ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 109, No. 3 (2022), p. 195–202
DOI 10.13080/z-a.2022.109.025

Impact of sward formation on soil organic carbon variation and relations with soil microbial activity

Regina SKUODIENĖ[1], Jūratė ALEINIKOVIENĖ[1,2], Donata TOMCHUK[1], Gintaras ŠIAUDINIS[1]

[1]Lithuanian Research Centre for Agriculture and Forestry
Gargždų g. 29, Vėžaičiai, Klaipėda distr., Lithuania
E-mail: regina.skuodiene@lammc.lt

[2]Vytautas Magnus University Agriculture Academy
Studentų g. 11, Akademija, Kaunas distr., Lithuania

Abstract

The aim of the study was to estimate the impact of temporal sward formation on soil organic carbon (SOC) variation and soil microbial biomass carbon (MBC) content in rhizosphere. The soil of experimental sites was *Bathygleyic Dystric Glossic Retisol*, where the soil acidity (pH) was 3.9 and 5.0, and four mixtures of legumes and grasses were cultivated. The mixtures were composed of one legume variety and two grass varieties – timothy (*Phleum pratense* L.) 35% and meadow grass (*Poa pratensis* L.) 15%, 50% of each: 1) red clover (*Trifolium pratense* L.), 2) white clover (*Trifolium repens* L.), 3) hybrid clover (*Trifolium hybridum* L.), and 4) alfalfa (*Medicago sativa* L.). In the soil of different swards, SOC was more accumulated at 0–10 cm depth. The species composition of the sward did not have any significant influence on the studied indicators. In the soil of swards with pH 5.0, the SOC content in three years increased by 3.3%, SOC stock by 8.5%, and the C to N ratio was favourable for the SOC accumulation. In the naturally acidic soil (pH 3.9), the SOC content and SOC stock decreased by 12.0% and 3.3%, respectively. In the 4th year of sward formation, irrespective of the soil acidity, the SOC stock decreased, but it was similar as in the beginning of sward formation. Sward formation had a significant impact on the soil MBC. In the 2nd year of sward formation, the MBC content increased by 1.7 times in the soil with pH 5.0 and by 2 times in the naturally acidic soil compared to the year of sowing and remained relatively stable in the 3rd and the 4th year of sward formation irrespective of the soil acidity.

Keywords: carbon accumulation, C to N ratio, sward development, microbial biomass carbon, perennials.

Introduction

One of the most important processes for saving the productivity of agroecosystem is the accumulation of soil organic carbon (SOC). The SOC stock reflects the relation between carbon insertion into the soil with residues of plants and organisms and carbon mineralisation as well as CO_2 emission (Bell, Lawrence, 2009). Together with changing environmental conditions, soil carbon changes as well until the balance between the insertion of organic residues to the soil and mineralisation is reached. To increase the SOC stock, it is not enough only to increase the amount of inserted organic matter and reduce its loss because of complicated processes in the whole ecosystem as well as biotic and abiotic factors (McGranahan et al., 2014). Therefore, the quantification of regional SOC changes in agricultural landscapes is crucial for maintaining sustainable agriculture and mitigating global climate change (Fan et al., 2022).

Due to the well-developed grass root system, swards improve soil with organic matter (Rasmussen et al., 2010) and affect soil biochemical processes (Lambers et al., 2009). Swards accumulate almost twice as much carbon in the soil as the intensively cultivated land (Guo, Gifford, 2002). In ecosystems of swards up to 98% of the total carbon content are sequestrated in the underground part. Carbon sequestrated in this form is distinguished by a smaller variability than in the overground (Ryals et al., 2014). SOC sequestration could even increase when growing mixtures of legumes and grasses (Skinner, Dell, 2016). According to the research data of Soussana et al. (2004), assessing carbon stock in the soil changes can reach up to 0.25 t ha^{-1} C through the year only because of an intensive use of the sward. SOC stock cannot increase consistently and without the end. The initial SOC content increase after the change of the land use is rapid; however, after some time it slows down and at the certain moment reaches the balance (Stockmann et al., 2013). Choosing relevant sward use technologies and increasing sward productivity it is possible to increase the amount

Please use the following format when citing the article:
Skuodienė R., Aleinikovienė J., Tomchuk D., Šiaudinis G. 2022. Impact of sward formation on soil organic carbon variation and relations with soil microbial activity. Zemdirbyste-Agriculture, 109 (3): 195–202. https://doi.org/10.13080/z-a.2022.109.025

of carbon accumulated in the soil (Osoro, 2014). Natural land-uses had a better soil quality indicating that SOC controls the soil quality and varies in response to land use (Atwell, Wuddivira, 2022).

The indirect soil pH effect on the SOC content is not consistent. Rousk et al. (2009) indicated that the amount of soil organic matter increased when the soil pH reached 5.0, whereas Kemmitt et al. (2006) stated that the amount of soil organic matter decreased when the soil pH increased from 3.5 to 6.5 together with the increased amount of microbial biomass carbon (MBC). The activity of microorganisms increases because they use SOC as the source of energy. Also, the processes of organic matter transformation in the soil directly depend on the C to N ratio (Jokubauskaite et al., 2014). Soils of the long-term (25 years) swards are distinguished for their greater C to N ratio than traditional intensively managed agricultural ecosystems (Šlepetienė et al., 2007).

The soil organic matter distinguished for its greater C to N ratio remains in the soil for longer time, and when this ratio diminishes, the processes are going on towards the direction of mineralisation (Crème et al., 2018). For the stable formation of soil organic combinations, the C to N ratio value in the ecosystem soil should be over 10 which means that processes of hummus formation in the soil are going on. The values below 10 signals about the processes of organic matter degradation (Hadas et al., 2004). A sufficient amount of nitrogen fosters soil biological processes, but the lack of carbon reduces the activity of microorganisms. Literature indicates (Rousk et al., 2009) that microorganisms use carbon combinations for energy and nutrition, while nitrogen is essential for the DNA protein synthesis and enzymes.

