

Article



# **Properties of Humic Acids in Meadow Soils Irrigated with the Slope-and-Flooding System**

Magdalena Banach-Szott<sup>1,\*</sup>, Andrzej Dziamski<sup>2</sup> and Maciej Markiewicz<sup>3</sup>

- <sup>1</sup> Department of Biogeochemistry and Soil Science, Bydgoszcz University of Science and Technology, 6 Bernardynska St., 85-029 Bydgoszcz, Poland
- <sup>2</sup> Department of Botany and Ecology, Bydgoszcz University of Science and Technology, 7 Kaliskiego St., 85-796 Bydgoszcz, Poland; andrzej.dziamski@gmail.com
- <sup>3</sup> Department of Soil Science and Landscape Management, Nicolaus Copernicus University in Toruń, 1 Lwowska St., 87-100 Toruń, Poland; mawicz@umk.pl
- \* Correspondence: mbanach@pbs.edu.pl

Abstract: The still-advancing soil degradation and the related losses of soil organic carbon stocks due to the limited inflow of organic residues in agro-ecosystems encourage more and more soil protection. Establishing meadow ecosystems is one of the key methods of agricultural land use preventing losses of organic carbon in soils. Based on the research on the properties of humic acids, it is possible to determine the advancement of the processes of transformation and decomposition of soil organic matter. The obtained results may allow for the development of a soil protection strategy and more effective sequestration of organic carbon. Therefore, the aim of the research was to determine the properties of humic acids defining the quality of organic matter of meadow soils irrigated for 150 years with the slope-and-flooding system. The research was performed based on the soils (Albic Brunic Arenosol) sampled from Europe's unique complex of permanent irrigated grasslands (the same irrigation management for 150 years), applying the slope-and-flooding system: the Czerskie Meadows. The soil samples were assayed for the content of total organic carbon (TOC) and the particle size distribution. HAs were extracted with the Schnitzer method and analysed for the elemental composition, spectrometric parameters in the UV-VIS (ultraviolet-visible) range, hydrophilic and hydrophobic properties and the infrared spectra. The research results showed that the HAs properties depend on the depth and the distance from the irrigation ditch. The HAs of soils sampled from the depth of 0-10 cm were identified with a lower "degree of maturity" as compared with the HAs of soils sampled from the depth of 20-30 cm, reflected by the values of atomic ratios (H/C, O/C, O/H), absorbance coefficients, and the FT-IR (Fourier transform infrared) spectra. The mean values of the H/C ratio in the HAs molecules of soils sampled from the depth of 20–30 cm were lower by 8.2% than those from the depth of 0–10 cm. The mean values of the absorbance coefficient A4/6 in the HAs molecules of soils sampled from the depth of 20-30 cm were lower by 9.6% than in the HAs molecules of soils sampled from the depth of 0–10 cm. The HAs molecules of the soils sampled 25 m from the irrigation ditch were identified with a higher degree of humification, as compared with the HAs of the soils sampled 5 m from the irrigation ditch. The results identified that humic acids produced in the many-year irrigated sandy soils were identified with a high degree of humification, which proves the relative stability of the soil's organic matter. It confirms the importance of meadow soils for the carbon sequestration process. It should also be emphasized that the research area is interesting, although hardly described in terms of organic matter properties. Further and more detailed applicable research is planned, e.g., monitoring of total organic carbon content and comparing the properties of irrigated and non-irrigated meadow soils. Continuity of research is necessary to assess the direction of the soil organic matter transformation in such a unique ecosystem. The obtained results may allow for the development of, inter alia, models of agricultural practices that increase carbon sequestration in soils. In the long term, this will allow for greater environmental benefits and, thus, also increased financial benefits.



Citation: Banach-Szott, M.; Dziamski, A.; Markiewicz, M. Properties of Humic Acids in Meadow Soils Irrigated with the Slope-and-Flooding System. *Agronomy* 2021, *11*, 2553. https:// doi.org/10.3390/agronomy11122553

Academic Editor: Diego Pizzeghello

Received: 27 October 2021 Accepted: 11 December 2021 Published: 15 December 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Keywords: meadow soil; carbon sequestration; elemental composition; UV-VIS; HPLC

## 1. Introduction

Humus substances are produced as a result of the processes of transformation and decomposition, together referred to as "humification". The term "humus substances" is used for the scientific description of specific nutrients of a complex structure which can be isolated and fractioned in many ways [1]. Those compounds take part in all the processes in soil, and they affect its physical, chemical and biological properties, for example, improving soil buffering capacity, supplying plants with available micronutrients, and immobilizing organic contaminants and metals [2–5]. Humus substances also determine the production potential of soil by taking part in global carbon cycling; they perform environmental functions related to carbon sequestration and the release of  $CO_2$  into the atmosphere [6,7]. With the research of the properties of humic acids, one can determine the advancement of transformation and decomposition processes.

Research on the properties of humic substances, characterized by a complex structure, therefore requires several instrumental methods [8].

To evaluate the properties of humic acids, the elemental composition and the values of H/C, O/C, O/H and N/C atomic ratios determined from them can be used [9–12]: the values of absorbance of their solutions in the UV-VIS spectrum  $A_{280}$ ,  $A_{465}$ , and  $A_{665}$  and the coefficients of absorbance  $A_{2/4}$ ,  $A_{2/6}$ ,  $A_{4/6}$ , and  $\Delta \log K$  [13–20], as well as the hydrophilic and hydrophobic properties [21–27].

The structural characteristics of humic acids, especially identifying the functional groups, are assayed from the infrared spectra (FTIR) [13,14,28,29].

Thanks to the studies of humic acids' properties, one can determine the advancement of the processes of transformation and decomposition of organic matter, which is the source of organic carbon.

Meadow soils provide good conditions for the accumulation and immobilisation of organic matter [30]. For this reason, these ecosystems represent a land use preventing the organic carbon (OC) losses in soils. The OC losses, as a result of the soil processes, are due to  $CO_2$  and  $CH_4$  gas emissions as well as soluble organic carbon leaking into groundwaters. It was demonstrated that meadow ecosystems help limit OC losses from soil, and they are important for a balanced management of that element in the environment [31–35]. Besides, permanent grassland soils are an important reservoir of organic carbon (OC), and its resources are similar to or higher than the carbon resources in forest soils.

In Poland, agricultural land and woodland account for a considerable share (around 90%) in the land management structure:

- Agricultural land—61% (including arable land—73%, grassland—20%, orchards—2%, and other land—5%),
- Forests and woodlands—31%,
- Other land—8%.

The native soils show a very low average content of organic matter (OM) at 1.7% and organic carbon (Corg) at 2.2% in dry matter of soil [36]. In the case of typical Brunic Arenosols used for agricultural purposes (e.g., arable land), the soil organic carbon content is, on average, 7 g·kg<sup>-1</sup> to 23 g·kg<sup>-1</sup> [37]. According to the international standards, a content of 2.0% Corg stands for soil degradation process. For that reason, to increase the carbon sequestration in the environment, a formation of permanent grasslands is required. In addition, a legal framework of protection should be sought for, in particular, such natural environments as forest and meadowland, degraded soils and soils under agricultural usage [34,38]. In the vast majority of this part of Europe, such ecosystems are formed on wetlands. On the other hand, the research on the organic matter of mineral soils developed as permanent grasslands, which, if possible, is transformed into arable fields or used alternately, is still a niche concept. However, as for meadow soils low in

carbon, only long-term use enhances permanent accumulation. An example of mineral soils managed as perennial grasslands can be provided by Poland and Europe's unique complex of permanent grasslands specified as the "Czersk Meadow Complex". The name "Czersk Meadow Complex" covers a historic term for perennial grasslands with a total area of almost 2000 hectares, considered to be meadows and pastureland and arranged in various parts of the Tuchola Forest (in Northwest Poland) [39]. The economic potential of the Tuchola Forest was discovered once it was incorporated into the Prussian Empire in 1772 as a result of the partitioning of Poland. The forest became a base from which timber was derived for many investments both in the farmer Polish areas as well as in other parts of then Prussia. It was also the time of the greatest changes in the vegetation structure, related to introducing pine monoculture. Despite the loss of the basic documents related to the construction of those structures, the history of the creation of the Czersk Meadow Complex is best described by Papke [40] and Sabiniarz [39]. The intensive forest economy during the Partitions period resulted in an increase in employment in forestry. At the same time, a demand for agricultural products increased, which, with an inconsiderable acreage of soils applicable for agriculture in the Tuchola Forest, required intensifying the production as well as increasing the agricultural acreage. The Tuchola Forest was also of strategic military significance for the Prussians; the Forest was located in close vicinity to Russia (one of the three empires which made the Partition of Poland) and the areas could be used for hay production for a huge army.

In terms of science, a closer interest in the Czersk Meadow Complex was thanks to the research performed by the IMUZ in Bydgoszcz and in Falenty [41,42] to increase meadow productivity. For the first time, the soils were classified, and their moisture content and the state of vegetation were specified. The results of the works demonstrate that more than a hundred-year-long irrigation of meadows with the slope-and-flooding system has resulted in the formation of a perennial turf. In the soil profiles, the humus horizon, with the average thickness of 10–20 cm, was identified. A regular irrigation of loose sandr sands facilitated the formation of varied plant communities depending on the moisture content. This translated into an increase in the processes related to the humus horizon formation in the surface soil layers, enriching the soil with organic matter and increasing its fertility as well as other environmental functions [39,43,44].

