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Land use effects on soil organic carbon sequestration in calcareous leptosols in former pastureland – a case study from the Tatra Mountains (Poland)

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Abstract

The purpose of the paper is to show SOC sequestration rates in calcareous shallow soils in reforested areas in Tatra Mts. with a particular focus on the different forms of organic matter (OM) storage. Three plant communities creating a mosaic on the slopes of the valley were taken into account.

After 50 years since the conversion of pastureland to grassland, dwarf pine shrub, and larch forest on soils, the development of genetic soil horizons as well as SOC sequestration in soil occur despite the steepness of slopes. SOC stock is the highest in soils under larch forest (63.5 mg ha^{-1} , SD 16.3), while in soil under grassland and under dwarf pine shrub, this value is smaller (47.5 mg ha^{-1} , SD 13.3 and 42.9 mg ha^{-1} , SD 22.0 respectively).

The highest amount of mineral-associated OM inside stable microaggregates (MOM FF3) is found in grassland soil (21.9–27.1% of SOC), less under dwarf pine shrub (16.3–19.3% of SOC) and larch forest (15.3–17.7% of SOC). The pool of mineral-associated OM inside transitional macroaggregates (MOM FF2) is found in soil under dwarf pine shrub (39.2–59.2% of SOC), with less under larch forest (43.8–44.7% of SOC) and the least in grassland soil (37.9–41.6% of SOC). The highest amount of the free light particulate fraction (POM LF1) is found in soil under dwarf pine shrub (6.6–10.3% of SOC), with less under larch forest (2.6–6.2% of SOC) and the least in grassland soil (1.7–4.8% of SOC).

1 Introduction

It is known that land use changes affect soil organic carbon (SOC) stocks. While undergoing natural or human-affected changes, an ecosystem can work as a carbon sink or source depending on the direction of the conversion. This problem is important and widely discussed in the context of soil degradation, as well as CO_2 emissions and from a purely academic point of view (Post and Kwon, 2000; Laganière et al., 2010; Fialho

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and Zinn, 2012; Corral-Fernández et al., 2013; Lozano-García and Parras-Alcántara, 2013; Batjes, 2014).

Studies on the impact of reforestation on SOC dynamics are particularly interesting as reforestation affects large areas of grassland in mountain areas all over the world (Didier, 2001; Paul et al., 2002; Halliday et al., 2003; Seeber and Seeber, 2005; Barua and Haque, 2013). On the other hand, research on SOC storage in mountain regions is rare (Prichard et al., 2000).

In the case of reforested agricultural land, the impact of land use changes on SOC stocks is rather well-known and not called into question. Long-term research has shown that SOC is found to accumulate following reforestation because of increased influx of organic matter (OM) and decelerating decomposition in the forest microclimate (Guo and Gifford, 2002; Murty et al., 2002; Paul et al., 2003; Wang et al., 2011).

In the case of the transition of grassland (pastureland and meadow) into forest, the situation is not clear. In most cases, reforestation is said to cause a decline in SOC stocks in soils found across former pastureland (Alfredsson et al., 1998; Corre et al., 1999; Tate et al., 2000; Guo and Gifford, 2002; Paul et al., 2002), which can be explained by the fact that grasses and herbaceous plants deliver a large amount of biodegradable roots to the soil, which causes the accumulation of OM in humus A-horizons in grassland (Oades, 1988). Other analyses have shown that changes in SOC stocks in reforested pastures provide inconclusive results, and the pattern of changes depends largely on local conditions (Murty et al., 2002; Johnson et al., 2003; Laganière et al., 2010; Poeplau and Don, 2013). Debasish-Saha et al. (2014) described higher SOC stocks in soils under forest than grassland in the subtropical hills of the Lower Himalayas. Laganière et al. (2010) state that in cold humid climates, changes in SOC storage are usually negative (loss of SOC) following reforestation, while in temperate marine climates, changes are clearly positive (SOC accumulation). This trend can be explained by the fact that the slow growth of trees in harsh climate conditions makes the increase in SOC content possible only after a long period of time, while most studies cited by Laganière et al. (2010) were carried out at relatively young plantations. An

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analysis of SOC changes must take into account the complexity of the carbon cycle in the forest ecosystem – including the accumulation of SOC in soil organic O-horizons (Johnson et al., 2003; Seeber and Seeber, 2005; Poeplau and Don, 2013).

Attempts to explain the forms of OM stabilization during accumulation have been undertaken by many researchers (Oades, 1984, 1988; Jastrow, 1996; Six et al., 2001, 2002; Deneff et al., 2004; Lützw et al., 2006; Plante et al., 2006; Steward et al., 2008), but in the context of land use, this problem is usually studied in agricultural soils (Lützw et al., 2002; Deneff et al., 2004; Pikul et al., 2007; Barbera et al., 2012; Jaiarree et al., 2014; Srinivasa et al., 2014) or in soils converted from cropland to grassland or forest (Don et al., 2009; Leifeld and Kögel-Knabner, 2005). Some data on differences in mechanisms associated with soil organic matter (SOM) stabilization in forest soils were provided by Laganière et al. (2011). There is little data available for mountain soils, and our understanding of the effect of environmental factors on SOM turnover is limited (Leifeld et al., 2009; Budge et al., 2011; Martinsen et al., 2011).

