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Influence of Liming Intensity on Fractions of Humified Organic Carbon in Acid Soil: A Case Study

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Abstract: Soil pH influences the composition and activities of microbial communities, which are driving the transformations of soil organic carbon (SOC). However, the effect of liming on SOC sequestration remains controversial. Evaluation of the effect of liming on SOC and humified carbon fractions was one of the most important tasks of this work. Studies were carried out in the long-term field experiment using slaked-lime rates of 0.5 or 2.0, depending on the soil hydrolytic acidity. It was determined that liming did not increase SOC content, but led to redistribution of SOC. The amount of mobile humic acids in the soil decreased while the intensity of liming increased. When liming at the 2.0 rate, the lowest HA1 level (0.251 g kg⁻¹ C) and highest amount of HA2 (0.21 g kg⁻¹ C) were found. It could be stated that soil liming, as a measure, allows the stabilization of HA and FA by the cation bridging mechanism. Liming resulted in slower mineralization of SOC and at the same time it also resulted in carbon sequestration in the form of valuable humic acids. An important consistent pattern of carbon transformation was identified in this study and can be applied in selecting of the most suitable combination of agricultural practices, promoting carbon sequestration and soil fertility.

Keywords: carbon sequestration; humic substances; qualitative characteristics of SOC; lime; acidification

1. Introduction

Soil organic carbon (SOC) plays a key role in agronomic aspects and environmental sustainability due to its contribution to biological, chemical, and physical soil properties. Soils accumulate three times more carbon than the amount found in the atmosphere and terrestrial vegetation (carrying approximately 1500 Pg C to a depth of 1 m) [1,2]. SOC sequestration has the potential to cause significant impact on atmospheric carbon levels [3]. Compared to natural soils, agricultural soil and its organic carbon level is more vulnerable to climate change, natural environmental conditions (climate, precipitation, and vegetation), the global carbon cycle and agricultural practices (tillage and fertilization) [4,5]. The study of mechanisms underlying carbon storage and release from soils has become increasingly important in the light of predicted changes in the global climate and the potential for soils to act as either a source or sink of atmospheric CO₂ [6–9]. Furthermore, easy-to-use technologies are required to support and increase stocks of SOC [10,11].

Humic substances (HS) are operationally classified as humic acid (HA), fulvic acid (FA), and humin (HU) based on their solubility in acid and base. They are still objects of intense discussion, research, and even controversy regarding their formation, composition, and structural aspects [12]. These are polymeric molecules with complex structure containing carboxyl, hydroxyl, phenolic, and quinone groups distributed in both solid and liquid phases [13]. As natural ligands, adsorbents, and transporters, HS have a key role in defining the characteristics, structure, and fertility of soils, as well as influencing plant growth.
increase of HS concentration is an indicator for the stability of organic matter and its maturity [14]. Different portions of SOC differ from each other in carbon turnover rates, biological stability, and biochemical composition. The labile fraction of SOC has been used as a sensitivity indicator of changes in soil quality. Due to significant interactions in soil systems, labile organic compounds have fast turnover rates as they serve as readily available substrates for soil microorganisms. Obviously, SOC dynamics in various fractions are driven by both the chemical composition of organic inputs and soil nutrient availability because they both have a hand in regulating microbial metabolisms [15–18].

