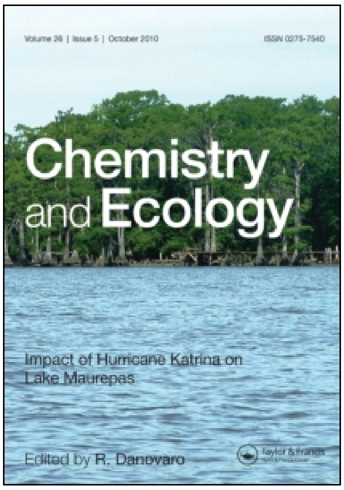

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The influence of reduced tillage, winter crops and ecologically managed long-term mono- and multi-component swards on soil humic substances

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The influence of reduced tillage, winter crops and ecologically managed long-term mono- and multi-component swards on soil humic substances

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Chemical analyses of soil humic substances were performed at the Chemical Research Laboratory of the LIA. A field experiment (Experiment 1) was established on a glacio-lacustrine clay loam on a silty clay Endocalcari–Endohypogleyic Cambisol. The study investigated two soil tillage systems: conventional (CT) and reduced (RT). The study also encompassed crop rotations with different structures: 0, 25, 50, 75 and 100% winter crops in a crop rotation. Experiment 2 compared the influence of long-term legume swards on soil humic substances in an Epicalcari–Endohypogleic Cambisol. The swards were used for five years under ecological management. No fertilisers or pesticides were used. There were 2.6 times more labile humic acids (HA) in the topsoil in RT compared with CT. A significant effect on the increase of labile HA content was determined in crop rotations with $\geq$ 50% winter crops. The highest amount of mobile HA accumulated in soil under a four-component sward (*Galega*+*Trifolium repens*+*Onobrychis*+*Festulolium*). Reduced soil use resulted in qualitative changes in HA determined by Fourier transform infrared (FT-IR) spectroscopy. The analytical methods used are appropriate for evaluating sensitivity to changes in soil management and are helpful for the development of reduced soil use systems. However, labile and mobile carbon pools are more sensitive than humus.

Keywords: soil; humus; mobile HS; labile HS; FT-IR; reduced tillage; winter crops; swards; ecological management

1. Introduction

Soil organic matter (SOM) has been increasingly considered as an indicator of soil quality, one of the components of biosphere sustainability and stability [1,2]. Maintaining and increasing organic carbon retention in soil is crucial, due to the importance of organic carbon (C) in the preservation of soil fertility, physical properties and biological activity. A growing consensus therefore regards the reduction of soil management intensity, such as reduced tillage, as a viable means to increase SOM and limit mineralisation and CO_2 emission [3,4]. The problem of evaluating the reserves and balance of organic matter, C and humic substances in various components of ecosystems is primarily related to growing concern about global warming and rapid climatic change [5].

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The quantity and quality of SOM and its major component, humus, are influenced by management practices, fertilisers [6–8,9] and soil tillage [2,10]. The depth distribution of SOM and soil organic carbon (SOC) varies with tillage. The effect of C management is reflected by correlations between aggregation, C inputs, soil C content and SOM fractions, and plant residue composition [11,12]. No-till systems result in quantitative and qualitative improvements in SOM [13]. Humus composition changes under different agricultural practices [14], but there is no consistent opinion on the effect of reduced tillage on the mobile and labile C pool.

In short-term soil tillage experiments, no effect of minimum tillage on humic substances (HS) has been identified [15]. Conversely, in long-term experiments, minimum tillage increased soil humus content [10]. Therefore, long-term soil tillage experiments are needed to understand soil chemical and biochemical processes and provide consistent experimental results. Long-term experiments require considerable resources, time and labour, but they offer the best practical means of understanding many of the problems facing farmers, ecologists and policy-makers [16].

HS in the soil are traditionally defined according to their solubility [17,18]. Chemical extractions of SOM have not been widely used to elucidate SOM dynamics in field settings [19]. Rarely, chemical extractions of soil mobile and labile HS have been amply used to detect HS changes in field experiments, particularly to address issues of maintenance of reduced soil status. Different fractionation systems, as well as many chemical extractants, have been used to obtain different SOM fractions. NaOH solution is a popular chemical extractant because it generally extracts appreciable or even large quantities of humic material. Humic acids (HA) not bound to polyvalent cations contributes mobile humic substances, presumed to be composed of younger less-humified materials [20]. Dissolved organic C is the most labile and mobile form of C in SOM [8].

The agronomic value and environmental effects of HS, especially HA, are well known. Numerous laboratory studies have shown that HS have significant impacts on plant development. Optimum plant growth is obtained with combined effects of HS and mineral nutrients. Mobile HA plays an important role in governing nutrient availability in soils. The correlations established between the content of mobile humic substances in soil and crop yield demonstrate the agronomic importance of this research [21].

In Lithuania, the area of land under certified organic production is increasing rapidly. Growing interest in ecological management has also increased the role of legumes in forage production. Red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) are the most important legumes in Lithuania [22]. Under Lithuania's climatic conditions, fodder galega is the most long-lived legume exhibiting the best overwinter survival. Fodder galega (*Galega orientalis* Lam.) is a perennial fast-growing forage legume with a strong stem and root system. The plant is deep-rooted with much subsurface root biomass and, depending on plant age and management, can account for $\leq$80% of total plant biomass [23].

The effects of individual agricultural practices on SOM have been comprehensively investigated [2,6,9,24–27]. However, there is little research evidence on the impact of ecologically managed wintering crop rotations and long-lived mono-component swards and their multi-component mixtures on humus, mobile and labile HS, spectral characteristics of HA and soil quality. It is also of major importance to ascertain the sensitivity and suitability of the analytical methods applied in this study for the assessment and comparison of HS in the development of reduced soil use systems.

The main task of this study was to verify the hypothesis that if conventional soil tillage systems are replaced by reduced, less-intensive systems, this will maintain or increase the quantity and quality of humic substances. Moreover, it is postulated that long-term mono-component and multi-component swards growing in ecological crop rotations sustain soil and associated SOM.

