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Response of soil nitrogen and carbon to organic management of legume swards

Jonas SLEPETYS, Alvyra SLEPETIENE

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: jonas.slepetys@lzi.lt, alvyra@lzi.lt

Abstract

Parallel to traditional function of grasslands to support livestock production, other important functions include carbon (C) sequestration as well as nitrogen (N) accumulation in the soil. During the experimental period, from 2001 to 2006, the content of nitrogen in the soil increased by 75–88% (in relative values). The experiment compared the influence of legume swards grown under organic management on soil organic carbon (SOC), humified carbon fractions, nitrogen, C/N ratio in the 0–10, 10–20, 20–30 cm layers of an *Endocalcari-Endohypogleyic Cambisol* (*CMg-n-w-can*). The study encompassed seven legume swards and their mixtures used for five years. The highest amount of SOC accumulated in the soil under the four-component sward (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*). Management of legume swards had the greatest effect on the carbon of humic acids' fraction C_{HA3}, especially on its buildup in the 20–30 cm layer. The highest content of biochemically most protected carbon was formed here. Multi-component swards exerted a positive effect on the increase in the carbon content of this fraction's humic acids, which can be qualified as strengthening of C sequestration. The highest content of total humified carbon (ΣC_{HA}) 4.05 g kg^{-1} (on average in the 0–30 cm layer) after five experimental years was recorded in the four-component sward (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium)*. In the 0–30 cm soil layer, all legume mixtures accumulated more humified carbon (ΣC_{HA}) (3.82–4.05 g kg^{-1}), compared to mono-component sward.

Key words: *Cambisol*, SOC, nitrogen, *Galega orientalis*, *Medicago sativa*, *Onobrychis viciifolia*, organic management, multi-component swards.

Introduction

Contemporary agricultural development is increasingly focused on economical, effective, sustainable systems that allow reduction of use of various resources, protect the environment, maintain sustainable landscape, and provide safe and high quality food to consumers. Policy changes in European agriculture are now creating an environment that should encourage greater adoption of legume-based grazing systems. However, this represents a fundamental modification to forage-based systems of livestock production, necessitating a move towards greater extensification and more attention to the management of fodder resources and livestock in both time and space (Rochon et al., 2003).

Maintaining and increasing organic carbon (OC) retention in the soil is crucial, due to the importance of OC in the preservation of soil fertility. A growing consensus therefore regards the reduction of soil management intensity as a viable means to increase soil organic matter (SOM) and limit mineralization and carbon dioxide (CO_2) emission (Schlesinger, 1977; 1995). Agricultural soils have the potential to make a contribution towards reducing net emissions of greenhouse gases – CO_2 and methane (CH_4) to the atmosphere through increased C sequestration in soil organic matter (Laird et al., 2001). Converting agricultural land to a more natural or restorative land use essentially reverses some of the effects responsible for SOC losses that occur upon conversion of natural to managed ecosystems. The term "soil C sequestration" implies removal of atmospheric CO_2 by plants and storage of fixed C as soil organic matter (Lal, 2004). Applying ecological concepts to the management of natural resources (e.g., nutrient cycling <...> enhanced soil biodiversity) may be an important factor in improving soil quality and SOC sequestration (Lavelle, 2000).

The strategy is to increase SOC density in the soil, improve depth distribution of SOC and stabilize SOC by encapsulating it within stable micro-aggregates so that C is protected from microbial processes or as recalcitrant C with long turnover time. In this context, managing agro-ecosystems is an important strategy for SOC/terrestrial sequestration (Lal, 2004; 2011; Smith et al., 2008). When estimating cropping systems in the context of C sequestration, one of the essential factors is C transformation during the formation process of humified C compounds that have a relatively longer retention time, compared with non-humified ones and in this way reduce emitting of carbon from the soil in the form of CO_2. Also the observed relationship between clay mineralogy and the chemical nature of the associated humic substances indicates that soil clay minerology strongly influences the humification process and formation of the clay-humic complexes (Laird et al., 2001).

In agriculture, SOM and SOC accumulation mainly originates from above ground organic inputs, root residues and exudates, and also residues remaining in the soil (Puttaso et al., 2011). Various authors' findings about this are rather controversial. Research done in Lithuania shows that in agricultural soils the amount of plant residues left by various crops in the topsoil and on the soil surface differed up to 9-fold: 1.75–15.4 t ha^{-1}, peas – 1.75 t ha^{-1}, perennial grasses – 15.4 t ha^{-1}, lucerne – 10.5 t ha^{-1}, clover-timothy of the 1st year of use – 6.85 t ha^{-1} (Magyla et al., 1994). The decomposition rate of such organic residues is governed by the chemical composition of the organic residues, presence of the decomposer organisms, environmental conditions and soil characteristics (Swift et al., 1979). Natural organic materials in soils consist of a complex mixture of different biochemicals exhibiting numerous morphologies and stages of biological oxidation. A continuum of decomposability exists based on chemical structure; however, this continuum can be altered by interactions with minerals within matrices capable of stabilising potentially labile organic matter against biological oxidation. Protection is not considered to equate to a permanent and complete removal of organic C from decomposition, but rather to a reduced decomposition rate relative to similar unprotected materials. The stabilisation of organic materials in soils by the soil matrix is a function of the chemical nature of the soil mineral fraction and the presence of multivalent cations, the presence of mineral surfaces capable of adsorbing organic materials, and the architecture of the soil matrix. The degree and amount of protection offered by each mechanism depends on the chemical and physical properties of the mineral matrix and the morphology and chemical structure of the organic matter (Baldock, Skjemstad, 2000).