Lützw et al. (2006) point out several mechanisms causing OM stabilization in the soil environment depending on its form as well as the rate of linkage with the mineral part of the soil. Particulate organic matter (POM) is claimed to be a potential source of available carbon for decomposers and it is more mineralizable than mineral-associated organic matter (MOM). POM can be protected for a few years because of its primary recalcitrance caused by its specific chemical structure (high lignin, waxes, fats), but its residence time is short. According to radiocarbon measurements, the mean residence time of POM fractions ranges from 1 to 10 years (Lützw et al., 2006), although in mountain soils, it can be as high as 100 years at elevations above 2000 m because of harsh climate conditions (Leifeld et al., 2009; Budge et al., 2011).

The process that can reduce SOM susceptibility to decomposition is occlusion by aggregation. The primary agents controlling the formation of macroaggregates (> 250 µm) are stabilization by POM, enmeshment by plant roots and fungal hyphae, as well as in casts and feces promoted by the hydrophobicity of surfaces. Macroaggregation is claimed to be sensitive to farming practices (Oades, 1984; Lützw et al., 2006).

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Turnover of OM is much slower in microaggregates (Lützwow et al., 2006) whose OM is protected from enzyme attack by microbial hydrophobic slime and glue as well as by negligible porosity that limits access to bacteria. OM occluded in microaggregates in soils in temperate climates can exist in the soil for about 100 years (vs. 10 years in the case of POM) (Lützwow et al., 2006). The residence time of MOM fractions is longer than 100 years, because it is humified, which makes it less available for decomposers (Lützwow et al., 2006) and it is also protected against degradation and decomposition by chemical binding; for example, by polyvalent cation bridges in the presence of clay minerals with expandable layer silicates (e.g. smectite, vermiculite, illite) and binding in the presence of metal cations (Ca^{2+} , Al^{3+} , Fe^{3+} and heavy metals), e.g. by complexation (Lützwow et al., 2006; Grünberg et al., 2013; Jindaluang et al., 2013). According to Leifeld et al. (2009), the mean residence time for MOM fractions in alpine soils can reach between 200 and 2200 years depending on elevation and soil depth.

The aim of our research is to determine SOC sequestration in calcareous shallow soils (Leptosols) in a renaturalized area in Jaworzynka Valley (Tatra Mts.) as well as to estimate OM distribution in soil physical fractions with respect to different types of land use. We are not aware of any studies focused on SOC sequestration having been conducted in soils formed on carbonate parent material and containing carbonates from the surface of the mineral part of the soil profile.

2 Methods

2.1 Study area and experimental design

Our research study was conducted in Jaworzynka Valley in the Tatra Mts. in southern Poland – a mountain range located in Central Europe and belonging to the Alpine mountain system (Fig. 1a and b). The process of reforestation and afforestation provides in the Tatra Mts. an opportunity to see how SOC accumulation has changed in calcareous soils due to different types of land use. Reforestation started here in the

1960s when sheep grazing was banned in the interest of nature conservation. Since then some abandoned pastureland has undergone natural succession, while some has been afforested as part of a major government afforestation program in Poland.

5 Animals (mainly sheep) had grazed in Jaworzynka Valley since the 16th century (Fig. 2). Grazing was banned after 1963. The soils on the sides of the valley became heavily eroded. In light of the risk of further erosion, dwarf pine shrub (*Pinetum mughii*) and larch (*Larix* sp.) forest were planted (1962–1963). Afforestation efforts stopped in 1970. Some places have been left as mountain grasslands (clearings). Today, Ja-
10 worzynka Valley features a mosaic of the aforementioned plant communities (Figs. 1b and 3).

The valley is built of dolomitic limestone (Anisian, Ladinian) (Sokołowski and Jaczynowska, 1979). The mean annual air temperature in the study area (data for the nearest station: Hala Gasienicowa) is 2.4 °C. The mean annual air temperature of the warmest month (August) is 10.8 °C (min. –4.1 °C; max. 17.6 °C). The mean annual air
15 temperature of the coldest month (February) is –5.3 °C (min. –21.4 °C; max. 7.2 °C). The valley's mean annual precipitation is 1661 mm (Błażejczyk et al., 2013).

The research study was conducted on three plots (30 m × 30 m) located close (Fig. 1b) to each other to avoid differences connected with slope position and exposure, which can affect SOC stock variability (Fernández-Romero et al., 2014) as well as geological and soil heterogeneity that can affect SOC stocks and forms of OM (Baldock and Skjemstad, 2000; Parras-Alcántara et al., 2014). Plot No. 1 represents a mountain
20 grassland (high mountain calcareous grassland: *Carici sempervirentis-Festucetum tatrae* association). Plot No. 2 represents thickets of dwarf pine *Pinetum mughii*. Plot No. 3 represents sparse larch (*Larix* sp.) forest (ca. 400 trees ha⁻¹) with a dense cover of grass *Calamagrostis* sp. on the forest floor (lower montane beech forest habitat: *Dentario glandulosae-Fagetum Calamagrostietosum arundinaceae*) (Fig. 4). The three
25 plots are located at an elevation between 1200 and 1220 m (location: 49°15'32" N, 19°59'35" E) on a uniformly inclined slope (linear mountain sideslopes) of 25° SW.