The following experimental objectives were pursued: using chemical methods and Fourier transform infrared (FT-IR) spectroscopy to examine the status of soil mobile and labile HS. The studied influences were conventional and reduced tillage, using different crop rotation structures during two crop rotations periods, and long-term swards grown in a pure stand and in a mixture with other legumes and grasses in an ecological crop rotation.

2. Materials and methods

2.1. *Site, soil characteristics and sampling*

Experiment 1 was conducted at the Joniskelis Research Station of the Institute of Agriculture. The station in the northern part of the lowlands of Central Lithuania (56°21′N, 24°10′E) on a drained, clay loam on silty clay with a deeper lying sandy loam Endocalcari–Endohypogleyic Cambisol [28]. The parent material is glacio-lacustrine clay. Clay particles (<0.002 mm) in the A_a horizon (0–30 cm) constitute 27.0%, in the B_1 horizon (52–76 cm) 51.6%, in the C_1 horizon (77–105 cm) 10.7%, and in the C_2 horizon (106–135 cm) 11.0%. Before the experiment, soil humus content (0–25 cm layer) was 2.20%, pH (KCl 1 M, w/v 1:2.5) was 6.6–6.8, available phosphorus (P_2O_5) was 154 mg $\cdot$ kg^{-1} and available potassium (K_2O) was 304 mg $\cdot$ kg^{-1}, determined in a solution of ammonium acetate–lactate, the soil/extractant ratio was 1:20, by the Egner–Riehm–Domingo (A–L) method [29]. Fractions of humic substances and the FT-IR spectra of humic acids in the long-term agricultural experiment were studied at the Chemical Research Laboratory of the Institute of Agriculture. Detailed studies of humic substances were conducted in the last (second) crop rotation of the experiment (2004 and 2006). Soil samples were collected from 0–15 and 15–25 cm depths, three field replicates were investigated.

Experiment 2 was conducted at the Institute of Agriculture at Akademija, near Kedainiai. According to FAO/UNESCO (1997), the soil is Epicalcari–Endohypogleic Cambisol (CMg-n-w-cap), with a clay content of 11.9%, a silt content of 34.2% and a sand content of 53.9%. Before the experiment, plough-layer pH (KCl 1 M, w/v 1:2.5) was 7.0, soil total N, determined by the Kjeldahl method was 1.40 g $\cdot$ kg^{-1}, available phosphorus (P_2O_5), determined by Egner–Riehm–Domingo (A–L) method was 128 mg $\cdot$ kg^{-1}, and available potassium (K_2O) was 211 mg $\cdot$ kg^{-1}. In 2006, soil samples were collected from 0–10, 10–20 and 20–30 cm depths with 6–8 boreholes per replicated plot. In both Experiments 1 and 2, three field replicates were investigated.

2.2. *Experimental design*

Experiment 1 was designed to study the feasibility of extending the winter crop area in the crop rotation and soil-tillage systems. The experiment was established in 1998 using the fully expanded crop rotation method. All crops were grown every year in four replicates. The plots were arranged in blocks. The basic area was 90 m^2, and the area of the record plot was 34.5 m^2 for cereals and 44 m^2 for grasses. The design was Factor A, crop rotations with different areas of winter and spring crops were as follows:

(1) Without winter crops – (i) Mixture of spring vetch (*Vicia sativa* L.) cv. Kursiai and spring oat (*Avena sativa* L.) cv. Jaugila; (ii) spring wheat (*Triticum aestivum* L.) cv. Munk; (iii) spring triticale (× *Triticosecale* Wittm.) cv. Gabo; (iv) spring barley (*Hordeum vulgare* L.) cv. Ūla.
(2) Twenty-five percent winter crops – (i) Mixture of red clover (*Trifolium pratense* L.) cv. Vyliai and timothy (*Phleum pratense* L.) cv. Gintaras II; (ii) spring wheat; (iii) spring triticale; (iv) spring barley, undersown crop.
(3) Fifty percent winter crops – (i) Mixture of red clover and timothy; (ii) winter wheat (*Triticum aestivum* Host.) cv. Sirvinta; (iii) spring triticale; (iv) spring barley, undersown crop.
(4) Seventy-five percent winter crops – (i) Mixture of red clover and timothy; (ii) winter wheat; (iii) winter triticale (× *Triticosecale* Wittm.) cv. Tewo; (iv) spring barley, undersown crop.

(5) One hunderd percent winter crops – (i) Mixture of red clover and timothy; (ii) winter wheat; (iii) winter triticale; (iv) winter barley (*Hordeum vulgare* L.) cv. Moldavskyj-16, undersown crop.

Factor B, soil tillage systems were as follows:

(1) Conventional (primary soil tillage is mouldboard ploughing).
(2) Reduced (after grasses, the soil for wheat is ploughed using a mouldboard plough; after cereals, ploughless soil tillage is applied for all crops).

Mineral fertilisation provided all rotations with the same optimal fertiliser amount.

Experiment 2 explored the effects of seven swards grown under ecological management on the accumulation of humic substances in different soil layers. The following long-lived swards and their mixtures were investigated:

(1) *Galega orientalis* cv. Gale (100% of legumes);
(2) *Medicago sativa* cv. Birute (100% of legumes);
(3) *Onobrychis viciifolia* cv. Meduviai (100% of legumes);
(4) *Galega+Medicago+Festulolium* cv. Punia (80% of legumes);
(5) *Galega+Onobrychis+Festulolium* (80% of legumes);
(6) *Galega+Medicago+T. pratense* cv. Arimaiciai+Festulolium (80% of legumes);
(7) *Galega+T. repens* cv. Atoliai+*Onobrychis+Festulolium* (80% of legumes).

The experiment was laid out as a randomised complete block with four replications and a plot size of 35 m^2. Swards were cut three times during the growing season and were used for five years. No fertilisers or pesticides were used.