Western Lithuania significantly differs from the other Lithuanian regions for its soil and climatic conditions. We hypothesised that the formation of sward ecosystem differs because of the sward plant and microbe associations and root microbial biomass fluctuation. During the research, the traditional temporary sward of red clover was compared to rarely sown longer-term swards of white or hybrid clover and alfalfa grasses. Besides, the major Poaceae plant grass timothy, a slower developing and resistant to acid soils meadow-grass, was included in the mixture.

The aim of the study was to estimate the impact of temporal sward formation on the soil organic carbon variation and soil microbial biomass carbon content in rhizosphere.

Material and methods

Site description. Experiments were carried out in Western Lithuania, at the Vėžaičiai Branch of the Institute of Agriculture of Lithuanian Research Centre for Agriculture and Forestry during the 2014–2017 period. The location of the experiment was the following: lat. 55°70′ N, long. 21°49′ E. The soil of the experimental site was *Bathygleyic Dystric Glossic Retisol* (WRB, 2015) in the plough horizon with a texture of loam consisting of 8.0% clay, 45.1% silt, and 46.9% sand. Regarding the pH_{KCl} index the soil with a smooth, highly acidic profile up to 300 cm is with a very high mobile aluminium (Al^{3+}) content up to 300 mg kg^{-1} (Repsiene, Karcauskiene, 2016). The soil is of a medium phosphorus, high potassium, and medium nitrogen content having an average content of soil organic carbon (SOC). The soil agrochemical properties of analysis are presented in Table 1.

Table 1. Agrochemical properties of the soil at the experimental site in 2014

Soil properties	Soil pH_{KCl} 3.9		Soil pH_{KCl} 5.0	
	Depth cm			
	0–10	10–20	0–10	10–20
Soil acidity (pH_{KCl})	4.01 ± 0.03	3.85 ± 0.03	5.48 ± 0.06	4.67 ± 0.05
Mobile Al^{3+} mg kg^{-1}	88.6 ± 11.73	89.4 ± 12.58	3.9 ± 2.22	43.3 ± 6.95
Mobile P_2O_5 mg kg^{-1}	162 ± 10.33	161 ± 8.88	135 ± 6.49	135 ± 6.96
Mobile K_2O mg kg^{-1}	201 ± 5.68	200 ± 3.80	195 ± 6.69	192 ± 5.83
Total N %	0.128 ± 0.00	0.128 ± 0.00	0.134 ± 0.00	0.135 ± 0.00

Mean values ± standard deviation

In 2014, the amount of precipitation in spring reached 92%, in summer 84%, and in autumn 53% of the standard climate normal (SCN) (Figure 1). Soil humidity conditions for the growth of perennials were bad during the whole growing season. In 2015, the amount of precipitation in spring reached 135%, in summer 90%, and in autumn 79% of the SCN. In 2016, the amount of precipitation in spring reached 109%, in summer 144%, and in autumn 64% of the SCN. Soil humidity conditions for the growth of perennials were optimal during the whole growing season. In 2017, although the amount of precipitation per growing season reached 133% of the SCN, its distribution was uneven: in spring it was by 25% lower, in summer by 18% lower, and in autumn by 117% higher than the SCN.

Experimental design and treatments. Two different pH levels of the soil were formed: one part of the experimental site was left naturally acidic (pH_{KCl} 3.9), and the second part was limed with dolomite once in autumn of 2013 before the sward trial establishment. Liming at rate 1.0 (7 t ha^{-1} $CaCO_3$) by hydrolytic acidity increased the soil pH_{KCl} to 5.0.

Factor A. Soil acidity (pH): 1) soil pH_{KCl} 5.0; 2) soil pH_{KCl} 3.9. Factor B. Legume-grass swards: 1) red clover (*Trifolium pratense* L.) cultivar 'Vyčiai' 12 kg ha^{-1} (TP) – control; 2) white clover (*Trifolium repens* L.) cultivar 'Nemuniai' 8 kg ha^{-1} (TR); 3) hybrid clover (*Trifolium hybridum* L.) cultivar 'Lomiai' 10 kg ha^{-1} (TH); 4) alfalfa (*Medicago sativa* L.) cultivar 'Birutė' 12 kg ha^{-1} (each of 50%) (MS), combined with timothy (*Phleum pratense* L.) cultivar 'Dubingiai' 10 kg ha^{-1} (35%) and meadow-grass (*Poa pratensis* L.) cultivar 'Rusnė' 10 kg ha^{-1} (15%).

The experiment was established in the arable land in four replications. The treatments were arranged randomly. The area of swards trial field was 24 m^2 (3.0 × 8.0 m). In the year of sward sowing (26 May 2014), cover crop barley was fertilised with $N_{60}P_{60}K_{90}$. In the experimental site, the following fertilisers were used: complex fertiliser $N_{16}P_{16}K_{16}$ 300 kg ha^{-1} and $N_4P_{16}K_{34}$ 100 kg ha^{-1}, and NH_4NO_3 (ammonium nitrate) 23.5 kg ha^{-1}. In 2015–2017, the swards were fertilised with $P_{60}K_{90}$. As phosphorus fertiliser, single superphosphate was used, 300 kg ha^{-1}. The source of potassium (K) was 160 kg ha^{-1} KCl. Each year, the fertilisation was done prior to the