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3 Field methods

Nine soil profiles were excavated in each plot; therefore, the soil material was collected from 27 pits. Soil profiles were excavated down to the lithic contact and described according to Schoeneberger et al. (2002). A reference profile was selected at each study site – the soil profile nearest to the central point of the plot (Table 1).

Unlike in the case of many other research studies, it was decided to collect samples from the genetic horizons of the soil, and not from the intervals. Organic O-horizons are well-developed in mountain soils (Kubienna, 1953; Drewnik, 2006); therefore, it is necessary to take into account the boundary between the organic O-horizon and the A horizon in research focused on the mechanisms of OM storage in soil.

One large (mineral sample – approx. 2 kg, organic sample – approx. 0.3 kg; moist), representative bulk sample was collected from each studied genetic soil horizon, then placed in sterile polyethylene bags. In addition, undisturbed soil samples were collected from the reference soil profiles in order to determine the bulk density of fine soils. In this case, due to a very large quantity of stone and gravel, a steel frame (20 × 20 cm) was used to obtain a large sample in the form of a rectangular prism with a volume ranging from 4000 to 8000 cm³, depending on the horizon. The studied soils were classified according to the WRB system (IUSS Working Group WRB, 2007).

3.1 Laboratory methods

Bulk soil samples taken from A horizons and mineral B-horizons were air-dried, gently crushed using a wooden rolling pin, and sieved using a 2 mm sieve. Live roots were removed. Soil samples from O horizons were milled after the living parts of plants in the samples had been removed.

Stone and gravel content (particles > 2 mm) was determined by weighing. Bulk density of fine soil (BD(*f*)) (mass volume⁻¹) was calculated according to Don et al. (2007)

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as follows:

$$BD(f) = \frac{\text{mass}_{\text{sample}} - \text{mass}_{\text{particles}>2\text{ mm}}}{\text{volume}_{\text{sample}} - \frac{\text{mass}_{\text{particles}>2\text{ mm}}}{\text{density}_{\text{particles}>2\text{ mm}}}} \quad (1)$$

Texture was determined by wet sieving (sand fractions) and the hydrometer method (silt and clay fractions) (Gee and Bauder, 1986). The concentration of total carbon (TC) and nitrogen (N) was determined by dry combustion gas chromatography using a CHNS analyzer (Micro Elementar Analyzer Vario Cube). CO₂ content obtained from carbonates (CO_{2(carb)}) was determined using the volumetric calcimeter method. Each sample's pH was measured in deionized water (1 : 2.5 soil/water ratio) (Thomas, 1996). Soil color was described in the moist state using Munsell Soil Color Charts (Oyama and Takehara, 2002).

SOC was calculated by subtracting inorganic carbon (SIC – carbon from CO_{2(carb)}) from TC. SOC stocks were calculated according to Don et al. (2007), as follows:

$$\text{SOC} = \sum_{t=0}^n BD(f) \times \text{SOC} \times \text{depth_volume} \quad (2)$$

In the equation, “depth_volume” is the depth of the horizon minus the volume of particles $\varnothing > 2$ mm. This calculation excludes particles $\varnothing > 2$ mm as they are not a component of bulk density.

Physical fractionation of the soil was carried out according to the Leifeld and Kögel-Knabner (2005) method to obtain several OM fractions: free light particulate fraction (POM LF1), light fraction occluded in macroaggregates (POM LF2), residual fraction occluded in microaggregates (ROM), and MOM: MOM fraction outside water-stable aggregates (MOM FF1), MOM fraction inside macroaggregates released after their disruption (MOM FF2) and MOM fraction inside microaggregates released after their disruption (MOM FF3). A 30 gram sample of air-dried soil (< 2 mm) was immersed in deionized water on a 20 μm mesh. After 5 min, the material was gently sieved to obtain

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a mineral fraction outside water-stable aggregates $< 20 \mu\text{m}$ (FF1). The sieved material was air-dried at 40°C and weighed. It was then transferred into a 100 mL centrifuge beaker and sodium polytungstate solution (1.8 g cm^{-3}) was added. The resulting material was gently stirred, left for 10 min to settle, and centrifuged for 10 min (2320 g). POM LF1 was decanted and washed with deionized water using a $20 \mu\text{m}$ mesh. The residual soil material was dispersed ultrasonically (Sonics 750) with an energy of 22 J mL^{-1} to break down macroaggregates. The dispersed soil was sieved at $20 \mu\text{m}$ mesh to obtain a mineral fraction $< 20 \mu\text{m}$ inside macroaggregates (FF2), dried, and POM LF2 was separated as described for POM LF1. The residual soil material was dispersed ultrasonically with an energy of 450 J mL^{-1} to break down microaggregates. The material was sieved using a $20 \mu\text{m}$ mesh to obtain a residual fraction (ROM) and obtain a mineral fraction $< 20 \mu\text{m}$ inside macroaggregates (FF3). The mineral fractions $< 20 \mu\text{m}$ (FF1, FF2, FF3) were collected after each dispersion step, washed by centrifugation, and weighed. The mass of these fractions relative to the total mass of the $< 20 \mu\text{m}$ fraction was taken as a sign of soil aggregate stability.

The concentration of TC, N and $\text{CO}_2(\text{carb})$ as well as the content of particles $\text{O} > 2 \text{ mm}$ were determined for 106 samples from all the studied soil profiles, while the texture, bulk density, pH, and OM fractionation were determined for samples from reference profiles (Table 1). Only material from A horizons was fractionated, because the soil material from O horizons was poorly decomposed without signs of mixing with mineral matter.

