anaerobic digestion by-product digestate, recovered from the agricultural waste, were removed from the reactor and both output streams (solid and liquid phases). Approximately 5 kg of separated solid digestate and 5 L of separated liquid digestate were collected and delivered to the laboratory. Digestate samples were mixed and stored at −20 °C until analysis. Soil samples were collected at 0–10, 10–20, 20–30 and 30–40 cm depths in 2019 and 2020. For each soil sample, five cores were randomly collected from each plot, assembled in depth on the field, and taken to the laboratory. The soil samples were air dried and ground to pass through a 2 mm sieve; the sub-samples were then finely ground to pass through a 0.25 mm sieve.

2.3. Chemical Analyses

Soil chemical analyses were done at the Chemical Research Laboratory of LAMMC, Institute of Agriculture. Coarse ground soil samples were used to determine pH. The finely ground samples were used to determine SOC, MHS and MHA. The pH of the soil was determined by the potentiometric method in 1 M KCl (1:2.5, *w/v*) extract. The content of SOC was determined by a spectrophotometric measurement method at a wavelength of 590 nm using glucose as a standard after wet combustion according to Nikitin [25]. MHS were extracted with 0.1 M NaOH [12]. The suspension was periodically shaken at ambient temperature for 24 h, after which 10 mL of a saturated Na_2SO_4 solution was added and MHS was separated by centrifugation at 3800 rpm (Universal 32, Hettich, Germany) for 10 min. For MHS determination, an aliquot of the extract was evaporated to dry mass and quantified spectrophotometrically. For the determination of MHA, an aliquot of the extract was acidified to pH 1.3–1.5 with 1 M H_2SO_4 and heated at +68–70 °C to precipitate the MHA. The MHA were determined after wet-combustion by spectrophotometric measurement as for SOC. The reagents of a recognized analytical grade (GR and AR) and only distilled water or water of equivalent purity were used during the analysis. Humification degree of OM was calculated according to the formula: HD = (MHA/SOC × 100).

2.4. Chemical Composition of the Digestate

Digestate from anaerobic digestion plant can be processed or used directly as a fertilizer in agriculture. Digestate can be used raw but can be additionally prepared using separation technique. Typically, the digestate from the bioreactor is further separated into the solid and the liquid fractions. In our study the solid–liquid separation step was performed via the screw press (Börger Bioselect separator) in the biogas plant. The organic carbon content and mobile humic and fulvic acids were determined after wet-combustion by spectrophotometric measurement at the wavelength of 590 nm using glucose as the standard. The total solids (TS) content of whole, solid and liquid digestate was evaluated after drying to constant weight; the content of organic matter (OM) by loss on ignition at 550 °C for 24 h. For the total and liquid digestate, the pH was measured immediately after the homogenization of the fresh sample. In the solid digestate, the pH in deionized water extract (1:5, *w/v*) was measured. The total Kjeldahl nitrogen (TKN) content was evaluated in fresh samples.

2.5. Statistical Analysis

The statistical software package SAS 7.4 was used to calculate mean values and standard errors. Data were subjected to the one-way analysis of variance (ANOVA) according to the treatment structure. Mean values were compared by Duncan's multiple range tests at the probability level of $p < 0.05$. The standard error values were used to construct error bars.

3. Results and Discussion

3.1. Chemical Composition of Different Phases of Separated Digestates

The quality of the digestate is determined by its chemical composition. The chemical composition of the digestate used for the experiment is presented in Table 1. Separated liquid digestate had the low TS content: (4.8–5.8 % in Vievis, 2.3–3.6% in Krekenava biogas plant), while separated solid digestate had the higher TS content (23.3–29.1% in Vievis, 29.7–31.3 in Krekenava biogas plant). Depending on the amount of TS, the different phases of the digestate, liquid and solid, differed in average 5–10 times. The difference in the amount of organic C_{org} in the different digestate phases was also determined. The separated solid digestate was 17–28 times richer in C_{org} than the liquid digestate.

Table 1. Chemical composition of the digestate used for fertilization, *2019*, *2020*.

Location of Biogas Plant	Date of Sampling	Indicators						
		pH	TS, %	OM, %	N g kg^{-1}	C_{org} g kg^{-1}	MHS g kg^{-1}	MHA g kg^{-1}
Liquid digestate								
Vievis	26 April 2019	7.8	4.8	4.7	4.7	20.2	7.5	2.3
	30 April 2020	7.6	5.8	5.7	6.2	31.3	19.1	2.6
Krekenava	29 April 2019	7.9	3.6	3.5	3.9	9.6	3.1	0.5
	15 May 2020	7.4	2.3	2.3	3.9	9.7	4.2	0.8
Solid digestate								
Vievis	26 April 2019	8.6	29.1	87.4	25.7	489.4	18.4	2.2
	30 April 2020	8.2	26.3	86.1	21.1	465.3	57.5	0.7
Krekenava	29 April 2019	8.4	31.3	75.2	17.1	426.4	18.5	0.8
	15 May 2020	8.9	29.7	87.8	15.7	526.6	24.4	0.8

Our data show that both the liquid and the solid fractions of the digestate contain valuable elements that are observed by the plants during the vegetation period. However, the liquid digestate fraction contains less TS. As a result, liquid digestate has more potential to migrate into deeper soil layers compared to the solid digestate fraction. Contrary, the solid fraction of digestate can be used an organic amendment due to the high OM and C_{org} content and the high stability once processed through the anaerobic digestion [26].

3.2. SOC Amounts in Different Soil Types and Land-Use

The integrated concept of the organic farming and the biogas production from the agricultural feedstocks has been suggested as a way of achieving carbon (C) neutrality in Europe. However, as the long-term effects of C removal for methane production on SOC are unclear, organic farmers have questioned whether the biogas production on farm will have a positive effect on soil fertility [27]. According to Witing et al., the inclusion of biogas production into the agricultural system modifies crop management, and as a result the SOC cycle of the agricultural landscape [28]. The results of our research show that digestate, when used as a bio-fertilizer in a low fertility soil in a crop rotation field, changes the SOC content in it, depending on the digestate fraction, fertilization rate and soil layer (Figure 2).

The greatest enrichment with SOC in Fluvisol in the first year of the experiment occurred using SD and increased with an increase of the fertilization rate. The concentration of SOC increased more in 0–10 cm layer using SD fertilization, and SOC increase was observed in deeper layers using LD fertilization. Significant SOC changes in the crop rotation field have not been recorded. This is linked to the results of that Barłóg et al., obtained in a crop rotation field during the 4-year experiment, when only a slightly higher SOC level was recorded in the digestate-treated soil. The authors stated that the lowest SOC content was recorded in the NPK and control treatments, regardless of the depth of soil sampling [29].

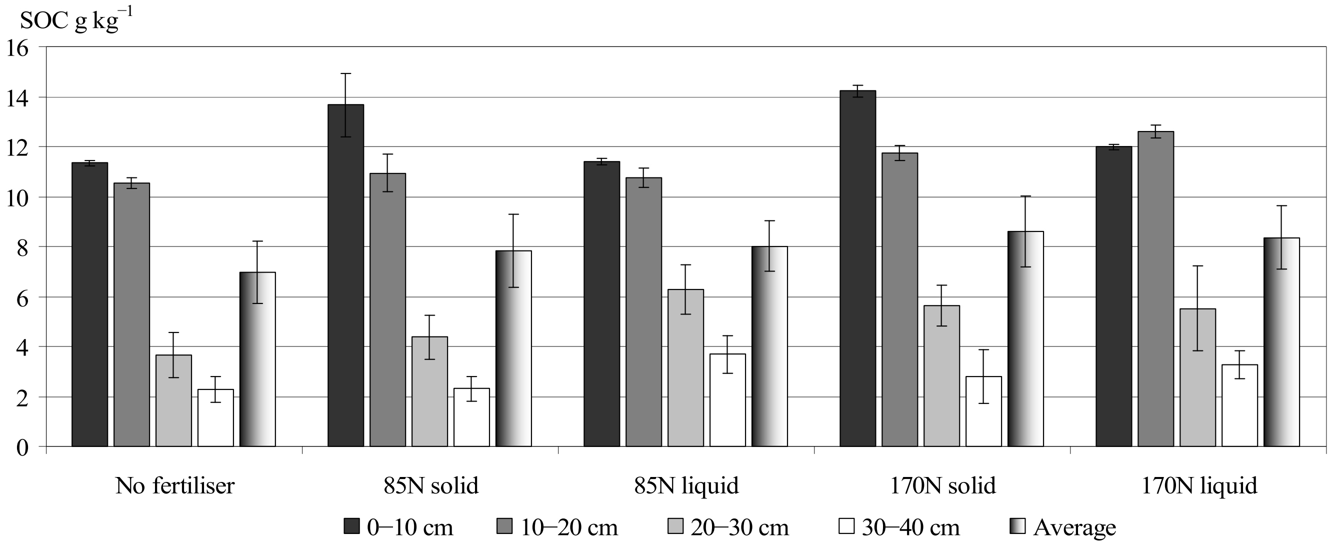

Figure 2. SOC content in the crop rotation field in Fluvisol, 2019.