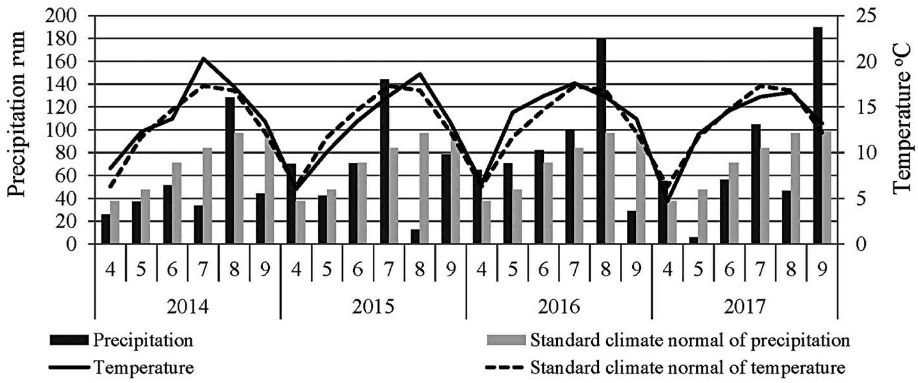


Figure 1. Mean monthly precipitation (mm) and temperature (°C) data for the growing seasons from April (4) to September (9) in 2014–2017 (data of Vėžaičiai Automatic Meteorological Station)

beginning of vegetation. The grasses were cut 2–3 times per year depending on the grass development stages. The 2nd-year sward was harvested at the beginning of flowering of Fabaceae plants. The 3rd- and the 4th-year sward was harvested at the beginning of heading of Poaceae plants.

Soil sampling and methods of analysis. For chemical analyses, in the months of September or October, soil samples were collected at 0–10 and 10–20 cm depth with a drill from each plot. All samples were air-dried, and visible roots and plant residues were removed manually. Then the samples were crushed, sieved through a 2 mm sieve, and homogeneously mixed. For the analyses of SOC content and composition, the soil samples were passed through a 0.2 mm sieve. Soil agrochemical characteristics were determined by these methods: soil pH_{KCl} was measured according to ISO 10390:2005 (Soil quality – Determination of pH); soil total nitrogen (%) – by the Kjeldahl method, soil mobile phosphorus (P_2O_5) and potassium (K_2O) (mg kg^{-1}) – by the Egner-Riehm-Domingo (A-L) method (LVP D-07:2016), and SOC content (%) – by the dry combustion Dumas method. Mobile aluminium (Al^{3+}) content (mg kg^{-1}) in the soil was determined according to the Sokolov methods ISO11260:1994 and ISO14254:2018 (Soil quality - Determination of exchangeable acidity using barium chloride solution as extractant); hydrolytic acidity – by the Kappen method modified by CINAO.

SOC stock was calculated according to the formula (Sanderman et al., 2011):

SOC stock (Mg ha^{-1}) = SOC content (%) × soil bulk density (kg m^{-3}) × soil depth (m) × 1 hectare (100 000 m^2).

SOC stock was calculated at two depths: 0–10 and 10–20 cm. To estimate the aeration and moisture conditions, the soil bulk density (kg m^{-3}) at 0–10 and 10–20 cm depth was determined. The density was determined with a 100 cm^3 cylindrical drill (by the Kachinsky method); the soil moisture content was expressed on a dry weight basis after oven drying to a constant weight at 105°C temperature.

Soil microbial biomass carbon (MBC) content was determined by the chloroform fumigation and extraction method according to Joergensen et al. (2011). The sample MBC content was estimated using the following equation:

$$MBC = CE / 0.35,$$

where CE is the difference between organic C extracted from fumigated and nonfumigated treated samples. The amount of MBC was expressed (mg C dry weight) on the air-dry soil basis and represents the average of three determinations (repeated three times on a single sample).

Statistical analysis. Significance of the differences between the means was determined according to the Fisher's protected least significant difference (LSD) at 0.05 probability level. The experimental data were subjected to the two-factor analysis of variance (ANOVA). The relations of characteristics were evaluated using the correlation-regression analysis with the software STAT (Raudonius, 2017).

Results and discussion

SOC variation. In the years of sward sowing, no significant differences were estimated between the SOC content of different acidity soils, soil C to N ratio, and SOC stock. At 0–10 cm soil depth, the SOC content ranged from 1.37% to 1.64%, and the accumulation of SOC reached 16.7–21.6 t ha^{-1} (Table 2).

Table 2. Soil organic carbon (SOC) content, C to N ratio, microbial biomass carbon (MBC) content, and SOC stock in the sowing year of perennials at 0–10 cm depth in 2014

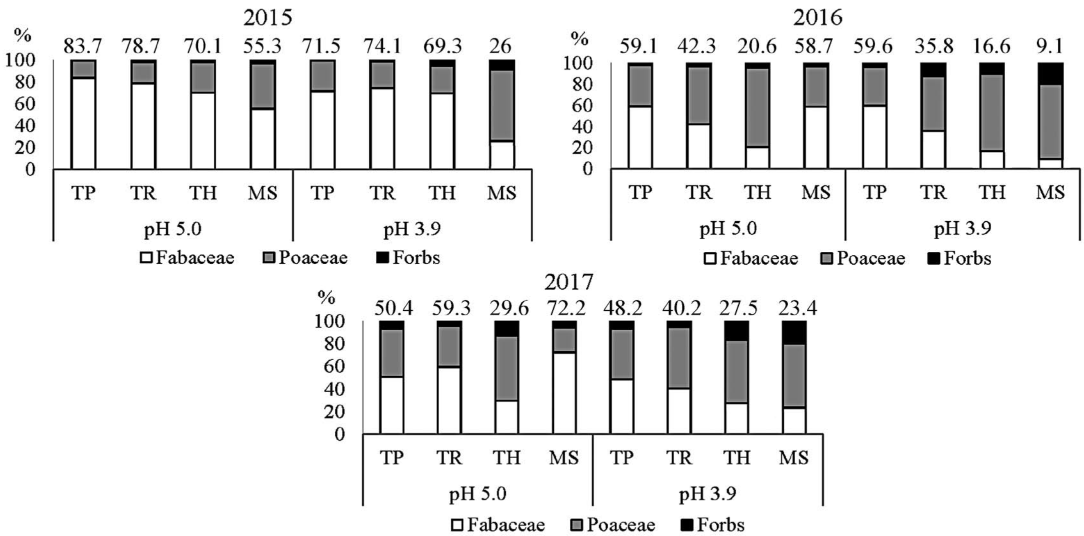
Factors Soil pH / sward with	SOC %	C:N	MBC µg g^{-1} C	SOC stock t ha^{-1}
		pH 5.0		
Trifolium pratense	1.61 a	12 a	135 b	20.8 a
Trifolium repens	1.64 a	12 a	130 b	21.6 a
Trifolium hybridum	1.46 a	12 a	116 c	18.7 a
Medicago sativa	1.37 a	11 a	117 bc	18.4 a
		pH 3.9		
Trifolium pratense	1.37 a	11 a	147 ab	16.7 a
Trifolium repens	1.46 a	11 a	134 b	18.4 a
Trifolium hybridum	1.41 a	11 a	160 a	18.3 a
Medicago sativa	1.44 a	11 a	128 bc	18.5 a