Soil management practices alter humification conditions such as soil acidity, exchangeable cation content and composition, humus-forming agent content, and biological activity, and these factors influence the rate and direction of the humification processes, but there is no consensus on the impact of liming on HS [19]. Ca$^{2+}$ addition with lime, according to Rocha et al. [20], is critical for SOC stabilization. One of the most important methods for increasing SOC protection and storage in acidic soils is the interaction of SOC with mineral particles via polyvalent cations. Seeking to manage SOC in acid soils, management techniques for reducing soil acidity, neutralizing toxic elements, and promoting polyvalent cations such as Ca$^{2+}$ and Mg$^{2+}$ throughout the soil profile are necessary. The potential of surface lime application to increase SOC storage and accumulation in acid soil by enhancing plant growth and SOC input have been shown in several long-term studies [20,21]. However, there is some scientific evidence indicating that application of lime enhances SOC mineralization, especially in acid soil. Moreover, Garbuio et al. [22] highlighted the importance and necessity of conducting experiments to better understand the long-term effects of soil amendments on a variety of SOC pools, especially humic compounds, which play a critical role in a soil fertility. Liming, which is already an established agronomic practice, could potentially be a management tool to mediate pH effects on SOC-sequestration, but better understanding of the resulting net effects is needed to allow, for example, the integration of pH effects in predictive models of soil carbon sequestration. Nonetheless, while many studies have investigated the different characteristics of HA and FA, the specific effects of lime on humus fractional composition and different humification process indices have not been well reported.

In this study, we predicted that acid soils with their inherent properties would not only create unfavorable conditions for the accumulation of organic carbon, but also promote its mineralization. We also predicted that it was likely that the concentration of carbon fractions of different stabilities will change during the carbon transformation processes. The goal of the study was to investigate the changes in soil organic carbon chemical fractions and to identify the factors that characterize humification processes in natural acid Dystric Glossic Retisol as influenced by different intensities of liming.

2. Materials and Methods

2.1. Study Site Description

The experiment was conducted at the Vėžaičiai Branch of the Lithuanian Research Centre for Agriculture and Forestry during the 2015–2019 period (Figure 1). The experimental site is located on the eastern fringe of the coastal lowland (western Lithuania; 55°41′37.7″ N 21°29′57.4″ E). The soil of the experimental site was naturally acid moraine loam, Bathygleyic Dystric Glossic Retisol (WRB, 2014), with a clay content (<0.002 mm) of 15.0%. Prior to the establishment of the experiment in 1949, the following agricultural parameters were evaluated. The experimental site chemical characteristics were as follows: pH$_{\text{KCl}}$ −4.2–4.4, plant available phosphorus −50–60 mg kg$^{-1}$, plant available potassium −130–180 mg kg$^{-1}$, and humus −2.6–2.9% (carbonates were lying more than 2 m below the surface).
Studies were conducted in a long-term field experiment, which was started in 1949, to investigate the influence of long-term liming on soil organic carbon qualitative indicators. In 1949, primary liming was carried out using slaked-lime rates of 0.5 or 2.0, depending on the hydrolytic acidity of the soil. The effect of primary liming was observed until 1964, when the experiment was split into two strips. In the first strip, the effect of primary liming continued to be observed. In 1965, the soil was repeatedly limed in the second strip at a rate of 0.5 based on the soil hydrolytic acidity (3.3 t ha\(^{-1}\)). With this background of primary and repeated liming, periodic liming began in 1985 with pulverized limestone (92.5 percent CaCO\(_3\)). Periodic liming at a rate of 0.5 t ha\(^{-1}\) CaCO\(_3\) was performed every 7 years and liming at a rate of 2.0 t ha\(^{-1}\) CaCO\(_3\) every 3–4 years (Figure 2). Lime was spread on the soil surface and applied to the soil on the same day with a cultivator at 7–12 cm depth.

**Figure 1.** The location of the field experimental site.

**Figure 2.** The scheme of the experiment (I–III shows replicates of the treatments).
Until 2005, the soil was limed on a constant schedule. The soil was not limed from 2005 to 2019, and the changes in soil parameters were observed. Using the long-term liming systems—primary (1949), repeated (1965), and periodic (1985–2005) from 1949 to 2005, various soil pH levels were generated (Table 1).