Thanks to irrigation, a better water supply can lead, on the one hand, to increased biomass production and, thus, a greater input of carbon to soil in a form of roots and dead plant material. On the other hand, a higher soil moisture increases soil microbiological activity, which can result in increased decomposition of organic matter of soil and thus in an increase in CO<sub>2</sub> emissions [45,46]. Increased microbiological decomposition of organic matter of soil, on the other hand, can lower the content of organic carbon in soil [47]. The effect of irrigation on SOC was, indeed, observed to vary according to local conditions and specific water and soil management [48–50]. Irrigation is essential for crop production in many regions of the world [46,48,51–54]. Reports are also available on the effect of meadow irrigation on SOC content [46,55] and on soil chemical and microbial parameters [56,57]. However, there are only a few research reports available on the properties of humus substances (including humic acids), one of the key fractions of organic matter [58–60]. Due to increasing soil degradation and total organic carbon (TOC) losses due to changes in soil use and intensified agricultural production, local, regional and global soil protection has become one of the key goals of the European Community policies [61]. Importantly, due to a specific biodiversity, meadow ecosystems, including the ones in which the research was performed, are one of the key methods of the agricultural land use preventing the losses of organic carbon in soils. With that in mind, the aim of this paper was to determine the properties of humic acids determining the quality of organic matter in soils of the unique Czersk Meadow Complex irrigated with the slope-and-flooding system. The research area is hardly described in terms of organic matter properties. Continuity of research is necessary to assess the direction of soil organic matter transformation in such a unique ecosystem.

## 2. Materials and Methods

# 2.1. Materials

Soil was sampled from perennial grasslands of the unique "Czersk Meadow Complex" (Poland), continuously irrigated for about 150 years with the slope-and-flooding system. Over the construction of respective districts of the complex (1842–1860), vast areas with woodlands were exposed to complete reclamation and cleaning from the redundant vegetation. For meadow irrigation, there were built two irrigation canals (the Wda Canal—24 km and the Grand Brda Canal—21 km), supplied with the waters of the two biggest rivers of the Tuchola Forest; the Wda River and the Brda River. Due to the fact that meadows are situated in the sandr areas of the upper Wda River and Brda River, where loose sands dominate, and the natural level of groundwaters occurs at over 2 m deep, the only possible way for their irrigation was the application of the slope-and-flooding system. Interestingly, the waters from irrigation ditches were distributed across the meadows with a system of canals, ditches and overflow furrows, creating a unique irrigation system. To do so, an entire system of ditches, partitions, culverts and seven aqueducts was constructed. Meadow quarters were carefully profiled and formed to create smooth slopes with about a 1-5% descension rate, from the irrigation canal and distributing furrows from which the water flows down freely. The irrigation ditches depth ranged from about 30 cm to 50 cm, and the distance between the ditches was about 50 m to avoid the standing waters and flooding. Below the areas, there were also created so-called supplying and discharging devices. The area prepared was sown with various species of grasses and herbs, and the meadow sward was still maintained. Initially, sheep grazed on the land; currently the land is mostly used as hay meadows. Meadows are irrigated over the vegetation period in the form of few-day flooding every 2–3 weeks, with a break for haymaking. However, it happens that, due to the low water level, water is missing, even in the supplying ditches, which makes irrigation impossible. The canal infrastructure, despite the considerable present day neglect, is still maintained and, for the most part, still operating continuously. However, as a result of the changes in the property structure, especially over the last 20–30 years, meadow complexes have undergone transformations. The meadows are partially the property of private owners or leased from state institutions. The mean annual NPK fertilisation was 120 kg N ha<sup>-1</sup>, 66.4 kg K ha<sup>-1</sup> and 22.2 kg P ha<sup>-1</sup>, which guaranteed two abundant swaths of green forage and hay of good quality. Mineral fertilisation, however, resulted in a change in the structure of vegetation of the meadow sward, which was related to the disappearance of species referred to as herbs, to the benefit of tall grasses. The flora and phytosociology studies have shown that in that area, there have formed plant communities which, in terms of phytosociology, are different subtypes of ryegrass meadows: the class Molinio –Arrherenatheretea and the order Arrherenatheretalia.

For the purpose of the research, there were selected the areas with mineral soils and only those objects which, undisturbed since the arrangement of the areas, have been maintaining the meadow sward, thanks to irrigation with the waters of the rivers, and are used, as originally, to acquire hay. The historic names of the respective areas of the Czersk Meadows (Kamionna, Podlesie, Cegielnia) were applied. The selection of the soil sampling locations (including the distances) was made from the state of vegetation supported with phytosociological studies of vegetation of the meadow sward developed in different soil moisture conditions, resulting from the slope of 1–5% and the quarter size [39,40].

Soil was sampled (in three replications), and the samples were taken in spring from the humus horizon from the depths of 0–10 cm, 10–20 cm and 20–30 cm. The mean annual temperature (in 2008–2012) was 7.9 °C, and the mean total annual precipitation was 648.6 mm. The soils (*Albic Brunic Arenosol*) were sampled in three replications at 5, 15 and 25 m away from the irrigation ditch in the areas of the Kamionna (53.842544, 18.150556), Cegielnia (53.879982, 18.138737) and Podlesie (53.867234, 18.146794) quarters. The distance between the replications was not more than 2 m. The distance between Cegielnia and Podlesie was 1.5 km, and between Podlesie and Kamionna was2.7 km (Figure 1). The soil



samples were dried at room temperature and sieved (2 mm). In total, the research covered 9 soil profiles for each of the objects (a total of 27 soil samples).

Figure 1. Soil sampling locations.

2.2. Methods

2.2.1. Soil Analysis

For air-dried soil samples, the following analyses were made:

The contents of total organic carbon (TOC) and total nitrogen (Nt) were assayed with the Vario Max CN analyser provided by Elementar (Germany). The content of TOC and the content of Nt were expressed in g kg<sup>-1</sup> of d.w. of soil [62].

The pH was assessed in the suspension of distilled water and soil with the pH meter MultiCal pH 540 GLP WTW [63].

The particle size composition was determined applying the areometric method [64].

The bulk density was determined with the gravimetric method, using 100 cm<sup>3</sup> steel rings [65].

2.2.2. Extraction of Humic Acids

Humic acids (HAs) were extracted and purified with standard methods using the following procedure [22]:

Decalcification was performed (24 h) with 0.05 M HCl (1:10 w/v). After centrifugation, the residue was washed with distilled water until neutral.

Then, we performed extraction (24 h) of the remaining solid with 0.5 M NaOH (1:10 w/v), with occasional mixing, followed by centrifugation.

Precipitation (24 h) of the humic acids was performed from the resulting alkaline extract with 2 M HCl to pH = 2 and centrifugation.

This was followed by purification of the resulting humic acids: the humic acid residue was treated with a mixture of HCl/HF (950 mL H<sub>2</sub>O, 5 mL HCl, 5 mL HF) over a 24 h period, followed by centrifugation. The residue was also treated with distilled water until zero reaction to chloride was achieved.

The preparations were lyophilised and powdered in agate mortar. The ash content in the humic acids preparations was lower than 2%.

#### 2.2.3. Characteristics of Humic Acids

In the separated humic acids, the following were assayed.

For the elemental composition (Perkin Elmer 2400 CHN analyser), the H/C, O/C, O/H, and N/C atomic ratios as well as  $\omega$  (internal oxidation degree) were calculated according to formula (1):

$$\omega = (2O + 3N - H) : C \tag{1}$$

where

O, N, H, C—content in atomic %.

The hydrophilic and hydrophobic properties were determined with liquid chromatograph HPLC Series 200 with a DAD detector supplied by Perkin-Elmer. The separation involved the use of column X-Terra C18, 5  $\mu$ m, 250 × 4.6 mm. The solutions of the humic acids were applied in 0.01 mol/L NaOH of the concentration of 2 mg/mL, with injection of the sample at 100  $\mu$ L with the solvent, acetonitrile water. The solvents flow was in the gradient (ratio H<sub>2</sub>O:ACN (v/v) over 0–6 min.—99.5:0.5, 7–13 min., 70:30, 13–20 min.—10:90) and detection occurred at the wavelength of 254 nm. Based on the areas determined under the peaks, the share of hydrophilic (HIL) and hydrophobic ( $\Sigma$ HOB = HOB1 + HOB2) fractions in humic acid molecules and parameter HIL/ $\Sigma$ HOB were determined [23,25,26].

