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Evaluation of soil organic carbon stability in grasslands of protected areas and arable lands applying chemo-destructive fractionation

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Abstract

The soils of Natura 2000 protected areas (*Endocalcari-Endohypergleyic Cambisols*, *Fluvi-Eutric Fluvisol* and *Endohypogleyi-Eutric Fluvisols*) and agrarian lands overgrown with grasses (*Endocalcari-Endohypogleyic Cambisols*) were investigated in this research in 2012. The soil organic carbon (SOC) concentrations decreased with the depth in all treatments, and the highest values were measured at 0–10 cm soil layer in pre-mainland section of middle reaches of the Nevėžis (76.8 g kg⁻¹) and in old semi-natural pasture (49.5 g kg⁻¹). The exclusive importance of the agrarian soil occupied with grasslands and long-lived swards and soils of protected areas occupied with natural meadows for environmental quality was revealed, because organic carbon was accumulated and sequestered in the form of stable compounds. Natural encroachment of wood pasture by forest plant communities in the northern part of Klampūtė wood pasture significantly increased SOC. The content of SOC was higher in all soil layers in wood pasture under restoration compared with surviving wood pasture. The carbon transformation processes in wood pastures differed depending on the depth: organic carbon was more stabilized in the 10–20 and 20–30 cm layers in the surviving wood pasture than that in wood pasture under restoration. It was established that the most intensive transformation and accumulation of organic carbon take place at 0–10 cm soil layer. The soils of protected areas and agricultural lands were characterized by differences in organic carbon stability. After applying the method of chemo-destructive fractionation the largest amount of residual organic carbon, which represents the resistance to degradation and the possibility to sequestration, was established in the soils under long-term use of grassland (pre-mainland section and old semi-natural pasture). The most unfavourable status of SOC, and thus all the soil organic matter (SOM), is observed in the riverside section. The labile carbon was dominant there, and the content of organic carbon resistant to chemo-destruction was very low in all soil layers.

Key words: labile carbon, Natura 2000, organic carbon, protected areas, residual carbon.

Introduction

Soil, which is a complex and continuously developing part of many ecosystems, including grassland, plays an especially important role in the protection of natural environment and use of its resources. The main functions of the soil – biomass production and biodiversity, source of raw materials, storing, filtering and transforming nutrients, substances and water, physical and cultural environment for humans – are directly dependent on carbon pool. The establishment of the Natura 2000 Network is one of the main actions undertaken at the European level to contribute to the maintenance of biodiversity (Bartula et al., 2011). In Northern Europe, traditional management of forests has frequently been connected with hay-making, therefore Fennoscandian wood pastures and Baltic meadows have a habitat tradition of 4,000 years and more, and such land-use systems have been part of the cultural history throughout Europe from prehistoric to present times (Bergmeier et al., 2010). This type of land-use

always involves grazing animals and trees or shrubs, and sometimes grass cutting.

One of the major sources of soil organic carbon (SOC) is plant residues, the highest content of which is left in the soil by perennial grasses, especially legumes (Smith, 2008). Grassland soils contain significant amounts of carbon because grasses transfer a large proportion of their products of photosynthesis belowground (Baker et al., 2007). Land conversion from grassland or forest to cropland resulted in rapid loss of soil carbon, which indirectly enhances global warming.

Soil organic matter (SOM), the main constituent of which is organic carbon, has been increasingly considered as one of the components of biosphere sustainability and stability (Šlepetiene, Šlepetys, 2005; Smith, 2008). SOM is derived from a complicated mixture of fresh organic materials from plants, soil fauna, root exudates, microbial residues and chemically or physically protected substrates, which generally

consist of labile and recalcitrant pools (von Lützow et al., 2007). To be sequestered, SOC should be converted from the active pools to less reactive intermediate or passive pools, and, consequently, sustained in the soil for decades or longer (Silveira et al., 2008). As SOM is a heterogeneous mixture of organic substances with different composition and lability (turnover time), it has been convenient to partition the organic carbon content of a soil into different pools (Chan et al., 2002): a) labile SOM – a quickly reactive labile organic matter, which provides energy and nutrients for soil microorganisms and releases part of the nutrients for plant usage; its half-life is between days and few years; b) stable SOM – a reservoir of less decomposable organic matter. The main and the most important function of this pool is its cation-exchange capacity; its half-life is between years and decades; c) inert SOM – an almost non-reactive organic matter which affects the physical properties of the soil; this pool is physico-chemically protected against decomposition; its half-life is between decades and centuries (Six et al., 2002).

Total SOC is not a useful indicator for monitoring purposes, where the changes in land-use are not drastic. In the last decades, more attention has been paid to the SOC of various lability (Schulz, 2004; Kolář et al., 2009), which has been acknowledged as a good indicator of soil quality and environmental health (Strosser, 2010). Most conventional methods used in total SOC determination have been developed to maximize oxidation and recovery of carbon. Carbon distribution in labile or stable SOM fractions could have implications in changes of physical, chemical, and biological soil properties (Guimarães et al., 2013). Thus, in order to identify in which SOC fractions the organic carbon is accumulated, Popov and Tcyplenkov (1994) modified the SOM oxidation method, dividing SOM into 11 pools with different degrees of carbon oxidizability, and called the method “chemo-destructive fractionation”. Chan et al. (2001) and Strosser (2010) have proposed a similar method by which SOM is divided into four fractions, and called the method “sequential oxidation”. The compounds that are part of the labile SOC fraction are biochemically active; they are related to material and energy transformations in the soil, as well as appreciably affect soil fertility (Popov et al., 2004).