In this study, it was considered justified to use descriptive statistics for presenting the value structure of variables resulting from measurements. The use of statistical inference to assess the significance of differences between the characteristics of three different sampled areas was not considered warranted given the inadequate number of samples (3×9 profiles).

4 Results

4.1 Soil morphology, physical and chemical properties of soils

According to the WRB system (IUSS Working Group WRB, 2007), reference profiles No. 1 and 3 were classified as Rendzic Hyperskeletal Leptosols (Humic, Eutric), while Profile No. 2 was classified as a Folic Hyperskeletal Leptosol (Calcaric, Humic) (Table 1).

O horizons (Oi) in Profile No. 2 and 3 have a thickness of 20 and 2 cm, respectively. The content of $\varnothing > 2$ mm particles increases with depth from 0% to 70–90% at a depth of 30 cm. Fine soil has a dark color in the range of 10 YR 2–4/1–4 and silt loam texture (Table 1). The studied soils are characterized by a very low bulk density of approx. 0.02–0.03 mgm^{-3} in the O horizons and approx. 0.18–0.43 mgm^{-3} in the A horizons and B horizons. Carbonates were found to be present in the fine soils. Carbonate content is very low (0.00–33.5 g kg^{-1} of carbonate CO_2) in organic O-horizons and increases with depth from 250 g kg^{-1} of carbonate CO_2 in A horizons to 300–400 g kg^{-1} of carbonate CO_2 in B horizons (Table 1). Soil pH values change with depth. The pH value measured in distilled water is 4.3–5.1 (profile No. 2) and 6.3 (profile No. 3) in O horizons, and 7.5–7.7 in A horizons, and approx. 7.8 in B horizons (Table 1).

4.2 SOC concentration, SOC stock and C/N ratio

Mean SOC concentration is the highest in O horizons (465.3 g kg^{-1} in soils under dwarf pine and 351.7 g kg^{-1} in soils under larch forest), medium in A horizons (56.8–65.5 g kg^{-1}), and the lowest in B horizons (15.9 g kg^{-1} under larch forest and 21.7 g kg^{-1} under grassland) (Table 2).

The SOC stock is 63.5 mg ha^{-1} (SD 16.3) in soil under larch forest, 47.5 mg ha^{-1} (SD 13.3) in grassland soil, and 42.9 mg ha^{-1} (SD 22.0) in soil under dwarf pine shrub (Fig. 5). In all plots, SOC is accumulated mainly in the mineral part of soil (A horizons

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and B horizons): 100 % of SOC in grassland, 94 % in larch forest, and 67 % in soil under dwarf-pine.

The C/N ratio varies depending on the type of soil horizon. It is 41.9 in the O horizon in soils under dwarf pine shrub, and 28.5 in soils under larch forest, while it ranges from 10.7 to 13.5 in A horizons and B horizons in all soils (Table 2).

4.3 Characteristics of individual fractions

The mass of the FF2 fraction accounts for more than 60 % of the < 20 μ m fraction total in soils under dwarf pine shrub and larch forest and more than 52 % of the < 20 μ m fraction total in soil under grassland (Table 3). The mass of the FF3 fraction of the < 20 μ m fraction total ranges from 27.9 to 31.6 % in soil under dwarf pine shrub and larch forest, while it ranges from 43.6 to 44.8 % in soil under grassland. The mass of the FF1 fraction of the < 20 μ m fraction total is the lowest in all the studied soil profiles (account for 0.9 % in the A2 horizon in grassland soil to 9.6 % in the A1 horizon in soil under dwarf pine).

In A horizons, SOC content in MOM fractions accounts for 67.6 to 85.8 % of total SOC, while in POM+ROM fractions, it ranges from 12.4 to 32.4 % of total SOC (Table 4).

The SOC concentration of the two POM fractions (POM LF1 and POM LF2) ranges from 227.4 to 308.3 g SOC kg⁻¹ (Table 4). The highest content of SOC of POM LF1 is found in the A1 horizon of soil under dwarf pine shrub (10.3 % of SOC), and the lowest in the A2 horizon in soil under grassland (1.7 % of SOC). SOC content of POM LF2 is the highest in the A1 horizon in soil under dwarf pine shrub (18.3 % of SOC) and the lowest in the A2 horizon in soil under larch forest (5.3 % of SOC) (Table 4). SOC concentration in MOM fractions ranges from 35.6 to 97.1 g SOC kg⁻¹. The SOC content in MOM FF2 is the highest among all MOM fractions, and ranges from 37.9 % of SOC in the A1 horizon in grassland soil to 59.2 % of SOC in the A2 horizon soil under dwarf pine shrub. SOC content in MOM FF3 accounts for 15.3 % of SOC in the A2 horizon under larch forest to 27.1 % of SOC in the A1 horizon in soil under grassland (Table 4).