UV-VIS absorption spectra (Perkin Elmer UV-VIS Spectrometer, Lambda 20) were determined. The VIS spectra were obtained from 0.02% humic acids solutions in 0.1 M NaOH and UV-spectra—after fivefold dilution. Absorbance, measured at 280 nm (A<sub>280</sub>), 400 nm (A<sub>400</sub>), 465 nm (A<sub>465</sub>), 600 nm (A<sub>600</sub>) and 665 nm (A<sub>665</sub>), was used to calculate the coefficient values [45]:

 $A_{2/4}$ —280 nm and 465 nm absorbance ratio

 $A_{2/6}$ —280 nm and 665 nm absorbance ratio

 $A_{4/6}$ —465 nm and 665 nm absorbance ratio

$$\Delta \log \mathbf{K} = \log \mathbf{A}_{400} - \log \mathbf{A}_{600} ; \tag{2}$$

and infrared spectra (Perkin-Elmer FT-IR Spectrometer, Spectrum BX) over 400–4400 cm<sup>-1</sup> were produced for HAs (3 mg) in KBr (800 mg). Deconvolution was applied, with a filter making the bands of  $\gamma = 4$  narrower, using the process of smoothing, for which the length parameter was l = 80% [28].

## 2.2.4. Statistical Analysis

The statistical analyses of the soil properties and humic acids involved the analysis of variance of the data derived from a single two-factor experiment in a split-plot design. The tables present the mean values from three replications. The significance of differences was evaluated using the Fisher test (LSD) at a significance level of  $\alpha = 0.05$ . The significance of differences was evaluated (ANOVA) using the Tukey test. Pearson's correlation was also analysed at a significance level of p < 0.05. Similarities across the objects were evaluated using cluster analysis. The method involves dividing the data set into groups to produce clusters in which the elements are similar to one another and, at the same time, different from the elements from the other groups. The groups of similar treatments are presented

in a form of dendrogram. In a given group, the smaller the Euclidean distance, the more similar the objects. Data clustering was performed with the Ward method [66]. The analysis was made after data standardisation. The cluster analysis was performed based on the elemental composition (H/C, O/H, O/C,  $\omega$ ), spectrometric parameters (A<sub>2/4</sub>, A<sub>2/6</sub>, A<sub>4/6</sub> and  $\Delta$ logK) and the HIL/ $\Sigma$ HOB parameter. The method involves dividing the data set into groups to produce clusters where the elements are similar to one another and, at the same time, different from the elements of the other groups. The relationships were determined using the Statistica MS 12.0 statistics software.

## 3. Results and Discussion

# 3.1. Basic Parameters of Soils

The soil pH values ranged from 5.3 to 7.0 (Table 1) and they were similar to the optimal pH value of soil for meadow vegetation [67]. It was found that the greater the depth, the greater the pH values; nevertheless, the increase was non-significant (Table 2). The soil samples differed in bulk density: in the 0–10 cm layer, it was 1.29 g cm<sup>-3</sup>, and in the 20–30 cm layer, it was 1.52 g cm<sup>-3</sup> (Table 2). The soils showed a high sand fraction (2.0–0.05 mm), which ranged from 87.3% to 92.8% and a very low clay fraction (<0.002 mm) ranging from 0.1% to 1.1% (Table 1). It was observed that the soil sampled from the depth of 0–10 cm was identified with a significantly higher mean percentage of the sand fraction as compared with the soil sampled from the depth of 10-20 cm and 20-30 cm, by 1.3%and 2.2%, respectively (p = 0.009 and p = 0.004, Table 2). The opposite dependencies were recorded for the percentage of the silt fraction (p = 0.011 and p = 0.0001, Table 2). There were found no definite tendencies in the changes in the particle size composition for the thickness of 0–30 cm. There was a negative, however, non-significant, correlation between the percentage of the sand fraction and the content of TOC (r = -0.12, for p < 0.05). Interestingly, the soil sampled from the depth of 0–10 cm demonstrated significantly lower mean contents of TOC than the soil sampled from the depths of 10–20 cm and 20–30 cm, by 70.3% and 57.9%, respectively (p = 0.0001 and p = 0.0001, Table 2). In the 10–20 cm layer, higher contents were reported: from 44.6 g kg<sup>-1</sup> to 56.5 g kg<sup>-1</sup> (Table 3). Lower TOC contents in the 0-10 layer can result from the advantage of the processes of mineralisation over humification, which is enhanced by a higher sand fraction and a lower bulk density, as compared with deeper soil layers. The importance of microbiological activity in the 0–20 cm layer of irrigated soils is stressed by, for example, Chenu et al. [50] and Trost et al. [55]. The contents of Nt in the soils under study, similar to the content of TOC, were highest in the soil sampled from the depth of 10–20 cm, and they ranged from  $3.81 \text{ g kg}^{-1}$  to  $4.91 \text{ g kg}^{-1}$ , with the lowest being in the soils of the 0–10 cm layer (from  $0.77 \text{ g kg}^{-1}$  to  $1.51 \text{ g kg}^{-1}$ ) (Table 3).

Surface irrigation of soils with the slope-and-flooding system could create more favourable conditions for the development of grasses and thus for biomass production and carbon sequestration [68]. Interestingly, in reference to the results of research of the meadow soils with a similar thickness of the humus horizon presented by Drag et al. [69] and Jończak [70], the samples under study were identified with much higher contents of TOC and Nt. According to Trost et al. [46,55] and Sun et al. [71], flooding is generally beneficial for carbon input and may increase the TOC and Nt content. One thus assumes that higher contents of TOC and Nt in the 10–20 cm and 20–30 cm soil layers point to favourable conditions for organic matter accumulation. The importance of the processes enhancing the enrichment in organic matter in the upper layers of the meadow soil profile is also stressed [72–74]. The content of TOC and Nt in the sand of Kamionna and Podlesie depended on the distance: soil sampled furthest from the irrigation ditch showed the highest contents of TOC and Nt (Table 3). The soil sampled from those objects 25 m away from the irrigation ditch recorded the pH value closest to neutral and the lowest percentage of the sand fraction (Table 1).

Fa	Factor		Sand (%)	Silt (%)	Clay (%)						
	Kamionna										
	5	5.5-6.4	90.8	8.8	0.4						
Distance (m)	15	5.6-6.3	91.0	8.6	0.4						
()	25	6.2–6.9	90.0	9.6	0.4						
L	SD	-	0.816	0.827	ns						
	0–10	5.5-6.2	92.5	7.4	0.1						
Depth (cm)	10-20	5.6–6.3	91.8	8.1	0.1						
(ent)	20–30	6.3–6.9	87.3	11.6	1.1						
L	.SD	-	0.307	0.318	0.141						
		Ceg	ielnia								
	5	6.7–7.0	88.0	11.7	0.3						
Distance (m)	15	6.0–6.8	90.4	9.4	0.2						
	25	6.9–7.0	91.0	8.9	0.1						
L	SD	-	0.144	0.118	0.051						
	0–10	6.0–6.7	90.7	9.2	0.1						
Depth (cm)	10-20	6.3–7.0	88.8	11.1	0.1						
(0)	20–30	6.8–7.0	90.0	9.6	0.4						
L	SD	-	0.161	0.161	0.101						
		Pod	llesie								
	5	5.3–6.5	91.4	8.2	0.4						
Distance (m)	15	5.8-6.4	91.8	7.9	0.3						
()	25	6.1–6.5	91.2	8.7	0.1						
L	SD	-	0.235	0.177	0.112						
	0–10	5.3–6.1	92.8	7.0	0.2						
Depth (cm)	10-20	6.0–6.3	91.7	8.2	0.1						
()	20–30	6.4–6.5	89.9	9.7	0.4						
L	SD	-	0.249	0.129	0.163						

**Table 1.** The pH and particle size composition of the soil samples.

LSD (Least significant difference).

# Table 2. Analysis of variance (ANOVA) with the Tukey test.

Parameter	0–10 cm n = 27	10–20 cm n = 27	Significant Level (p) *	0–10 cm n = 27	20–30 cm n = 27	Significant Level (p) *
TOC $(g kg^{-1})$	14.7	49.5	0.0001 *	14.7	34.9	0.0001 *
Nt (g kg <sup>-1</sup> )	1.09	4.30	0.0001 *	1.09	2.51	0.0001 *
TOC/Nt	15.2	11.6	0.0002 *	15.2	14.7	0.689
Sand (%)	92.0	90.8	0.009 *	92.0	90.0	0.004 *
Silt (%)	7.92	9.13	0.011 *	7.92	10.3	0.0001 *
Clay (%)	0.18	0.13	0.145	0.18	0.73	0.0001 *
pН	6.01	6.28	0.268	6.01	6.64	0.006 *
Bulk density (g cm <sup>-3</sup> )	1.29	1.43	0.0002 *	1.29	1.52	0.0002 *
H/C	1.34	1.30	0.005 *	1.34	1.23	0.0001 *
ω	0.97	0.136	0.014 *	0.97	0.164	0.0001 *
A <sub>4/6</sub>	5.00	4.71	0.015 *	5.00	4.52	0.0002 *
ΔLogk	0.678	0.638	0.024 *	0.678	0.581	0.0001 *

Table 2. Cont.

Parameter	0–10 cm n = 27	10–20 cm n = 27	Significant Level ( <i>p</i> ) *	0–10 cm n = 27	20–30 cm n = 27	Significant Level (p) *
HIL (%)	38.73	39.85	0.011 *	38.73	40.53	0.0005 *
HIL/ΣHOB	0.633	0.663	0.011 *	0.633	0.684	0.0004 *
* significant level.						

Table 3. Results of the analysis of variance for the basic chemical parameters of soils.