Lithuanian researchers have extensively investigated the influence of tillage, fertilization and cultivation of various crops on SOM and humus (Slepetiene, Slepetys, 2005; Tripolskaja et al., 2012). In line with the new trends in global research, since increasingly more investigations and quantitative determinations of carbon flows are performed in order to reduce the negative impact of human activity on the environment, more attention in research has been given to carbon compounds transformations in agricultural soils (Liaudanskienė et al., 2011). Agrarian and forest soils are comprehensively investigated in Lithuania, but there is no detailed comparison with the soils of protected areas.

We hypothesize that differences in the amount and type of the organic matter inputs, soil orders and land-uses affect the stability of SOC. Our research attempts to investigate and to compare the changes of oxidizable soil

carbon fractions in different natural (protected areas) and agrarian ecosystems. The obtained data will be important both theoretically and practically for the assessment of carbon accumulation in the soils. The main task of this research was to investigate the distribution of various SOC fractions with different oxidizable ability in the grassland soils of protected areas and agricultural lands in Central Lithuania.

Materials and methods

Details of experimental site and soil sampling.

The soils of Natura 2000 protected areas and Krekenava Regional Park (reserve of middle reaches of the Nevėžis), and also agrarian lands overgrown with grasses were investigated in this research. A detailed description of sites is given in Table. Soil samples were taken in three replications in each investigated site in 2012. Six sub-samples per plot were taken randomly by using a steel auger. Each soil sample core was separated into 0–10, 10–20 and 20–30 cm depth, and combined across sub-samples by depth for each plot. The vegetation was recorded using the Braun-Blanquet scale (Braun-Blanquet et al., 1965). Plant communities were evaluated according to the national classification (Balevičienė et al., 1998). Latin names of plants are provided in accordance with botanical names' digest (Gudžinskas, 1999). The soils were named on the basis of soil taxonomical classification (Lietuvos dirvožemiai, 2001). The exact details of the investigated soil pH_{KCl} and other parameters in different layers are available in Slepetienė et al. (2013).

EU's network of protected areas Natura 2000 was developed to preserve and restore habitats of European importance under natural habitats, wild flora and fauna Council Directive 92/43/EEC (1992). One of these habitats is Fenoscandian wood pastures 9070, which is rapidly disappearing throughout Lithuania. Forests with fragments of natural meadows in Klampūtė area are in the Nevėžis C XI moraine plain according to physical geographical classification of Lithuania, and occupy an area of 12 hectares. According to the current state, the wood pasture area was divided into two parts: northern and southern. The southern part of the area has preserved the vegetation structure and species composition typical of the Fennoscandian wood pastures. Single old oak (*Quercus robur* L.) trees, large hawthorn (*Crataegus* spp.) shrubs, including intrusive herbaceous plant communities grow there. The northern part of the area is overgrown with a dense forest, which has replaced the previous Fennoscandian wood pastures. The meadow herbaceous plants have been superseded by the species inherent to forest plant communities. The fact that this area had been used as a pasture before can be judged by the deployment of the isolated old trees (*Quercus robur* L., *Tilia cordata* Mill., *Fraxinus excelsior* L.). Tree crowns were matted, and growing stock closeness was 95% against the clearance. *Picea abies* (L.) H. Karst. is quite widespread here. Plants specific to acidic soils and tolerant of shade can grow in this area. These are *Oxalis acetosella* L., mosses *Eurhynchium angustirete* (Broth.) T. J. Kop., *Pleurozium schreberi* (Brid.) Mitt. and others, calcium-loving plants are almost nonexistent.

It will be interesting to observe the changes taking place in vegetation and soil pH after introduction of livestock grazing in the area, since young trees and firs non-specific to Fenoscandian wood pasture habitats had been cut down. Only old trees were left, mostly *Quercus robur* L., *Fraxinus excelsior* L., *Ulmus minor* Mill., *Tilia cordata* Mill. It is likely that due to the soil properties and other edaphic conditions, *Quercus robur*, *Malus sylvestris* (L.) Mill. and old shrubs have survived in the northern part of Klampūtė: *Crategus rhipidophylla* Gand., *Coryllus avellana* L. and *Euonymus europaeus* L. The coverage with trees and shrubs accounted for 40% before the start

of wood pasture restoration. Meadow flora was abundant in the glades with many herbaceous plant species, including *Prunella vulgaris* L., *Ranunculus polyanthemos* L., *Succisa pratensis* Moench, *Veronica chamaedrys* L., *V. officinalis* L., *Alchemilla* L., *Dactylis glomerata* L., *Geum rivale* L., *Agrostis capillaris* L., *Agrimonia eupatoria* L., *Galium boreale* L., *G. mollugo* L., *G. album* Mill., *Phleum pratense* L. var. *nodosum* (L.) Huds., *Pimpinella major* (L.) Huds., *Filipendula vulgaris* Moench, *Briza media* L., *Trifolium montanum* L. A total of 69 species were growing in the meadows (Table).