In our research in the crop rotation field, SOC stratification was recorded when SOC accumulates in the upper layers of 0–10,10–20 cm (Figures 2 and 3).

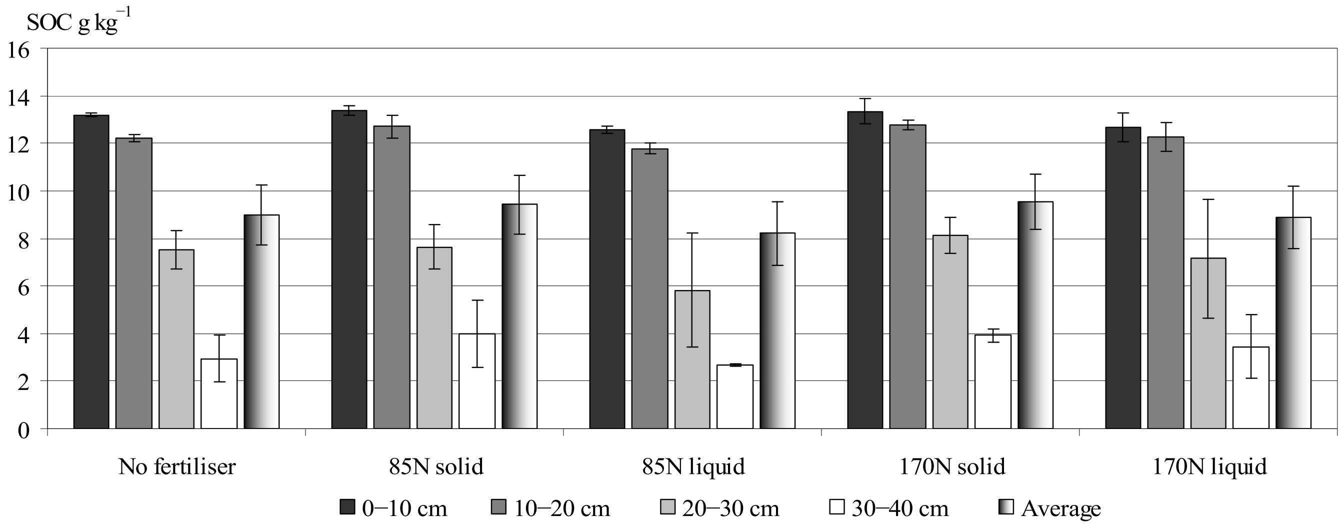

Figure 3. SOC content in the crop rotation field in Fluvisol, 2020.

In the following year of the experiment (2020) a similar, but weaker trends of SOC increase in the crop rotation field of Fluvisol were identified using SD fertilization (Figure 3).

In Fluvisol in semi-natural grassland, a positive effect of SOC increase was observed in 10–20 cm layer (Figure 4). This was particularly apparent in the first year of research. In the long-term experiment Levin showed that clover-grass leys have a remarkably positive effect on SOC, increasing it by 0.004% for every year a ley is grown, even if the above ground biomass is removed for the renewable energy production and is not returned as the organic fertilizer. Returning the nutrients and part of the organic matter as the biogas digestate increased biomass production and, in turn, SOC even further, by 0.017% for every 1 t ha^{-1} of the digestate C applied. When row crops were undersown with clover or clover-grass, this also had a positive effect on SOC, increasing it by 0.002% every year undersown crops were grown [27]. Digestate fertilization gives organic farmers the opportunity to control the timing and quantity of N fertilization, improving yields of other crops and hence increasing C inputs to soil. Our results therefore support scenarios for sustainable farming involving the anaerobic digestion of agricultural feedstocks.

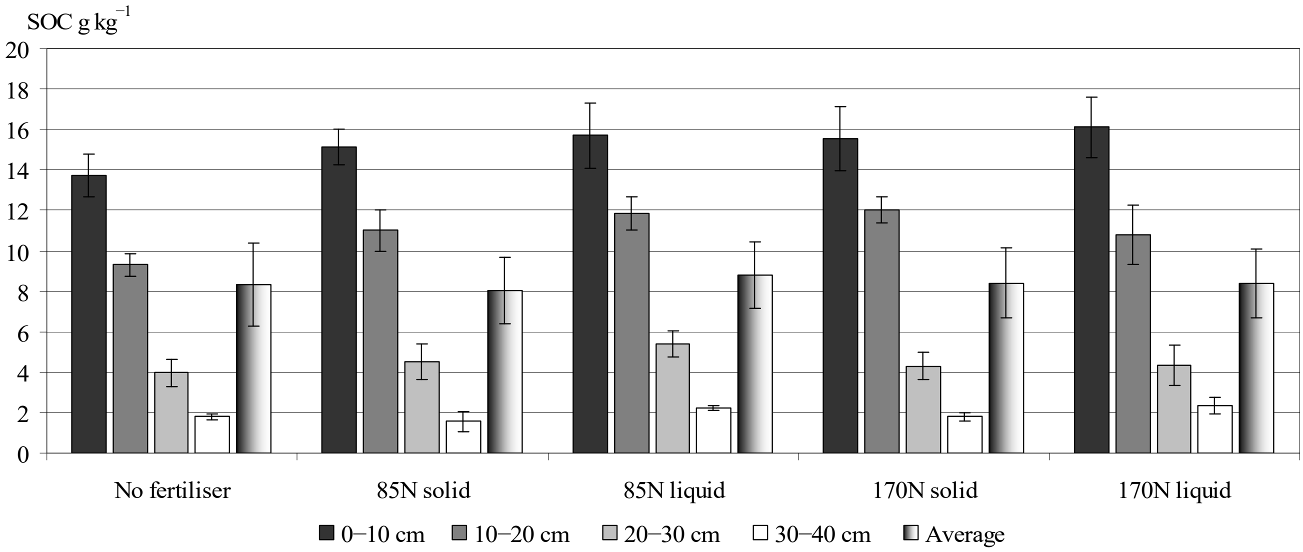

Figure 4. SOC content in grassland in Fluvisol, 2019.

In both soils investigated, SOC distribution in the soil layers was typical of grasslands (Figures 5 and 6). In grassland soils, SOC accumulates mainly in the upper layer of the soil. In our experiment, maximum concentration of SOC was found in 0–10 cm layer, while in the deeper layers the amount of SOC proportionally decreased. In the solid digestate-treated Retisol by the highest recommended fertilization rate (170 kg ha^{-1} N) the maximum SOC accumulation was found in a layer of 0–10 cm (12.88 g kg^{-1}). There was also a slight increase in SOC content in 10–20 cm layer (7.14 g kg^{-1}), but in 20–30 cm layer, the SOC content remained unchanged.