About two-thirds of terrestrial C is below ground and this pool generally has much slower turnover rates than aboveground C (Schlesinger, 1977). Organic matter shows differential resistance to microbial decomposition depending on the complexity of the organic compounds, with some of them showing resistance for hours and some lignified materials remaining for thousands years (Schlesinger, 1977; Van Veen, Paul, 1981). In sward ecosystems, up to 98% of the total C content is sequestered in the underground part, and carbon sequestered in this form is characterised by a lower variability level than carbon in the above-ground part. Any practice that increases net primary productivity or reduces the rate of heterotrophic respiration will increase C storage. Also, planting more trees, for example, or reducing the intensity of tillage on cropland, or restoring grasslands on degraded lands will all increase C storage in plants, soil, or both (Janzen, 2004). The conversion from arable cropping to grassland is regarded an effective means to increase soil carbon stocks (Guo, Gifford, 2002). However, swards differ markedly in biological properties, amount and composition of plant residues that depend on their management and cultivation technologies. As a result, their participation in C transformation processes is different. One of the ways to estimate the effects of different mixtures of swards on SOC accumulation and retention is to establish their influence on humified carbon compounds.

The search is on for new leguminous plants that are longevous and not demanding in terms of growing conditions. Grain and forage legumes are grown on some 180 million ha, or 12% to 15% of the Earth's arable surface (Graham, Vance, 2003). The economic and ecological benefits of forage legumes are well known. Symbiotically fixed nitrogen accumulated by legumes can be useful in various aspects: it produces protein-rich forage and completes nutrient balance in soils (Ledgard, 2001; Arlauskienė et al., 2011). However, some legumes have also undesirable characteristics, one of which is their short persistence in sward, which is not always true (Frame, 1992). On an organic farm, where no mineral or organic nitrogen fertilisers are applied it is very important that legumes persist as long as possible in the sward, therefore it is necessary to look for more persistent, long living legumes that could replace red clover at least partly. In Lithuania, the land area under certified organic production is rapidly increasing. A growing interest in organic management has also increased the role of legumes in forage production. Red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) are the most important legumes in Lithuania. For the current study, we selected a mixture of legumes, less commonly used than clover: common sainfoin (*Onobrychis viciifolia* Scop.), fodder galega (*Galega orientalis* Lam.) and lucerne (*Medicago sativa* L.). Under Lithuania's climatic conditions, fodder galega (*Galega orientalis* Lam.) is the most long lived legume exhibiting the best overwinter survival (Balezentiene, Mikulioniene, 2006). Introducing grass species, with higher productivity or C allocation to deeper roots, converting plants to deeper rooting species has been shown to increase soil C (Rochon et al., 2003; Smith, 2004; Smith et al., 2008). 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 ≤ 80% of the total plant biomass. In Lithuania, the area under fodder galega approximates 3000 ha and tends to increase. Efforts are made to establish fodder galega on extensively used land to prevent weed spread. Fodder galega can be used as a forage, exotic long-flowering and amenity plant. Its application is also possible in apiculture and energy production. However, little is known about its cultivation peculiarities and effects on soil properties (Balezentiene, Mikulioniene, 2006). There is no research evidence on the performance of mixtures including fodder galega, their comparison with other sward mixtures and effects on soil chemical composition.

Although there exist some valuable data on the influence of swards on soil properties, they are limited solely to the short-term effects (Karki, Goodman, 2011). In 1–2 years experiments it is not always possible to establish the impact of the practices tested. Conversely, in longer experiments, the effect is more evident, and the differences between the practices are more distinct. So far we have not found any research publications dealing with the effects of fodder galega and swards including it and other organically grown legume mixtures on carbon accumulation and humification in the soil during a 5-year period. As a result, comprehensive soil research is instrumental in order to fill this gap.

The objective of the current study was to explore the influence of legume swards in a pure crop and in mixtures with other legumes under organic management regime on SOC, N, C/N ratio and humified carbon fractions in the tested soil.

Materials and methods

Site, soil characteristics and sampling. The experiment was conducted in 2001 at the Lithuanian Institute of Agriculture (55º24′ N, 23º52′ E). The soil is *Endocalcari-Endohypogleyic Cambisol* (*CMg-n-w-can*) according to FAO/UNESCO (1997), with a clay content of 11.9%, silt 34.2% and sand 53.9%. Before the experiment, topsoil pH (KCl 1M, w/v 1:2.5) was 7.0, available phosphorus (P_2O_5) determined by Egner-Riem-Domingo (A-L) method: 128 mg kg^{-1} and available potassium (K_2O) 211 mg kg^{-1}.