### Table 1. Scheme and total amounts of CaCO$_3$ applied for the different liming intensities.

| Liming Intensity | Total Amount of CaCO$_3$ Applied, t ha$^{-1}$, 1949–2005 | $\text{pH}_{\text{KCl}}$ |
|------------------|----------------------------------------------------------|------------------|
| Unlimed          | 0                                                        | 4.2              |
| Liming using ×0.5 of the liming rate every 7 years | 3.3 | 3.4 | 11.4 | 18.1 | 5.6 |
| Liming using ×2.0 of the liming rate every 3–4 years | 13.2 | 1.7 | 90.0 | 104.9 | 6.7 |

#### 2.2. Soil Sampling and Methods of Analysis

Soil samples were collected every year, in the autumn (2015–2019, September) after the harvesting. The five-field crop rotation were applied as follows: barley with perennial grasses, perennial grasses, winter triticale, and spring oilseed rape. Soil samples ($n = 45$) were collected with a steel auger from three replicates of topsoil (0–20 cm). The initial size of each treatment was 74.75 m$^2$ (length 11.5 m, width 6.5 m). All samples were air-dried before being physically removed of any visible roots or plant debris. The samples were crushed and homogenized after being sieved through a 2 mm sieve. The soil samples were passed through a 0.2 mm sieve for humus content and composition measurement. The sieved soil was dried in a 105 $^\circ$C oven for 16 h.

Chemical analyses were conducted at the Chemical Research Laboratory of the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Soil reaction was determined in 1M KCl according to the standard ISO 10390:2005 (soil-solution ratio 1:2.5), mobile aluminum-ISO 14254:2018, and $N_{\text{total}}$-ISO 10694:1996. Plant-available phosphorus (P$_2$O$_5$) and potassium (K$_2$O) were determined according to the standard LVP D-07:2016. Soil organic-carbon content was determined photometrically at the wavelength of 590 nm using the UV–VIS spectrophotometer Cary 50 (Varian) using the glucose as a standard after wet combustion according to Nikitin (1999).

**Determination of humus fractional composition.** Humus is composed of unique humic compounds such as humic acids, fulvic acids, and humin, which cannot be hydrolyzed. The fractional composition of humus is determined by the interaction of its individual constituent compounds with the mineral part of the soil. The solubilities of the humus fractions are unequal and can be determined in dilute acid and alkali solutions. The humic substances are dissolved in sodium hydroxide solution, and in the resulting extracts the humic acids are separated from the fulvic acids by precipitating the humic acids with mineral acid solutions. The concentration of mobile, calcium, and clay mineral-bonded fractions of humic (HA) and fulvic (FA) acids, as well as the humic-to-fulvic acids ratio (HA:FA), can be calculated using this approach.

**The humification degree (HD).** The relative proportion of humic acids’ carbon in total soil organic carbon is expressed as a percentage and is the degree of humification of the organic matter [23]:

$$\text{HD} (\%) = \frac{\text{HA}}{\text{SOC}} \times 100$$  

where $\text{HA}$ is the humic acids carbon content (g kg$^{-1}$) and SOC is a soil organic carbon content (g kg$^{-1}$).
The relative proportion of the most aggressive FA1a fraction of the carbon content in total humic substances’ (HA + FA) carbon content is used to determine the aggressiveness of HS:

\[ \text{Aggressiveness} \% = \frac{FA_{1a}}{HA + FA} \times 100 \]  

where FA1a is the carbon content of the aggressive fulvic acids’ fraction (g kg\(^{-1}\)), HA is the humic acids’ carbon content (g kg\(^{-1}\)), and FA is the fulvic acids’ carbon content (g kg\(^{-1}\)).

The coefficient of humus mobility. The ratio between the carbon content of mobile and calcium-bound humic and fulvic acids:

\[ \text{Mobility} = \frac{HA1 + FA1}{HA2 + FA2} \]  

where HA1 and FA1 are the carbon content of mobile humic and fulvic acids (g kg\(^{-1}\)), and HA2 and FA2 are the carbon content of calcium-bound humic and fulvic acids (g kg\(^{-1}\)).