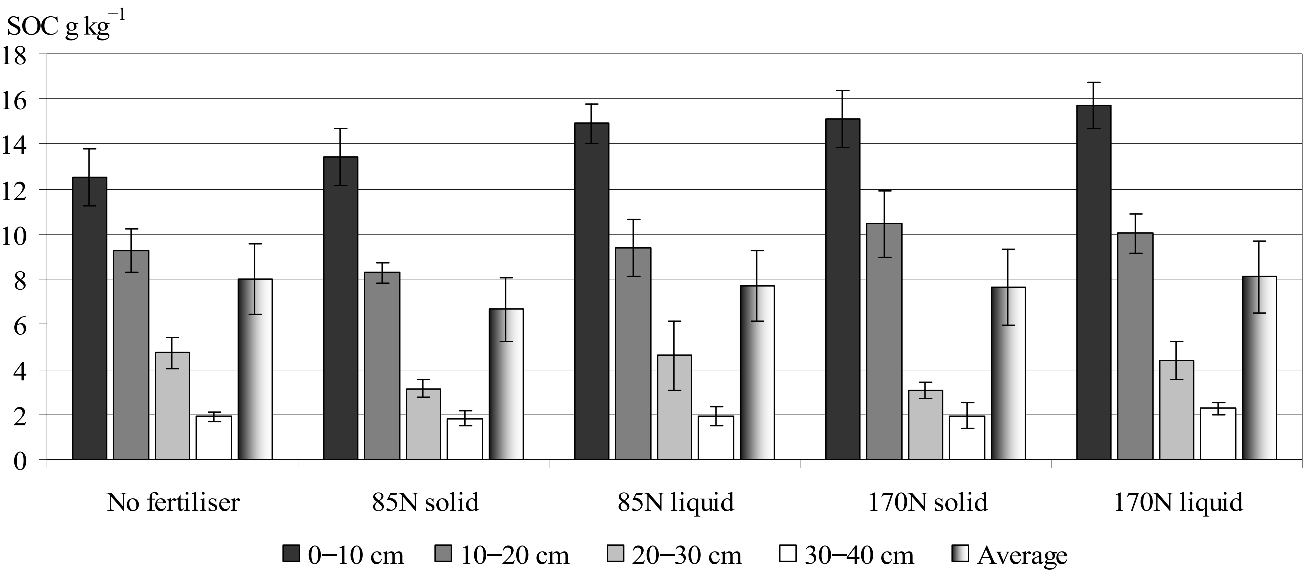

Figure 5. SOC content in grassland soil in Fluvisol, 2020.

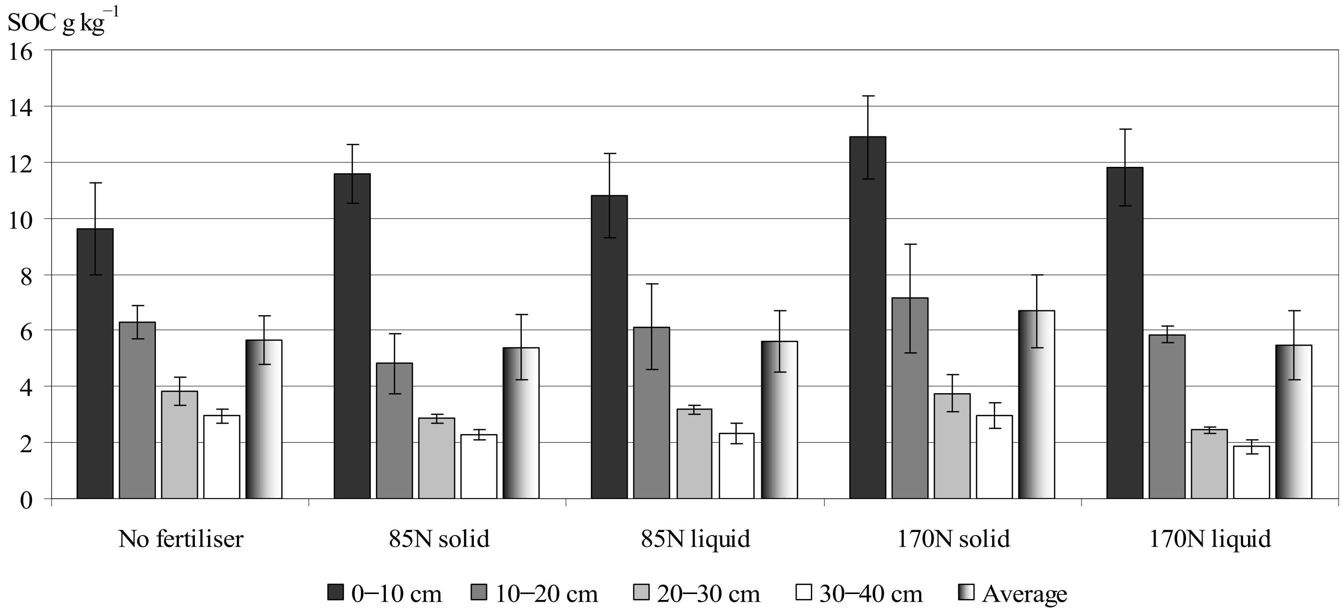

Figure 6. SOC content in grassland in Retisol, 2019.

SOC distribution in soil layers in Retisol (Figure 7) was also typical of grassland soils and was similar to that in Fluvisol. In this soil, the influence of fertilization on SOC in 0–10 cm layer became more pronounced compared to Fluvisol.

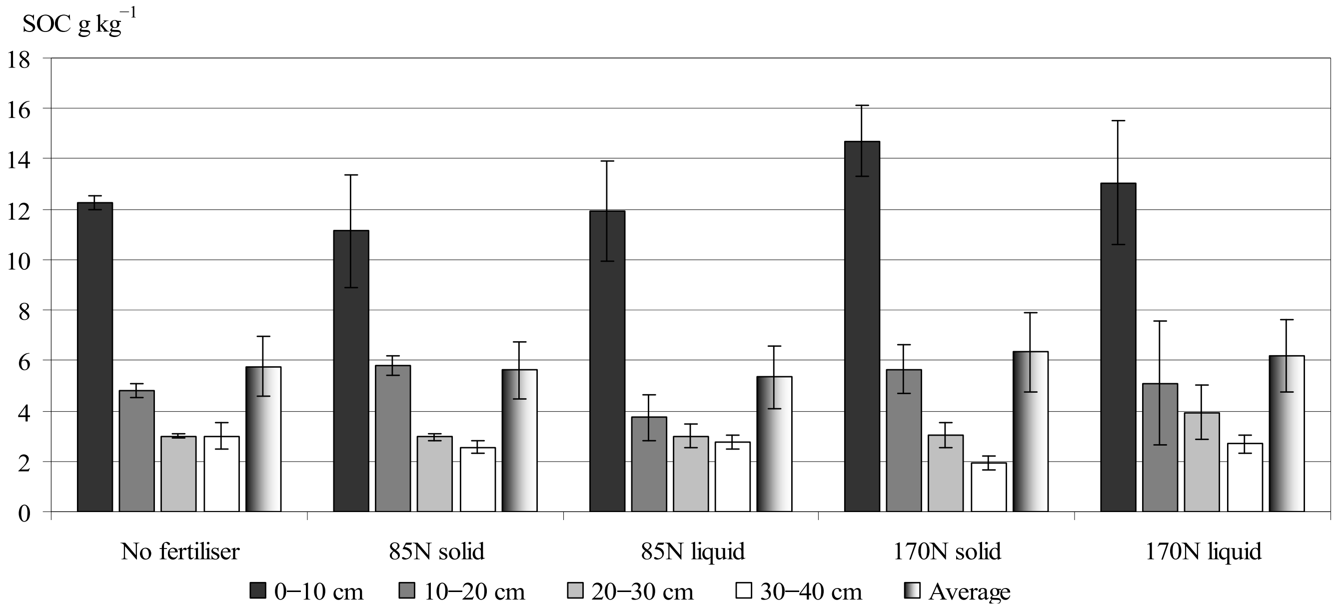

Figure 7. SOC content in grassland in Retisol, 2020.

The change in the distribution of SOC in the soil layers is usually only observed during the longer time period due to the certain soil management practices. According to Hobley and Wilson SOC depth depletion appeared to be driven predominately by land-management and, to a lesser extent, site factors, with climate only playing a minor role. This supports the hypotheses that land use is the main driver of SOC depth distribution [30].

Our studies show that the digestate fertilization approach increases the SOC concentration in the soil regardless of the soil type. However, the factors of soil genetic properties and other local conditions are important for the distribution of SOC in the soil layers. In the Retisol soil with long-term grassland biomass grown, the SOC content in the 0–10 cm layer was significantly higher compared to the 10–20 cm layer (Figures 6 and 7). Meanwhile, in Fluvisol soil with long-term grassland biomass grown, the differences between SOC in the 0–10 and 10–20 cm layers were smaller (Figures 4 and 5).