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Larch forest is sparse with dense cover of *Calamagrostis sp.* on its floor (Fig. 4). No biomass measurements were done as part of the study, but it may be assumed, that tall *Calamagrostis sp.* grass delivers to the soil a higher amount of plant tissue than do short xerothermic grassland plants. Guzman et al. (2014) found that after three years of reclamation of mine area soils using tall prairie grasses, these soils had received significantly more biomass than soils reclaimed using cool-season forage grass. In the Jaworzynka Valley, the relatively higher SOC stock in larch forest (Fig. 5) can be also explained by a specific forest microclimate affecting forest floor species' composition and OM decomposition rates and the fall of needles, which does not occur in grassland areas (cf. Kim, 2000; Seeber and Seeber, 2005; Rigueiro-Rodríguez et al., 2012). Soils covered with larch forest are not studied very often; however, the SOC stock in Jaworzynka Valley soils in plot No. 3 is in the range given for soils found in larch forests (formerly agriculturally used Luvisols) in China (Wang et al., 2011).

While the SOC stock is quite similar throughout the entire soil profile in soils found under dwarf pine shrub and soils found under grassland, it is grassland soils that possess a significantly higher amount of SOC sequestered in the A horizon rather than dwarf-pine shrub soils (Fig. 5). This finding is partly consistent with other results cited by Laganière et al. (2010) and Poeplau and Don (2013), giving evidence to decreasing SOC stocks following grassland reforestation in the first several years after the conversion. This is most likely due to constraints on the delivery of very rapidly decomposable grass roots in forest ecosystems in comparison with grassland ecosystems (Oades, 1988).

In coniferous forests ecosystems, thick O horizons develop (Table 1) as a result of a large amount of litter delivered to the soil as well as a specific forest microclimate and acidification caused as a result of needle decomposition (Bochter and Zech, 1985; Seeber and Seeber, 2005; Drewnik, 2006). The development of O horizons can rarely offset SOC depletion caused by the limited delivery of grass roots (Kammer et al., 2009). In the studied soils under dwarf pine shrub, the SOC stock in the O horizon comprises only one third of the entire SOC stock despite its substantial thickness,

which is similar to the results obtained in forest soils in the Stołowe Mts. in southwestern Poland (Gałka et al., 2014).

The relatively small SOC stock in dwarf pine shrub soils may be the result of the fact that it is a relatively young plant community and large amounts of OM – derived from the roots of dead trees, as observed by Debasish-Saha et al. (2014) in the soils of the Lower Himalayan hills – have not yet appeared.

The SOC stock determined for individual plots in our study area (Fig. 5) is significantly lower than that found in similar mountain environments in the temperate climate zone (Kammer et al., 2009; Gałka et al., 2014). The most important reason for the relatively small SOC stock is the small depth of the soil and a very large number of $\emptyset > 2$ mm particles (Table 1). This leads to a small SOC stock despite the high concentration of these elements in the local soil mass (fine particles) (Table 2). Studies on SOC stocks have not been carried out in the Tatra Mts. so far, but results obtained by Skiba (1983) and Miechówka (2000) indicate that soils developed on calcareous parent material under similar conditions are characterized by a similar or higher concentration of SOC, greater thickness as well as a smaller number of $\emptyset > 2$ mm particles, which allows to suppose that the SOC stock would also be higher in typical Tatra soils than that in the studied soils.

The lower SOC stock determined for the studied soils in comparison with Haplic Cambisols in the Urals (Kammer et al., 2009) can be explained by greater biological activity in calcareous soils or more favorable climate conditions devoid of drought periods. The SOC stock determined for our study area is significantly smaller than that found in a similar environment in the Stołowe Mts., which are part of the Sudeten Mts. in southwestern Poland (Gałka et al., 2014). This can be explained by the fact that the soils in the studied area are much more shallow.

5.3 Land use effects on OM fractions in soil

In the studied soils, OM is mainly associated with the mineral part of the soil (Table 4), which is consistent with research results obtained for other soils in temperate

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climate zones, where more than 60 % of the total SOC fraction is associated with the mineral part of soil (Jastrow, 1996; Don et al., 2009). OM in the soils studied can be divided into two main groups: (1) MOM FF fractions (MOM FF1, MOM FF2, MOM FF3) with a low C/N ratio suggesting a relatively high contribution of humified as well as microorganisms-derived OM, which is believed to serve as a factor gluing soil particles together inside microaggregates (Oades, 1984; Six et al., 2001), and (2) POM fractions (POM LF1, POM LF2) with a high C/N ratio, which suggests relatively weakly decomposed plant-derived material (Table 5).

The highest mass of FF2 fractions in relation to the mass of the FF1 fraction in all the studied soils (Table 3) refers (Leifeld and Kögel-Knabner, 2005) to the relatively high structural stability linked with the development of water-stable aggregates in the studied soils, in comparison with sandy soils investigated by Leifeld and Kögel-Knabner (2005). This can be an effect of the calcium and magnesium carbonate content as well as the relatively high content of mineral colloids, which promote structural stability in soils (Oades, 1988; Muneer and Oades, 1989; Deneff et al., 2004; Lützow et al., 2006; Grünberg et al., 2013). Similarly, in the studied soils, OM associated with the mineral part of the soil occurs mainly in macroaggregates (MOM FF2) and microaggregates (MOM FF3) (Tables 3 and 4). In all the studied soils, the amount of SOC outside of water-stable aggregates (MOM FF1) is very small. The highest concentration of SOC in macroaggregates in the studied soils is consistent with results obtained by Jastrow (1996) and Debasish-Saha et al. (2014). The researchers independently concluded that this is the result of the transitional nature of macroaggregates, in comparison with microaggregates, which contain a relatively passive pool of OM because of strong bonds with clay minerals.