Table. Site characteristics, Central Lithuania, 2012

Site	Soil	Vegetation type
Protected area of Natura 2000, Klampūtė, Kėdainiai district		
Surviving wood pasture, the southern part (55°17'39.85" N, 23°53'18.37" E, 53 m a.s.l.)	<i>Endocalcari-Endohypergleyic Cambisol</i> (CMg-n-h-can)	The typical structure and species composition of Fenoscandian wooded pastures 9070 has survived: <i>Quercus robur</i> L., <i>Fraxinus excelsior</i> L., <i>Tilia cordata</i> Mill., <i>Prunella vulgaris</i> L., <i>Ranunculus polyanthemos</i> L., <i>Succisa pratensis</i> Moench, <i>Veronica chamaedrys</i> L., <i>V. officinalis</i> L.
Wood pasture under restoration, the northern part (55°17'42.16" N, 23°53'20.80" E, 53 m a.s.l.)	<i>Endocalcari-Endohypergleyic Cambisol</i> (CMg-n-h-can)	The mixed forest vegetation had established before clearing. Few meadow plant species have survived. Tree crowns were matted and closeness of trees reached 95%. <i>Picea abies</i> (L.) H. Karst. species was abundant here.
Old semi-natural Valinava pasture, Kėdainiai district (55°22'48.66" N, 23°51'58.42" E, 60 m a.s.l.)	<i>Endocalcari-Endohypogleyic Cambisol</i> (CMg-n-w-can)	Currently predominant species: <i>Festuca rubra</i> L., <i>Dactylis glomerata</i> L., <i>Phleum pratense</i> L., <i>Alopecurus pratensis</i> L., <i>Elytrigia repens</i> (L.) Desv. ex Nevski, <i>Taraxachum officinale</i> F. H. Wigg., etc.
Sown long-lived sward fodder galega, Akademija, Kėdainiai district (55°23'57.15" N, 23°51'54.69" E, 64 m a.s.l.)	<i>Endocalcari-Endohypogleyic Cambisol</i> (CMg-n-w-can)	Although pure galega was initially sown, <i>Dactylis glomerata</i> L., <i>Festuca pratensis</i> Huds., <i>Taraxachum officinale</i> have become established.
Protected area of middle reaches of the Nevėžis, the floodplain near Dembava, Panevėžys district. This part of Krekenava Regional Park is already included in the list Natura 2000.		
Pre-mainland section (55°29'01.63" N, 24°03'25.05" E, 31 m a.s.l.)	<i>Fluvi-Eutric Fluvisol</i> (Fle-fv)	<i>Molinia</i> meadows 6410 were formed on calcareous, peaty or clayey silt-laden soil with typical flora: <i>Molinia caerulea</i> (L.) Moench, <i>Selinum carvifolia</i> L., <i>Inula salicina</i> L., <i>Potentilla erecta</i> (L.) Rausch., <i>Ophioglossum vulgatum</i> L.
Central section, meadow (55°28'59.43" N, 24°03'28.54" E, 31 m a.s.l.)	<i>Endohypogleyic-Eutric Fluvisol</i> (Fle-gln-w)	Formed semi-natural dry grasslands 6210 and scrubland facies on calcareous substrates with typical species <i>Fragaria viridis</i> Duchesne, <i>Filipendula vulgaris</i> Moench, <i>Medicago falcata</i> L.
Central section, former arable land (55°28'59.43" N, 24°03'28.54" E, 31 m a.s.l.)	<i>Endohypogleyic-Eutric Fluvisol</i> (Fle-gln-w)	Steppe grasslands begin to form and there is only narrow spectrum of the species typical of them. Almost mono-dominant overgrowths of <i>Phleum phleoides</i> (L.) H. Karst., <i>Tanacetum vulgare</i> L. predominated, and only single indicators of the following grasslands are observed. Neophyte, such as <i>Oenothera biennis</i> L. <i>rubricaulis</i> Klebe, <i>Silene tatarica</i> (L.) Pers., etc. settled.
Riverside section (55°28'57.65" N, 24°03'37.59" E, 31 m a.s.l.)	<i>Endohypogleyic-Eutric Fluvisol</i> (Fle-gln-w)	Semi-natural dry grasslands 6210 and scrubland facies are forming on calcareous substrates with typical species <i>Fragaria viridis</i> Duchesne, <i>Filipendula vulgaris</i> Moench, <i>Medicago falcata</i> L. Unlike in central part, species specific to sands occur here: <i>Sedum acre</i> L., <i>Festuca trachyphylla</i> (Hack.) <i>Krajina</i> , <i>Thymus pulegioides</i> L.

After World War II, the wood pastures were rapidly disappearing because of the ban on grazing in forests and large-scale land reclamation. Klampūtė area is included in the wood pastures remediation plan because its individual fragments corresponded to the structure inherent to this habitat. In 2011, restoration of the habitat

in Klampūtė area was launched. Young trees and those non-specific to the habitat were cut down during the winter. The grass was mown in 2012. It is planned to acquire cattle which will be grazed on the restored wood pastures in the future. Restoration success will depend on their rational use. The sward in Valinava pasture was

sown in 1946. The pasture sward has not been renovated up to the present day in the paddock occupying an area of 2.1 ha, where the soil samples were taken. This is one of the oldest pastures in Lithuania. Currently, medium early sward with white clover is formed there. This sward contains a small portion of early maturity grass *Alopecurus pratensis*. For many years, the sward had been used for grazing and forage, but currently it only cut for silage making, since there are no grazing cattle there.

Fodder galega is grown in organic crop rotation, in certified area. Estonian variety 'Gale' was sown at a seed rate of 30 kg ha⁻¹ with a cover crop of spring barley in 2001. Fodder galega did not receive any fertilization, and no chemicals were used. For the first five years, when the crop was used for forage, fodder galega was cut twice per season: the first cut was taken at the beginning of mass flowering (end of May – beginning of June), and the second cut was taken at the end of vegetation (end of October). Over the last seven years galega crop occupying an area of 1.1 ha has been used for seed.