Experimental design. The experiment examined the effects of seven swards grown under organic management on the accumulation of C, N and humified carbon fractions in different soil layers. In the spring 2001, pure or mixtures of herb species were sown under barley. The seed rate for pure crop was as follows: *Galega orientalis* 30 kg ha^{-1}, *Medicago sativa* 15 kg ha^{-1}, *Onobrychis viciifolia* 80 kg ha^{-1}, *Trifolium pratense* 15 kg ha^{-1}, *Trifolium repens* 10 kg ha^{-1}, *Festuliolium* 18 kg ha^{-1}. The following long-lived swards and their mixtures were investigated: 1) *Galega orientalis* cv. 'Gale' (100% of legumes), 2) *Medicago sativa* cv. 'Birutė' (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* + *Trifolium pratense* cv. 'Arimaičiai' + *Festulolium* (80% of legumes), 7) *Galega* + *Trifolium repens* cv. 'Atoliai' + *Onobrychis* + *Festulolium* (80% of legumes).

The experiment was laid out as a randomized complete block with four replications and a plot size of 35 m^2 (2.5 × 14 m). The swards were cut two or three times during the growing season and were used for five years. The first cut was taken at the beginning (3-cut management) or at mass flowering (2-cut management) stage. The second cut was taken in the middle of July (3-cut management) or beginning of August (2-cut management). The last cut was taken in the middle of October (3-cut management). No fertilisers and pesticides were used. In 2001 and 2006, soil samples were collected from the 0–10, 10–20 and 20–30 cm depths with 6 boreholes per replicated plot from three field replicates. For root biomass determination, two 25 × 25 × 25 cm soil cores with roots were dug from each experimental plot. The soil cores were transferred on meshed sieve (2 mm) and soil was washed from roots by running water. The roots were dried at 60ºC to a constant weight then weighed and expressed as DM (dry matter).

Analytical methods. For chemical analysis, visible roots and plant residues were removed and then the soil was sifted through a 0.25 mm sieve. Analyses were performed on air-dried samples. SOC content was determined by the Tyurin dichromate – oxidation method (Nikitin, 1999). Fractional composition was identified according to the Tyurin method modified by Ponomareva and Plotnikova (1980). For fractional composition, solutions of different NaOH concentrations were used for extraction: 0.1 M NaOH (room temperature); 0.02 M NaOH (hot extraction) also 0.05 M H_2SO_4 (for decalcitation, 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 68–70ºC and after that humic acids were separated by filtering. Separated humic acids were re- dissolved in 0.1 M NaOH solution. Some humic and fulvic acid solutions of each fraction were evaporated, oxidized and OC content determined in each fraction, using the same procedures as for soil samples. The following humic acids fractions were identified: HA1 – "free" and weakly bound with clay minerals and referred to as mobile humic acids fraction, HA2 – bound with calcium, HA3 – strongly bound with soil clay minerals; the fulvic acids fractions: FA1a – the so called "aggressive", FA1 – mobile, FA2 – bound with calcium, FA3 – bound with soil clay minerals fulvic acids fractions. A more detailed description of the fractionation methodology can be found in Ponomareva and Plotnikova (1980), Slepetiene and Slepetys (2005). This method allows calculation of the ratio of total humic acids' carbon (ΣC_{HA}) to total fulvic acids' carbon (ΣC_{FA}) in the soil as well as humification degree.

Statistical analyses. Experimental data were analysed by one-factor Analysis of Variance (*Anova*, *Statistica*, version 6.0) (Tarakanovas, Raudonius, 2003).

Results and discussion

Total nitrogen content in the soil. It is well known that legume swards accumulate nitrogen (N) in the soil. The experiment was conducted in the soil not rich in N (0.85 g kg^{-1} in 0–30 cm layer) (Table 1).

Table 1. The influence of legume swards on soil total nitrogen (N) content (g kg^{-1})

Legume sward	Soil depth cm			Mean across all depths	In relative values %
	0–10	10–20	20–30		
At the beginning of the experiment (2001)					
	0.98	0.93	0.63	0.85	100
After five years of legume sward cultivation (2006)					
1. *Galega orientalis*	1.58	1.58	1.31	1.49	175
2. *Medicago sativa*	1.78	1.50	1.24	1.51	178
3. *Onobrychis viciifolia*	1.63	1.54	1.33	1.50	176
4. *Galega* + *Medicago* + *Festulolium*	1.63	1.59	1.38	1.53	180
5. *Galega* + *Onobrychis* + *Festulolium*	1.71	1.63	1.46	1.60	188
6. *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium*	1.72	1.59	1.25	1.52	179
7. *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*	1.66	1.61	1.37	1.55	182
Mean across legume sward in 2006	1.67	1.58	1.33	1.53	
LSD_{05}	0.140	0.131	0.112		

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%. Preceding crop oats were grown in 2000.