In soil under grassland, the largest amount of SOC bound within MOM FF3 (microaggregates OM) is found in contrast to soils found under coniferous communities (dwarf pine shrub and larch forest) (Table 4). This corresponds with the highest mass of FF3 and the lowest mass of FF1 and refers (Leifeld and Kögel-Knabner, 2005) to high structural stability linked with the development of stable microaggregates in soils

under grassland. Conversely, the mass of the FF1 fraction is the largest in soils found under dwarf pine, while the largest part of SOM is stored in the MOM FF2 fraction. This shows that the soil structure is not very stable (quantity of FF1 fraction) and most of the SOM occurs in relatively unstable aggregates (quantity of FF2 fraction) (Leifeld and Kögel-Knabner, 2005; Debasish-Saha et al., 2014).

The C/N ratio in MOM does not change with depth along the soil profile (Table 4). The lowest C/N ratio is observed in the MOM FF1 fraction, which is most likely due to less protection of OM from microbial attack outside the water-stable aggregates (Lützwow et al., 2006).

Significantly higher content of SOC stored in the POM LF1 and POM LF2 fractions in the soil under dwarf pine shrub vs. that in soil under grassland and under larch forest is observed, both outside the aggregates (POM LF1), as well as occluded in macroaggregates (POM LF2) (Table 4). The highest amount of SOC stored in the POM LF1 fraction seems to confirm suppressing decomposition rates in soils under coniferous vegetation in contrast to grassland soils. This can be affected by the suppressing effect of coniferous plant material (lowered pH) on soil microbial activity (Drewnik, 2006), lower soil temperature that limits soil biological activity in forest ecosystems (Kim, 2000) or by the delivery of more recalcitrant plant material such as tree roots to the forest soil (Debasish-Saha et al., 2014). Budge et al. (2011) found in Alpine soils that the residence time of POM derived from dwarf shrubs was longer than the residence time of POM derived from grassland, although they do not settle, if this is the result of the suppressing effect of the plant community on microbial activity or higher recalcitrance of the material delivered to the soil.

OM occurring outside aggregates (POM LF1) is less decomposed than OM occluded in macroaggregates (POM LF2) in all the studied soils as evidenced by the highest C/N ratio (Table 5). This proves that relatively young organic material (poorly humified) is gradually incorporated into soil aggregates (cf. Oades, 1984; Six et al., 2001; Lützwow et al., 2006; Budge et al., 2011). The C/N ratio has been observed to increase in the POM LF1 fraction with depth in all the studied soils (Table 5). This suggests that the

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primary precursors of POM formation are plant roots because the decomposition rate of OM increases with depth within O horizons developed as a result of litter accumulation on the mineral surface of the soil (i.e. C/N ratio decreases) (Ussiri and Johnson, 2003; Budge et al., 2011).

In contrast, the C/N ratio in the POM LF2 fraction decreases with depth (Table 5), which indicates a higher decomposition rate of OM occluded in aggregates with depth. This can be the result of incorporation of weakly-decomposed, relatively fresh OM into the structures of aggregates, which occurs most rapidly in top of mineral part of soil (A1 horizon) in comparison with deeper horizons. This further suggests an abundance and significant activity of soil fauna in top soil horizons in comparison with deeper horizons (Zanella et al., 2011).

5.4 Management issues

Research in Jaworzynka Valley can show which method of renaturation is the most favorable in shallow eroded calcareous soils developed on steep slopes in a temperate humid climate. So, which is the more advantageous solution: (1) allowing grassland to remain grassland, (2) afforestation using sparse larch forest with a naturally developed dense cover of tall grass *Calamagrostis sp.* on the forest floor (lower montane beech forest habitat: *Dentario glandulosae-Fagetum Calamagrostietosum arundinaceae*), (3) planting dwarf pine shrub. All three methods effectively protect the soil against erosion, which is confirmed by horizonation of all the studied soils – seen to be experiencing soil stabilization (Targulian and Krasilnikov, 2007; Guzman et al., 2014).

The expected effect of soil reclamation and renaturation is SOC (OM) sequestration, especially in resistant (mineral-associated, aggregated) forms, which leads to increased soil fertility, soil stability, improved soil structure (resistance against erosion) and helps restrict CO₂ emission into the atmosphere (Oades, 1984; Debasish-Saha et al., 2014; Fernández-Romero et al., 2014; Guzman et al., 2014). In this context, afforestation with sparse larch forest is the most effective, because soils under larch forests sequester the highest amount of SOC (Fig. 5), and a significant part of OM oc-

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Table 1. Basic properties of reference soil profiles.