Fac	Factor $\begin{array}{cccc} TOC & Nt & TOC & Nt \\ (g  kg^{-1}) & (g  kg^{-1}) & TOC/Nt & (g  kg^{-1}) & (g  kg^{-1}) \end{array}$		TOC/Nt	TOC (g kg <sup>-1</sup> )	Nt (g kg <sup>-1</sup> )	TOC/Nt				
		Kamionna				Cegielnia			Podlesie	
	5	22.7	1.83	14.55	39.2	2.35	17.35	35.0	2.78	13.19
Distance (m)	15	29.4	2.02	16.87	27.3	2.46	12.76	34.2	2.97	11.71
(111) _	25	40.2	3.04	14.20	32.1	3.16	10.93	37.4	3.07	12.85
LS	SD	1.58	0.431	Ns	0.56	0.136	1.293	2.01	0.168	1.262
	0–10	14.2	0.99	17.00	12.3	0.77	16.06	17.8	1.51	12.44
Depth <sup>–</sup> (cm) <sub>–</sub>	10-20	47.5	4.17	11.43	44.6	3.81	11.81	56.5	4.91	11.52
	20–30	30.6	1.73	17.19	41.8	3.39	13.17	32.4	2.40	13.78
LS	SD	1.05	0.045	2.138	0.32	0.163	2.871	1.59	0.132	1.356

LSD (least significant difference).

The basic parameter determining the intensity of organic matter decomposition in soils, which translates into the properties of the humic acids produced, is the value of the ratio TOC/Nt. Under constant meadow irrigation, in general, the highest values of the TOC/Nt ratio (from 13.19 to 17.35) were reported for the soil sampled closer to the irrigation ditches, which is the key consequence below the nitrogen content in those samples (Table 3). In general, the greater the distance, the lower the value of the parameter; for the soil sampled 25 m away from the irrigation ditch, it ranged from 10.93 to 14.20. The values of the TOC/Nt ratio reflecting quantitative dependencies between the organic carbon and total nitrogen content depended on the depth. It was found that the lowest value of TOC/Nt was noted for the soils with the highest carbon content, i.e., sampled from a depth of 10–20 cm ranging from 11.43 to 11.81 (Table 3). The analysis of variance confirmed that the soil sampled from the 10–20 cm layer recorded significantly lower (by 23.7%) mean values of the ratio TOC/Nt, as compared with the soil sampled from the 0-10 cm layer (p = 0.0001) (Table 2). The values of the TOC/Nt ratio for the soil sampled 10–20 cm deep can be indicative of a high degree of transformation of organic matter present in the material, and of a prevalence of mineralisation over synthesis of organic nitrogen compounds [75].

## 3.2. Elemental Composition of Humic Acids

The basic indicators used in the assessment of humic acid properties include elemental composition and the values of atomic ratios calculated from the composition (Table 4). The H/C values for the humic acids ranged from 1.10 to 1.35, and they were similar to the values reported by Zdenek and Tesarova [76]. Humic acids showed a significant decrease in the value of the H/C ratio with depth. The value of the ratio to the HAs molecules of the soils sampled from the 0–10 cm layer was 1.34 and differed from humic acids of the soils sampled from the 10–20 cm and 20–30 cm layers, by 3% and 8.2%, respectively (p = 0.005 and p = 0.0001) (Table 2). The distance from the irrigation ditch affected the value of the ratio only in the HAs molecules of the soils in Cegielnia; an increase in the parameter value was recorded with an increase in the distance. With the values of atomic ratios H/C, one can determine the degree of condensation of aromatic rings (domination of the groups of

aromatic rings compared to aliphatic chains) and, as a result, the 'degree of maturity' of the molecules of humic acids (the term 'degree of maturity' provides information about, for example, the share of aliphatic structures which are typical of materials at the initial stage of the decomposition process). The literature reports [76-78] showed that a decrease in the value of the H/C ratio indicates an increase in the degree of the aromaticity of the humic acids, which, in turn, suggests an increase in their degree of humification (degree of maturity). Dergacheva et al. [79] stressed that the value of the H/C ratio depends on the conditions the humic acids originated in. The H/C values above 1.0 can point to the advantage of aliphatic structures in the molecules of humic acids [80]. The results show that the structure of the humic acids corresponds to the aromatic systems coupled with the aliphatic chain, containing up to 10 atoms of C [9]. The parameters used to describe the advancement of the process of humification also provide the degree of internal oxidation of the HAs molecules and the ratios of O/C, O/H, and N/C and parameter  $\omega$ . Higher  $\omega$ , O/C, and O/H values and lower H/C values correspond to the humic acids with a higher "degree of maturity" [81,82]. For the HAs molecules studied, the N/C values ranged from 0.054 to 0.081, the O/C values from 0.529 to 0.635, and the O/H values ranged from 0.435 to 0.515 (Table 4). The value of parameter  $\omega$  fell within the range from 0.041 to 0.208. The HAs molecules of soils, in general, identified an increase in the values of O/H and parameter  $\omega$  and a decrease in the value of the N/C ratio with depth, which points to a higher degree of maturity of the HAs' molecules of soils sampled from the greatest depth, as compared to the humic acids of soils sampled from the 0–10 cm layer. Another confirmation also comes from significant differences across the mean values of parameter  $\omega$  for HAs molecules. The mean values of this parameter in the HAs molecules of the soils sampled from the 0–10 cm layer was higher by 86% from humic acids of the soils sampled from the 10–20 cm layer and by 83.1% from humic acids of the soils sampled from the 20–30 cm layer (p = 0.014 and p = 0.0001) (Table 2). There were demonstrated no definite dependencies between the distance of the soil sampling from the watercourse. Only in the case of humic acids of the soils sampled in Podlesie, the values of the ratio O/C, O/H and parameter  $\omega$  were decreasing together with the distance from the watercourse, which could point to a decrease in the degree of maturity of HAs molecules with an increasing distance. However, in reference to the values of the ratio H/C (although non-significant), the opposite conclusion seems applicable, and so, based on the results of the elemental composition, one cannot make a definite statement about the dependence of the degree of maturity on the distance from the watercourse.

Fac	Factor		N/C	O/C	O/H	ω
			Kamionna			
	5	1.29	0.068	0.622	0.484	0.160
Distance (m)	15	1.27	0.067	0.635	0.501	0.198
	25	1.28	0.067	0.614	0.481	0.162
LS	LSD		ns	0.010	0.010	ns
	0–10	1.34	0.077	0.620	0.462	0.131
Depth (cm)	10-20	1.28	0.071	0.626	0.488	0.181
()	20-30	1.21	0.054	0.625	0.515	0.208
LS	LSD		0.006	ns	0.026	0.062
			Cegielnia			
	5	1.10	0.061	0.529	0.478	0.132
Distance (m)	15	1.24	0.071	0.582	0.464	0.121
	25	1.35	0.081	0.620	0.450	0.108
LS	SD	0.053	0.015	0.014	ns	ns

Table 4. Results of the analysis of variance for the values of atomic ratio of humic acids.

Fac	ctor	H/C	N/C	O/C	O/H	ω
	0–10	1.29	0.072	0.580	0.449	0.082
Depth (cm)	10–20	1.24	0.071	0.570	0.462	0.115
(ciri)	20-30	1.16	0.070	0.582	0.480	0.165
LS	SD	0.051	ns	ns	0.015	0.033
			Podlesie			
	5	1.27	0.068	0.609	0.473	0.145
Distance (m)	15	1.26	0.069	0.591	0.467	0.124
()	25	1.25	0.063	0.542	0.435	0.041
LS	SD	ns	ns	0.022	0.020	0.063
	0–10	1.32	0.073	0.586	0.444	0.080
Depth (cm)	10-20	1.28	0.070	0.595	0.459	0.111
(((((((((((((((((((((((((((((((((((((((	20-30	1.18	0.058	0.562	0.472	0.120
LSD		0.060	0.006	0.021	0.012	0.024

Table 4. Cont.

LSD (least significant difference).