2.3. *Analytical methods*

For chemical analysis, visible roots and plant residues were removed and soil was sifted through a 0.25 mm sieve. Analyses were performed on air-dried samples. Humus content was determined using the Tyurin method modified by Nikitin and calculated by multiplying organic carbon content by 1.724 [30]. This modified method of wet combustion with photometric carbon determination measures organic carbon content according to the dichromate approach using combustion in a thermostat at 160 °C for 30 min. Combustion time was measured from the moment when the temperature in the thermostat reached 160 °C. The combustion procedure in the thermostat allowed us to minimise temperature variations. After cooling, the content of the flasks was transferred to 50 mL measuring flasks and diluted, afterwards it was shaken and left overnight to settle. The next day, measurements were performed using the spectrophotometer Cary 50 (Varian). C was measured using glucose standards ($1\,mg\,C \cdot mL^{-1}$). Different amounts of standard glucose solution were evaporated and combusted as per the test samples, adding the same volume of chromium mixture. The obtained solutions were diluted to 50 mL. They were then shaken, left overnight to settle and used for photometry.

The SOM fractionation procedure commenced with a 0.1 M NaOH extraction, before the acid wash. The humic acid fraction recovered by this approach was designated the mobile humic acids fraction (MHA) in early work by Russian researchers [17,18] and was used in the recent studies of well-known humus researchers, such as Olk et al. [19,31,32]. According to the Ponomariova–Plotnikova [33] method, mobile humic substances (MHS) were extracted by 0.1 M NaOH solution (room temperature) at a soil solution ratio of 1:20. The extracted humic substances were then separated into humic and fulvic acid fractions by acidifying the extract to pH 1.3–1.5 using 0.5 M H_2SO_4 at 68–70 °C and mobile humic acids were separated by filtering [2]. Separated humic acids were re-dissolved in 0.1 M NaOH solution. Some humic acid solution was evaporated, oxidised and organic carbon content determined, using the same procedures as for soil samples. C content in the fraction was determined by the dichromate oxidation procedure [30,33]. The MHA fraction of humic acids is presented here as 'free and weakly bound with clay minerals'.

To determine labile OM, soil–water suspension (1:5) according to the VDLUFA method [34] was boiled with a reflux condenser for 1 h, then cooled to room temperature, centrifuged in plastic test tubes (Nalgene, USA) using a Universal 32 (Hettich, Germany) centrifuge at 3800 rpm. The extract was filtered through a 0.45 μm cellulose filter using a membrane pump (KNF, Germany) and a glass vacuum filtering instrument (Schleicher & Schuell, Germany).

In the extract obtained, soil C content was determined using an automatic spectrophotometer UV/VIS Cary 50 Conc (Varian, Germany) at a wavelength of 590 nm using glucose as a standard after wet digestion [30]. In these hot water extracts the concentrations of humic and fulvic acids in solution were measured spectrophotometrically, using formulae for calculation. In water soil extracts, the concentration of humic substances was not high, therefore increasing the concentration of extracts enabled an increase in the sensitivity and accuracy of measurements. Increasing the concentration of water extracts was performed following the Krasiukov–Lapin method using DEAE–cellulose (diethylaminoethtylcellulose) sorbent [35]. Sorbent with humic substances present on a filter was washed with 5–10 mL of 0.3 mol · L^{-1} NaOH. An alkaline solution of humic substances transferred to a 50 mL flat-bottomed flask was acidified using concentrated HCl solution to pH 4.0 and heated for 45 min in a water-bath (temperature 60 °C). Precipitated humic acids were filtered through a 0.45 μm cellulose filter. The filter with remaining fulvic acids was placed into a 25 mL measuring cylinder, and the pH was adjusted to 8–9 with 0.5 mol · L^{-1} NaOH solution. Afterwards, the precise volume of the solution was measured. Humic acids remaining on the filter were dissolved in 5–10 mL of 0.01 mol · L^{-1} NaOH solution, the obtained solution was transferred into a 25 mL measuring cylinder, the pH was adjusted to 8–9 using 0.5 mol · L^{-1} HCl solution, then the volume of the solution measured. Optical densities of the obtained humic and fulvic acid solutions were determined spectrophotometrically using a UV/VIS Cary 50 Conc (Varian, Germany) spectrophotometer at 330 and 360 nm wavelengths, respectively. The carbon content in humic acids is calculated according to the formula:

$$C_{hum} = \frac{(0.92 + 26.80D_{\mathrm{hum330}} + 7.50D_{\mathrm{hum360}})}{V_2}V_1,$$

where D_{hum330} is the optical density of humic acid solution at 330 nm wavelength; D_{hum360} is the optical density of humic acid solution at 360 nm wavelength; V_1 is the volume of concentrated humic acid solution (mL); V_2 is the volume of test sample (mL); and 0.92, 26.80 and 7.50 are coefficients.

The carbon content of fulvic acids is calculated according to the formula:

$$C_{fulv} = \frac{(26.80D_{\mathrm{fulv330}} - 7.00D_{\mathrm{fulv360}})}{V_2}V_1,$$

where D_{fulv330} is the optical density of fulvic acid solution at 330 nm wavelength; D_{fulv360} is the optical density of fulvic acid solution at 360 nm wavelength; V_1 is the volume of concentrated fulvic acid solution (mL); V_2 is the volume of test sample (mL); and 26.80 and 7.00 are coefficients.

Infrared radiation absorptions of humic acids were measured using a Nicolet FT-IR 380 (Thermo Fisher, USA) spectrometer. Spectra were recorded at 3000–1000 cm^{-1} wave number range. Peak resolutions of 4 cm^{-1} were used to obtain the spectrum. To record each spectrum, 32 scans were made using a multi-reflection horizontal attenuated total reflection accessory (HATR) with an integrated Zn–Se crystal, on whose surface the sample was poured, uniformly distributed and pressed well using a special spatula. Before measuring, the spectra of the baseline were measured to eliminate the effects of the instrument and ambient conditions on sample spectra. The spectra were processed using OMNIC™ spectroscopy software, Version 7.2 (Thermo Electron) [36]. Prior to analyses, spectra were normalised.

2.4. *Statistical analyses*

Experimental data were analysed by two-factor analysis of variance (ANOVA; Statistica, Version 6.0). In Experiment 1, factor A was type of crop rotation structure, and factor B type of tillage (conventional and reduced). In Experiment 2, factor A was type of grass, and factor B was soil depth (0–10, 10–20, 20–30 cm).

3. Results

3.1. *Humus content*

Data from Experiment 1 show the humus content in different soil layers as influenced by the tillage system and percentage of winter crops in the crop rotation (Table 1).