During the experimental period (from 2001 to 2006), although nitrogen fertilization was not applied, nitrogen content in the soil increased by 75–88% (in relative values) due to the N fixation by legumes. The highest soil nitrogen content 1.60 g kg^{-1} (on average in the 0–30 cm layer) after the five experimental years was established in the *Galega* + *Onobrychis* + *Festulolium* sward. Depending on the sward, N distributed unevenly in separate soil layers. In *Galega orientalis* mono-crop, the distribution of nitrogen in both 0–10 and 10–20 cm soil layers was even (1.58 g kg^{-1} N). For the rest of the crops, the highest N accumulation (1.63–1.78 g kg^{-1}) was recorded in the top soil layer (0–10 cm).

Soil organic carbon (SOC) content. Our research findings indicate that during the experimental period, all swards increased SOC accumulation by 13–19% (in relative values) compared with that present at trial establishment (Table 2). As a result, legume swards exerted a greater positive effect on nitrogen accumulation than on SOC. The 0–10 cm soil layer was the richest in SOC (15.0–15.6 g kg^{-1}).

Table 2. The influence of legume swards on soil organic carbon (SOC) content (g kg^{-1})

Legume sward	Soil depth cm			Mean across all depths	In relative values %
	0–10	10–20	20–30		
At the beginning of the experiment (2001)					
	13.1	12.9	9.74	11.9	100
After five years of legume sward cultivation (2006)					
1. *Galega orientalis*	15.1	13.9	11.6	13.5	113
2. *Medicago sativa*	15.3	13.9	12.1	13.8	116
3. *Onobrychis viciifolia*	15.2	14.8	12.3	14.1	119
4. *Galega* + *Medicago* + *Festulolium*	15.0	14.5	11.3	13.6	114
5. *Galega* + *Onobrychis* + *Festulolium*	15.6	14.0	11.3	13.6	114
6. *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium*	15.6	14.0	11.5	13.7	115
7. *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*	15.2	14.2	11.6	13.7	115
Mean across legume sward	15.3	14.2	11.7	13.7	
LSD_{05}	0.25	0.16	0.20		

Note. Explanations under Table 1.

Soil C/N ratio. The soil C/N ratio is often used to explain different turnover rates for early residue decomposition. The C/N ratios of legumes are narrow. N can be easily mineralised when the C/N ratio is less than 20:1. According to Mikkelsen and Hartz (2008) the C/N ratio of added organic materials is a good, but not an absolute, predictor of whether N immobilization is likely (C/N ratio > 25:1) or if mineralization is likely (C/N ratio < 20:1). In our study, the mean of C/N of soil across all depths of legume swards was narrow (8.8–9.4), below 10 in all soil layers (Table 3).

Table 3. The influence of legume swards on soil C/N ratio, 2006

Legume swards	Soil depth cm			Mean across all depths
	0–10	10–20	20–30	
1. *Galega orientalis*	9.6	8.8	8.8	9.1
2. *Medicago sativa*	8.6	9.3	9.8	9.2
3. *Onobrychis viciifolia*	9.3	9.6	9.2	9.4
4. *Galega* + *Medicago* + *Festulolium*	9.2	9.1	8.2	8.8
5. *Galega* + *Onobrychis* + *Festulolium*	9.1	8.6	7.7	9.0
6. *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium*	9.1	8.8	9.2	9.0
7. *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*	9.2	8.8	8.5	8.8
Mean across legume sward	9.2	9.0	8.8	9.0

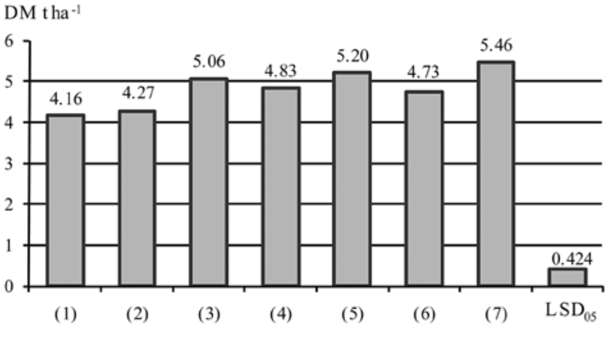
Carbon fractions. Data show, that OC distributed unevenly within humic acids' fractions (Table 4).

There was the lowest content of carbon of mobile humic acids (C_{HA1}), slightly higher content of carbon of humic acids bound with calcium (C_{HA2}), and the highest content of carbon of strongly bound humic acids (C_{HA3}). The soil of all legume mixtures had a higher accumulation of humified carbon (ΣC_{HA}) (3.82–4.05 g kg^{-1}) compared with mono swards (3.59–3.63 g kg^{-1} at the 0–30cm soil depth). Legume sward management exerted the greatest effect on C_{HA3} accumulation compared with other fractions' carbon, especially at the 20–30 cm depth. A significant increase in this fraction's carbon was established in the multi-component swards (4–7 treatments) and in the *Onobrychis viciifolia* sward. Multi-component swards had a significant positive effect on the increase in the content of this fraction's humic acids carbon, which can be judged as strengthening of C sequestration. The highest content of humified carbon (ΣC_{HA}) 4.05 g kg^{-1} (on average at the 0–30 cm layer) after five experimental years was detected in the four-component sward (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*) and in the three-component sward (*Galega* + *Medicago* + *Festulolium*). This suggests that the highest content of biochemically protected carbon formed in the soil of this four-component sward. This, in turn, can be linked to the highest root mass 5.46 t ha^{-1} determined in the mentioned treatment (Fig. 1). The root mass of all multi-component swards was higher than that of pure swards.