## 3.3. Spectrometric Parameters of Humic Acids in the UV-Vis Range

Based on absorbance values of humic acids in the UV-VIS range and the coefficients of absorbance  $A_{2/4}$ ,  $A_{2/6}$ ,  $A_{4/6}$ , and  $\Delta \log K$ , the following properties can be determined: the degree of advancement of organic material humification as well as changes in the properties of humic acids which occur due to various anthropogenic factors [14,16–20]. In the study reported, quite low values of the coefficients of absorbance  $A_{2/4}$ ,  $A_{2/6}$ , and A<sub>4/6</sub> (4.41–6.45, 19.39–33.81, and 4.39–5.28, respectively) were recorded (Table 5). They are indicative of high humic acid 'maturity', thus suggesting that the molecular mass and aromatic centre condensation were high [83]. Additionally, the values of the coefficient  $\Delta \log K$  (ranging from 0.535 to 0.700) indicate a high level of humification [15]. Importantly, the values of the coefficients of absorbance  $A_{2/4}$ ,  $A_{2/6}$ ,  $A_{4/6}$  and  $\Delta logK$  were lower than the values for the HAs molecules of meadow soil reported by Drag et al. [69]. Similarly, Jończak [70], investigating, for example, the humic acids of meadow soils sampled in the Slawno Plain (Poland), and Kobierski et al. [84] for the molecules of HAs of the soils sampled from the floodplain between the Vistula riverbed (Poland) and the flood embankment, recorded higher values of the coefficient of absorbance  $A_{4/6}$ . All that confirms a high degree of transformation of organic matter in soils, and thus a high humus quality. The HAs molecules were identified with a decrease in the values of  $A_{2/4}$ ,  $A_{2/6}$ ,  $A_{4/6}$ , and  $\Delta \log K$  with depth (Table 4). With the analysis of variance, it was demonstrated that the values of parameter  $A_{4/6}$  and  $\Delta \log K$  for HAs molecules of the soils sampled from the depth of 0–10 cm were significantly higher than for the molecules of HAs of the soils sampled from the 10–20 cm layer (p = 0.015 and p = 0.024) as well as the HAs molecules of the soils sampled from the layer of 20–30 cm (p = 0.0002 and p = 0.0001) (Table 2). Drawing on literature reports [85,86], it was found that the humic acids of the soils of the layer 0–10 cm revealed a lower degree of humification as compared with the humic acids of deeper horizons. The distance factor had a significant effect on the value of spectrophotometric parameters of the HAs molecules in the soils sampled in Cegielnia and in Kamionna (Table 5). In Kamionna, values of the coefficients of absorbance of the HAs sampled 5 m away from the irrigation ditch were higher as compared with the sites further away from the watercourse, whereas in Cegielnia, with an increasing distance, only a significant decrease in the value  $\Delta \log K$  was recorded. The values of the coefficients of absorbance  $A_{2/6}$  and  $A_{4/6}$  in this location were also decreasing; however, they were non-significant. The results suggest that the further from the watercourse, the higher the degree of humification in the molecules of HAs in the soil sampled from those locations. As for the humic acids of the soils sampled in Podlesie, there were observed decreased, although non-significant, values of the coefficients of absorbance  $A_{4/6}$  and  $\Delta \log K$  with an increase in the distance, which can suggest an increase in the degree of humification of the molecules of humic acids.

Fa	ctor	$A_{2/4}$	A <sub>2/6</sub>	$A_{4/6}$	ΔlogK
		Kami	ionna		
Distance	5	6.45	33.81	5.23	0.675
(m)	15	5.25	24.49	4.66	0.628
	25	5.28	23.51	4.46	0.632
L	SD	0.417	3.60	0.63	0.025
Depth	0–10	6.05	29.59	4.86	0.688
(cm)	10-20	5.55	26.50	4.73	0.654
	20-30	5.38	25.72	4.75	0.593
L	SD	0.409	Ns	ns	0.051
		Cegi	elnia		
Distance	5	5.21	24.72	4.74	0.697
(m)	15	4.94	23.37	4.72	0.614
	25	5.33	24.19	4.53	0.535
L	SD	0.288	Ns	ns	0.042
Depth	0–10	5.54	27.06	4.88	0.645
(cm)	10–20	5.14	24.11	4.69	0.614
	20-30	4.79	21.11	4.42	0.588
L	SD	0.230	0.994	0.420	0.028
		Pod	lesie		
Distanco	5	5.05	24.47	4.90	0.671
(m)	15	5.19	24.90	4.71	0.629
	25	5.18	25.12	4.77	0.610
LSD		0.128	Ns	ns	ns
Denth	0–10	5.87	30.94	5.28	0.700
(cm)	10-20	5.12	24.16	4.71	0.647
	20–30	4.41	19.39	4.39	0.564
L	SD	0.195	2.44	0.32	0.031

Table 5. Results of the analysis of variance for the coefficients of absorbance of humic acids.

LSD (least significant difference).

## 3.4. Analysis of the Fourier Transform Infrared (FTIR) Spectra of Humic Acids

The FTIR spectra of the HAs (Figure 2) revealed the presence of absorption bands, the ranges of which as well as their corresponding functional groups are given in Table 6 [87–89]. The intensity of the absorption bands in the molecules of HAs depended on the depth and, to a little extent, on the distance from the irrigation ditch. The FT-IR spectra of humic acids of the soils in Kamionna, Podlesie and in Cegielnia showed a lowered intensity of the bands in the range of 3300 cm<sup>-1</sup> and 2920–2960 cm<sup>-1</sup> and bands with a low intensity at 2850 cm<sup>-1</sup> deep down the soil profile. With depth, there was observed an increase in the intensity of the bands within the range of 1730–1710 cm<sup>-1</sup>, pointing to an increase in the share of carbonyl groups in acids and ketones. Interestingly, the deeper down the soil profile, the greater the gradual disappearance of the bands of 1660–1620 cm<sup>-1</sup> related to, for example, the presence of amide nitrogen structures in humic molecules. The intensity of the bands in the range of 1460–1440 cm<sup>-1</sup> and 1420–1400 cm<sup>-1</sup> were decreasing with the sampling depth. Interpreting the FTIR spectra in terms of the effect on the properties of humic acids, a considerable variation was found. Generally, the FT-IR spectra of the

HAs molecules of soils showed an increase in the intensity of the band in the range of  $1730-1710 \text{ cm}^{-1}$  together with the distance from the watercourse.



**Figure 2.** FT–IR spectra of humic acids of the soils sampled in Podlesie 25 m away from the watercourse (depths: 0–10 cm, 10–20 cm and 20–30 cm).

Wavenumber (cm <sup>-1</sup> )	Assignment <sup>a</sup>
3400–3100	O-H stretching of alcohols, phenols and acids, N-H stretching
3100-3000	C-H groups of aromatic and alicyclic compounds
2960–2920; 2850	asymmetric and symmetric C-H stretching of $\rm CH_3$ and $\rm CH_2$ group
1730–1710	C=O stretching of carboxyl, aldehyde, ketone group
1660–1620	C=O of stretching of amide groups; N-H deformation
1610–1600	C-C stretching of aromatic rings
1550-1530	N-H deformation, C=N stretching (amide II bands)
1520-1500	C-C stretching of aromatic rings
1460–1440	C-H asymmetric of CH <sub>3</sub> and CH <sub>2</sub>
1420–1400	C-O stretching and OH deformation of phenols
1380–1320	C-N aromatic amine, COO-, C-H stretching
1280–1200	C-O stretching of aryl ethers, esters and phenols
1160–1030	C-O stretching alcohols, ethers and polysaccharides

Table 6. List of peaks (bands) present in FT-IR spectra.

<sup>a</sup> Enev et al. <sup>87</sup> Zhang et al. <sup>88</sup>, Hayes and Swift <sup>89</sup>.

# 3.5. Hydrophilic and Hydrophobic Nature of Humic Acids

The degree of organic matter humification is also related with the hydrophilic–hydrophobic properties of humic acids determined with the chromatographic analysis. Similar to that reported by Debska et al. [22,26,77], Preuße et al. [23] and Woelki et al. [25], a separation into fractions was recorded. Fractions of retention time of 3.0–8.0 min show greater hydrophilic properties, whereas the fractions of a retention time between 12.0 min and 25.0 min become more and more hydrophobic (Figure 3). The share of hydrophilic fraction ranged from 37.71% to 40.76%. The share of hydrophobic fraction was higher, and it ranged

from 59.24% to 62.29% (Table 7). In Kamionna and in Cegielnia, the share of the fractions in the HAs molecules depended significantly on the soil sampling depth. It was found that the share of hydrophilic fraction was increasing, and the share of hydrophobic fraction was decreasing deep down the soil profile; a similar tendency was observed in Podlesie (Table 7). The same was confirmed by the analysis of variance based on which it was found that the share of fraction HIL was highest in the HAs molecules of the soils sampled from the depth of 20–30 cm, and it was, on average, 40.53% (Table 2). The distance from the watercourse, on the other hand, did not have a significant effect on the share of HIL and HOB fractions. The changes in the share of respective fractions resulted in changes in the value of the HIL/ $\Sigma$ HOB ratio. The values of parameter HIL/ $\Sigma$ HOB ranged from 0.606 to 0.690 (Table 7). With the analysis of variance, it was found that the values of the parameters depended on the depth; the HAs molecules of the soils collected at depths of 10–20 cm and 20–30 cm demonstrated higher values of the ratio of HIL/ $\Sigma$ HOB than the HAs molecules of soils collected at a depth of 0-10 cm (p = 0.011 and p = 0.0004, respectively) (Table 2). Parameter HIL/ $\Sigma$ HOB, as shown by, for example, Debska et al. [22,26,78], is connected with the degree of organic matter humification; the value of the parameter increases with an increase in the degree of the maturity of the molecules of humic acids. The results point to the lowest degree of maturity of the HAs of soils collected at a depth of 0–10 cm. The effect of the distance on the the hydrophilic-hydrophobic properties was significant for HAs in Cegielnia. However, in general, the humic acids of the soils sampled 25 m away from the watercourse identified a higher degree of humification, as compared with the humic acids of the soils sampled 5 m away from the irrigation ditch. Similar dependencies were reported by Banach-Szott et al. [90] for the humic acids of the Fluvisols sampled in the flood plains of the Vistula River; the values of the ratio HIL/ $\Sigma$ HOB were increasing with the distance from the riverbed. Interestingly, the values of that parameter recorded by Banach-Szott et al. [90] were slightly higher, ranging from 0.72 to 0.81. Thus, the HAs molecules were identified with a slightly lower degree of maturity than the humic acids of Fluvisols.