The landscape reserve of the middle reaches of the Nevėžis, occupying an area of 366 ha, is part of Krekenava Regional Park, and is already included in the list of Natura 2000. The soil samples were taken in four representative places in the alluvial grassland with an area of 4.2 ha near Dembava, Panevėžys district. The vegetation typical of natural grassland has survived there. We attempted to relate soil chemical parameters to the flora and vegetation composition in our investigation. The Cl. *Molinio-Arrhenatheretea elatioris* R. Tx. 1937 and Ass. *Molinietum caeruleae* W. Koch 1926 communities (*Molinia caerulea* (L.) Moench, *Selinum carvifolia* (L.) L., *Inula salicina* L., *Potentilla erecta* (L.) Rausch., *Ophioglossum vulgatum* L. (Table) were formed in the lower part of pre-mainland section, where one soil sample was taken. Two soil samples were taken in the central section of floodplain of the Nevėžis (meadow and former arable land). Water floods the central section of the floodplain irregularly. The following steppe grassland communities have formed there: Cl. *Festuco-Brometea erecti* ass. *Pulsatillo-Phleotum phleoidis* Passarge 1959 (Balevičienė et al., 1998). These communities are more common in warm areas and calcareous soils. The soil pH in the respective soil layers, in both meadow and former arable land was very similar due to deep ploughing of the light textured soil (Šlepetienė et al., 2013). Steppe grasslands begin to form, *Phleum phleoides* (L.) H. Karst and tansy *Tanacetum vulgare* L. overgrowths predominate there. Neophytes such as *Oenothera biennis* L., *O. rubricaulis* Kleb., and *Silene tatarica* (L.) Pers., etc. have become established. The plant communities of sands previously prevailed in the riverside section, but the steppe grassland vegetation has already formed there. Only some species from the former sands' vegetation have survived: *Festuca trachyphylla* (Hack.) *Krajina*, *Thymus pulegioides*, *Sedum acre* L.

Methods of analyses. All samples were air-dried, visible roots and plant residues were manually removed. Then the samples were crushed, sieved through a 2-mm sieve and homogeneously mixed. For the soil organic carbon (SOC) determination and chemo-destructive fractionation an aliquot of the soil samples

was passed through a 0.25-mm sieve. SOC content was determined by dry combustion (Dumas) method using a fully automatic analyzer Vario EL III ("Elementar", Germany).

Chemo-destructive fractionation is classified as physico-chemical method, and based on different resistance of SOC to the action of oxidant in the form of solutions with the identical concentration of oxidizer K₂Cr₂O₇, but with growing oxidizing ability. Oxidizing ability of these solutions depends on the concentration of H⁺, provided with the different amount of H₂SO₄. The method allows determining up to 11 SOC pools differing in resistance to oxidation (Popov, Tcyplenkov, 1994). As this method is insufficiently elucidated in literature, we are presenting a detailed description of the procedure of chemo-destruction of SOC. Contents equal by weight (250 ± 0.1 mg) of investigated soil sample are placed into 11 heat-resistant flasks (volume of 50–100 mL); then all flasks are poured by 5 mL 0.8 N water solution of K₂Cr₂O₇. Whereupon 5 mL of distilled water is added to the 1st flask, 5 mL of 10% solution of H₂SO₄ – to the 2nd flask, 5 mL of 20% solution of H₂SO₄ – to the 3rd flask and so on – consistently different flasks are poured by solutions of H₂SO₄ with a concentration increase by 10%, and finally 5 mL of concentrated H₂SO₄ – into the 11th last flask. All flasks are placed in a thermostat heated to 150°C and maintained for 20 minutes, starting from the moment when the temperature again reaches 150°C. The amount of oxidized SOC was determined by a photometric procedure at the wavelength of 590 nm using the spectrophotometer Cary 50 ("Varian", Germany) equipped with software, and glucose as a standard (by intensity of the green colouring, predefined by Cr³⁺). It is convenient to interpret the results of carbon measurements presented in differential form. Therefore the difference between the current (higher oxidizing ability) and the previous (lower oxidizing ability) determination is calculated, starting from the 2nd pool. Analyzing the quantitative – qualitative distribution of SOM by intensity of chemo-destruction, Popov et al. (2004) combined the isolated 11 pools of SOC into three fractions: labile fraction F1 (1–4 pools), moderately labile fraction F2 (5–7 pools), and stable fraction F3 (8–11 pools). The fourth (F4) residual fraction of organic carbon is defined as the difference between the total SOC determined by dry combustion method and organic carbon after reaction with 98% H₂SO₄.

Statistical analysis. Experimental data of SOC chemical analyses were processed ($P < 0.05$) by the statistical programme *STAT ENG* for *Excel* version 1.55 (Tarakanovas, Raudonius 2003). Each variable ($n = 3$) was displayed as mean ± standard error of the mean.

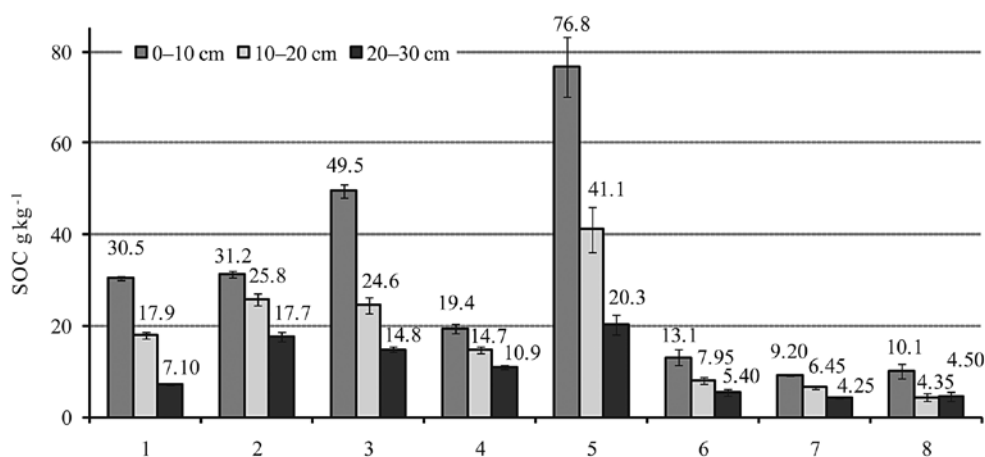
Results and discussion

In Lithuania, like in other countries, there is little research on the preservation processes of organic carbon, the key element of organic matter in differently-aged protected areas and no comparison with agrarian land. Changes in SOC content can be used as an indicator of changes in the ability of soils to maintain yields and other functions (King et al., 2005). Our findings showed that quantitative distribution of SOC accumulated in separate