2.3. Statistical Analysis

The statistical analysis was carried out using SAS Enterprise, version 7.1 (SAS Institute Inc., USA). Analysis of variance was used in the determination of any differences between the experimental treatments (one-way ANOVA). The means of the treatments were compared using Fisher’s LSD test, which determined the least significant differences at the 0.05 probability level.

3. Results and Discussion

3.1. Alteration of Soil Chemical Properties under Different Liming Intensities

Soil acidity is the major soil chemical constraint that limits soil fertility and agricultural productivity. Plants are poisoned by acid soils due to nutritional problems; the lack of key elements such as Ca, Mg, P, and Mo; and the toxicity of Al, Mn, and H activity. In acid soils, excess Al primarily injures the root apex and inhibits root elongation causing reduced water and nutrient uptake, and as a result, crops cultivated on acid soils have limited access to nutrients and water, decreasing crop growth and yield. To solve such problems, the proper application of lime is fundamental. The increase in soil pH following lime application is linked to a larger concentration of ions in the soil solution, which may reduce exchangeable acidity (H + Al) and Al\(^{3+}\) concentration in the soil. [24]. The data presented in the Table 2, show that chemical properties of Dystric Glossic Retisol depended on the amount of incorporated lime. Seventy years after the establishment of the experiment (1949), the soil acidity in the unlimed control treatment was still on the increase.

| Treatments | Unlimed | Limed at 0.5 Rate | Limed at 2.0 Rate |
|------------|---------|------------------|------------------|
| pH\(_{KCl}\) | 3.95 ± 0.072 a | 5.15 ± 0.956 b | 6.74 ± 0.457 c |
| Al\(^{3+}\), mg kg\(^{-1}\) | 99.86 ± 3.581 b | 23.75 ± 2.699 a | 0.18 ± 0.023 c |
| K\(_2\)O, mg kg\(^{-1}\) | 108.2 ± 2.63 a | 89.52 ± 9.64 bc | 82.05 ± 7.38 b |
| P\(_2\)O\(_5\), mg kg\(^{-1}\) | 160.1 ± 17.97 a | 138.5 ± 15.31 ab | 117.9 ± 16.69 b |
| Exchangable Ca, % | 0.001 ± 0.0006 b | 0.002 ± 0.0011 ab | 0.006 ± 0.0003 a |
| Exchangable Mg, % | 0.200 ± 0.0261a | 0.281 ± 0.0396b | 0.295 ± 0.0111 b |
| N\(_{total}\), % | 0.110 ± 0.0126a | 0.115 ± 0.0214a | 0.116 ± 0.0169a |

Note: Means followed by different lowercase letters indicate significant differences at p < 0.05 level based on the least significant difference (LSD) test.

In comparison to the unlimed soil, the pH of the long-term periodically limed soil was significantly higher (5.15–6.74). This result corroborated the findings of Bossofolani et al. [25] and Getachew et al. [26], who found that liming dramatically elevated soil pH. The unlimed soil had the highest concentration of mobile aluminum (99.86 mg kg\(^{-1}\)).
The process of mobile aluminum returning to its previous level slowed as the lime rate was increased, and the greater contents were immobilized and associated with other compounds. The amount of mobile aluminum in the soil decreased as the acidity decreased from 3.95 to 6.74. According to the results of soil liming based on changes in plant nutrients, intensive soil liming considerably reduced the level of plant-available phosphorus and potassium, which contradicts the findings of other studies [26–28]. On the other hand, these findings are validated with Haynes’ [29] findings, which stated that the precipitation of insoluble calcium phosphates could limit P availability at high soil pH and low Al\(^{3+}\) content. Exchangeable Ca and Mg differed significantly (\(p < 0.01\)) between unlimed and intensive (at the 2.0 rate) liming treatments. According to Souri and Hatamian [30], Jafer and Gebresilassie [28], and Getachew et al. [26], lime use tends to increase exchangeable Ca and Mg. Lime application in acid soils, according to these scientists, enhances exchangeable bases while lowering some micronutrients (Fe, Zn). Total nitrogen (N\(_{\text{total}}\)) did not differ significantly between treatments when various lime rates and intensities were applied. This is consistent with the results of other researchers [31,32].