**Figure 3.** RP-HPLC chromatogram of humic acids of the soils sampled in Podlesie 25 m away from the watercourse.

To acquire complete information on the differences (similarities) in the chemical composition of HAs, depending on the depth and the distance from the irrigation ditch, the cluster analysis was applied based on the elemental composition, and spectrometric parameters as well as on the HIL–HOB properties, dividing HAs into two groups (Figure 4a). In the first group, similar properties were found for the HAs molecules of the soils sampled from 20–30 cm in Podlesie and in Cegielnia, while in the second group, there were identified two subgroups. The first subgroup covered the humic acids of the soils collected from 0–10 cm in Kamionna and in Podlesie. In the second subgroup, similar properties were found for the HAs molecules of the soils sampled from 10–20 cm in Podlesie and in Cegielnia, the HAs of the soils sampled from 10–20 cm and 20–30 cm in Kamionna, and the humic acids of the soils collected from 0–10 cm in Cegielnia. As seen from the diagram, the soil sampling depth was the determining factor of the properties of HAs.

**Table 7.** Results of the analysis of variance for the share (%) of hydrophilic (HIL) and hydrophobic (HOB) fractions in the molecules of humic acids.

Fact	or	HIL	ΣΗΟΒ	ΗΙΙ/ΣΗΟΒ	HIL	ΣΗΟΒ	HIL/ΣΗΟΒ	HIL	ΣΗΟΒ	HIL/ΣΗΟΒ
		Kamior	ina			Cegieln	ia		Podles	ie
D: /	5	38.88	61.12	0.637	39.62	60.38	0.657	39.85	60.15	0.663
(m)	15	40.04	59.96	0.668	38.18	61.82	0.620	40.16	59.84	0.671
	25	39.81	60.19	0.662	40.42	59.58	0.680	40.48	59.52	0.682
LSD		ns	ns	ns	ns	ns	0.057	ns	Ns	ns
	0–10	38.57	61.43	0.628	37.71	62.29	0.606	39.90	60.10	0.664
(cm)	10–20	39.83	60.17	0.662	39.92	60.08	0.664	39.82	60.18	0.690
	20–30	40.34	59.66	0.676	40.59	59.41	0.686	40.76	59.24	0.663
LSI		1.374	1.374	0.037	2.264	2.264	0.061	ns	Ns	ns

LSD (least significant difference).



**Figure 4.** Cluster analysis determined based on humic acids parameters: H/C, O/H, O/C,  $\omega$ , A2/4, A2/6,  $\Delta \log K$ , and  $HIL/\Sigma HOB$  (**a**) for HAs of all the sampling locations, (**b**) for HAs of the soils in Kamionna, (**c**) for HAs of the soils in Cegielnia, (**d**) for HAs of the soils of Podlesie (K—Kamionna, C—Cegielnia, P—Podlesie; 1—depth 0–10 cm, 2—depth 10–20 cm, 3—depth 20–30 cm; 5, 15 and 25—distance away from the irrigation ditch).

A cluster analysis was performed based on the properties of humic acids of soils for each of the sampling sites (Figure 4b–d). The dendrograms definitely point to the HAs isolated from the soil sampled 5 m away from the irrigation ditch in Kamionna and Cegielnia as being significantly different from the soils sampled 15 m and 25 m away. As for the Podlesie sampling site, the distance was not the factor which definitely determined the humic acid properties.

### 4. Conclusions

The soils under study represent sandy soils with a very low percentage content of the clay fraction. It was found that meadow soils demonstrated relatively high contents of TOC and Nt, whereas for the soils sampled from the depth of 10–20 cm, the highest contents of those elements were noted. This can suggest an advantage of the mineralisation processes over the processes of humification in the 0–10 cm layer. It also points to an increase in the bulk density of the soils and a decrease in the percentage content of the sand fraction with depth. The atomic ratios, and spectrometric properties as well as the hydrophilic–hydrophobic properties point to a high maturity of molecules of the humic acids of meadow soils continuously irrigated for more than 150 years. The molecules of humic acids demonstrated an increase in the degree of humification with increased depth. The distance from the watercourse determined the time of the water retention and, as a consequence, the humic acids of the soils sampled furthest from the irrigation ditch, in general, showed a higher degree of humification as compared with the humic acids of the soils sampled closest.

The results identified that in soils low in organic matter, such as Arenosols, humic acids with a high degree of humification are formed as a result of long-term irrigation and use as a grassland, which proves the relative stability of the soil's organic matter. It confirms the importance of meadow soils for the carbon sequestration process.

The results also point to the fact that the humic horizon (0–30 cm) in such a type of soil with a relatively high content of humus and a dominating sand fraction in the grain size composition must be assayed in reference to the respective layers due to the variation in the values of parameters confirmed with a statistical analysis. It is, therefore, an indication for researchers that for providing the characteristics in mineral meadow soils, one must sample the soil from the depths proposed.

**Author Contributions:** Conceptualisation, M.B.-S. and A.D.; data curation, M.B.-S. and M.M.; formal analysis, M.B.-S.; funding acquisition, A.D.; investigation, M.B.-S. and A.D.; methodology, M.B.-S.; writing—original draft, M.B.-S. and M.M.; writing—review and editing, M.B.-S., M.M. and A.D. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** The research was made as part of 1047/B/P01/2010/39 research project, financed by the Ministry of Science and Higher Education (Poland).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

Conflicts of Interest: The authors declare that they have no conflict of interest.

## References

- Hayes, M.H.B.; Swift, R.S. Vindication of humic substances as a key component of organic matter in soil and water. *Adv. Agron.* 2020, 163, 1–37. [CrossRef]
- Lanyi, K. Assessment of the relations between the spectroscopic characteristics of soils and their ability to adsorb organic pollutants. *Microchem. J.* 2005, 79, 249–256. [CrossRef]
- 3. McCarthy, P. The principles of humic substances. *Soil Sci.* 2001, *166*, 738–751. [CrossRef]