Note. Different letters indicate significant ($p < 0.05$) differences between the means.

According to the research data of Šlepetienė et al. (2013), the SOC content in the soil of swards ranges from 0.73% in previously cultivated fields to 8.31% in flooded-meadows. The soil C to N ratio was estimated to range from 11 to 12 and was favourable for the SOC accumulation. Mačiulytė-Mikučionienė (2010) indicated that the C to N ratio of soil should be 10–12 for SOC stabilisation in the agroecosystem. The age of sward has a significant influence on the C to N ratio of soil, as the sward formation process lasts for two years (Tomchuk, 2018). The root mass of grasses mostly develops in the 2nd year of their growth. The root mass in the naturally acidic soil increased by 3.2 times, and in the soil with pH 5.0 by 8.8 times (Skuodienė et al., 2017; Tomchuk, 2018).

In each year of sward formation, the amount of legumes in dry matter yield (DMY) depended on the soil pH and plant biological characteristics. In the 2nd year of sward formation, the amount of red clover in DMY reached 83.7% when the soil pH was 5.0 and 71.5% at the soil pH of 3.9 (Figure 2). Similar amounts (78.7% and 74.1%, also 70.1% and 69.3%) were determined in the mixtures of white and hybrid clover with grass sward in soils of different acidity. The amount of slower developing legume grass alfalfa in DMY reached 55.3% (equal to the seed ratio in the mixture) when the soil pH was 5.0 and only 26.0% at the soil pH of 3.9.

In the 2nd year of sward formation, when the root system is being developed, the SOC content, C to N ratio, and SOC stock at 0–10 and 10–20 cm depth significantly depended on the soil pH index (Table 3).



TP – *Trifolium pratense*, TR – *Trifolium repens*, TH – *Trifolium hybridum*, MS – *Medicago sativa*

Figure 2. Botanical composition (%) of dry matter yield in the second (2015), third (2016), and fourth (2017) years of the temporary sward formation

In the soil with pH 5.0, the accumulation of SOC content is significantly greater compared to that in the naturally acidic soil. During two experimental years, at 0–10 cm depth of the soil with pH 5.0, the SOC content averagely increased by 2.6%; however, in the naturally acidic soil (pH 3.9) it decreased by 14.1%. The species composition of sward did not have any significant influence on this index.

The soil C to N ratio was significantly or by 16.7% and 23.1% lower in the soil with pH 3.9 compared to that with pH 5.0 at 0–10 and 10–20 cm depth, respectively. In the soil with pH 5.0, the C to N ratio ranged from 11 to 14 and was relevant for the SOC accumulation. On the average, in the soil with pH 5.0, SOC was accumulated by 30.1% and 40.8% more compared to that of the naturally acidic soil at 0–10 and 10–20 cm depth, respectively. A medium strong correlation between the pH index and carbon stock was estimated at 0–10 cm (r = 0.411**, $p < 0.01$) and 10–20 cm (r = 0.665**, $p < 0.01$) soil depth. In the 2nd and the 3rd year of age, morphological properties of the roots of most legumes are changing, so the conditioning functions of the ecosystem elements are related to the roots (Brock et al., 2000).

In the 3rd year of sward formation, irrespective of the soil acidity, the amount of red clover in DMY of the sward was averagely by 1.3 times and the amount of white clover by 2.0 times lower compared to those in the 2nd year of sward formation (Figure 2). Being more sensitive to the environmental conditions, especially to the soil humidity, the hybrid clover thinned out. In the soil with pH 5.0, the amount of hybrid clover decreased by 3.4 times and in the naturally acid soil by 4.2 times. In the 3rd year of sward formation, at the soil pH of 5.0, the alfalfa became denser later and reached 58.7% in the sward, i.e., 3.4 percentage units more than in the 2nd year of sward formation. However, in the naturally acid soil, the amount of alfalfa reached only 9.1%. A strong correlation (r = 0.873**; $p < 0.01$) was determined between the amount of alfalfa in DMY of the sward and soil acidity. The correlation analysis showed a weak dependence of the clover amount in DMY on the soil pH in the 2nd (r = 0.355*; $p < 0.05$ and in the 3rd (r = 0.333*; $p < 0.05$) year of age: the soil pH determined the amount of clover in DMY, 12.6% and 11.1%, respectively. The regression analysis makes the assumption that together with the pH index increase by 1 unit the amount of clover in DMY increases by 10.8–15.3% (Tomchuk, 2018).