Table 4. The effect of legume swards on the humic acids' fractions at different soil depths, 2006

Legume swards	Soil depth	C_{HA1}	C_{HA2}	C_{HA3}	ΣC_{HA}
	cm	C g kg^{-1}			
1. *Galega orientalis Lam.*	0–10	0.41	1.64	1.94	3.99
	10–20	0.45	1.51	1.89	3.85
	20–30	0.32	1.33	1.41	3.06
2. *Medicago sativa*	0–10	0.46	1.49	1.90	3.85
	10–20	0.42	1.43	1.86	3.71
	20–30	0.32	1.35	1.53	3.20
3. *Onobrychis viciifolia*	0–10	0.42	1.54	2.13	4.09
	10–20	0.40	1.61	1.91	3.92
	20–30	0.36	1.43	1.66**	3.45
4. *Galega* + *Medicago* + *Festulolium*	0–10	0.43	1.69	2.20	4.32
	10–20	0.47	1.57	1.98	4.02
	20–30	0.35	1.51	1.71**	3.57
5. *Galega* + *Onobrychis* + *Festulolium*	0–10	0.49	1.53	2.24	4.26
	10–20	0.41	1.57	2.14	4.12
	20–30	0.26	1.36	1.61**	3.23
6. *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium*	0–10	0.51	1.60	2.18	4.29
	10–20	0.42	1.56	2.10	4.08
	20–30	0.23	1.26	1.61**	3.10
7. *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*	0–10	0.58	1.64	2.45*	4.67
	10–20	0.49	1.51	2.09	4.09
	20–30	0.31	1.42	1.66**	3.39
Mean across all depths	1.	0.39	1.49	1.75	3.63
	2.	0.40	1.42	1.77	3.59
	3.	0.39	1.53	1.90	3.82
	4.	0.42	1.59	1.96	3.97
	5.	0.39	1.49	1.96	3.84
	6.	0.39	1.47	1.96	3.82
	7.	0.46	1.52	2.07	4.05
Mean across legume sward	0–10	0.45	1.59	2.15	4.19
	10–20	0.43	1.53	1.99	3.96
	20–30	0.31	1.38	1.58	3.27

Notes. C_{HA1} – carbon of humic acids, extracted with 0.1 M NaOH and separated by acidifying at pH 1.3–1.5; C_{HA2} – carbon of humic acids, bound with calcium, extracted with 0.1 M NaOH after removing calcium and separated from fulvic acids by acidifying solution at pH 1.3–1.5; C_{HA3} – carbon of humic acids strongly bound with soil clay minerals; ΣC_{HA} – sum of humic acids fractions. Treatment effects were tested for significance by one-way *ANOVA*. Significant differences between legume sward treatment means are indicated by $^{**}P < 0.01$ and $^{*}P < 0.05$.



(1) *Galega orientalis*, (2) *Medicago sativa*, (3) *Onobrychis viciifolia*, (4) *Galega* + *Medicago* + *Festulolium*, (5) *Galega* + *Onobrychis* + *Festulolium*, (6) *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium*, (7) *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*

Figure 1. Root biomass of swards, 2006

SOC distribution in the fulvic acids' fractions was varied (Table 5). However, the similarities in the distribution of mobile and stable forms in fulvic acids' fractions were similar to those of humic acids. There were the lowest amounts of C_{FA1a} and C_{FA1} fractions of fulvic acids, attributed to mobile, slightly higher content of C_{FA2}, and the highest content of C_{FA3}. Legume swards management had the greatest influence on C_{FA3} accumulation in the 20–30 cm layer (2.09–2.56 g kg^{-1}). Significant (at $P < 0.01$) increase in C_{FA3} in this layer was determined in the four-component swards *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium* (2.56 g kg^{-1}) and *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium* (2.53 g kg^{-1}).

SOC is divided into pools having different properties and turnover rates depending on stabilisation mechanisms. The labile and mobile fractions play a very important role in the formation of aggregates, and because of their rapid turnover time they are the most sensitive to changes in soil management. Carbon pool, associated with soil mineral part, has a longer turnover time and is important in preventing mineralization of carbon in the soil. The biochemically protected humified carbon pool turns over very slowly and affects long time carbon sequestration in the soil.

Ratio of carbon of humic acids to carbon of fulvic acids. One of the major quality indicators of soil organic matter and its humified part is the ratio of carbon of humic acids to carbon of fulvic acids ($\Sigma C_{HA}:\Sigma C_{FA}$). The $\Sigma C_{HA}:\Sigma C_{FA} < 1$ ratio showed that the bigger part of carbon was present in fulvic acids' composition compared with humic acids (Fig. 2).