Reduced soil tillage significantly increased humus content in both topsoil and subsoil compared with conventional tillage. Reduced soil tillage, especially in combination with growing of winter crops, in comparison with conventional tillage, significantly increased the amount of humus in the 0–25 cm soil layer [37]. In the conventional tillage system, the humus content accumulated in the topsoil (0–15 cm) was from 19.8–20.6 $g \cdot kg^{-1}$ in the crop rotation with 0–50% of winter crops to 21.0 $g \cdot kg^{-1}$ in the crop rotation with 75–100% of winter crops. In the reduced soil tillage system, humus content was significantly higher in the topsoil (0–15 cm) from 22.0–22.8 $g \cdot kg^{-1}$ (0–50% of winter crops in the crop rotation) to 23.6 $g \cdot kg^{-1}$ (100% of winter crops in the crop rotation). The highest humus content was in the reduced soil tillage system, having increased the

Table 1. The influence of soil tillage systems and proportion of winter crops in the crop rotation on the content of humus ($g \cdot kg^{-1}$). Mean data from 2004 and 2006.

Proportion of winter crops, % (A)	Depth (cm)	Soil tillage system (B): Conventional	Soil tillage system (B): Reduced	Mean across proportion of winter crops (A)
0	0–15	20.6	22.8	21.7
	15–25	18.9	20.4	19.7
25	0–15	19.8	22.6	21.2
	15–25	20.4	22.0	21.2
50	0–15	20.4	22.6	21.5
	15–25	19.9	21.5	20.7
75	0–15	21.0	22.5	21.8
	15–25	21.0	20.7	20.8
100	0–15	21.0	23.6	22.3
	15–25	21.1	22.8	22.0
Mean across tillage (B)	0–15	20.6	22.8	
	15–25	20.2	21.5	
LSD 0.05		0–15 cm	15–25 cm	
A		0.74	1.73	
B		0.37	0.87	
AB		1.10	2.60	

Note: Significance at $p < 0.05$.

Table 2. The influence of grass species on the content of humus ($g \cdot kg^{-1}$) in soil. Mean data from 2006.

Grass (A)	Soil depth (B) (cm) 0–10	10–20	20–30	Mean across all depths
1. *Galega orientalis*	24.8	24.5	19.8	23.0
2. *Medicago sativa*	26.0	23.8	20.0	23.2
3. *Onobrychis viciifolia*	26.4	23.9	20.9	23.7
4. *Galega+Medicago+Festulolium*	26.2	25.5	21.2	24.3
5. *Galega+Onobrychis+Festulolium*	25.8	25.0	19.5	23.4
6. *Galega+Medicago+T. pratense +Festulolium*	26.8	24.0	19.5	23.4
7. *Galega+T. repens +Onobrychis+Festulolium*	26.8	24.0	19.9	23.6
Mean across grasses	26.1	24.4	20.1	
LSD 0.05				
A	1.10			
B	0.64			
AB	2.01			

Notes: 1. *Galega orientalis* 100%; 2. *Medicago sativa* 100%; 3. *Onobrychis viciifolia* 100%; 4. *Galega orientalis* 40%, *Medicago sativa* 40%, *Festulolium* 20%; 5. *Galega orientalis* 40%, *Onobrychis viciifolia* 40%, *Festulolium* 20%; 6. *Galega orientalis* 40%, *Medicago sativa* 20%, *Trifolium pratense* 20%, *Festulolium* 20%; 7. *Galega orientalis* 40%, *Trifolium repens* 20%, *Onobrychis viciifolia* 20%, *Festulolium* 20%. Humus content at the beginning of the experiment in 2001 was: 22.6 $g \cdot kg^{-1}$ at 0–10 cm; 22.3 $g \cdot kg^{-1}$ at 10–20 cm; 1.68 $g \cdot kg^{-1}$ at 20–30 cm. Significance at $p < 0.05$.

share of cereals in the crop rotation structure to 100%. Similar regularities were identified in the 15–25 cm layer. These results demonstrate the role of wintering crops in conserving SOM.

The data from Experiment 2 show the humus content in different soil layers as influenced by sward type (Table 2).

The soil under long-lived swards after five years of cultivation was rich in humus: in all soil layers 0–10, 10–20, 20–30 cm, the humus content had mean values of 26.1, 24.4 and 20.1 $g \cdot kg^{-1}$, respectively. Multi-component swards (throughout the 0–30 cm layer) tended to increase humus content more than mono-component swards.

3.2. ***Fractions of humic substances***

3.2.1. *Mobile fractions*

Significant changes in SOM often require the application of long-term and diverse measures. Some researchers suggest that humus substances in fractions are better indicators of changes in SOM than total humus content. The data presented in Table 3 show the content of mobile humic substances carbon dissolved in 0.1 M NaOH in different soil layers as affected by tillage system and share of wintering crops in the crop rotation. Estimated fractions of mobile humic substances (MHS) enabled us to better evaluate the effects of the percentage of winter crops. A significant increase in MHS carbon in both soil layers was established in the crop rotation with 100% winter crops. Similar trends were shown in the total humus content data. Compared with conventional tillage, reduced tillage significantly increased the MHS content of topsoil. An increase in the percentage of winter crops in the crop rotation from 75 to 100% strengthened this effect and the highest MHS content (2.51–2.42 $g \cdot kg^{-1}$ C) was identified here. Conversely, using conventional tillage, the crop rotation without winter crops had as little as 1.81 $g \cdot kg^{-1}$ C of MHA in the 15–25 cm soil layer.

The data presented in Table S1 (available online only) show the variation in humified C, representing mobile humic acids (MHA). Statistically significant topsoil increases in MHA were obtained in the crop rotation with 100% winter crops, compared with the rotation composed solely

Table 3. The influence of soil tillage systems and proportion of winter crops in the crop rotation on the content of mobile soil carbon ($C\,g \cdot kg^{-1}$). Mean data from 2004 and 2006.