In the 3rd year of sward formation, the SOC content remained significantly greater in the soil with pH 5.0. At the 0–10 cm soil depth, the SOC content of red and white clover-grass sward was determined substantially higher compared with that of other swards. In the naturally acidic soil, the SOC content was estimated to be averagely 21.0% lower in the soil depths studied

Table 3. Soil organic carbon (SOC) content, C to N ratio, microbial biomass carbon (MBC) content, and SOC stock at 0–10 and 10–20 cm depth in 2015–2017

Factors Soil pH / sward with	SOC %		C:N		MBC µg g^{-1} C		SOC stock t ha^{-1}	
	0–10	10–20	0–10	10–20	0–10	10–20	0–10	10–20
Perennials of the 2nd year of development (2015)								
pH 5.0								
Trifolium pratense	1.65 a	1.51 a	13 a	12 b	231 c	235 c	21.9 a	20.5 a
Trifolium repens	1.57 a	1.59 a	12 a	13 a	202 d	248 c	21.5 a	22.0 a
Trifolium hybridum	1.55 a	1.48 a	14 a	13 a	192 d	215 d	21.0 a	20.6 a
Medicago sativa	1.47 a	1.45 a	11b	14 a	160 e	175 e	20.2 a	19.7 b
pH 3.9								
Trifolium pratense	1.32 ab	1.16 b	11 b	11 b	324 a	398 ab	17.4 b	15.2 c
Trifolium repens	1.18 b	1.09 b	10 b	10 c	327 a	379 b	16.4 b	14.2 c
Trifolium hybridum	1.27ab	1.13 b	11 b	9 c	307 ab	403 a	16.9 b	15.2 c
Medicago sativa	1.12 b	1.10 b	9 b	10 c	283 b	340 c	14.5 c	14.3 c
Perennials of the 3rd year of development (2016)								
pH 5.0								
Trifolium pratense	1.69 a	1.55 a	11 ab	12 a	226 a	236 a	22.5 a	20.9 a
Trifolium repens	1.67 a	1.57 a	12 a	12 a	183 a	267 a	22.7 a	21.7 a
Trifolium hybridum	1.53 b	1.47 a	12 a	12 a	252 a	274 a	21.4 a	20.6 a
Medicago sativa	1.41 b	1.47 a	11 ab	11 ab	232 a	266 a	19.8 b	20.2 a
pH 3.9								
Trifolium pratense	1.28 c	1.22 b	10 b	9 b	180 a	231 a	17.4 cd	16.2 b
Trifolium repens	1.25 c	1.16 b	10 b	10 b	111 b	220 a	17.6 cd	15.9 b
Trifolium hibridum	1.22 c	1.19 b	10 b	10 b	273 a	269 a	16.4 d	16.0 b
Medicago sativa	1.26 c	1.25 b	10 b	11 ab	195 a	185 a	18.0 c	17.5 b
Perennials of the 4th year of development (2017)								
pH 5.0								
Trifolium pratense	1.48 a	1.31 a	11 a	10 b	302 b	274 b	18.8 a	17.4 b
Trifolium repens	1.46 a	1.40 a	10 a	12 a	349 a	283 ab	21.0 a	19.3 a
Trifolium hybridum	1.48 a	1.31 a	11 a	11 ab	326 ab	270 b	20.1 a	17.8 a
Medicago sativa	1.36 a	1.22 a	10 a	10 b	309 b	244 c	19.5 a	16.1 b
pH 3.9								
Trifolium pratense	1.47 a	1.20 b	11 a	10 b	322 ab	270 b	18.2 a	16.3 b
Trifolium repens	1.26 a	1.11 b	9 a	10 b	328 ab	295 ab	17.3 b	15.0 c
Trifolium hybridum	1.31 a	1.15 b	10 a	10 b	318 ab	289 ab	17.0 b	15.4 c
Medicago sativa	1.28 a	1.12 b	11 a	10 b	338 ab	307 a	18.2 a	16.0 b

Note. Different letters indicate significant ($p < 0.05$) differences between the means.

compared to that of the soil with pH 5.0. According to Repsiene and Karcauskiene (2016), in the limed soil, plants have more favourable conditions for their growth, so the SOC content significantly increases.

A significantly lower C to N ratio (9–11) at 0–20 cm depth was estimated in the soil with pH 3.9, while in the soil with pH 5.0, the C to N ratio reached 11–12 (Table 3). The C to N ratio evaluation showed that the nutrition conditions for the soil microorganisms were stable and favourable. Favourable meteorological conditions positively affected the growth of perennials determining a better growth of roots, vitality, initiating the use of soil resources, and accelerating soil organic matter mineralisation (Tomchuk, 2018).

In the soil with pH 5.0, SOC was accumulated significantly or by 24.1% and 26.8% more compared to that of the naturally acidic soil at 0–10 and 10–20 cm depth, respectively. In three experimental years, in the soil with pH 5.0, the SOC stock increased averagely from 19.9 to 21.6 t ha^{-1}. The species composition of the sward did not have any significant influence on the SOC stock. Medium strong and very strong correlations were determined between the soil pH index and SOC stock at 0–10 cm ($r = 0.642^{**}$; $p < 0.01$) and 10–20 cm ($r = 0.749^{**}$; $p < 0.01$) depth. Together with changing environment conditions soil carbon changes as well, until the balance between the introduction of organic residues into the soil and mineralisation is reached (McGranahan et al., 2014). Under the established and consistent plant cover, the ecosystem regulates the stabilisation even of carbon stocks in the soil (Kaisermann et al., 2018).

In the 4th year of sward formation, along the legume-grass sward successions (irrespective of the soil pH, the amount of red clover in the sward decreased, while white and hybrid clover as well as alfalfa increased), significant differences of the SOC content, C to N ratio, and SOC stock were determined only at 10–20 cm soil depth between the soils of different acidity. A decrease of the SOC content and SOC stock was observed (Table 3). Such decrease could be conditioned by the level of soil acidity which affects mineralisation processes and the amount of carbon that is used for the nutrition of microorganisms (Jokubauskaitė, 2016).

The plant development is genetically determined, and the properties of the soil are modified by processes occurring during plant growth. Particularly important changes are impacted by soil biological properties determining both the sward soil ecosystem stability and durability (Skuodienė et al., 2017). Decomposition of the organic residues to the stable SOC is a microbiological process, and it is influenced by the activity of microorganisms. The biomass and activity of soil microorganisms of agrarian origin depend on the ecotope (Rousk et al., 2009; Muraškienė et al., 2020), while the quality of organic matter is related to growing grass species because they directly influence the quality of organic residues (De Deyn et al., 2011).