3.3. Humic Substances in Different Soil Types and Land-Uses as Influenced by Different Phases of Digestate

MHS tended to increase in the crop rotation field in digestate-treated Fluvisol. In all treatments in the crop rotation field, MHS accumulated in 0–10 and 10–20 cm soil layers (Figures 8–11). A significantly higher accumulation of MHS in a 0–10 cm layer was similar to the accumulation of SOC.

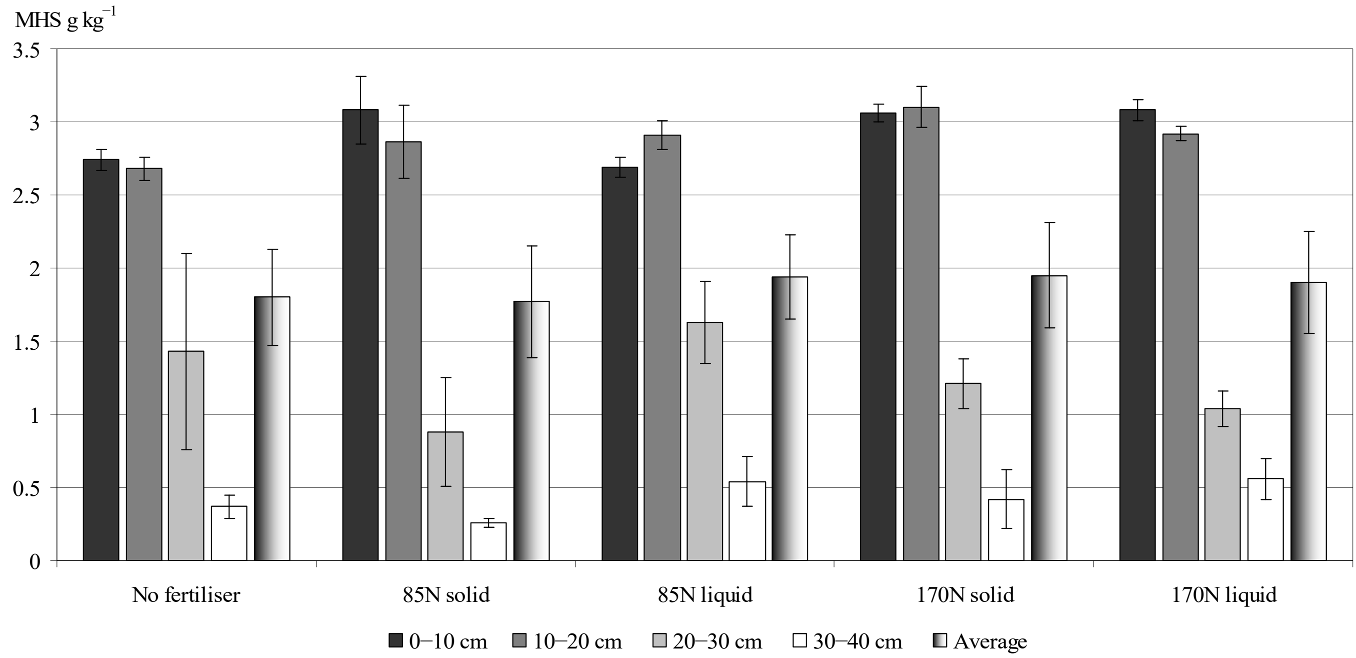

Figure 8. MHS content in the crop rotation field in Fluvisol, 2019.

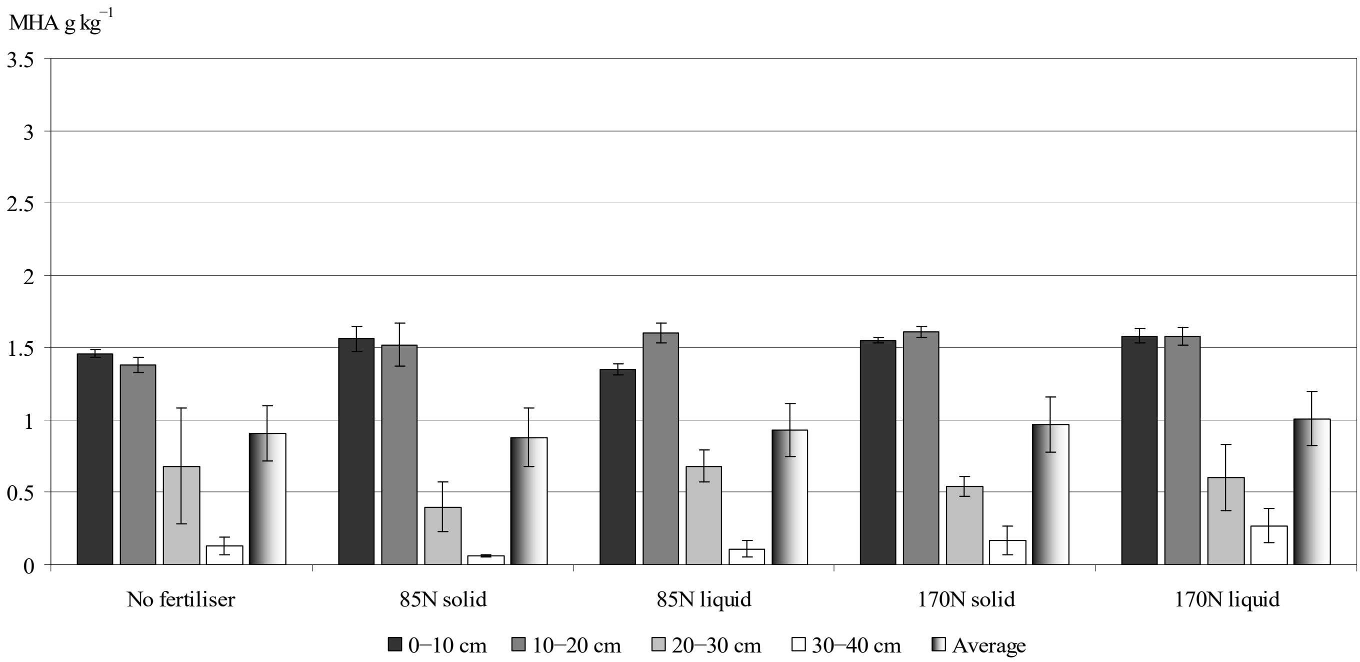

Figure 9. MHA content in the crop rotation field in Fluvisol, 2019.

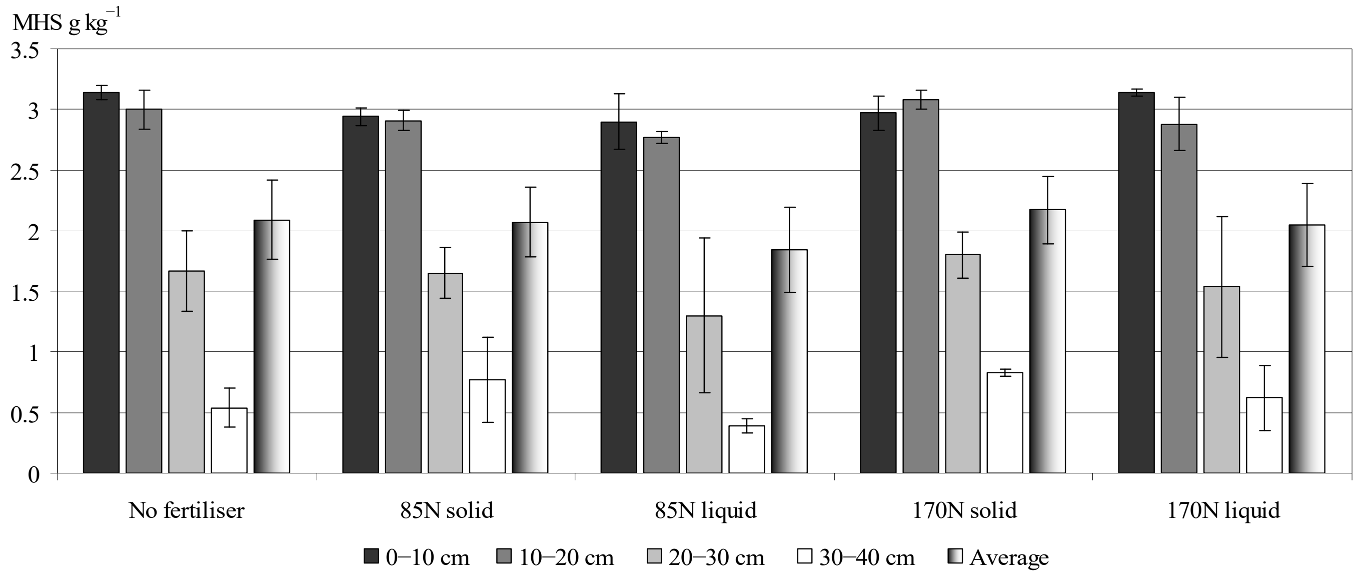

Figure 10. MHS content in the crop rotation field in Fluvisol, 2020.