- Yamashita, Y.; Jaffé, R.; Maie, N.; Tanoue, E. Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). *Limnol. Oceanogr.* 2008, 53, 1900–1908. [CrossRef]
- 5. Canellas, L.P.; Piccolo, A.; Dobbss, L.B.; Spaccini, R.; Olivares, F.L.; Zandonadi, D.B.; Façanha, A.R. Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. *Chemosphere* **2010**, *78*, 457–466. [CrossRef]
- Lal, R. Soil Carbon Sequestration in Latin America. In *Carbon Sequestration in Soils of Latin America*; Lal, R., Cerri, C.C., Bernoux, M., Etcheves, J., Cerri, E., Eds.; Food Products Press: New York, NY, USA, 2006; pp. 49–64.
- 7. Hayes, M.H.B.; Clapp, C.E. Humic substances: Considerations of compositions, aspects of structure, and environmental influences. *Soil Sci.* 2001, *166*, 723–737. [CrossRef]
- 8. Weber, J.; Chen, Y.; Jamroz, E.; Miano, T. Preface: Humic substances in the environment. *J. Soils Sediments* **2018**, *18*, 2665–2667. [CrossRef]
- 9. Visser, S.A. Application of van Krevelen's graphical statistical method for the study of aquatic humic material. *Environ. Sci. Technol.* **1983**, *17*, 412–417. [CrossRef] [PubMed]
- Fuentes, M.; Baigorri, R.; González-Gaitano, G.; García-Mina, J.M. The complementary use of 1H NMR, 13C NMR, FTIR and size exclusion chromatography to investigate the principal structural changes associated with composting of organic materials with diverse origin. Org. Geochem. 2007, 38, 2012–2023. [CrossRef]
- 11. Trubetskaya, O.E.; Trubetskoj, O.A.; Voyard, G.; Richard, C. Determination of hydrophobicity and optical properties of soil humic acids isolated by different methods. *J. Geochem. Explor.* **2013**, *132*, 84–89. [CrossRef]
- Boguta, P.; D'Orazio, V.; Sokołowska, Z.; Senesi, N. Effects of selected chemical and physicochemical properties of humic acids from peat soils on their interaction mechanisms with copper ions at various pHs. *J. Geochem. Explor.* 2016, 168, 119–126. [CrossRef]
   Tan, K.H. *Principles of Soil Chemistry*; CRC Press: Boca Raton, FL, USA, 2010.
- 14. Polak, J.; Bartoszek, M.; Żądło, M.; Kos, A.; Sułkowski, W.W. The spectroscopic studies of humic acid extracted from sediment collected at different seasons. *Chemosphere* **2011**, *84*, 1548–1555. [CrossRef] [PubMed]
- 15. Kumada, K. Chemistry of Soil Organic Matter; Elsevier: Amsterdam, The Netherlands, 1988.
- 16. Gonet, S.S.; Debska, B. Properties of humic acids produced during decomposition of plant residues in soil. *Rostl. Vyroba* **1999**, 45, 455–460.
- Vieyra, F.E.M.; Palazzi, V.I.; de Pinto, M.I.S.; Borsarelli, C.D. Combined UV–Vis absorbance and fluorescence properties of extracted humic substances-like for characterization of composting evolution of domestic solid wastes. *Geoderma* 2009, 151, 61–67. [CrossRef]
- Weishaar, J.L.; Aiken, G.R.; Bergamaschi, B.A.; Fram, M.S.; Fujii, R.; Mopper, K. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* 2003, *37*, 4702–4708. [CrossRef] [PubMed]
- 19. Chen, J.; Gu, B.; LeBoeuf, E.J.; Pan, H.; Dai, S. Spectroscopic characterization of the structural and functional properties of natural organic matter fractions. *Chemosphere* **2002**, *48*, 59–68. [CrossRef]
- 20. Rodriguez, F.J.; Schlenger, P.; García-Valverde, M. Monitoring changes in the structure and properties of humic substances following ozonation using UV-Vis, FTIR and H NMR techniques. *Sci. Total Environ.* **2016**, *541*, 626–637. [CrossRef]
- Banach-Szott, M.; Debska, B. Chromatographic Separation of Humic Acids of a Forest Soil. In *Humic Substances-Linking Structure* to Functions; Frimmel, F.H., Abbt-Braun, G., Eds.; Repository KITopen: Karlsruhe, Germany, 2006; pp. 225–228.
- 22. Debska, B.; Banach-Szott, M.; Dziamski, A.; Gonet, S.S. Chromatographic characteristics (HPLC, HPSEC) of humic acids of soil fertilised with various organic fertilisers. *Chem. Ecol.* **2010**, *26*, 49–57. [CrossRef]
- 23. Preuße, G.; Friedrich, S.; Salzer, R. Retention behavior of humic substances in reversed phase HPLC. *Fresenius J. Anal. Chem.* 2000, 368, 268–273. [CrossRef]
- 24. Sierra, M.; Giovanela, M.; Parlanti, E.; Soriano-Sierra, E.J. 3D-fluorescence spectroscopic analysis of HPLC fractionated estuarine fulvic and humic acids. *J. Brazilian Chem. Soc.* 2006, *17*, 113–124. [CrossRef]
- 25. Woelki, G.; Friedrich, S.; Hanschmann, G.; Salzer, R. HPLC fractionation and structural dynamics of humic acids. *Fresenius J. Anal. Chem.* **1997**, 357, 548–552. [CrossRef]
- 26. Debska, B.; Drag, M.; Banach-Szott, M. Molecular size distribution and hydrophilic and hydrophobic properties of humic acids isolated from forest soil. *Soil Water Res.* **2007**, *2*, 45–53. [CrossRef]
- 27. Debska, B.; Gonet, I. Share of hydrophilic and hydrophobic fractions in humic acids formed as a result of post-harvest residue decomposition. *Pol. J. Soil Sci.* 2007, 40, 57–65.
- Cocozza, C.; Miano, T. Structural Resolution of Metal-Humic Acids Interactions through Deconvolution FT-IR Spectroscopy. In Proceedings of the 11th IHSS Meeting, Boston, MA, USA, 21–26 July 2002; Davis, G., Ghabbour, E.A., Eds.; IHSS: Boston, MA, USA, 2002; pp. 264–266.
- 29. Pajączkowska, J.; Sułkowska, A.; Sułkowski, W.W.; Jędrzejczyk, M. Spectroscopic study of the humification process during sewage sludge treatment. *J. Mol. Struct.* 2003, 651, 141–149. [CrossRef]
- Wiesmeier, M.; Urbanski, L.; Hobley, E.; Lang, B.; von Lutzow, M.; Marin-Spiotta, E.; van Wesemael, B.; Rabot, E.; Lieb, M.; Garcia-Franco, N.; et al. Soil organic carbon storage as a key function of soil—A review of drivers and indicators at various scales. *Geoderma* 2019, 333, 149–162. [CrossRef]

- Minami, K.; Goudriaan, J.E.A.; Lantinga, E.A.; Kimura, T. Significance of Grasslands in Emission and Absorption of Greenhouse Gases. In Proceedings of the XVIIth International Grassland Congress, Palmerston North, Hamilton, Lincoln, New Zealand, 13–16 February 1993; pp. 1231–1238.
- Conant, R.T.; Paustian, K.; Elliott, E.T. Grassland management and conversion into grassland: Effects on soil carbon. *Ecol. Appl.* 2001, *11*, 343–355. [CrossRef]
- 33. Mannetje, L.T. Advances in grassland science. NJAS Wagen. J. Life Sci. 2003, 50, 195–221. [CrossRef]
- 34. Lal, R. Sequestering carbon in soils of agro-ecosystems. Food Policy 2011, 36, 533–539. [CrossRef]
- 35. Kämpf, I.; Hölzel, N.; Störrle, M.; Broll, G.; Kiehl, K. Potential of temperate agricultural soils for carbon sequestration: A metaanalysis of land-use effects. *Sci. Total Environ.* **2016**, *566*, 428–435. [CrossRef]
- 36. Environmental Protection. Study of the Central Statistical Office 2019. Available online: https://stat.gov.pl/obszary-tematyczne/ srodowisko-energia/srodowisko/ochrona-srodowiska-2019,1,20.html (accessed on 29 November 2019). (In Polish)
- 37. Kondratowicz-Maciejewska, K.; Kobierski, M.; Murawska, A. Effect of Brunic Arenosols use on selected physicochemical properties in organic matter. *Soil Sci. Ann.* **2012**, *63*, 19–24. [CrossRef]
- EIP-AGRI, Focus Group. Profitability of Permanent Grassland 2019. Available online: https://ec.europa.eu/eip/agriculture/ sites/default/files/fg9\_permanent\_grassland\_profitability\_starting\_paper\_2014\_en.pdf (accessed on 28 May 2014).
- 39. Sabiniarz, A. Historic outline of the Czersk Meadows. Grassl. Sci. Pol. 2006, 9, 184–194. (In Polish)
- 40. Papke, R. The formation of plant communities of the Czerskie Meadows depending on water conditions. *Zesz. Probl. Post. Nauk Roln.* **1958**, *13*, 97–117. (In Polish)
- 41. Grzyb, S. Characteristics of soils and vegetation in Czerskie Meadows. Mater. Semin. 1969, 8, 14–26. (In Polish)
- 42. Lorenc, K. Changes in the structure of plant communities in Czerskie Meadows in terms of phytosociology. IMUZ Falenty. *Mat. Konf.* **1969**, *8*, 79–85. (In Polish)
- 43. Sabiniarz, A.; Kozłowski, S. Czerskie Meadows in the landscape aspect. Grassl. Sci. Pol. 2009, 12, 141–154. (In Polish)
- 44. Sabiniarz, A.; Kozłowski, S. Łąki Czerskie w aspekcie paszowym (Czerskie Meadows in terms of forage). Łąkarstwo w Polsce. *Grassl. Sci. Pol.* **2009**, *12*, 155–163. (In Polish)
- 45. Kochsiek, A.E.; Knops, J.M.H.; Walters, D.T.; Arkebauer, T.J. Impacts of management on decomposition balance in irrigated and rainfed no till agricultural systems. *Agr. Forest Meteorol.* **2009**, *149*, 1983–1993. [CrossRef]
- 46. Trost, B.; Prochnow, A.; Drastig, K.; Meyer-Aurich, A.; Ellmer, F.; Baumecker, M. Irrigation, soil organic carbon and N<sub>2</sub>O emissions. A review. *Agron. Sustain. Dev.* **2013**, *33*, 733–749. [CrossRef]
- Getaneh, F.; Deressa, A.; Negassa, W. Influence of Small Scale Irrigation on Selected Soil Chemical Properties. In Proceedings of the Tropentag, Witzenhausen, Germany, 9–11 October 2007.
- Nunes, J.M.; López-Piñeiro, A.; Albarrán, A.; Muñoz, A.; Coelho, J. Changes in selected soil properties caused by 30 years of continuous irrigation under Mediterranean conditions. *Geoderma* 2007, 139, 321–328. [CrossRef]
- 49. Costantini, E.A.C.; Lorenzetti, R. Soil degradation processes in the Italian agricultural and forest ecosystems. *Ital. J. Agron.* 2013, *8*, e28. [CrossRef]
- 50. Chenu, C.; Angers, D.A.; Barré, P.; Derrien, D.; Arrouays, D.; Balesdent, J. Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations. *Soil Tillage Res.* **2019**, *188*, 41–52. [CrossRef]
- Howell, T.A. Irrigation's Role in Enhancing Water Use Efficiency. In National Irrigation Symposium, Proceedings of the 4th Decennial Symposium, Phoenix, AZ, USA, 14–16 November 2000; Evans, R.G., Benham, B.L., Trooien, T.P., Eds.; American Society of Agricultural Engineers: Phoenix, AZ, USA, 2000; pp. 66–80.
- 52. Fallahzade, J.; Hajabbasi, M.A. The effects of irrigation and cultivation on the quality of desert soil in central Iran. *Land Degrad. Dev.* **2012**, *23*, 53–61. [CrossRef]
- Follett, R.F.; Jantalia, C.P.; Halvorson, A.D. Soil carbon dynamics for irrigated corn under two tillage systems. *Soil Sci. Soc. Am. J.* 2013, 77, 951–963. [CrossRef]
- 54. Entry, J.A.; Sojka, R.E.; Shewmaker, G.E. Management of irrigated agriculture to increase organic carbon storage in soils. *Soil Sci. Soc. Am. J.* 2002, *66*, 1957–1964. [CrossRef]
- 55. Trost, B.; Ellmer, F.; Baumecker, M.; Meyer-Aurich, A.; Prochnow, A.; Drastig, K. Effects of irrigation and nitrogen fertilizer on yield, carbon inputs from above ground harvest residues and soil organic carbon contents of a sandy soil in Germany. *Soil Use Manag.* 2014, *30*, 209–218. [CrossRef]
- 56. Kenngott, K.G.; Riess, K.; Muñoz, K.; Schaumann, G.E.; Buhk, C.; Diehl, D. Flood Pulse Irrigation of Meadows Shapes Soil Chemical and Microbial Parameters More Than Mineral Fertilization. *Soil Syst.* **2021**, *5*, 24. [CrossRef]
- 57. Buhk, C.; Schirmel, J.; Rebekka, G.; Frör, O. Traditional Water Meadows: A Sustainable Management Type for the Future? In *Irrigation in Agroecosystems*; IntechOpen: London, UK, 2018.
- 58. Stevenson, F.J. Humus Chemistry: Genesis, Composition Reactions; Wiley: Chichester, UK, 1994.
- 59. Senesi, N.; Miano, T.M. *Humic Substances in the Global Enviro nment and Implications on Human Health;* Elsevier: Amsterdam, The Netherlands, 1994.
- 60. Schlesinger, W.H. Biogeochemistry: An Analysis of Global Change; Academic Press: San Diego, CA, USA, 1997.
- COM 231 Final. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions; Thematic Strategy for Soil Protection: Croatia, Turkey, 2006. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52006DC0231&from=EN (accessed on 26 September 2006).