Soil MBC content. As the soil pH index in the year of grass sowing more influenced the spread of sward root system (Skuodienė et al., 2017), this also influenced the changes of MBC content and the variety of microorganisms (Lauber et al., 2008). Along with the sward formation, in the year of sowing at 0–10 cm depth and the 2nd year of sward formation at 0–20 cm depth, the MBC content was determined to be significantly greater by 1.1 and 1.7 times, respectively, in the soil with pH 3.9 compared to that in the soil with pH 5.0. In the 1st and the 2nd year of sward formation, significant differences were determined between diverse legumes-grass swards. The greatest MBC stock at 0–20 cm soil depth was determined in red clover-grass and hybrid clover-grass swards. Not only growing agricultural plants but also microorganisms are influenced by soil liming (Muraškienė et al., 2020). In the 2nd year of sward formation, the MBC content increased by 1.7 times in the soil with pH 5.0 and by 2.0 times in the naturally acidic soil compared to the year of sowing (Tables 2 and 3). The MBC content in the sward root zone (at 0–20 cm soil depth) ranged averagely from 335 to 721 µg g^{-1} C. Maková et al. (2011) reported that the MBC content in swards may accumulate more than 258 and 460 µg g^{-1} C. The extent of microbial biomass had the tendency to be higher at 10–20 cm soil depth of plant rooting (in some extent, reaching from 175 to 403 µg g^{-1} C) (Table 3). However, it could be observed that the growth of microorganism biomass in soils indicates that the activity of microorganisms and possibilities for organic matter degradation are greater. Therefore, this indicator is also important in assessing the accumulation of organic matter in the soil (Tomchuk, 2018). In the 3rd year of sward formation, the correlation (r = 0.305–0.324*; $p < 0.05$) was determined between the SOC stock and MBC content.

Due to ecological sward changes, in the 3rd year of sward formation, the MBC content was determined to be 1.2 times greater in the soil with pH 5.0. In the 4th year of sward formation, the MBC content in different acidity soil was determined to be similar; however, it was greater by 21.5% and 48.0% compared to the 3rd year of sward formation in the soil with pH 5.0 and 3.9, respectively (Table 3). The correlation analysis indicated a strong negative correlation (r = −0.868**; $p < 0.01$) between the MBC content and soil pH at 0–20 cm depth.

It could be also indicated that during the sward formation (in our experiment, in the year of sowing and in the 2nd year of sward formation) the MBC content, first, is more effected by the soil pH (Rosenzweig et al., 2016). Thus, the soil pH is even directly controlling the microbial community diversity, richness, and activity (Chowdhury et al., 2011). However, during the following sward formation stages, sward grasses are producing greater root biomass and increasing with active soil organic matter contributing more to the SOC in the soil (Chen et al., 2016). This indicates that with the increase in sward grass root biomass the MBC pool increases (Pilon et al., 2013).

We hypothesised that succession in legume-grass swards changes their ecological characteristics. Literature indicates (Eilers et al., 2010) that the chemical composition of roots secretion and residues depends on the plants growing in swards, because plant species influence organic combinations that are emitted to the environment. Therefore, the conditions for microorganisms accompanying plants changed in the ecotope soil as well (Armolaitis et al., 2013). Usually, SOC changes can be observed after decades. The results of our experiment show that the SOC variations were not remarkable but related with sward formation within the swards succession time.

Conclusions

1. In the sowing years of sward ecosystem, there were no significant differences determined between the soils of different acidity, soil organic carbon (SOC) content, C to N ratio, and SOC stock. However, in the 2nd and the 3rd year of sward formation, the SOC content, C to N ratio, and SOC stock were significantly influenced by the soil pH. A significantly greater carbon accumulation was determined in the soil with pH 5.0. In two experimental years, the accumulation of SOC here increased by 8.5%. Due to the ecological fluctuation, in the 4th year of sward development, the SOC stock was determined to be similar as in the sward sowing year.

2. Soil acidity as well as the species composition of sward had a significant impact on the content of soil microbial biomass carbon (MBC) in the 1st and the 2nd year of sward formation. Starting from the 3rd year of sward development after changes in the botanical composition have started, the influence of the above-mentioned factors remained inconsiderable.

3. SOC variation depended on the activity of soil microorganisms. In the 2nd year of sward development, in the naturally acidic soil, the accumulation of soil MBC content increased together with decreasing the amount of SOC.

Acknowledgements

This work was supported by the Programme "Plant Biopotential and Quality for Multifunctional Practice and Sustainability of Agroecosystems" funded by LAMMC and by the Project "Are Mixed Species Systems Fostering Belowground C Inputs and C Sequestration?" (MIXROOT-C) that was funded by the European Union (Grant No. 862696).

Received 08 04 2022
Accepted 08 06 2022

References

Armolaitis K., Aleinikovienė J., Lubytė J., Žėkaitė V., Garbaravičius P. 2013. Stability of soil organic carbon in agro and forest ecosystems on *Arenosols*. Zemdirbyste-Agriculture, 100 (3): 227–234. https://doi.org/10.13080/z-a.2013.100.029

Atwell M. A., Wuddivira M. N. 2022. Soil organic carbon characterization in a tropical ecosystem under different land uses using proximal soil sensing technique. Archives of Agronomy and Soil Science, 68 (3): 297–310. https://doi.org/10.1080/03650340.2020.1831693

Bell M., Lawrence D. 2009. Soil carbon sequestration – myths and mysteries. Tropical Grasslands, 43 (4): 227–231.