Proportion of winter crops, % (A)	Depth (cm)	Soil tillage system (B) Conventional	Soil tillage system (B) Reduced	Mean across proportion of winter crops (A)
0	0–15	2.00	2.16	2.08
	15–25	1.81	2.10	1.95
25	0–15	1.94	2.38	2.16
	15–25	2.06	2.05	2.06
50	0–15	2.02	2.27	2.15
	15–25	2.01	2.29	2.15
75	0–15	2.01	2.51	2.26
	15–25	2.24	2.06	2.15
100	0–15	2.13	2.42	2.28
	15–25	2.10	2.38	2.24
Mean across tillage (B)	0–15	2.02	2.34	
	15–25	2.04	2.18	
LSD 0.05		0–15 cm	15–25 cm	
A		0.192	0.223	
B		0.096	0.111	
AB		0.288	0.334	

Note: Significance at $p < 0.05$.

of spring crops. MHS accumulation in soil depended on swards and their mixtures' biological characteristics (Tables S2 and S3 – available online only). The soluble C content ($1.72\,g \cdot kg^{-1}$) was lowest in the four-component sward (*Galega+T. repens+Onobrychis+Festulolium)*. Conversely, a higher humified carbon content representing MHA was determined in this sward, because of the reduction in the proportion of mobile fulvic acids (MFA), which are considered agronomically less valuable. The MFA findings determined in the experiment are discussed elsewhere [38]. The increase in MHA content and the reduction in MFA demonstrate improvements in humic acid composition. The use of mono- and multi-component swards during the 5-year experimental period showed significant increases in MHS. Compared with 2001 data, the content of humified C (represented by MHA), increased more than two-fold. In the four-component sward (*Galega+ T. repens+Onobrychis+Festulolium*), the 0–30 cm soil layer had more MHS ($0.42\,g \cdot kg^{-1}$ C), compared with the mono-sward (*Galega orientalis;* $0.39\,g \cdot kg^{-1}$ C). The increase in MHS (7.7%) in multi-component swards corresponded to the determined increase in humus content in this treatment, but was stronger than in humus (2.6%). Large amounts of under- and above-ground biomass and high yields produced by this perennial legume leave abundant biomass in and on the soil to decompose and humify. In Experiment 2, the ratio of grass root to above-ground dry mass of grasses of the first year of use was very diverse: *Trifolium pratense*, 0.95; *Medicago sativa*, 0.67; *Trifolium repens*, 2.09; and *Galega orientalis*, 1.39. Stronger humification processes, estimated according to MHA accumulation, were identified in soil under the sward composed of plants with a wider under- to above-ground mass ratio.

3.2.2. *Labile fractions*

The data reported in Table S4 (available online only) suggest the effects of tillage systems and proportion of winter crops in the crop rotation on hot-water-soluble (labile) C content in the soil.

Labile soil C content in topsoil was significantly influenced by increasing the area of winter crops from 50 to 100% and a similar trend was observed in the subsoil. In both soil layers, reduced tillage significantly increased labile soil C content. The use of reduced tillage and increasing the proportion of winter crops to 100% in the crop rotation, estimated as the result of interaction of these two factors, resulted in an increase in labile C of $\leq 0.79\,g \cdot kg^{-1}$ in the topsoil and $\leq 0.74\,g \cdot kg^{-1}$ in the subsoil. Thus, the use of both agricultural practices: RT and increasing the winter crop area in the crop rotation up to 100% can improve soil quality. This regularity was evident in the entire 0–25 cm plough-layer.

Figure 1 shows how the concentration of humic acids extracted in hot water varied in relation to tillage system, proportion of winter crops in the crop rotation and soil layer. Figure 2 shows the data for fulvic acid concentrations. The humified C content in the topsoil in the reduced tillage system was more than twice that in the conventional system (Figure 1). The effects of both tillage and winter crop share in the crop rotation were clearly revealed by differences in topsoil humified C content. This shows the sensitivity of the method used to determine humified C in the labile fraction of SOM. We concluded that the analytical protocols are suitable for assessment of the impact of tillage and plants on ‘young’ organic matter at the initial stages of formation (Figure 1). The accumulation of fulvic acids was influenced more by tillage than by plants (Figure 2). Reduced soil tillage significantly increased the concentration of fulvic acids, but this effect was much weaker than seen for humified C in this fraction.

The porportion of winter crops grown in the crop rotation exerted positive effects on the content of labile humified C. The application of reduced tillage significantly increased the amounts of hot-water-soluble labile humic acids and labile fulvic acids, compared with conventional tillage. The labile humic acid content of water extracts was $\leq$2.6 times higher and labile fulvic acid carbon was $\leq$14% higher, respectively, than the conventional tillage system. Compared with the crop rotation without winter crops, significant increases were determined where winter crops accounted for $\geq$50% of the crop rotation.

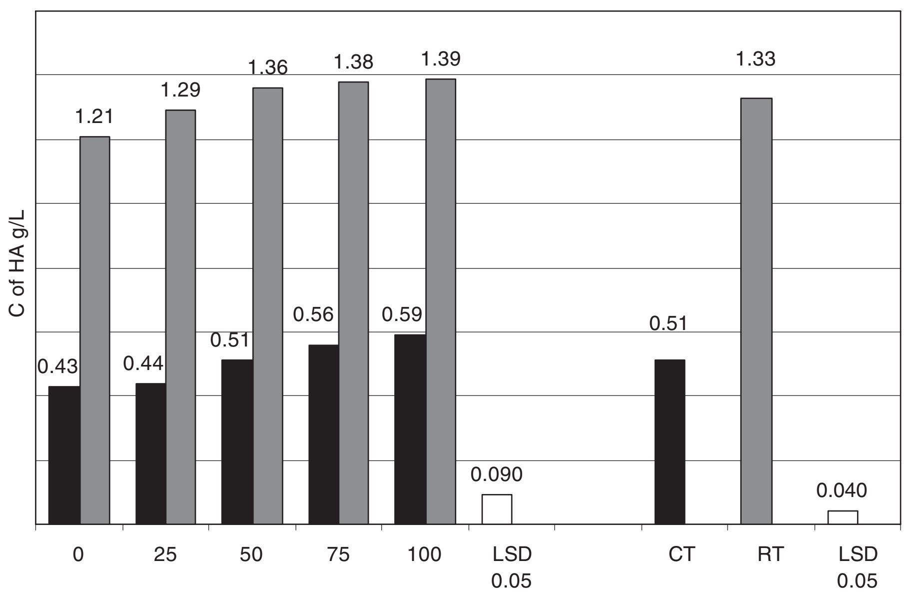

Figure 1. The influence of soil tillage systems and proportion of winter crops in the crop rotation on the content of humic acids in hot water extract in topsoil (mean data from 2004 and 2006).