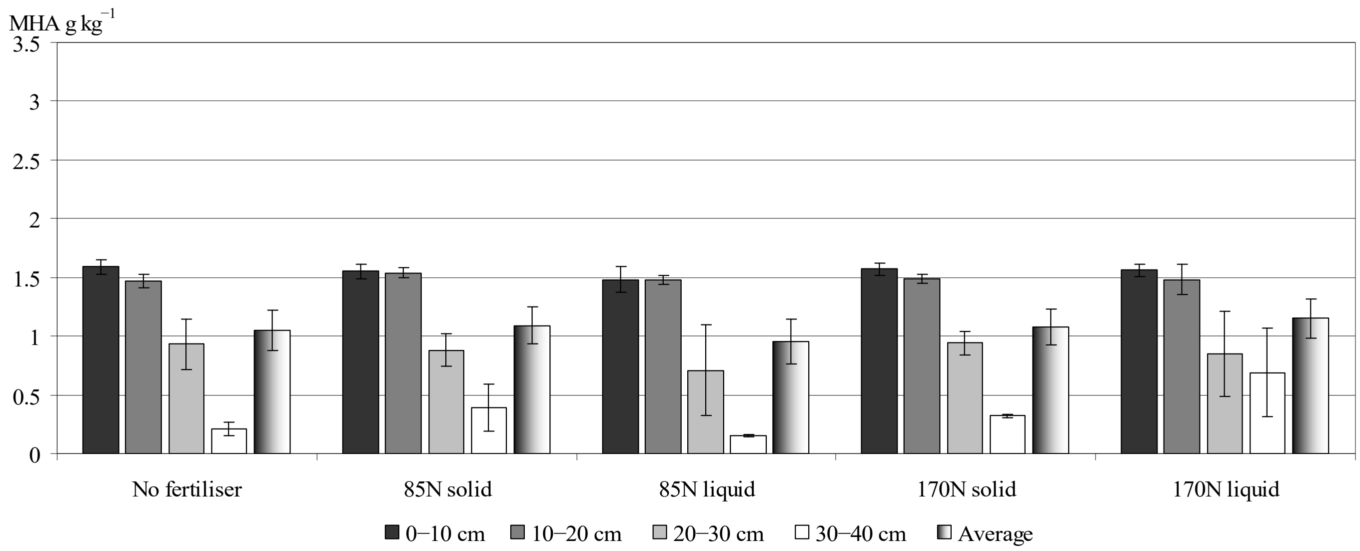

Figure 11. MHA content in the crop rotation field in Fluvisol, 2020.

In both grassland soils investigated, MHS and MHA were more concentrated in the upper 0–10 cm soil layer and decreased evenly in the deeper layers (Figures 12–19). This uneven distribution of MHS and MHA in the soil layers in the crop rotation field and grassland reflects the influence of tillage, where both upper layers of soil were mixed each year.

According to Wang et al., HA are naturally formed in the digestion process, so with the by-product of biogas production, digestate, they enter the soil and therefore enrich it [16]. In the soil, the usual processes of organic matter transformation also take place. The process of HA formation, also called humification, involves various microorganism-dominated biological and biochemical processes [31]. As a result, both HS, including HA, entering the soil and their changes in natural processes in the soil lead to changes in the organic part of the soil. In the crop rotation field, changes in MHA took place in the two upper 0–10 and 10–20 cm layers of Fluvisol (Figures 9 and 11). Changes in grassland of this type of soil were more noticeable in the upper 0–10 cm layer (Figures 13 and 15). This is in line with previous data where the positive effect of grasses on the accumulation of SOC and MHA was determined. The highest SOC and MHA contents accumulated in the soil that had not been tilled for a long time, compared with arable soils. In grassland soil the SOC and MHA tented to accumulate in the topsoil. Additionally, the long-term use of swards as pastures increased SOC content in the 0−10 cm soil layer by as many as 2−2.5 times [32].

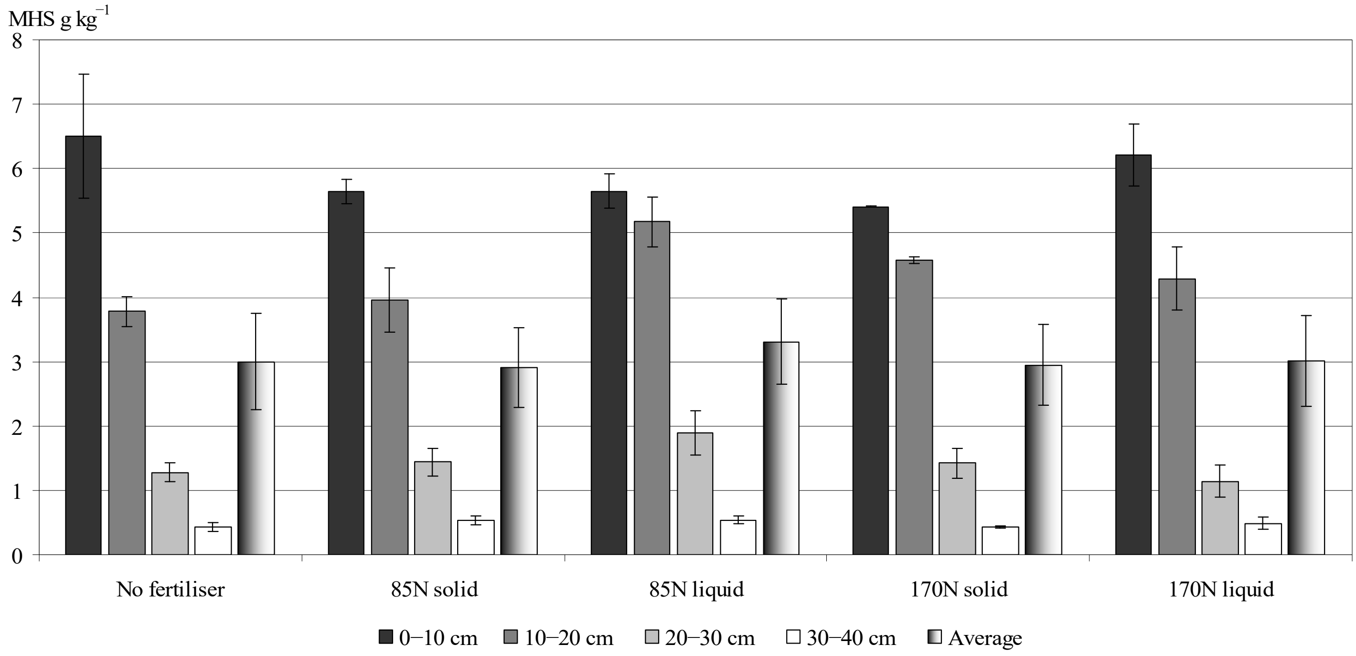

Figure 12. MHS content in the grassland in Fluvisol, 2019.