3.2. The Content of Organic Carbon in the Soil

Many decades of research have shown that soil organic matter, the main element of which is SOC, plays an important role in a wide range of soil properties. It is determined that liming positively influences soil chemistry, improving plant growth [33], but its effect on SOC is highly soil-type specific, and the net effects remain poorly understood. The neutralizing effect of lime on acid soils may accelerate mineralization and CO\(_2\) emission [34] but increasing biomass production can also enhance SOC formation and generate a net carbon sink in certain conditions [35]. While some authors reported an increase of SOC content after long-term liming [36,37], SOC was significantly decreased in this research (Figure 3). The current results were in line with findings of studies by Luo et al. [38].

\(\text{Figure 3. The mean differences in SOC content (g kg}^{-1}\) in the topsoil under different intensities of liming (mean ± standard deviation). Bars with different lowercase letters indicate significant differences at \(p < 0.05\) level based on the least significant difference (LSD) test.\)

The content of SOC was 13.1 g kg\(^{-1}\) in the unlimed treatment, while in soil periodically limed at the 2.0 liming rate every 3–4 years it was approximately by 0.7 g per kilogram lower. Based on the data obtained, we can state that liming was associated with higher rates of mineralization across all liming treatments. These findings show that liming to increase soil pH may make it easier for bacteria to mineralize both fresh and existing organic matter. As a result, lime is expected to prevent SOC mineralization by increasing aggregate protection and binding to Ca\(^{2+}\) and clay surfaces. Despite the fact that aggregation and binding give some protection, microbial use of SOC continues. Dumale et al. [39] highlighted, that
the addition of lime could increase mineralization of SOC in light clay soils, where clay content in the soil is less than 20%. However, these findings highlight the significance of addressing both soil biological and soil chemical characteristics when attempting to modify soil carbon dynamics.

3.3. Changes in the Humus Fractional Composition under Different Intensities of Liming

Based on the literature, it is known that lime can affect SOC in two ways. Lime use can improve SOC quality, but this does not necessarily increase the SOC pools. We can assume that liming created favorable pH conditions that increased plant productivity resulting in greater organic matter inputs in the form of dead roots and decaying crop residues and that consequently, this led to changes, generally, in the organic matter transformation processes, and humification of SOC, specifically. Data obtained from our study, allow us to conclude that long-term liming at different intensities did not positively affect total SOC content but led to redistribution of carbon into the humus fraction.

The HA1 fraction consists of the mobile, most soluble humic acids that are most likely to form and mineralize most rapidly. During the mineralization process, these humic acids enrich the soil with nutrients necessary for plant development and the formation of above-ground mass. The highest HA1 fraction humic acids were found in the unlimed soil (0.278% g kg\(^{-1}\) C) (Figure 4). In such soils with high levels of mobile humic acids (HA1), the stability of organic carbon decreases and the potential for leaching, soil degradation and erosion of plant nutrients increases. High levels of mobile humic acids can be viewed negatively, especially when the organic carbon content of the soil is low. Based on the obtained results, it can be argued that the concentration of mobile humic acids in the arable layer decreased during the liming of the soil. The content of mobile humic acids varied from 0.278 g kg\(^{-1}\) C in non-limed soil to 0.251 g kg\(^{-1}\) C in soil limed every 3–4 years at the 2.0 rate. The amount of mobile humic acids in the soil decreased while the intensity of the liming increased. This indicates greater stability of the organic matter.