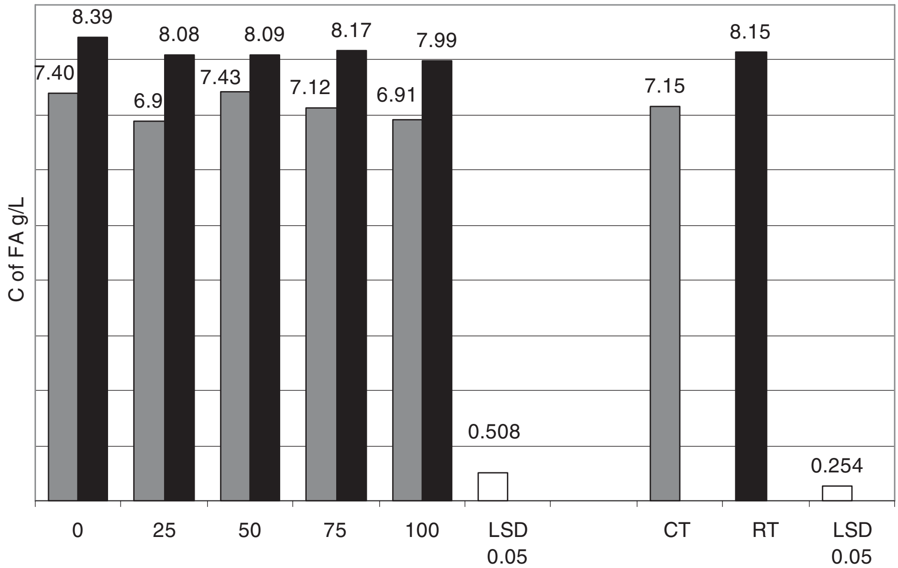

Figure 2. The influence of soil-tillage systems and proportion of winter crops in the crop rotation on the content of fulvic acids in hot water extract in topsoil (mean data from 2004 and 2006).

3.2.3. *FT-IR spectra of humic acids*

Figure 3 presents HA quality indicators measured using FT-IR spectroscopy methods. The application of reduced soil tillage resulted in qualitative changes in HA. Structural fragments of HA become more hydrophobic and more resistant to mineralisation [39]. The main absorbance peaks of the FT-IR spectra were at the following wavelengths: 2990–2920 cm^{-1} (C−H stretch of $-CH_2$ and $-CH_3$), 1460–1440 cm^{-1} (C−H deformation of CH_2 or CH_3 groups) and 1880–1830 cm^{-1} (C=C in aromatic structure). The aromatic character of the compounds and the presence of

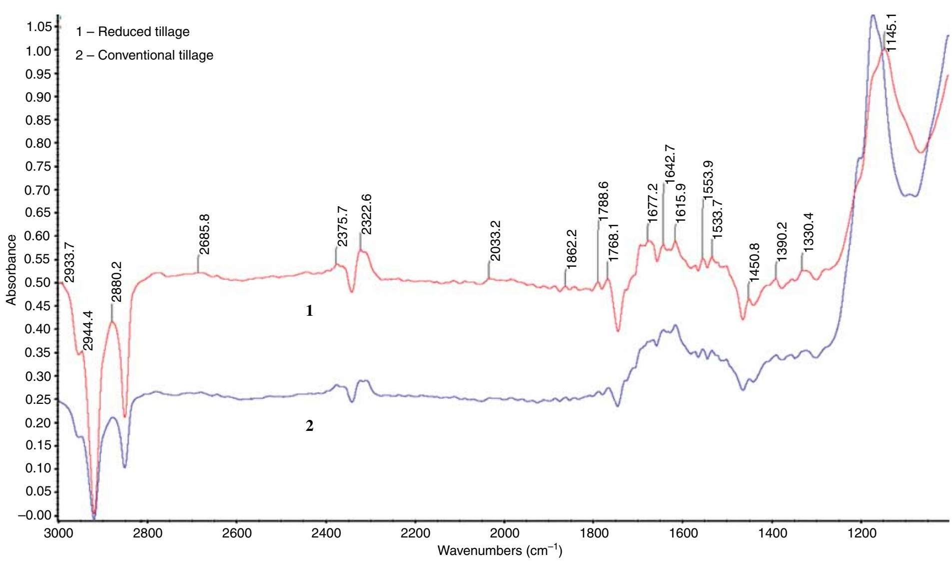

Figure 3. FT-IR absorbance spectra of humic acids. Experiment 1.

metil- and metilen- groups define their hydrophobic nature. Hydrophobic organic matter is more resistant to microbial degradation. Analysis of FT-IR spectra suggests that the soil under the reduced tillage system tended to accumulate HA fractions resistant to mineralisation.

4. Discussion

Experimental evidence suggests that soil tillage systems affected the amount of labile water-soluble humified C in soil. In the reduced soil tillage treatments in topsoil (0–15 cm), there was 2.6 times more water-soluble HA carbon and 14% FA carbon compared with conventional soil tillage. Mobile and labile humic acid fractions, despite their appreciable quantities, were sensitive to nutrient cycling. The MHA fraction is a young pool of SOM. It is less recalcitrant than other humic acids owing to a lack of complexation with cations and it has higher nitrogen (N) and C contents than more humified material. The decomposable C pool was measured using the parameter of hot-water-extractable C as the very easily decomposable fraction of SOM. This fraction contained more readily available substances, such as organic N compounds, carbohydrates, phenols and lignin monomers [34].