The $\Sigma C_{HA}:\Sigma C_{FA}$ ratio was the highest (0.726) in the organically managed 4-component sward (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*) (80% of legumes). In the 0–10 cm soil layer, in the multi-component swards mixtures with *Galega orientalis* Lam., the $\Sigma C_{HA}:\Sigma C_{FA}$ ratio was higher than that in the mono-component swards (0.665 and 0.680–0.745), respectively. Similar trend was identified also in the 10–20 cm layer.

Table 5. The effect of legume swards on the fulvic acids' fractions at different soil depths
Mean data from 2006

Legume swards	Soil depth cm	C_{FA1a}	C_{FA1}	C_{FA2}	C_{FA3}	ΣC_{FA}	ΣC_{HA} + ΣC_{FA}
		g kg^{-1}					
1. *Galega orientalis*	0–10	0.98	0.74	1.45	2.82	5.98	1.00
	10–20	1.00	0.81	1.47	2.67	5.94	0.98
	20–30	0.85	0.64	1.23	2.09	4.80	0.79
2. *Medicago sativa*	0–10	0.96	0.82	1.36	2.82	5.96	0.98
	10–20	0.93	0.68	1.40	2.58	5.58	0.93
	20–30	0.82	0.49	1.23	2.14	4.66	0.78
3. *Onobrychis viciifolia*	0–10	0.90	0.69	1.48	2.90	5.96	1.00
	10–20	0.89*	0.77	1.24	2.77	5.65	0.96
	20–30	0.85	0.51	1.29	2.29	4.93	0.84
4. *Galega* + *Medicago* + *Festulolium*	0–10	0.87	0.68	1.34	3.00	5.88	1.02
	10–20	0.86*	0.80	1.39	2.87	5.91	0.99
	20–30	0.78*	0.54	1.13	2.39*	4.83	0.84
5. *Galega* + *Onobrychis* + *Festulolium*	0–10	0.86	0.75	1.48	3.14	6.22	1.05
	10–20	0.84*	0.77	1.32	2.97	5.90	1.00
	20–30	0.73*	0.48	1.31	2.32	4.84	0.80
6. *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium*	0–10	0.75*	0.80	1.29	3.26	6.09	1.04
	10–20	0.72*	0.75	1.33	3.16*	5.95	1.00
	20–30	0.62*	0.48	1.27	2.56**	4.93	0.80
7. *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*	0–10	0.76*	0.67	1.34	3.35*	6.12	1.07
	10–20	0.71*	0.64	1.25	2.96	5.55	0.96
	20–30	0.63*	0.50	1.24	2.53**	4.88	0.83
Mean across depth	1.	0.73	1.38	1.38	2.53	5.57	0.92
	2.	0.66	1.33	1.33	2.51	5.40	0.90
	3.	0.66	1.34	1.34	2.65	5.51	0.93
	4.	0.67	1.29	1.29	2.75	5.54	0.95
	5.	0.67	1.37	1.37	2.81	5.65	0.95
	6.	0.68	1.30	1.30	2.99	5.66	0.95
	7.	0.60	1.28	1.28	2.95	5.52	0.95
Mean across legume sward	0–10 cm	0.87	0.74	1.39	3.04	6.03	1.02
	10–20 cm	0.85	0.75	1.34	2.85	5.78	0.96
	20–30 cm	0.75	0.44	1.24	2.33	4.84	0.81