layers of soils of protected areas and agricultural lands was very different (Fig. 1). The upper (0–10 cm) soil layer was obviously the richest in SOC in all investigated treatments. The largest amounts of SOC were established in the soil of meadow of pre-mainland section of the Nevėžis. The peat-formation processes had already started there, and SOC content in upper soil layer was more than 4-fold higher than that in arable land under long-lived fodder galega, and also higher compared with old semi-natural pasture and wood pastures. SOC content in soil of wood pasture under restoration was higher in all soil layers compared with surviving wood pasture: 31.2 and 30.5 g kg⁻¹ in the 0–10 cm layer, 25.8 and 17.9 g kg⁻¹ in the 10–20 cm layer, 17.7 and 7.1 g kg⁻¹ in the 20–30 cm layer, respectively. Natural encroachment of forest plant communities to wood pasture in northern part of Klamputė significantly increased SOC content, especially in the deeper soil layers. One of the possible explanations for this is that the residues and roots of grasses decompose

faster than residues and roots of broadleaf trees, likely due to the lower lignin found in grasses (Pinno, Wilson, 2011). In addition, the soil was more humid and more acidic in the area which was prevailed by forest plants (Šlepetienė et al., 2013), therefore the conditions were unfavourable for complete mineralization of organic residues.

The type and diversity of plant species in grasslands play an important role for carbon transfer into the soil (Steinbeiss et al., 2008). Significantly higher SOC amount was determined in 0–10 cm soil layer in the old semi-natural pasture (49.5 g kg⁻¹), compared with long-lived fodder galega sward (19.4 g kg⁻¹), and this is the result of a higher input of organic residues, especially from the belowground grass root biomass. Herbaceous plants have a well-developed root system, which is densely distributed in topsoil, and fodder galega has a deep root system. With the depth, the difference in SOC between the following treatments decreased.



Notes. 1) surviving wood pasture, southern part of Klamputė; 2) wood pasture under restoration, northern part of Klamputė; 3) old semi-natural pasture; 4) sown long-lived fodder galega sward; 5) pre-mainland section of middle reaches of the Nevėžis; 6) central section of middle reaches of the Nevėžis, meadow; 7) central section of middle reaches of the Nevėžis, former arable land; 8) riverside section of middle reaches of the Nevėžis. Vertical bars indicate standard error of means, n = 3.

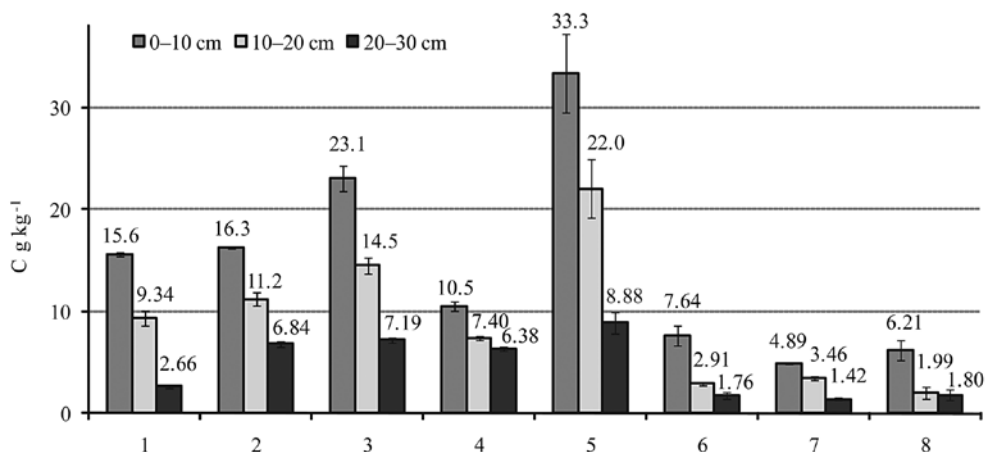
Figure 1. Soil organic carbon (SOC) content in different (0–10, 10–20 and 20–30 cm) soil layers, 2012

It is evident that quantities of SOC accumulated under different land-uses are different. In order to identify in which carbon fraction SOC is accumulated, we proposed SOC fractionating according to Popov and Tcyplenkov (1994).

The fractions of organic carbon extracted under a gradient in oxidizing conditions were different among the different treatments (Figs 2–5). When SOC content is high, the proportion of readily oxidized carbon fraction is also high in the soil (Figs 1 and 2). Significant differences in the carbon content of most readily oxidizable SOC fraction were found among the investigated treatments in the upper soil layer (Fig. 2). Labile carbon content varied from 33.3 g kg⁻¹ in pre-mainland section of the Nevėžis to 4.89 g kg⁻¹ in the central section of former arable land. In the soils of two parts of wood pasture, labile carbon content was similar (15.6 and 16.3 g kg⁻¹), but about a third less than that in old semi-natural pasture (23.1 g kg⁻¹). The amount of labile carbon in the soil of long-lived fodder galega was significantly lower than that in soils of native grasslands of pre-mainland section and