Variation in environmental factors, including temperature, moisture, composition of the decomposer community and residue composition influence plant residue decomposition [26]. In our study, we found that a long-lived multi-component sward consisting of 80% legumes tended to increase humus and MHA levels in the soil. C/N ratios are often used to explain different turnover rates for early residue decomposition. The C/N ratios of legumes are narrow. Different sward compositions and plant residue biochemistry control their decomposition rates, soil C cycling and the formation of humic substances. There is little research information on herbaceous plant decomposition in the soil, decomposition rate or transformation. Root mortality is an important ecosystem event with the subsequent decomposition of roots playing major roles in the input of carbon and nutrients to soil [40]. The data suggest that most *T. repens* roots were short-lived with, for example, 73% of *T. repens* roots surviving for <21 days. This study shows unequivocally that roots of *T. repens* can be relatively short-lived, and suggests that root mortality is likely to be a significant route by which carbon and nutrients enter the soil. The four-component sward mixture created preconditions for plant biomass humification and the formation of humified carbon in the soil.

Determination of different HS fractions lays a platform for discussions on their significance and use. In general, HS contents, characteristics and distributions within fractions can be treated as first indicators of positive and negative changes occurring in the soil. Research into mobile and labile HS is important from the agronomic viewpoint. The content of these substances is related to crop productivity. Simultaneously, humified compounds are well preserved in the soil, participate in HS transformation processes and closely relate to soil and environment quality. However, it is not always clear which of the test methods is more suitable for estimation of changes in OM. The MHA fraction is a younger, more labile SOM pool that directly responds, both in terms of quality and quantity, to crop management. However, the response of the more humified, polyvalent cation-bound CaHA fraction to crop management is less pronounced. There are few studies that have applied MHA fractionation procedures toward gaining a process-level understanding of how crop management affects SOM properties, and how altered SOM properties in turn affect soil. Greater sensitivity of MHA than CaHA to cropping intensity was noted for elemental analysis and spectroscopic analysis by FT-IR [31,32]. Reported findings agree with the regularities of the suitability of methods for the assessment of studied changes in soil organic properties. Even less evidence is found on the responsiveness of labile C compounds, particularly their humified part, to crop management and tillage system assessment. Thus, we can conclude that the analytical methods chosen for this study are applicable for the assessment and comparison of changes in soil humic substances in the development of reduced agricultural soil systems. All reported analytical methods are appropriate for evaluating sensitivity to changes in management. However, labile and mobile C pools are more sensitive than humus. Further studies are needed to examine the effectiveness of the labile humified C pool. It is necessary to compare analytical methods and their modifications for the analysis of labile organic matter and especially the humified component.

5. Conclusions

Reduced soil tillage significantly increased humus content, both in topsoil and subsoil, compared with conventional tillage. The highest humus content was established in the reduced tillage system having increased the proportion of winter crops to 100%. Over a five year period, humus content over the whole 0–30 cm layer increased more in multi-component swards than in mono-component swards. Compared with conventional tillage, reduced tillage significantly increased the MHA content in topsoil. Increasing the winter crop area in the crop rotation to 100% tended to increase this effect.

The investigated agricultural means influenced the content of labile hot-water-soluble humified carbon in soil. In the reduced tillage system, the topsoil 0–15 cm layer contained 2.6 times more labile HA in the water extract and 4% more labile FA than in conventional tillage. Labile HA content increased significantly when the proportion of winter crops in the crop rotation was $\geq$50%. The highest amount of MHA accumulated in soil under multi-component swards of four long-term grasses (*Galega*+*T. repens*+*Onobrychis*+*Festulolium*) grown in ecological farming. In soil under the sward composed of plants with wider ratios of under- to above-ground plant mass, when estimated according to MHA accumulation, humification processes were found to be very active.

Using FT-IR spectroscopy it was determined that reduced tillage influenced changes in the quality of soil humic acids: the fragments that constitute HA became more hydrophobic and resistant to mineralisation compared with those in the conventional tillage system. The analytical methods chosen to determine soluble humified C content and spectra measurements by FT-IR spectroscopy proved to be effective in the analysis of reduced soil management practices.

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References

[1] D. Jodaugiene, A. Motuzas, A. Stancevičius, and S. Raudonius, *Different soil tillage and humus quality, clay fraction content and its organic and mineral composition in Endohypogleic–Eutric Planasols*, Agr. Sci. 4 (2001), pp. 3–11 (in Lithuanian with English summary).

[2] A. Slepetiene and J. Slepetys, *Status of humus in soil under various long-term tillage systems*, Geoderma 127 (2005), pp. 207–215.

[3] D.C. Reicosky, *Crop residue management: soil, crop, climate interaction*, in *Crop Residue Management*, J.L. Hatfield and B.A. Stewart, eds., Lewis Publisher, Boca Raton FL, 1994.

[4] W.H. Schlesinger, *Carbon sequestration in soils*, Science 284 (1999), p. 2095.

[5] V. Rojkov, D. Konjushkov, and B. Kogut, *Distribution of the reserves of organic matter in the soil cover of Russia*, 17th WCSS Symposium, Thailand, 2002.

[6] R.H. Ellerbrock, A. Höhn, and J. Rogasik, *Influence of management practice on soil organic matter composition*, in *The Role of Humic Substances in the Ecosystems and in Environmental Protection*, IHSS – Polish Society of Humic Substances, J. Drozd, S.S. Gonet, N. Senesi, and J. Weber, eds., Zaklad Poligraficzny Abedik, Wroclaw, Poland, 1997, pp. 233–238.