![Figure 4. The influence of the different liming intensities on the mean differences in carbon content in the humic acid fractions (g kg\(^{-1}\) C). C\(_{HA1}\), mobile humic acids; C\(_{HA2}\), humic acids bounded with calcium; and C\(_{HA3}\), humic acids bounded with soil minerals. Bars with different lowercase letters indicate significant differences at \(p < 0.05\) level based on the least significant difference (LSD) test.](image)

The HA2 fraction consists of calcium-bounded humic acids. It is believed that calcium humates are involved in the formation of the soil fragments, and that their depletion leads to the formation of an agronomically low-value structure. An increase in this carbon fraction (0.21 percentage points) was found in the soil limed at the 2.0 rate every 3–4 years...
compared to the unlimed soil (0.124 g kg\(^{-1}\) C). Such an increase in the combination of humic acids with calcium can be influenced by the amount of lime added when high levels of calcium enter in the soil. It was determined that after soil liming, the main soil chemical parameters (mainly soil acidity and mobile aluminum) change, resulting a re-group of the composition of humic substances, with the mobile acids replenishing the calcium-bound fraction \([40,41]\). Our findings support these statements—under long-term liming at the 2.0 rate the lowest HA1 level (0.251 g kg\(^{-1}\) C) and highest amount of HA2 (0.21 g kg\(^{-1}\) C) were found. It can be stated that soil liming is a measure that makes it possible to stabilize HA and FA by the cation bridge mechanism.

The HA3 fraction consists of hardly soluble humic acids bounded to the mineral part of the soil—the clay minerals—which are one of the fractions that ensure a stable soil structure. Some studies \([42–44]\) have shown that the amount of carbon associated with clay particles (HA3) largely depends on the soil texture and not on the input of organic or mineral fertilizers to the soil. A significant increase in this carbon fraction (0.096 percentage points) has been defined in soil limed at the 2.0 rate every 3–4 years (Figure 4). The content of humic acids bound to clay minerals in the limed soil, using different liming intensities, varied in the range of 0.137–0.236 g kg\(^{-1}\) C. Litvinovich et al. \([40]\) and Barreto et al. \([45]\) reported that in the presence of calcium, the adsorption of humic compounds by the soil’s clay minerals increases. This is in line with our findings and shows that calcium may play a major role in SOC sequestration in acid moraine loam soil.

The data in Figure 5 show the effect of soil liming on the amount of fulvic acids. During the humification process, different liming intensities result in varying amounts of fulvic acid, which alter the chemical and physical aspects of the soil. FA is soluble at all pH levels, excels more than HA in soils with higher acidity, and degrades soil clay minerals, which is especially typical for FA1a fraction. FA1a is the fraction of fulvic acids that is characterized by active degradation of soil minerals, as well as a negative impact on plant growth and productivity. In the unlimed soil, the FA1a fraction (0.114 g kg\(^{-1}\) C) was dominant. The predominance of the FA1a fraction was associated with a low content of organic carbon, which disrupts the humification processes in the soil. A reduction of this fraction was found in all experimental variants compared to the control.

![Figure 5. The effect of different liming intensities on the mean differences of carbon content in fulvic acid fractions (g kg\(^{-1}\) C). C\(_{FA1a}\), mobile, aggressive fulvic acids; C\(_{FA1}\), mobile fulvic acids bounded with HA1 fraction; C\(_{FA2}\), fulvic acids bounded with calcium; and C\(_{FA3}\), fulvic acids bounded with soil minerals. Bars with different lowercase letters indicate significant differences at \(p < 0.05\) level based on the least significant difference (LSD) test.](image-url)