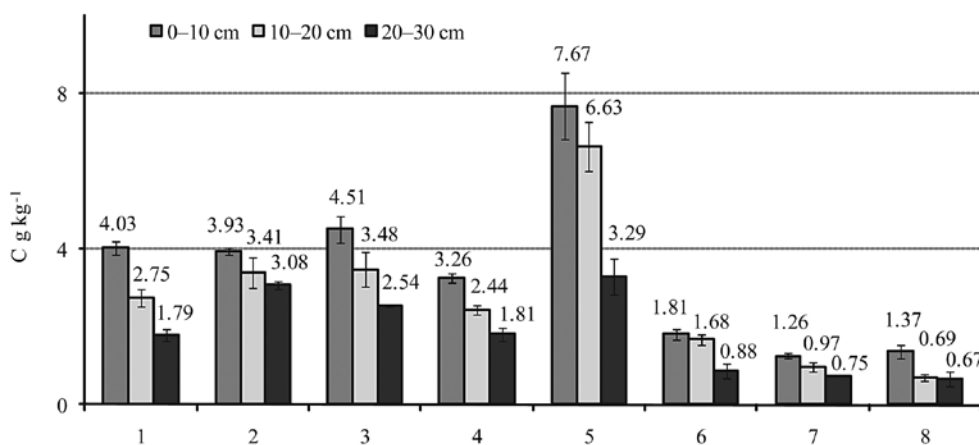
wood pasture. This agrees with the findings reported by Cardelli et al. (2012), who found a decrease in the content of very active organic carbon fraction in cultivated soils, relative to undisturbed areas. Carbon of this fraction is linked to supply of organic residues in the soil (Chan et al., 2001), which explains greater differences of this fraction between treatments. However, the magnitude of the differences decreased with depth. The part of labile carbon (F1 fraction) in SOC was between 43% and 61% in 0–10 cm soil layer, between 36% and 58% in 10–20 cm layer, and between 32% and 58% in 20–30 cm layer. In all treatments, except for long-lived fodder galega, part of labile carbon in SOC decreased with depth.

In Figure 3, we can see the same trend as in Figure 2: the treatment with higher content of SOC has a higher carbon content of moderately labile SOC fraction (F2), but F2 carbon content in all treatments was from 3.3 to 5 times lower in comparison with F1 carbon content. The highest F2 carbon content in 0–10 cm layer was determined in soil of pre-mainland section (7.67 g kg⁻¹), and the lowest – in the central section of former arable



Explanations under Figure 1

Figure 2. Carbon (C) content of labile soil organic carbon (SOC) fraction (F1) in different (0–10, 10–20 and 20–30 cm) soil layers, 2012



Explanations under Figure 1

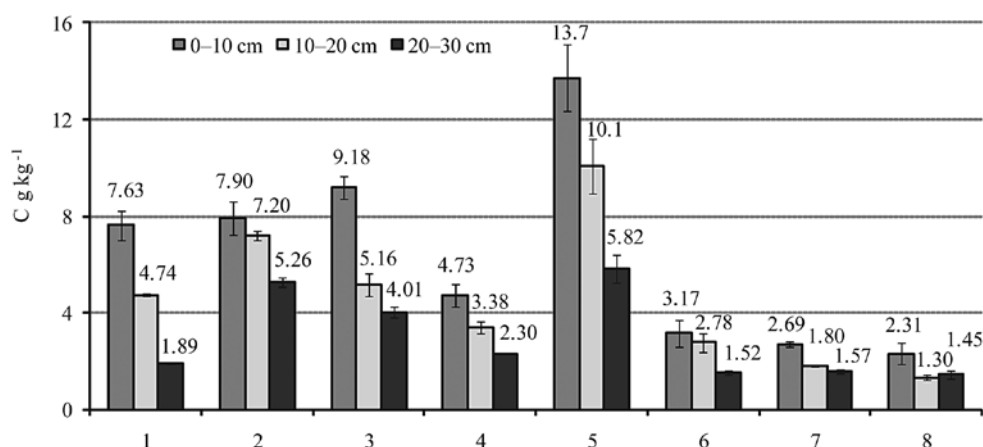
Figure 3. Carbon (C) content of moderately labile soil organic carbon (SOC) fraction (F2) in different (0–10, 10–20 and 20–30 cm) soil layers, 2012

land (1.26 g kg⁻¹). Among the moderately labile carbon contents in two parts of wood pasture (4.03 and 3.93 g kg⁻¹), old semi-natural pasture (4.51 g kg⁻¹), and long-lived fodder galega (3.26 g kg⁻¹) the differences were not very big. The part of moderately labile carbon in SOC was between 9% and 16% in 0–10 cm soil layer, between 13% and 21% in 10–20 cm layer, and between 15% and 25% in 20–30 cm layer. In long-lived fodder galega treatment the part of moderately labile carbon in SOC was 16.6–16.8% at all depths; in other treatments the part of moderately labile carbon increased consistently with depth.

The most obvious differences of stable fraction (F3) carbon content between the investigated treatments were identified at 0–10 cm depth, and the differences decreased with depth (Fig. 4). The difference between the stable carbon contents in surviving wood pasture and wood pasture under restoration at 0–10 cm depth are slight. However, stable carbon contents in the soil of wood pasture under restoration in 10–20 and 20–30 cm layers were 7.20 and 5.26 g kg⁻¹, compared with 4.74 and 1.89 g kg⁻¹ in surviving wood pasture. Natural encroachment of woody species helped to create