[7] R.H. Ellerbrock, A. Höhn, and J. Rogasik, *Functional analysis of soil organic matter as affected by long-term manurial treatment*, Eur. J. Soil Sci. 50 (1999), pp. 65–71.
[8] J.L. Evans, I.J. Fernandez, L.E. Rustad, and S.A. Norton, *Methods for evaluating carbon fractions in forest soils: a review*, Maine AES Tech. Bull. 178 (2001), pp. 3–27.
[9] T.O. West and W.M. Post, *Soil organic carbon sequestration rates by tillage and crop rotation. A global data analysis*, Soil Sci. Soc. Am. J. 66 (2002), pp. 1930–1946.
[10] A.D. Michnovskaja, T.P. Kiritchenko, and D.G. Suleimanova, *Effect of soil tillage methods on the microbiological processes, content and quality of organic matter in Chernozems Lesostepi of Ukraine*, Res. Works AAS Leningrad (1986) (in Russian).
[11] D.A. Angers, *Water-stable aggregation of Québec silty clay soils: some factors controlling its dynamics*, Soil Till. Res. 47(1–2) (1998), pp. 91–96.
[12] D.A. Martens, *Plant residue biochemistry regulates soil carbon cycling and carbon sequestration*, Soil Biol. Biochem. 32 (2000), pp. 361–369.
[13] M.A. Arshad, M. Schnitzer, D.A. Angers, and J.A. Ripmeester, *Effect of till vs no-till on the quality of soil organic matter*, Soil Biol. Biochem. 22 (1990), pp. 595–599.
[14] M.V. Novitskii and V.A. Ilyushchenko, *The content and composition of labile humus in the soddy-podzolic loamy sands as dependent on the degree of agricultural development*, Agr. Chem. (Agrokhimiya) 1(2) (1997), pp.165–169.
[15] B. Jankauskas, G. Jankauskienė, M.A. Fullen, C.A. Booth, and A. Slepetiene, *Soil organic matter changes in Lithuanian soils: experiences and results*, Zemdirbyste-Agriculture 96(1) (2009), pp. 85–97.
[16] P.R. Poulton, *The Rothamsted long-term experiments: are they still relevant?* Can. J. Plant Sci. 6. (1996), pp. 559–571.
[17] D.S. Orlov, *Humus Acids of Soils and General Humification Theory*, Moscow University Press, Moscow, 1990 (in Russian).
[18] D.S. Orlov and L.A. Grishina, *Practical Manual of Humus Chemistry*, Moscow University Press, Moscow, 1981 (in Russian).
[19] D.C. Olk, *A chemical fractionation for structure–function relations of soil organic matter in nutrient cycling*, Soil Sci. Soc. Am. J. 70 (2006), pp.1013–1022.
[20] N. Mahieu, D.C. Olk, and E.W. Randall, *Multinuclear magnetic resonance analysis of two humic acid fractions from lowland rice soils*, J. Environ. Qual. 31 (2002), pp. 421–430.
[21] A. Slepetiene and D. Simanskaite, *The relationship between the components of humic substances in the soil and crops grown*, 10th Nordic IHSS Symposium on 'Character of natural organic matter and its role in the environment,' University of Latvia, Riga, 2005, pp. 59–60.
[22] A. Arlauskienė and S. Maikštėnienė, *Biological value of leguminous plants as preceding crops in the agocenosis*, Agr. Sci. 1 (2001), pp. 22–30 (in Lithuanian with summary in English).
[23] J. Andrzejewska and S. Ignaczak, *Effectiveness of symbiosis between fodder galega (*Galega orientalis *Lam.) and* Rhizobium galegae *on fallow land*, Electron. J. Pol. Agr. Uni. 4(2) (2001), pp. 1–23.
[24] J. Álvaro-Fuentes, M.V. López, C. Cantero-Martinez, and J.L. Arrúe, *Tillage effects on soil organic carbon fractions in Mediterranean dryland agroecosystems*, Soil Sci. Am. J. 72 (2008), pp. 541–547.
[25] M. von Lützow, J. Leifeld, M. Kainz, I. Kögel-Knabner, and J.C. Munch, *Indication for soil organic matter quality in soils under different management*, Geoderma 105(3–4) (2002), pp. 243–258.
[26] J.F. Parr and R.I. Papendick, *Factors affecting the decomposition of crop residues by microorganisms*, in *Crop Residue Management Systems*, W.R. Oschwald, ed., American Society of Agronmy, Madison, WI, 1978, pp. 101–129.
[27] P. Smith, *Carbon sequestration in cropland: the potential in Europe and the global context*, Eur. J. Agron. 20(3) (2004), pp. 229–236.
[28] FAO/UNESCO, *Soil map of the world revised legend with corrections and updates*, Technical Paper 20. ISRIC Wageningen, 1997, pp. 1–140.
[29] H. Egner, H. Riem, and W.R. Domingo, *Untersuchungen über die chemische Bodenanalyse als Grundlage für Beurteilung der Nährzustandes der Böden*, Ann. R. Agr. College Sweden 26 (1960), pp. 199–215.
[30] B.A. Nikitin, *Methods for soil humus determination*, Agr. Chem. (Agrokhimiya) 3(2) (1999), pp. 156–158.
[31] D.C. Olk, G. Brunetti, and N. Senesi, *Decrease in humification of organic matter with intensified lowland rice cropping: a wet chemical and spectroscopic investigation*, Soil Sci. Soc. Am. J. 64 (2000), pp. 1337–1347.
[32] D.C. Olk, G. Brunetti, and N. Senesi, *Organic matter in double-cropped lowland rice soils: chemical and spectroscopic properties*, Soil Sci. 164 (1999), pp. 633–649.
[33] V.V. Ponomareva and T.A. Plotnikova, *Humus and Soil Formation*, Nauka, Leningrad, 1980 (in Russian).
[34] E. Schulz and M. Körschens, *Characterization of the decomposable part of soil organic matter and transformation processes by hot water extraction*. Eurasian Soil Sci. 31 (1998), pp. 809–813.
[35] V.N. Krasiukov and I.A. Lapin, *Method of humic substances determination in natural waters*, Description of invention SU 1385041, 1998 (in Russian).
[36] OMNIC v7.3 Release Notes. Thermo electron. Updated for service Pack 1. Available at: www.mmrc.caltech.edu/FTIR/Nicolet Software/Nicolet/ReleaseNotes/OMNIC Release Notes.pdf (accessed 1 June 2010).
[37] A. Velykis, A. Satkus, and A. Slepetiene, *Effect of biologization of agriculture and means of sustainability on soil organic matter changes*, Latvijas Universitatis Raksti (Acta Universitatis Latviensis) 692 (2005), pp. 165–174.
[38] J. Slepetys, *Long term swards for ecological farming. Scientific report*, Akademija (2007), pp. 1–39.
[39] B. Chefetz, T. Ilani, E. Schulz, and J. Chorover, *Wastewater dissolved organic matter: characteristics and sorptive capabilities*, Water Sci. Technol. 53(7) (2006), pp. 51–57.
[40] H. Persson, *Root dynamics in a young Scot's pine in Central Sweden*, Oikos, 630 (1978), pp. 508–519.