Horizon	Depth (cm)	Particles > 2 mm (%)	Color (moist)	Texture	BD(<i>f</i>) ^a (mg m ⁻³)	CO _{2(carb)} ^b (g kg ⁻¹)	TC ^c	SOC ^d	N ^e	pH (H ₂ O)	C/N ^f
Profile No. 1; plot No. 1; mountain meadow – grassland; Rendzic Hyperskeletic Leptosol (Humic, Eutric)											
A1	0–15	58	10YR2/2	silt loam	0.26	301.1	143.3	62.0	5.0	7.57	12.4
A2	15–32	81	10YR2/2	silt loam	0.18	262.4	133.6	62.7	4.9	7.67	12.8
B	32–42	90	10YR4/4	silt loam	0.43	334.0	118.1	27.9	2.5	7.87	11.2
Profile No. 2; plot No. 2; dwarf pine shrub (<i>Pinetum mughi</i>); Folic Hyperskeletic Leptosol (Calcaric, Humic)											
Oi1	0–10	0	organic material		0.02	0.0	477.9	477.9	10.8	4.30	44.3
Oi2	10–20	8	organic material		0.03	5.1	470.4	469.0	11.4	5.11	41.1
A1	20–25	45	10YR2/1	silt loam	0.18	249.3	147.2	79.9	6.0	7.47	13.3
A2	25–45	63	10YR2/1	silt loam	0.19	306.0	138.7	56.1	4.5	7.60	12.5
A3	45–50	70	10YR2/1	silt loam	0.19	308.1	135.5	52.3	4.5	7.66	11.6
Profile No. 3; plot No. 3; larch (<i>Larix</i> sp.) forest; Rendzic Hyperskeletic Leptosol (Humic, Eutric)											
Oi	0–2	0	organic material		0.03	33.5	436.4	427.3	11.2	6.35	38.2
A1	2–12	56	10YR3/2	silt loam	0.37	264.2	124.6	53.3	4.3	7.58	12.4
A2	12–22	58	10YR3/2	silt loam	0.39	294.8	121.2	41.6	2.7	7.72	15.4
B	22–30	77	10YR4/4	silt loam	0.40	396.5	116.5	9.4	0.8	7.80	11.8

^a bulk density of the fine soil.

^b CO_{2(carb)}–CO₂ from carbonates.

^c total carbon.

^d soil organic carbon.

^e total nitrogen.

^f SOC/N ratio.

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Table 2. The concentration of TC, SIC, SOC and C/N ratio in analyzed soils – mean values (SD in brackets.)

Plot	Type of soil horizon	<i>n</i> ^a	TC ^b (g kg ⁻¹)	SIC ^c	SOC ^d	C/N ratio ^e
1 mountain grassland	Humus A-horizons	18	135.7 (7.4)	76.5 (7.6)	59.2 (12.6)	12.9 (1.0)
	Mineral B-horizon	9	124.4 (5.9)	92.7 (29.3)	21.7 (9.5)	10.7 (1.7)
2 dwarf pine, <i>Pinetum mughii</i>	Organic O-horizons	13	467.3 (37.6)	2.1 (5.5)	465.3 (42.7)	41.9 (7.9)
	Humus A-horizons	20	136.7 (25.0)	71.3 (18.1)	65.5 (34.1)	13.0 (2.3)
3 larch (<i>Larix</i> sp.) forest	Organic O-horizons	20	362.8 (72.4)	11.1 (9.0)	351.7 (80.0)	28.5 (6.6)
	Humus A-horizons	18	120.3 (10.4)	63.4 (13.6)	56.8 (15.6)	12.8 (1.0)
	Mineral B-horizon	8	117.1 (7.4)	101.2 (13.2)	15.9 (7.8)	13.5 (1.3)

^a number of samples.

^b total carbon.

^c inorganic carbon (carbon from CO_{2(carb)}).

^d soil organic carbon.

^e SOC/N ratio.

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Table 3. Fraction of particles < 20 μm released after wet sieving and ultrasonification to the release of total fine fraction (FF).

Horizon	Depth (cm)	Wet sieving (FF1 fraction) (%)	Ultrasonification	
			22 J mL ⁻¹ (FF2 fraction)	450 J mL ⁻¹ (FF3 fraction)
Profile No. 1; plot No. 1; mountain meadow – grassland; Rendzic Hyperskeletal Leptosol (Humic, Eutric)				
A1	0–15	4.2	52.3	43.6
A2	15–32	0.9	54.3	44.8
Profile No. 2; plot No. 2; dwarf pine shrub (<i>Pinetum mugh</i>); Folic Hyperskeletal Leptosol (Calcaric, Humic)				
A1	20–25	9.6	59.5	30.9
A2	25–45	9.4	61.8	28.8
A3	45–50	7.6	64.5	27.9
Profile No. 3; plot No. 3; larch (<i>Larix</i> sp.) forest; Rendzic Hyperskeletal Leptosol (Humic, Eutric)				
A1	2–12	7.2	61.3	31.6
A2	12–22	7.6	63.6	28.7

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Table 4. Soil organic carbon (SOC) in fractions.

Horizon	Depth (cm)	SOC in fraction						Percent of SOC in soil					
		POM LF1 ^a	POM LF2 ^a	ROM ^a	MOM FF1 ^a	MOM FF2 ^a	MOM FF3 ^a	POM LF1 ^a	POM LF2 ^a	ROM ^a	MOM FF1 ^a	MOM FF2 ^a	MOM FF3 ^a
Profile No. 1; plot No. 1; mountain meadow – grassland; Rendzic Hyperskeletal Leptosol (Humic, Eutric)													
A1	0–15	289.5	248.5	10.6	45.8	75.2	64.5	4.8	11.5	5.9	1.8	37.9	27.1
A2	15–32	276.2	224.7	3.9	54.5	80.2	51.1	1.7	10.3	2.2	0.5	41.6	21.9
Profile No. 2; plot No. 2; dwarf pine shrub (<i>Pinetum mughl</i>); Folic Hyperskeletal Leptosol (Calcaric, Humic)													
A1	20–25	255.8	235.5	8.4	57.9	97.1	92.1	10.3	18.3	3.8	3.8	39.2	19.3
A2	25–45	284.2	308.3	7.8	36.4	97.0	57.5	6.6	12.5	5.7	3.4	59.2	16.3
A3	45–50	284.9	249.1	3.5	51.7	88.4	60.3	7.9	10.6	3.0	3.8	55.2	16.3
Profile No. 3; plot No. 3; larch (<i>Larix</i> sp.) forest; Rendzic Hyperskeletal Leptosol (Humic, Eutric)													
A1	2–12	227.4	247.1	9.9	35.6	69.9	55.0	6.2	11.4	7.7	2.6	43.8	17.7
A2	12–22	262.0	245.5	3.9	36.5	59.5	45.1	2.6	5.3	4.6	3.3	44.7	15.3