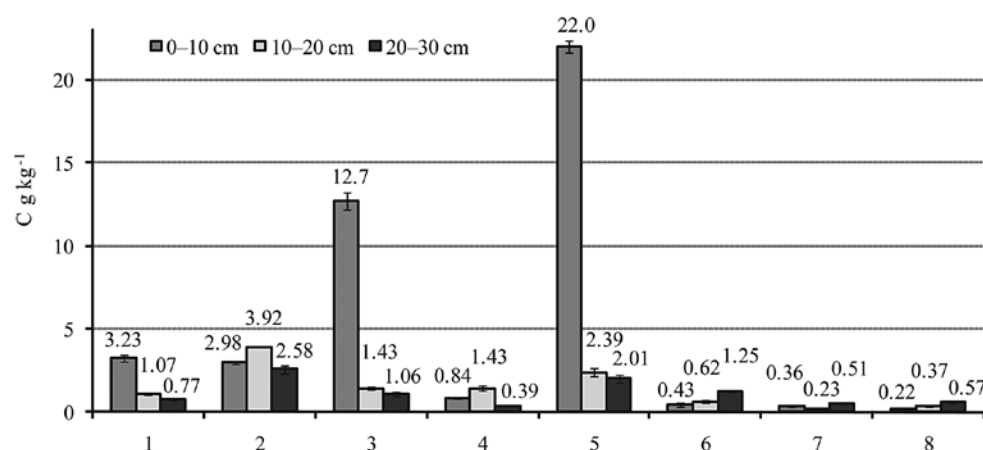
conditions (amount and type of plant residues, soil pH) for stabilization of organic carbon in deeper soil layers. For many plants as much as 30–50% of the carbon fixed in photosynthesis is initially trans-located belowground. Evidence indicates that belowground plant carbon is major source for subsequent conversion into more stable forms of SOC. Our data support this proposition because the stable carbon content in all soil layers of old semi-natural pasture was more than 1.5 times higher compared to the long-lived fodder galega. The part of stable carbon in SOC was between 17% and 29% in 0–10 cm soil layer, between 23% and 30% in 10–20 cm layer, and between 21% and 36% in 20–30 cm layer. In long-lived fodder galega treatment the part of stable carbon in SOC decreased from 24% in upper layer to 21% in 20–30 cm layer; in other treatments the part of stable carbon increased consistently with depth.

As with SOC and previously discussed soil carbon fractions, the residual carbon fraction (F4) also showed large variations between treatments (Fig. 5). The residual carbon content in 0–10 cm layer in soils of wood pastures, old semi-natural pasture and long-lived fodder



Explanations under Figure 1

Figure 4. Carbon (C) content of stable soil organic carbon (SOC) fraction (F3) in different (0–10, 10–20 and 20–30 cm) soil layers, 2012



Explanations under Figure 1

Figure 5. Carbon (C) content of residual soil organic carbon (SOC) fraction (F4) in different (0–10, 10–20 and 20–30 cm) soil layers, 2012

galega was significantly lower than in pre-mainland section, and in other layers the distribution varied. In wood pasture under restoration and long-lived fodder galega treatments, the highest content of residual carbon was determined in 10–20 cm layer. In soil of surviving wood pasture and old semi-natural pasture residual carbon content decreased with depth. The opposite trend was observed in soils of central and riverside sections of the middle reaches of the Nevėžis, where the residual carbon content increased with depth, but it was very low in all soil layers.

The part of residual carbon in SOC was between 2% and 28% in 0–10 cm soil layer, between 3.5% and 15% in 10–20 cm layer, and between 3.5% and 23% in 20–30 cm layer. According to the research data presented in Figure 5, the highest concentration of organic carbon protected by physico-chemically and biochemically mechanisms was found in soil at 0–10 cm layer of pre-mainland section – 22 g kg⁻¹. This accounted for 26.8% of SOC. The residual carbon content in the upper 0–10 cm layer of old semi-natural pasture was 12.7 g kg⁻¹, which

accounted for 25.6% of SOC. This suggests that in the soil occupied with long-lived grassland not only SOM accumulation due to continued persistent high input of organic matter took place but also the transformation of carbon compounds in the direction of stable and inert carbon pool increase because of the favourable conditions for its stabilization and transformation. The SOC exists as a continuum in soil from labile to strongly stabilized forms. The used method of chemo-destruction makes it possible to distinguish between active and passive SOC fractions. Our investigation of SOC pools in the soils of protected areas of Natura 2000 and agrarian lands indicated significant heterogeneity in the chemical lability between the treatments. Six et al. (2002) found that soil texture was important for organic carbon stabilization and accumulation in the soils. The soil of pre-mainland section of middle reaches of the Nevėžis stands out by a very high both SOC and different lability fractions carbon content among all the treatments. It could be explained by the fact that a lot of silt was banked up from the slopes to this section due to erosion and the lightest silt particles

settled here. Moreover, the pre-mainland section of river floodplain is often springy. Eventually, the process of neutral or weakly acidic peat formation can begin due to excess humidity (Gipiškis et al., 2007).

Differences in plant communities lead to alterations in soil microclimate, quality and quantity of carbon input, and the content, transformation and decomposition of organic carbon. The higher plant diversity in natural meadow of pre-mainland section leads to larger plant biomass, both aboveground and belowground (Davidson, Janssens, 2006), and therefore a larger biomass input into the soil compared with wood pastures, old semi-natural pasture, and long-lived fodder galega sward. After microorganisms decompose biomass, one part of released carbon is accumulated by silt particles in the upper soil layer, and the other part of released carbon is transported into soil solution. Water carries dissolved organic carbon to deeper soil horizons, where it is preserved from complete mineralization. The carbon transformation processes in wood pastures differed depending on the depth: organic carbon was more stable in the 10–20 and 20–30 cm layers in the surviving wood pasture than that in wood pasture under restoration.

The soil of riverside section of middle reaches of the Nevėžis stood out by a very low SOC content. Heavy coarse sediments such as leaching sand and sandy loam settle here. The following sands had a little of light silt particles, therefore they could not protect organic carbon from mineralization or from leaching into the deeper layers. The soils of protected areas and grasslands in Central Lithuania were characterized by differences in organic carbon stability. The relative amount of recalcitrant and labile SOC fractions is an important soil characteristic, which determines protection of SOC from decomposition. The easily oxidizable carbon fraction prevailed in the upper soil layer, indicating the presence of readily mineralizable organic compounds. However, major differences were found in this fraction. The labile SOC compounds are biochemically active. They are related to material and energy transformations in the soil and they appreciably affect soil fertility (Chan et al., 2001). Smaller differences between treatments were established in moderately labile, stable, and recalcitrant carbon fractions, which suggest that these fractions are less sensitive to environmental factors. The readily oxidizable carbon fraction could be applied to monitor the changes of SOC and soil quality.