The FA1 fraction consists of readily soluble fulvic acids combined with the HA1 fraction of humic acids. A significant reduction of fulvic acids in this fraction was found in
limed soils at rates of 2.0 every 3–4 years compared to unlimed soils. Similar results were obtained by Tobiašová [46] and Tobiašová et al. [47], when the fulvic acids of the FA1a and FA1 fractions predominated in the unlimed soil, and the content of these acids decreased in terms of organic carbon when applying both mineral and organic fertilization [46,47]. The FA2 fraction consists of fulvic acids bound to the calcium and HA2 fractions of humic acids. The amount of FA2 fraction varied from 0.031 g kg$^{-1}$ C to 0.046 g kg$^{-1}$ C depending on the applied liming intensity. These findings support the hypothesis of Litvinovich et al. [40] that calcium applied as lime materials interacts more actively with HA than FA, resulting in more resistant compounds. The FA3 fraction consists of fulvic acids bound to the clay minerals of the soil and the humic acids of the HA3 fraction. Soil liming had a significant effect on the content of fulvic acids in the soil of this fraction. Soil liming at the 0.5 rate mostly promoted the accumulation of fulvic acids in this fraction. When comparing the amount of fulvic acids in the humus fractional composition, we found that the FA3 content was higher than the fractions of other fulvic acids in all studied treatments. Pinskiy et al. [48] found that, at the beginning, clay particles adsorb FA more strongly as they are more chemically active having a higher content of functional groups.

This study found direct moderately statistically significant relationships between organic carbon content and carbon content in chemical fractions of humic substances (Table 3).

|          | $C_{org}$ | $C_{HA1}$ | $C_{HA2}$ | $C_{HA3}$ |
|----------|-----------|-----------|-----------|-----------|
| $C_{org}$|           | 0.411     | 0.317     | 0.396     |
| $C_{FA1}$| 0.178     |           |           |           |
| $C_{FA1a}$| 0.295    | 0.805     |           |           |
| $C_{FA2}$| 0.428     | 0.461     | 0.654     |           |
| $C_{FA3}$| 0.262     | 0.448     | 0.244     | 0.752     |

The dependence between $C_{HA1}$ and $C_{FA1}$ was strong, which have been due to the fact that at higher soil acidities, $C_{HA1}$ and $C_{FA1}$ are dominant. Furthermore, the strong dependence between $C_{HA3}$ and $C_{FA3}$ confirms the statement that FAs are adsorbed more strongly as they are more chemically active due to a higher content of functional groups. The HA3 and FA3 fractions accounted for the substantial portion of total humic and fulvic acids in the moraine loam soil.

3.4. Qualitative Characteristics of SOC under the Different Intensities of Liming

According to Orlov [49] and Tripolskaja [50], the main indicator of the quality of humus is the ratio of humic to fulvic acids; the higher it is, the better the quality of the humus and the greater the possibility of preserving the humus. Based on the D. Orlov [49], soil humus is the fulvic type if the HR/FR ratio is <0.5; when this ratio varies from 0.5 to 1.0, such humus is called the humic fulvic type, and when it is >1, it is the humic type. In our experiment, the ratio of humic and fulvic acids in the unlimed soil was 0.95 (Table 4), which indicates that the soil was dominated by fulvic acids and the type of humus formation was humic–fulvic. An increase in the ratio of humic to fulvic acids indicates an improvement in the quality of the humus. Liming of the soil at the 2.0 rate every 3–4 years significantly increased this ratio to 1.26. According to Radmanović et al. [51] and Lobanov et al. [52], the humification degree (HD) is influenced by soil conditions, with soil pH being one of the determining factors—the higher the soil pH, the greater the HD, and our findings fully support this statement. Consequently, during the liming of the soil, the accumulation of more valuable humic acids takes place. Based on these data, it can be stated that the liming of the soil led to an improvement in the quality of humus in the arable soil layer.
Table 4. Qualitative characteristics of SOC in the Retisol (0–20 cm depth), 2015–2019 average data.

| Treatments  | Unlimed | Limed at 0.5 Rate | Limed at 2.0 Rate |
|-------------|---------|------------------|------------------|
| HA/FA       | 0.95    | 1.19             | 1.26             |
| HD, %       | 29.9    | 32.7             | 38.0             |
| Aggressiveness | 11.74  | 9.107            | 7.857            |
| Mobility    | 2.93    | 2.119            | 1.509            |