Brock J. L., Albrecht K. A., Tilbrook J. C., Hay M. J. M. 2000. Morphology of white clover during development from seed to clonal populations in grazed pastures. Journal of Agricultural Science, 135 (2): 103–111. https://doi.org/10.1017/S0021859699008060

Chen S., Lin S., Reinsch T., Loges R., Hasler M., Taube F. 2016. Comparison of ingrowth core and sequential soil core methods for estimating belowground net primary production in grass-clover swards. Grass and Forage Science, 71 (3): 515–528. https://doi.org/10.1111/gfs.12214

Chowdhury N., Marschner P., Burns R. 2011. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil, 344 (1): 241–254. https://doi.org/10.1007/s11104-011-0743-9

Crème A., Rumpel C., Le Roux X., Romian A., Lan T., Chabbi A. 2018. Ley grassland under temperate climate had a legacy effect on soil organic matter quantity, biogeochemical signature and microbial activities. Soil Biology and Biochemistry, 122: 203–210. https://doi.org/10.1016/j.soilbio.2018.04.018

De Deyn G. B., Shiel R. S., Ostle N. J., McNamara N. P., Oakley S., Young I., Freeman C., Fenner N., Quirk H., Bardgett R. D. 2011. Additional carbon sequestration benefits of grassland diversity restoration. Journal of Applied Ecology, 48 (3): 600–608. https://doi.org/10.1111/j.1365-2664.2010.01925.x

Eilers K. G., Lauber C. L., Knight R., Fierer N. 2010. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. Soil Biology and Biochemistry, 42 (6): 896–903. https://doi.org/10.1016/j.soilbio.2010.02.003

Fan M., Margenot A. J., Zhang L., Lal R., Wu J., Chang N., Shaukat M., Chen F., Gao C. 2022. Soil organic carbon dynamics in intensively managed agricultural landscapes of eastern China. Archives of Agronomy and Soil Science, 68 (4): 503–515. https://doi.org/10.1080/03650340.2020.1842371

Guo L. B., Gifford R. M. 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology, 8 (4): 345–360. https://doi.org/10.1046/j.1354-1013.2002.00486.x

Hadas A., Kautsky L., Goek M., Kara E. E. 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biology and Biochemistry, 36: 255–266. https://doi.org/10.1016/j.soilbio.2003.09.012

Joergensen R. G., Wu J., Brookes P. C. 2011. Measuring soil microbial biomass using an automated procedure. Soil Biology and Biochemistry, 43 (5): 873–876. https://doi.org/10.1016/j.soilbio.2010.09.024

Jokubauskaitė I. 2016. Changes in dissolved and humified carbon in acid soil as influenced by different liming and fertilization systems: doctoral dissertation. Lithuanian Research Centre for Agriculture and Forestry, 155 p.

Jokubauskaite I., Slepetiene A., Karcauskiene D. 2014. Influence of different fertilization on the dissolved organic carbon, nitrogen and phosphorus accumulation in acid and limed soils. Eurasian Journal of Soil Science, 4 (2): 137–143. https://doi.org/10.18393/ejss.91434

Kaisermann A., Ogée J., Sauze J., Wohl S., Jones S. P., Gutierrez A., Wingate L. 2018. Disentangling the rates of carbonyl sulfide (COS) production and consumption and their dependency on soil properties across biomes and land use types. Atmospheric Chemistry and Physics, 18: 9425–9440. https://doi.org/10.5194/acp-18-9425-2018

Kemmitt S. J., Wright D., Goulding K. W., Jones D. L. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology and Biochemistry, 38 (5) :898–911. https://doi.org/10.1016/j.soilbio.2005.08.006

Lambers H., Mougel C., Jaillard B., Hinsinger P. 2009. Plant–microbe–soil interactions in the rhizosphere: an evolutionary perspective. Plant and Soil, 321 (1): 83–115. https://doi.org/10.1007/s11104-009-0042-x

Lauber C. L., Strickland M. S., Bradford M. A., Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry, 40 (9): 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021

Mačiulytė-Mikučionienė R. 2010. Integrated evaluation of the capacity and main properties of *Gleyic Luvisols* with different fertilization systems: doctoral dissertation. Lithuanian University of Agriculture, 83 p.

Maková J., Javoreková S., Medo J., Majerčík K. 2011. Characteristics of microbial biomass carbon and respiration activities in arable soil and pasture grassland soil. Journal of Central European Agriculture, 12 (4): 752–765. https://doi.org/10.5513/JCEA01/12.4.986

McGranahan D. A., Daigh A. L., Veenstra J. J., Engle D. M., Miller J. R., Debinski D. M. 2014. Connecting soil organic carbon and root biomass with land-use and vegetation in temperate grassland. The Scientific World Journal, 2014: 1–9. https://doi.org/10.1155/2014/487563

Muraškienė M., Aleinikovienė J., Skuodienė R., Tomchuk D., Armolaitis K. 2020. Soil respiration and microbial biomass peculiarities as influenced by different land use intensity. Zemdirbyste-Agriculture, 107 (1): 3–10. https://doi.org/10.13080/z-a.2020.107.001

Osoro K. 2014. Profitability of permanent grassland. EIP-AGRI Focus Group, 29 p.

Pilon R., Picon-Cochard C., Bloor J. M. G., Revaillot S., Kuhn E., Falcimagne R., Balandier P., Soussana J. F. 2013. Grassland root demography responses to multiple climate change drivers depend on root morphology. Plant and Soil, 364 (1): 395–408. https://doi.org/10.1007/s11104-012-1371-8

Rasmussen J., Eriksen J., Jensen E. S., Jensen H. H. 2010. Root size fractions of ryegrass and clover contribute differently to C and N inclusion in SOM. Biology and Fertility of Soils, 46 (3): 293–297. https://doi.org/10.1007/s00374-009-0430-7

Raudonius S. 2017. Application of statistics in plant and crop research: important issues. Zemdirbyste-Agriculture, 104 (4): 377–382. https://doi.org/10.13080/z-a.2017.104.048

Repsiene R., Karcauskiene D. 2016. Changes in the chemical properties of acid soil and aggregate stability in the whole profile under long-term management history. Acta Agriculturae Scandinavica, Section B: Soil and Plant Science, 66 (8): 671–676. https://doi.org/10.1080/09064710.2016.1200130