Conclusions

The data obtained in the experiment proved the importance of soils of protected areas and agricultural lands as a source of carbon accumulation under the agro-climatic conditions of Lithuania in the background of climate change.

1. The soil type and use as well as plant diversity play an important role in carbon transfer into the soil in grasslands. The soil organic carbon (SOC) concentrations decreased with the depth in all treatments, and the highest values were measured at 0–10 cm soil layer in pre-mainland section of middle reaches of the Nevėžis (76.8 g kg⁻¹) and in old semi-natural pasture Valinava

(49.5 g kg⁻¹). The exclusive importance of the agrarian soil occupied with grasslands and long-lived swards and soils of protected areas occupied with natural meadows on environment quality was revealed, because organic carbon was accumulated and sequestered in the form of stable compounds. Natural encroachment of wood pasture by forest plant communities in northern part of Klampūtė significantly increased SOC. The content of SOC was higher in all soil layers in wood pasture under restoration compared with surviving wood pasture: 31.2 and 30.5 g kg⁻¹ in the 0–10 cm layer, 25.8 and 17.9 g kg⁻¹ in the 10–20 cm layer, 17.7 and 7.1 g kg⁻¹ in the 20–30 cm layer, respectively. The most intensive transformation and accumulation of organic carbon was found to take place at 0–10 cm soil layer.

2. Excessively large proportion of labile carbon compounds in the soil indicates unfavourable processes occurring in the soil, trend towards leaching of nutrients, and thus to soil degradation. Such trends were identified in the meadow and former arable land of the central section of the middle reaches of the Nevėžis. However, the most unfavourable status of SOC, and thus all the soil organic matter (SOM), was observed in the riverside section. The labile carbon was dominant there, and the content of organic carbon resistant to chemo-destruction at 0–10 cm layer was very low – only 0.22 g kg⁻¹, at 10–20 cm layer – 0.37 g kg⁻¹, and at 20–30 cm layer – 0.57 g kg⁻¹. The carbon transformation processes in wood pastures differed depending on the depth: organic carbon was more stable in 10–20 and 20–30 cm layers in the wood pasture under restoration than that in surviving wood pasture.

3. Having applied chemo-destructive fractionation method, the largest amount of residual organic carbon, which represents the resistance to degradation and the possibility to sequestration, was established in the soils under long-term use of grassland (pre-mainland section and old semi-natural pasture). From the environmental point of view, the above mentioned soil use is very important in order to accumulate carbon in the soil, to reduce degradation of SOC, emission to the atmosphere in the form of CO₂, and thereby reduce the climate change.

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Dirvožemio organinės anglies stabilumo įvertinimas saugomų teritorijų ir dirbamoje žemėje taikant chemodestrukcinį frakcionavimą

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Santrauka

Tirti *Natura 2000* saugomų teritorijų dirvožemiai (giliau karbonatingi giliau glėjiniai rudžemiai ir salpžemiai: aliuvinis-deliuvinis pasotintasis bei giliau glėjiški pasotintieji) ir žemės ūkio paskirties dirvožemiai, užsėti žolynais (giliau karbonatingi giliau glėjiški rudžemiai). Visuose variantuose gilėjant dirvožemio sluoksniui dirvožemio organinės anglies (DOA) koncentracija mažėjo, o daugiausia DOA buvo nustatyta dirvožemio 0–10 cm sluoksnyje Nevėžio slėnio paterasio zonoje ($76,8 \text{ g kg}^{-1}$) ir ilgaamžėje pusiau natūralioje ganykloje ($49,5 \text{ g kg}^{-1}$). Išryškėjo žemės ūkio paskirties dirvožemių, kuriuose yra ganyklos bei ilgaamžiai žolynai, ir saugomų teritorijų dirvožemių su juose esančiomis natūraliomis pievomis ypatinga svarba aplinkos kokybei, nes juose stabilių anglies junginių pavidalu yra kaupiama ir sekvestruojama organinė anglis. Natūralus miško augalų bendrijų skverbimasis į šiaurinę Klamputės miško ganyklos dalį žymiai padidino DOA kiekį. Visuose atkuriamos miško ganyklos dirvožemio sluoksniuose buvo nustatyti didesni kiekiai DOA, lyginant su išlikusios miško ganyklos dirvožemiu. Anglies transformacijos procesai miško ganyklose skyrėsi priklausomai nuo gylio: organinė anglis buvo labiau stabilizuota išlikusios miško ganyklos dirvožemio 10–20 ir 20–30 cm sluoksniuose, lyginant su atkuriamos miško ganyklos dirvožemiu. Organinės anglies transformacija ir kaupimasis intensyviausiai vyko dirvožemio 0–10 cm sluoksnyje. Saugomų teritorijų ir žemės ūkio paskirties dirvožemiai Vidurio Lietuvoje pasižymėjo nevienodu organinės anglies stabilumu. Didžiausias nesuskaidytos organinės anglies, kuri rodo atsparumą degradacijai ir sekvestravimo galimybe, kiekis cheminio destrukcinio frakcionavimo metodu buvo nustatytas dirvožemiuose, kuriuose ilgą laiką nuolat augo žolynai (Nevėžio vidurupio slėnio paterasio zona ir ilgaamžė pusiau natūrali ganykla). Nustatyta, kad nepalankiausia DOA, kaip ir visos jo organinės medžiagos, būklė buvo Nevėžio vidurupio slėnio kranto zonoje. Jos dirvožemyje vyravo labili organinė anglis, o cheminei destrukcijai atsparios anglies koncentracija buvo itin maža visuose sluoksniuose.

Reikšminiai žodžiai: labili anglis, *Natura 2000*, nesuskaidoma anglis, organinė anglis, saugomos teritorijos.