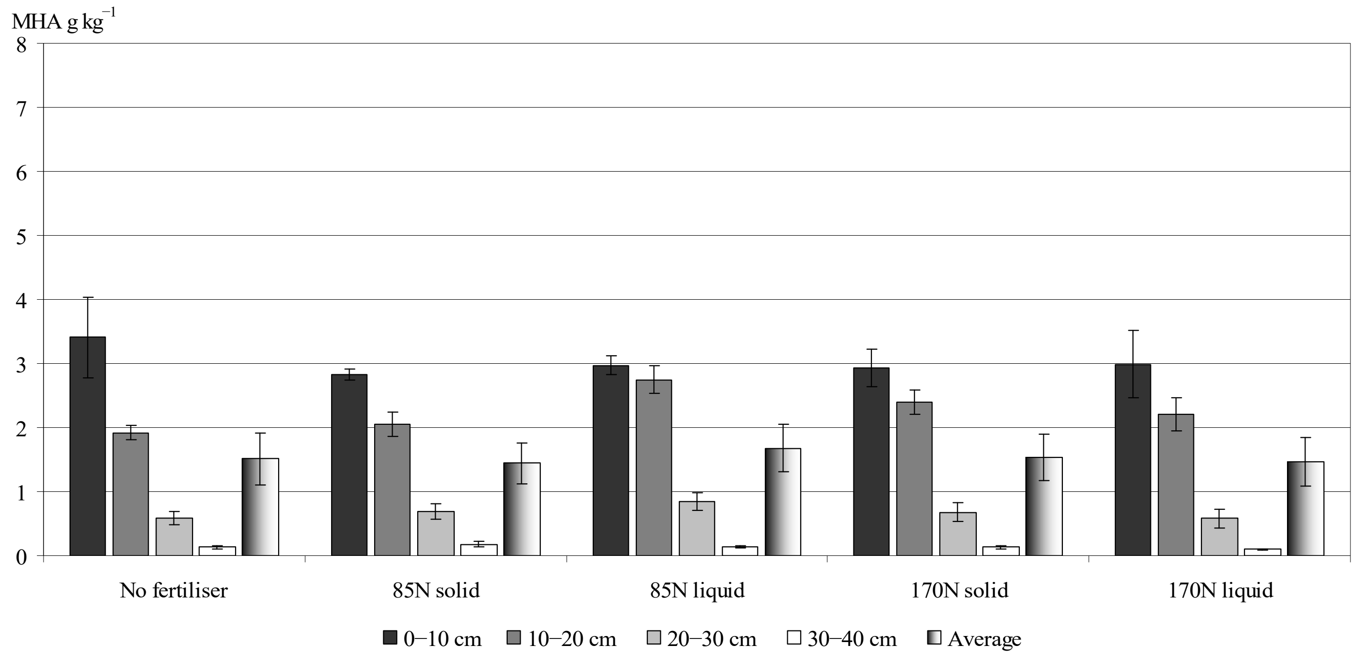

Figure 13. MHA content in the grassland in Fluvisol, 2019.

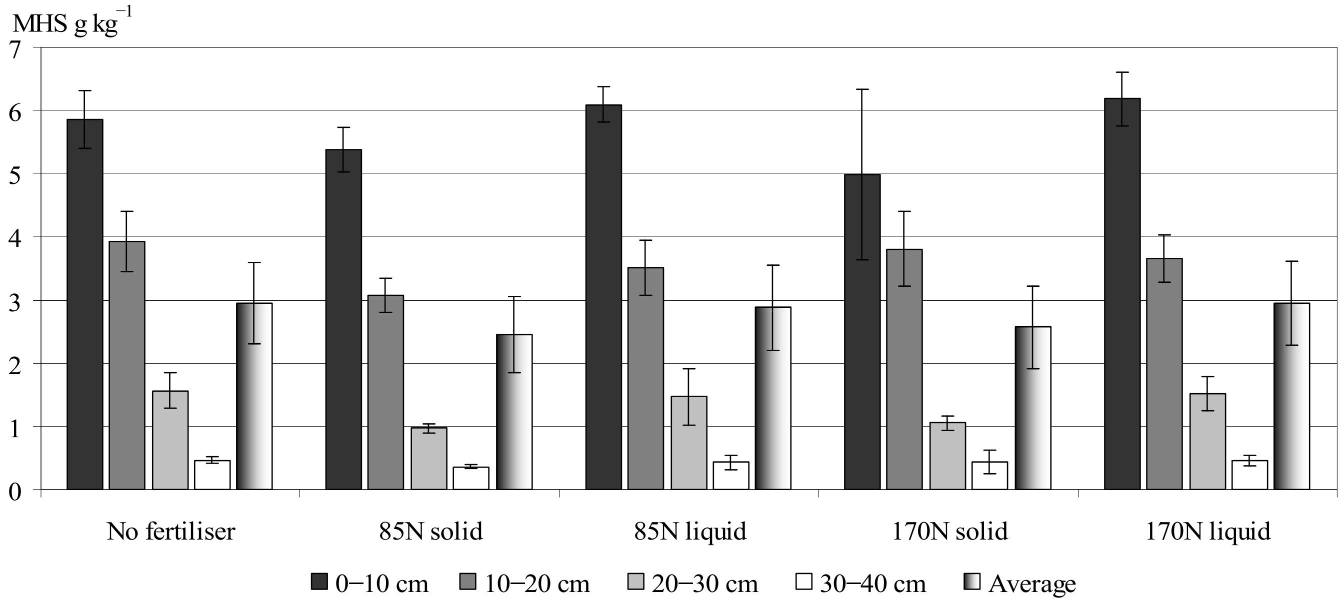

Figure 14. MHS content in the grassland in Fluvisol, 2020.

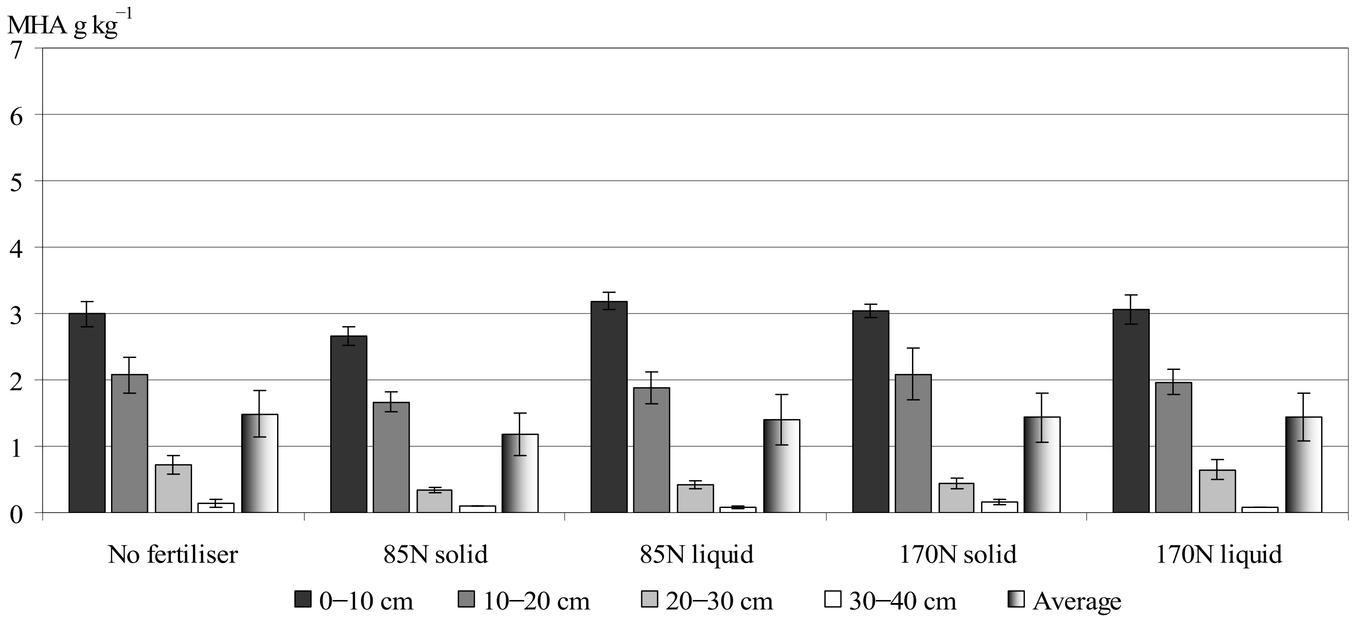

Figure 15. MHA content in the grassland in Fluvisol, 2020.