Rosenzweig S. T., Carson M. A., Baer S. G., Blair J. M. 2016. Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration. Applied Soil Ecology, 100: 186–194. https://doi.org/10.1016/j.apsoil.2016.01.001

Rousk J., Brookes P. C., Bååth E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology, 75 (6): 1589–1596. https://doi.org/10.1128/AEM.02775-08

Ryals R., Kaiser M., Torn M. S., Berhe A. A., Silver W. L. 2014. Impacts of organic matter amendments on carbon and nitrogen dynamics in grassland soils. Soil Biology and Biochemistry, 68: 52–61. https://doi.org/10.1016/j.soilbio.2013.09.011

Sanderman J., Baldock J., Hawke B., Macdonald L., Puccini A., Szarvas S. 2011. National Soil Carbon Research Programme: Field and Laboratory Methodologies. CSIRO Land and Water, Australia, 26 p.

Skinner R. H., Dell C. J. 2016. Yield and soil carbon sequestration in grazed pastures sown with two or five forage species. Crop Science, 56: 2035–2044. https://doi.org/10.2135/cropsci2015.11.0711

Skuodienė R., Tomchuk D., Aleinikovienė J. 2017. Plant root morphology and soil biological indicators under primary development of various swards. Acta Agriculture Scandinavica, Section B: Soil and Plant Science, 67 (5) :435–443. https://doi.org/10.1080/09064710.2017.1293724

Soussana J. F., Loiseau P., Vuichard N., Ceschia E., Balesdent J., Chevallier T., Arrouays D. 2004. Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management, 20 (2): 219–230. https://doi.org/10.1111/j.1475-2743.2004.tb00362.x

Stockmann U., Adams M. A., Crawford J. W. (…) Zimmermann M. 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agriculture, Ecosystems and Environment, 164: 80–99. https://doi.org/10.1016/j.agee.2012.10.001

Šlepetienė A., Šlepetys J., Kavoliutė F., Liaudanskienė I., Kadžiulienė Ž. 2007. Changes in carbon, nitrogen, phosphorus, and sulphur in West Samogitia natural and different agrarian land-use systems. Žemdirbyste-Agriculture, 94 (3): 90–99.

Šlepetienė A., Liaudanskienė I., Šlepetys J., Stukonis V., Jokubauskaitė I. 2013. Soil carbon, nitrogen and phosphorus distribution in grassland systems, important for landscape and environment. Journal of Environmental Engineering and Landscape Management, 21 (4): 263–272. https://doi.org/10.3846/16486897.2013.830973

Tomchuk D. 2018. Grassland belowground biomass and organic carbon accumulation in different terrain ecosystems: doctoral dissertation. Lithuanian Research Centre for Agriculture and Forestry, 132 p.

WRB. 2015. World reference base for soil resources. World Soil Resources Reports No. 106. FAO.

Žolyno formavimosi įtaka dirvožemio organinės anglies kaitai priklausomai nuo mikroorganizmų aktyvumo

R. Skuodienė[1], J. Aleinikovienė[1,2], D. Tomchuk[1], G. Šiaudinis[1]

[1]Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės instituto Vėžaičių filialas
[2]Vytauto Didžiojo universiteto Žemės ūkio akademija

Santrauka

Tyrimo tikslas – įvertinti trumpalaikio žolyno formavimosi įtaką dirvožemio mikroorganizmų biomasės anglies kiekiui rizosferoje ir organinės anglies kaitai. Tyrimas atliktas 2014–2017 m. Dirvožemis – natūraliai rūgštaus nepasotintojo balkšvažemio moreninis priemolis. Lygiame reljefe eksperimentas vykdytas skirtingo rūgštumo (pH 3,9 ir 5,0) dirvožemyje, kuriame auginti keturi pupinių ir miglinių – pašarinio motiejuko (*Phleum pratense* L.) 35 % ir pievinės miglės (*Poa pratensis* L.) 15 % – žolių mišiniai po 50 %: 1) raudonasis dobilas (*Trifolium pratense* L.), 2) baltasis dobilas (*Trifolium repens* L.), 3) rausvasis dobilas (*Trifolium h*ybridum L.) ir 4) mėlynžiedė liucerna (*Medicago sativa* L.). Skirtingų žolynų dirvožemyje organinė anglis labiau kaupėsi armens viršutiniame (0–10 cm) sluoksnyje. Žolynų rūšinė sudėtis tiriamiems rodikliams esminės įtakos neturėjo. Žolynų dirvožemyje, kurio pH 5,0, per trejus eksperimento metus organinės anglies kiekis padidėjo 3,3 %, organinės anglies sankaupos – 8,5 %, o C:N santykis buvo palankus organinės anglies kaupimuisi. Natūraliai rūgščiame (pH 3,9) dirvožemyje organinės anglies kiekis ir sankaupos sumažėjo atitinkamai 12,0 ir 3,3 %. Ketvirtaisiais žolynų formavimosi metais, nepriklausomai nuo dirvožemio rūgštumo, organinės anglies sankaupos sumažėjo, tačiau jų kiekis buvo panašus, kaip ir žolyno formavimosi pradžioje. Žolyno formavimasis turėjo esminės įtakos mikroorganizmų biomasės anglies kiekiui. Antraisiais žolyno formavimosi metais suminis mikroorganizmų biomasės anglies kiekis padidėjo 1,7 karto dirvožemyje, kurio pH 5,0, ir 2,0 kartus natūraliai rūgščiame dirvožemyje, palyginus su sėjos metais, ir išliko santykinai stabilus trečiaisiais bei ketvirtaisiais žolynų formavimosi metais, nepriklausomai nuo dirvožemio rūgštumo.

Reikšminiai žodžiai: anglies kaupimas, C ir N santykis, daugiametės žolės, mikroorganizmų biomasės anglis, žolynų vystymasis.