- 62. Debska, B.; Dlugosz, J.; Piotrowska-Dlugosz, A.; Banach-Szott, M. The impact of bio-fertilizer on the soil organic matter status and carbon sequestration—results from a fiel-scale study. *J. Soils Sediments* **2016**, *16*, 2335–2343. [CrossRef]
- 63. van Reeuwijk, L.P. *Procedures for Soil Analysis*; International Soil Reference and Information Centre: Wageningen, The Netherlands, 2002.
- 64. Kobierski, M. Texture of different types of soils formed from glacial till in the aspect of PTG 2008 classification system. *Soil Sci. Ann.* **2010**, *61*, 67–76. (In Polish)
- 65. Kobierski, M.; Kondratowicz-Maciejewska, K.; Kociniewska, K. Soil quality assessment of Phaeozems and Luvisols from the Kujawy region (central Poland). *Soil Sci. Ann.* **2015**, *66*, 111–118. [CrossRef]
- 66. Ward, J.H. Hierarchical grouping to optimise an objective function. J. Am. Stat. Assoc. 1963, 58, 236–244. [CrossRef]
- 67. Jadczyszyn, T.; Kowalczyk, J.; Lipiński, W. Fertilization recommendations for field crops and permanent grasslands. In *Teaching Materials*; IUNG-PIB: Puławy, Poland, 2010; Volume 95, p. 24. (In Polish)
- 68. Pizzeghello, D.; Francioso, O.; Concheri, G.; Muscolo, A.; Nardi, S. Land use affects the soil C sequestration in alpine environment. *NE Italy For.* **2017**, *8*, 197. [CrossRef]
- 69. Drag, M.; Debska, B.; Dziamski, A. Properties of humic substances of forest and meadow soil in the area of the Wierzchlas Reserve. *Humic Subst. Ecosys.* **2007**, *7*, 141–151.
- 70. Jonczak, J. Soil organic matter properties in stagnic luvisols under different land use types. Acta Agroph. 2013, 20, 565–576.
- 71. Sun, S.; Che, T.; Gentine, P.; Chen, Q.; Wang, L.; Yan, Z.; Chen, B.; Song, Z. Shallow groundwater inhibits soil respiration and favors carbon uptake in a wet alpine meadow ecosystem. *Agric. For. Meteorol.* **2021**, 297, 108254. [CrossRef]
- 72. Kuzyakov, Y. Review: Factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci. 2002, 165, 382–396. [CrossRef]
- 73. van Camp, G.; Gentile, A.; Bujarrabal, B.; Jones, R.; Montanarella, L.; Olazabal, C.; Selvaradjou, S. *Reports of the Technical Working Groups Establish under the Thematic Strategy for Soil Protection;* Vol. IV: Contamination and Land Management. EUR 21319 EN; Office for Official Publications of the European Communities: Luxembourg, 2004; p. 28870.
- 74. Khalid, M.; Soleman, N.; Jones, D.L. Grassland plants affect dissolved organic carbon and nitrogen dynamics in soil. *Soil Biol. Biochem.* **2007**, *39*, 378–381. [CrossRef]
- 75. Becher, M.; Banach-Szott, M.; Godlewska, A. Organic matter properties of spent button mushroom substrate in the context of soil organic matter reproduction. *Agronomy* **2021**, *11*, 204. [CrossRef]
- 76. Filip, Z.; Tesarova, M. Microbial degradation and transformation of humic acids from permanent meadow and forest soils. *Int. Biodeterior. Biodegr.* **2004**, *54*, 225–231. [CrossRef]
- 77. Orlov, D.S. Humus Acids of Soils; Balkema: Rotterdam, The Netherlands, 1986.
- 78. Debska, B.; Drag, M.; Tobiasova, E. Effect of post-harvest residue of maize, rapeseed, and sunflower on humic acids properties in various soils. *Pol. J. Environ. Stud.* **2012**, *21*, 603–613.
- Dergacheva, M.I.; Nekrasova, O.A.; Okoneshnikova, M.V.; Vasileva, D.I.; Gavrilov, D.A.; Ochur, K.O.; Ondar, E.E. Ratio of elements in humic acids as a source of information on the environment of soil formation. *Contemp. Probl. Ecol.* 2012, *5*, 497–504. [CrossRef]
- Amir, S.; Jouraiphy, A.; Meddich, A.; El Gharous, M.; Winterton, P.; Hafidi, M. Structural study of humic acids during composting of activated sludge-green waste: Elemental analysis, FTIR and <sup>13</sup>C NMR. J. Hazard. Mater. 2010, 177, 524–529. [CrossRef]
- Sanchez-Monedero, M.A.; Cegarra, J.; Garcia, D.; Roig, A. Chemical and structural evolution of humic acids during organic waste composting. *Biodegradation* 2002, 13, 361–371. [CrossRef]
- 82. Gonet, S.S.; Debska, B.; Zaujec, A.; Banach-Szott, M.; Szombathova, N. Effect of the Tree Species and Soil-and-Climate Conditions on the Properties of Humus in Forest Soils. In *Role of Organic Matter in the Environment*; Gonet, S.S., Markiewicz, M., Eds.; PTSH: Wroclaw, Poland, 2007; pp. 61–98.
- 83. Senesi, N.; D'Orazio, V.; Ricca, G. Humic acids in the first generation of Eurosoils. Geoderma 2003, 116, 325–344. [CrossRef]
- 84. Kobierski, M.; Kondratowicz-Maciejewska, K.; Banach-Szott, M.; Wojewódzki, P.; Castejón, J.M.P. Humic substances and aggregate stability in rhizospheric and non-rhizospheric soil. *J. Soils Sediments* **2018**, *18*, 2777–2789. [CrossRef]
- 85. Tinoco, P.; Almendros, G.; González-Vila, F.; Sanz, J.; González-Pérez, J. Revisiting molecular characteristics responsive for the aromaticity of soil humic acids. *J. Soils Sediment.* 2015, *15*, 781–791. [CrossRef]
- 86. Filcheva, E.; Hristova, M.; Nikolova, P.; Popova, T.; Chakalov, K.; Savov, V. Quantitative and qualitative characterisation of humic products with spectral parameters. *J. Soils Sediments* **2018**, *18*, 2863–2867. [CrossRef]
- Enev, V.; Pospisilova, L.; Klucakova, M.; Liptaj, T.; Doskocil, L. Spectral characterization of selected humic substances. *Soil Water Res.* 2014, 9, 9–17. [CrossRef]
- Zhang, J.; Wang, J.; An, T.; Wei, D.; Chi, F.; Zhou, B. Effects of long-term fertilization on soil humic acid composition and structure in Black Soil. *PLoS ONE* 2017, 12, e0186918. [CrossRef] [PubMed]
- Hayes, M.H.B.; Swift, R.S. An appreciation of the contribution of Frank Stevenson to the advancement of studies of soil organic matter and humic substances. J. Soils Sediments 2018, 18, 1212–1231. [CrossRef]
- Banach-Szott, M.; Kobierski, M.; Kondratowicz-Maciejewska, K. Humic substances in Fluvisols of the Lower Vistula floodplain, North Poland. *Environ. Sci. Pollut. Res.* 2018, 25, 23999–24002. [CrossRef] [PubMed]