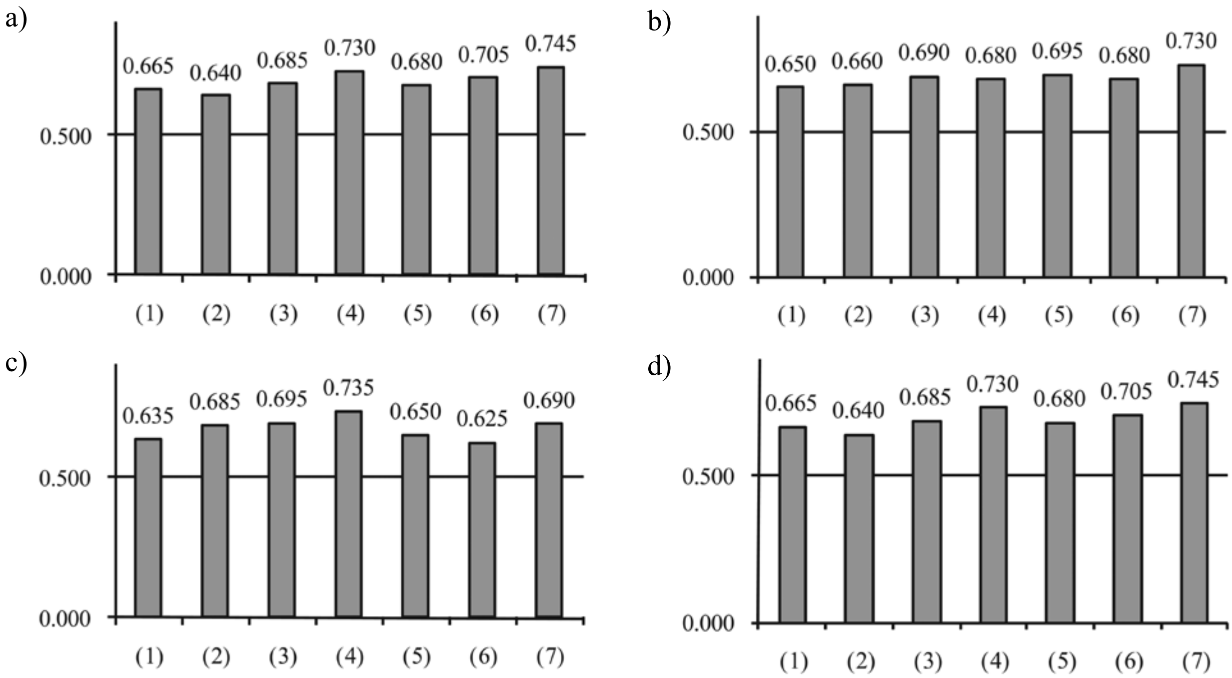
Notes. C_{FA1} – fulvic acids, extracted with 0.1 M NaOH solution minus fraction FA1a; C_{FA1a} – fulvic acids, extracted with 0.05 M H_2SO_4, so called "aggressive" fulvic acids; C_{FA2} – fulvic acids, extracted with 0.1 M NaOH solution after separation of humic acids, minus C_{FA1}; C_{FA3} – fulvic acids, extracted by hot solution of 0.02 M NaOH; ΣC_{HA} – sum of humic acid fractions; $\Sigma C_{HA} + \Sigma C_{FA}$ – sum of humic and fulvic acid fractions. Treatment effects were tested for significance by one-way *ANOVA*. Significant differences between legume sward treatment means are indicated by $^{**}P < 0.01$ and $^{*}P < 0.05$.

Correlations between investigated parameters of soil and swards. An increase in the sum of humic acids fractions (ΣC_{HA}) was influenced by carbon of humic acids strongly bound with soil mineral part C_{HA3} ($r = 0.945^{**}$l) (Table 6). Correlation analyses show, that SOC content in the 0–30 cm soil layer correlated significantly with experimental year's herbage yield ($r = 0.833^{*}$l), carbon content in herbage biomass ($r = 0.760^{*}$l), with humic to fulvic acids ratio ($r = 0.804^{*}$l) and with C_{HA2} ($r = 0.855^{*}$l).

The content of labile and mobile forms of these substances is related to crop productivity, since it correlates directly with plant yield (Slepetiene, Simanskaite, 2004). Humic substances incorporated into the soil increased grass production at the first cut during the growing season (Verlinden et al., 2010). Simultaneously, more stable humified carbon compounds such as C_{HA3} are well preserved in the soil, promote C sequestration and are closely related not only to soil quality, but also to environmental quality.

The significant correlation between C_{HA3} and ΣC_{HA} ($r = 0.945^{*}$l) shows that humified carbon strongly bound with soil mineral part (C_{HA3}) influenced soil quality improvement resulting from increasing ΣC_{HA}. The chemical-physical interaction between mineral part of soil and humic acids shows grassland soils' capacity to sequester SOC. Variations in environmental factors, including temperature, moisture, composition of the decomposer community and residue composition, influence plant residue decomposition (Parr, Papendick, 1978). In our research, it was found that long-lived legume mono- and multi-component swards during a 5-year period increased SOC content by 13–19% and nitrogen content by 75–88% (in relative values) due to N fixation by legumes. Different sward compositions and plant residue biochemistry control their decomposition rates, soil C cycling and carbon distribution in humified fractions. There is little research evidence on herbaceous plant decomposition in the soil, decomposition rate or transformation. It is thought that 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 (Persson, 1978).

According to many indicators, multi-component swards had a greater effect on SOC accumulation than mono-component ones. The four-component sward mixture created the best conditions for plant biomass humification and the formation of humified carbon in the soil. This was indicated by the ΣC_{HA}:ΣC_{FA} ratio, which was the highest in organically managed four legumes – grass sward (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*) (80% of legumes). Also, this sward accumulated significantly higher content of biochemically and physically protected carbon (total humified carbon ΣC_{HA} and C_{HA3} fraction bound with soil clay minerals). This sward was composed of two shallow-growing, fine-rooted (*T. repens*, *Festulolium*) and two deep-growing, large-rooted (*Galega*, *Onobrychis*) herbaceous plants, that optimally filled in the 0–30 cm soil layer with root tangle, leaving a lot of organic residues in the soil. The greatest root mass was established in this treatment.