The increase in the relative share of humic acids in relation to other humic substances is valuable from an agronomic and ecological point of view. The relative proportion of humic acid C in total SOC, expressed as %, is understood as the degree of humification of organic matter (HD) [49]. According to the data of our experiment (Table 4), the lowest degree of humification of organic matter (29.9) was in unlimed soil.Liming of the soil promoted humification of organic compounds in the soil. The highest degree of humification of organic matter (38.0) was determined using the soil liming at the 2.0 rate. This is in line with the literature, where the incorporation of the lime into the soil results in an increase in carboxylic, alkyl, and α-aromatic compounds in the soil, which promote the formation and humification of stable humic substances [53,54]. According to Orlov [49], the degree of humification is high at 30–40%, medium at 20–30%, and low at 10–20%. Based on the obtained results, it can be asserted that the degree of humification was high in all variants of the study, except for the unlimed (control), where the degree of humification was average. Therefore, we can conclude that liming resulted in slower mineralization of organic carbon. At the same time, there was a higher content of humic substances, which contributed to recalcitrant C pools, which is estimated as the C restoration potential in soil. The obtained results are confirmed by the data of other authors [55,56].

The relative proportion of the most aggressive FA1a fraction was used to determine the aggressiveness of fulvic acids. The significant influence of liming on this indicator was revealed in all limed treatments (at the 0.5 and 2.0 rates). It can be affirmed that in unlimed soil microbiological activity is high, resulting formation of new mobile HS, a particularly aggressive FA1a, and, as mentioned by Romanovskaja et al. [57], the use of lime reduced this index due to the aforementioned interaction of HS with calcium cations.

The mobility of humus compounds has been defined in order to assess the qualitative changes in humus as a result of the applied liming rates. HS mobility decreased with increasing liming intensity. The effect of liming at the 2.0 rate was strongest with HS mobility decreasing to 1.509 compared to the unlimed soil (2.93). Summarizing the obtained data, in moraine loam soil humic substances have a decreased mobility and a stronger bind due to an increased amount of humic acids bounded to the clay particles that interact with organic components and stabilize them.

4. Conclusions

The amount of lime applied affected the chemical properties of the Dystric Glossic Retisol. Compared to unlimed soil, soil liming at the highest intensities (0.5 rate every 7 years and 2.0 rate every 3–4 years) resulted in a slower recovery of pH and mobile aluminum concentration, as well as a considerable reduction in plant-available phosphorus and potassium. Periodical liming (at 0.5 and 2.0 liming rates) had a negative and statistically significant impact on SOC levels in the soil, but eventually resulted in C displacement into the humus fractional composition. Soil liming resulted in the accumulation of all humic acids as well as the FR2 and FR3 fulvic acid fractions in the topsoil. The use of lime in the soil led to a significant increase in the favorable humic acids. Compared to unlimed soil, the HR/FR ratio, which shows humus quality, increased to 1.26 as a result of the applied liming. The mobility of humic substances diminished as the liming intensity was increased. Furthermore, due to the interaction of humic acids with calcium cations, the use of lime reduced the aggressiveness of fulvic acids. Because organic carbon is sequestered and preserved in stable forms in lime-applied soils, the findings of this study emphasize the significance of these soils to environmental quality. This additional knowledge will add
significantly to the scientific assessment of the implications of liming at various intensities on the functioning of agro-ecosystems, as well as contribute to addressing climate change issues and the search for a long-term agricultural development model. However, the physical stability of SOC, considering how different mechanisms work together to protect SOC against mineralization and what their significances are, is still largely unclear. The physical aspect of soil aggregates and their interaction with carbon stability and soil liming remain uncertain. Future research is needed to make up for these knowledge gaps.

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