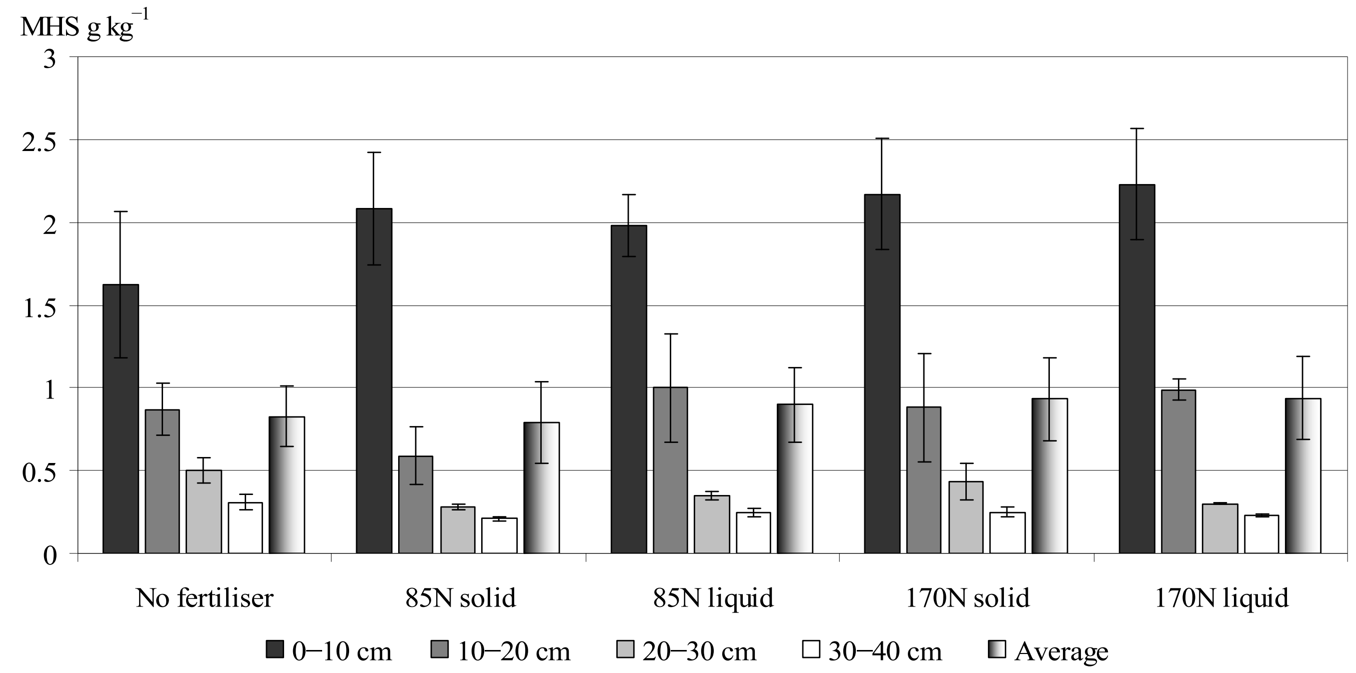

Figure 16. MHS content in grassland in Retisol, 2019.

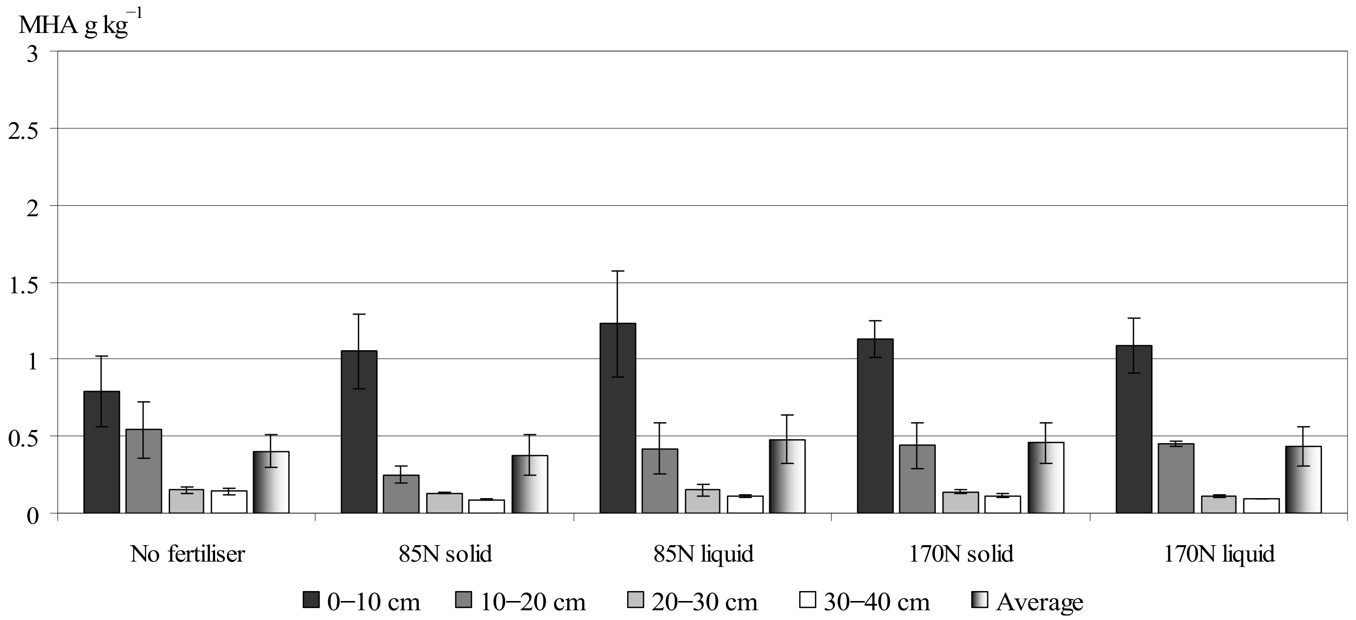

Figure 17. MHA content in grassland in Retisol, 2019.

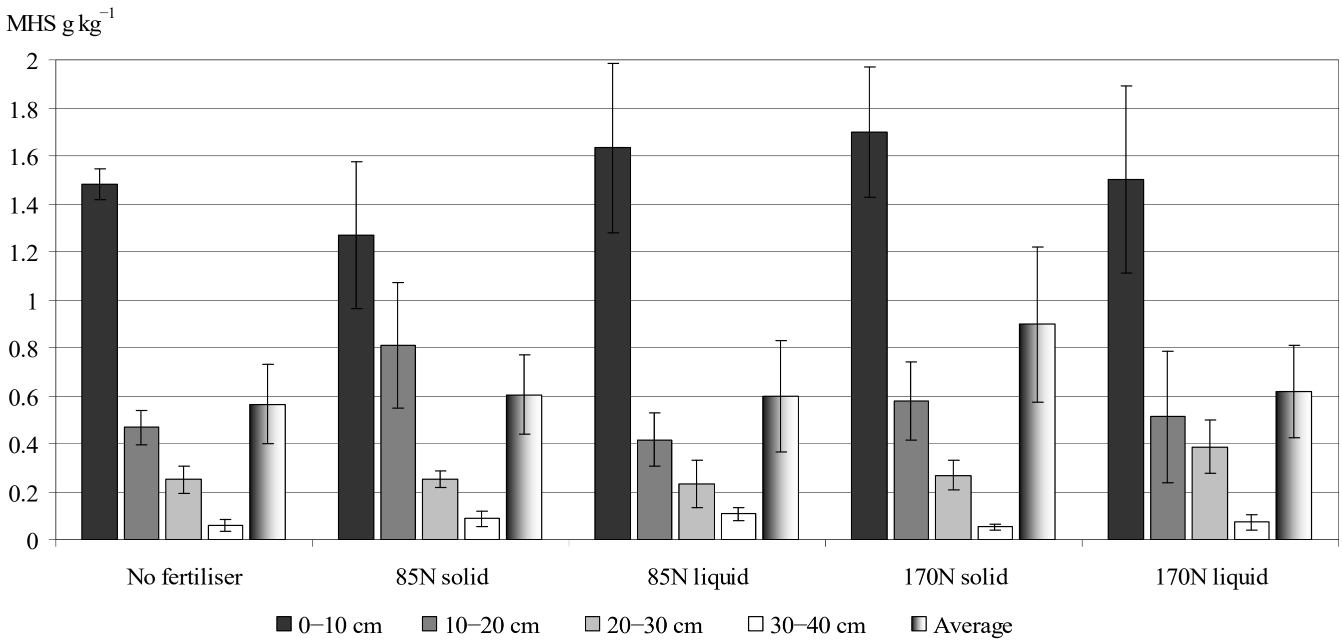

Figure 18. MHS content in grassland in Retisol, 2020.