(1) *Galega orientalis*, (2) *Medicago sativa*, (3) *Onobrychis viciifolia*, (4) *Galega* + *Medicago* + *Festulolium*, (5) *Galega* + *Onobrychis* + *Festulolium*, (6) *Galega* + *Medicago* + *Trifolium pratense* + *Festulolium*, (7) *Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*

Figure 2. The ΣC_{HA}:ΣC_{FA} ratio at different soil depths: a) 0–10 cm, b) 10–20 cm, c) 20–30 cm, d) 0–30 cm

Table 6. Linear correlations (*r*) between soil parameters (0–30 cm layer) and biomass of aboveground part of swards (n = 42)

	C of swards	C/N of swards	Sward yield DM t ha^{-1}	C_{HA2}	ΣC_{HA}	ΣC_{HA}: ΣC_{FA}	Soil N
SOC	0.760*		0.833*	0.850*	0.914*	0.804*	
C_{HA1}						0.834*	
C_{HA2}			0.855*				
C_{HA3}		0.908*			0.945*	0.913*	
ΣC_{HA}		0.779*					

* – significant at $P < 0.05$; C of sward-carbon content in sward DM; data of humified carbon fractions: C_{HA1}, C_{HA2}, C_{HA3}, ΣC_{HA}, ΣC_{HA}:ΣC_{FA} in the 0–30 cm soil layer

Conclusions

1. The investigated legume swards grown under organic management affected soil nitrogen and organic carbon (OC) in an *Endocalcari-Endohypogleic Cambisol* (*CMg-n-w-can*). During the experimental period, from 2001 to 2006, the content of nitrogen in the soil increased by 75–88% (in relative values) due to nitrogen (N) fixation by legumes, despite the fact that neither mineral nor organic nitrogen fertilisers were applied. The highest content of N 1.60 g kg^{-1} (on average in the 0–30 cm layer) after the five experimental years was established in the soil of *Galega* + *Onobrychis* + *Festulolium* sward.

2. According to many indicators, the multi-component swards exerted a more considerable effect on soil organic carbon (SOC) accumulation than mono-component ones. The highest amount of SOC accumulated in the soil under organically grown multi-component sward of four grasses (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*).

3. All investigated legume swards had the greatest effect on C_{HA3} fraction, especially its buildup in the 20–30 cm layer. The multi-component swards produced significant positive effect on the increase in carbon content of this humic acids' fraction, which can be regarded as C sequestration enhancement. All legume mixtures tended to increase humified carbon (ΣC_{HA}) accumulation (3.82–4.05 g kg^{-1}) more markedly than mono-swards in the 0–30 cm soil depth. The highest content of humified carbon (ΣC_{HA}) 4.05 g kg^{-1} (on average in the 0–30 cm layer) after the five experimental years was found in the four-component sward (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*) and in the three-component sward (*Galega* + *Medicago* + *Festulolium*). This treatment accumulated not only the highest total SOC content but also the highest biochemically and physically protected carbon level.

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Pupinių žolynų, augintų taikant organinę žemdirbystę, įtaka dirvožemio azotui ir angliai

J. Šlepetys, A. Šlepetienė

Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas

Santrauka

Greta žolynų tradicinės paskirties – tiekti pašarinę produkciją gyvulininkystei, kita jų atliekama svarbi funkcija yra dirvožemyje kaupti azotą (N) ir anglį (C). Tyrimų laikotarpiu (2001–2006 m.) N kiekis dirvožemyje padidėjo 75–88 % (santykiniais skaičiais). Tyrimų metu lyginta pupinių žolynų, augintų organinės žemdirbystės sąlygomis, įtaka dirvožemio azoto, organinės anglies, humifikuotos anglies frakcijų kiekiui ir C:N santykiui – giliau karbonatingo giliau glėjiško rudžemio (RDg4-k2) 0–10, 10–20 bei 20–30 cm sluoksniuose. Tirti septyni pupiniai žolynai ir jų mišiniai. Žolynai naudoti penkerius metus. Didžiausias organinės anglies kiekis susikaupė daugiakomponenčiame žolyne (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*). Pupinių žolynų naudojimas didžiausią įtaką turėjo huminių rūgščių C_{HA3} frakcijos kaupimuisi, ypač dirvožemio 20–30 cm sluoksnyje. Jame formavosi didžiausias kiekis biochemiškai labiausiai apsaugotos anglies. Daugiakomponenčio žolyno teigiama įtaka didinant šios frakcijos anglies kiekį gali būti vertinama kaip anglies sekvestracijos stiprinimas. Didžiausias suminės humifikuotos anglies (ΣC_{HA}) kiekis (4.05 g kg^{-1}) (vidutiniškai 0–30 cm sluoksnyje) nustatytas daugiakomponenčiame žolyne (*Galega* + *Trifolium repens* + *Onobrychis* + *Festulolium*). Dirvožemio 0–30 cm sluoksnyje geresnį humifikuotos anglies (ΣC_{HA}) kaupimąsi lėmė žolynų mišiniai (3,82–4,05 g kg^{-1}) nei monožolynai (3,59–3,63 g kg^{-1}).

Reikšminiai žodžiai: rudžemis, organinė anglis, azotas, *Galega orientalis* Lam., *Medicago sativa*, *Onobrychis viciifolia*, organinė žemdirbystė, daugiakomponenčiai žolynai.