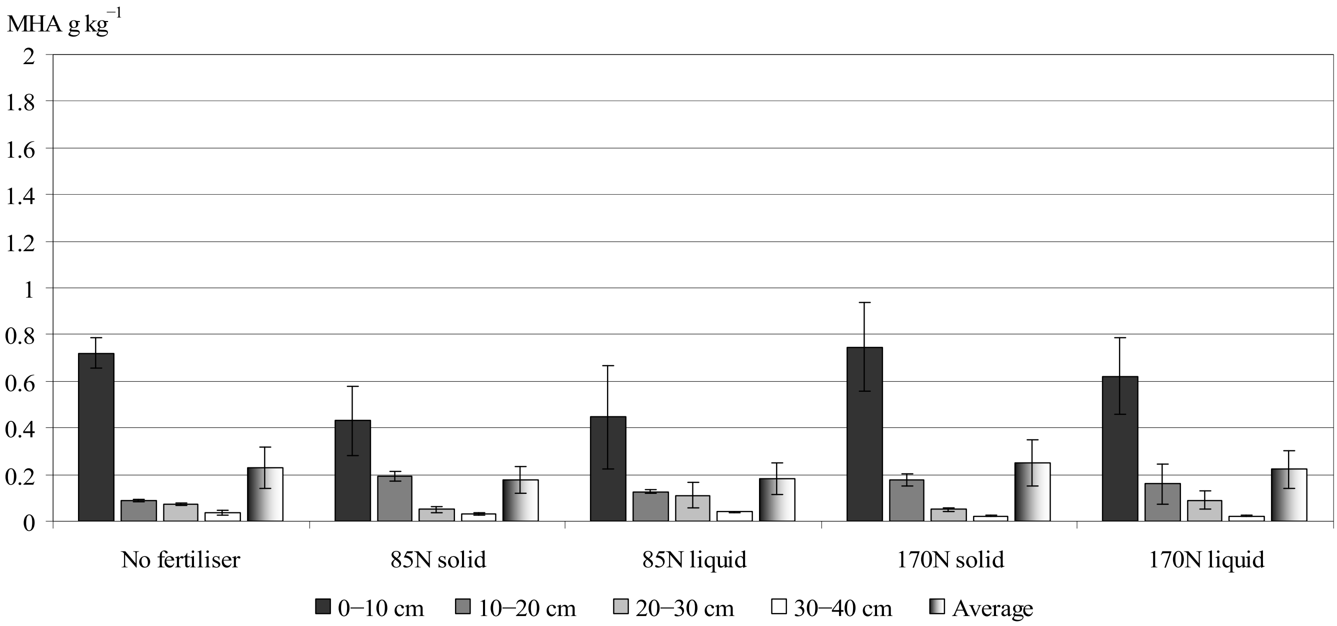

Figure 19. MHA content in grassland in Retisol, 2020.

Fertilization with different phases and rates of digestate in the first year of the study significantly increased the amount of MHS and MHA in the upper layer of Retisol (Figures 16 and 17). It is very important that valuable fractions of humic substances, MHS, increased in soil (Figures 17 and 19). The meteorological conditions during the year of the experiment were different, which was also reflected in the results obtained.

The results obtained in this study are related to the latest research by other researchers, such as Horta and Carneiro (2022) who aimed to evaluate the fertilizing value of the solid fraction of a digestate as an organic amendment and as a source of nitrogen to crops replacing mineral N. The digestate used in the fertilization of the vegetable crops showed a beneficial effect as a soil organic amendment increased the soil's carbon stock. The positive effect of solid digestate was found in the experiments which were completed in a high fertility soil [33]. Meanwhile, our study was conducted in low fertility and erosion-prone soils where the need for the soil's quality improvement is even greater. The obtained results demonstrate the effects of different digestate fractions on the humification process, which is very important for the restoration and improvement of the soil's properties.

We summarize that regular soil incorporation of transformed organic matter as an anaerobic digestion residue, including relatively stable carbon compounds, contained in digestates used, even in the short term may be important for SOC, MHS and MHA. This would encourage the preservation of SOM in the soil. Complex digestate and genetic properties of the soil and land use are important factors in this process. The present study suggests that soil type and land use significantly affect HD value, which reflects the humification process in the 0–40 cm soil layer. The largest HD was determined in grassland in Fluvisol (19.54–23.94%) (Table 2). In the same soil used as a crop rotation field, HD was nearly 2-fold lower (11.58–12.31%). In grassland Fluvisol (0–40 cm) this indicator was up to 4-fold higher compared to that in grassland Retisol. We concluded that long-term factors such as soil type and land use strongly affected the humification level expressed as HD (%) in the soil.

Table 2. Effect of land-use and fertilization by digestate on the humification degree (HD%) of OM, 2020. Averaged in 0–40 cm soil layer.

Land Use	Fertilization	HD, %
Crop rotation field in Fluvisol	No fertilizer	12.06
	85N solid	11.58
	85N liquid	11.78
	170N solid	11.77
	170N liquid	12.31
Grassland in Fluvisol	No fertilizer	23.94
	85N solid	19.84
	85N liquid	21.41
	170N solid	20.15
	170N liquid	19.54
Grassland in Retisol	No fertilizer	5.87
	85N solid	3.86
	85N liquid	3.75
	170N solid	5.08
	170N liquid	4.75

4. Conclusions

The use of digestates was associated not only with the plan to replace mineral fertilizers with bio-fertilizers, needed to produce agricultural crop production, but also in the hope that the soil would be enriched with SOC and SOM composition would be improved. The results of this study reveal a greater positive effect on the increase in SOC because of the use of maximum recommended fertilization rate of solid digestate. MHS tended to increase in the grasslands and in crop rotation field in the digestate-treated soil. In all experimental treatments, MHS in the crop rotation field accumulated in 0–10 and 10–20 cm soil layers. The uneven distribution of MHS and MHA in the soil layers in the crop rotation field and grassland reflects the influence of tillage. In grassland soils, SOC tends to accumulate mainly in the upper layer. In our experiment, the maximum concentration of SOC was found in the 0–10 cm layer, while in the deeper layers the amount of SOC as well as of MHS and MHA proportionally decreased. Soil type and land use significantly affected HD value, which reflects the humification process in the soil. The highest HD was determined in grassland Fluvisol (19.54–23.94%). Further research is needed to find out how the use of digestates, derived from different feedstocks used in the biogas production process, could be used as bio-fertilizers for agricultural crops and explore organic matter transformation and humification processes in more detail. Finally, the microbial activity in these processes would be useful to investigate as well.

Author Contributions: Study supervision, A.S. (Alvyra Slepetiene); Conceptualization, Methodology, Investigation, A.S. (Alvyra Slepetiene), A.M., L.J. and A.S. (Aida Skersiene); writing—original draft preparation A.S. (Alvyra Slepetiene), A.M. and L.J.; writing—review and editing A.M., L.J., A.S. (Alvyra Slepetiene), L.J., M.K. and O.B.; statistical analyses, M.K.; laboratory analyses, A.S. (Alvyra Slepetiene) and A.Sk. (Aida Skersiene). All authors have read and agreed to the published version of the manuscript.

Funding: This paper presents the results of the research obtained through the project "Quality diagnostics of biogas production by-product (digestate) for innovative use as a biofertilizer" No. DOTSUT-217 (01.2.2-LMT-K-718-01-0053) funded by the Research Council of Lithuania.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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