^a POM LF1 – particulate organic matter, free light fraction > 20 µm obtained by density fractionation (< 1.8 g cm⁻³); POM LF2 – particulate organic matter, light fraction > 20 µm occluded in macroaggregates obtained by dispersion with an energy of 22 J mL⁻¹ and density fractionation (< 1.8 g cm⁻³); ROM – residual fraction (> 20 µm) occluded in macroaggregates, particulate organic matter occluded in macroaggregates obtained with an energy of 450 J mL⁻¹; MOM FF1 – organic matter fraction associated with mineral part of soil, fraction < 20 µm outside water-stable aggregates, obtained by immersing and wet-sieving; MOM FF2 – organic matter fraction associated with mineral part of soil, fraction < 20 µm, obtained by dispersion with ultrasonic energy of 22 J mL⁻¹ and wet-sieving; MOM FF3 – organic matter fraction associated with mineral part of soil, fraction < 20 µm, obtained by dispersion with ultrasonic energy of 450 J mL⁻¹ and wet-sieving.

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Table 5. C/N ratio in fractions.

Horizon	Depth (cm)	POM LF1 ^a	POM LF2 ^a	ROM ^a	MOM FF1 ^a	MOM FF2 ^a	MOM FF3 ^a
Profile No. 1; plot No. 1; mountain meadow – grassland; Rendzic Hyperskeletal Leptosol (Humic, Eutric)							
A1	0–15	30.2	26.2	26.5	7.3	9.6	9.4
A2	15–32	34.1	18.7	9.8	8.7	9.8	8.8
Profile No. 2; plot No. 2; dwarf pine shrub (<i>Pinetum mughi</i>); Folic Hyperskeletal Leptosol (Calcaric, Humic)							
A1	20–25	27.8	19.3	21.0	9.1	9.3	11.5
A2	25–45	31.6	23.0	4.1	7.3	10.4	10.1
A3	45–50	33.5	17.1	2.9	7.2	8.8	9.6
Profile No. 3; plot No. 3; larch (<i>Larix</i> sp.) forest; Rendzic Hyperskeletal Leptosol (Humic, Eutric)							
A1	2–12	29.9	21.3	4.0	7.6	9.0	8.6
A2	12–22	34.0	19.5	3.9	7.2	8.4	8.5

^a explanation in Table 4.

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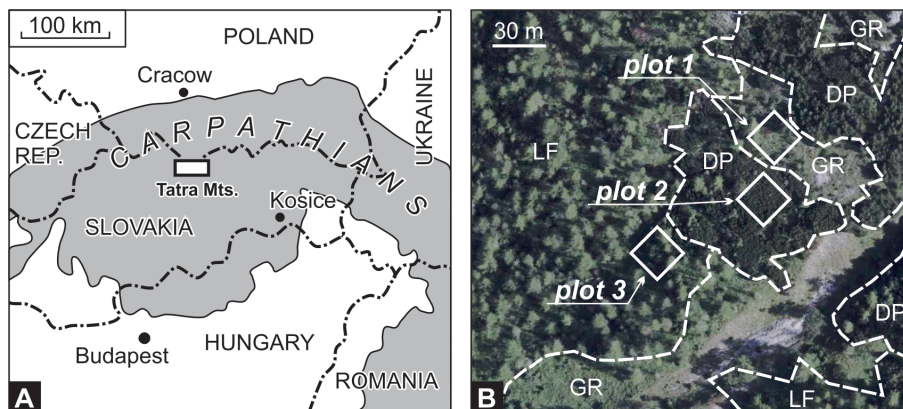


Figure 1. Location of the study area: **(a)** Tatra Mountains in the Carpathian mountain chain; **(b)** aerial photographs of the study area in Jaworzynka Valley with marked research plots: GR – mountain grassland (high mountain calcareous grassland: *Carici sempervirentis-Festucetum tatrae* association), DP – thickets of dwarf pine *Pinetum mughi*, LF – open (sparse) larch (*Larix* sp.) forest with a dense cover of grass *Calamagrostis* sp. on the forest floor.

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Figure 2. Sheep grazing in Jaworzynka Valley; visible are shepherds' huts (photo archive – Tatra Documentation Center, Tatra National Park, Zakopane, Poland).

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Figure 3. Present-day view of Jaworzynka Valley.

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Figure 4. Open Larch (*Larix* sp.) forest with a dense cover of grass *Calamagrostis* sp. on the forest floor – plot No. 3.

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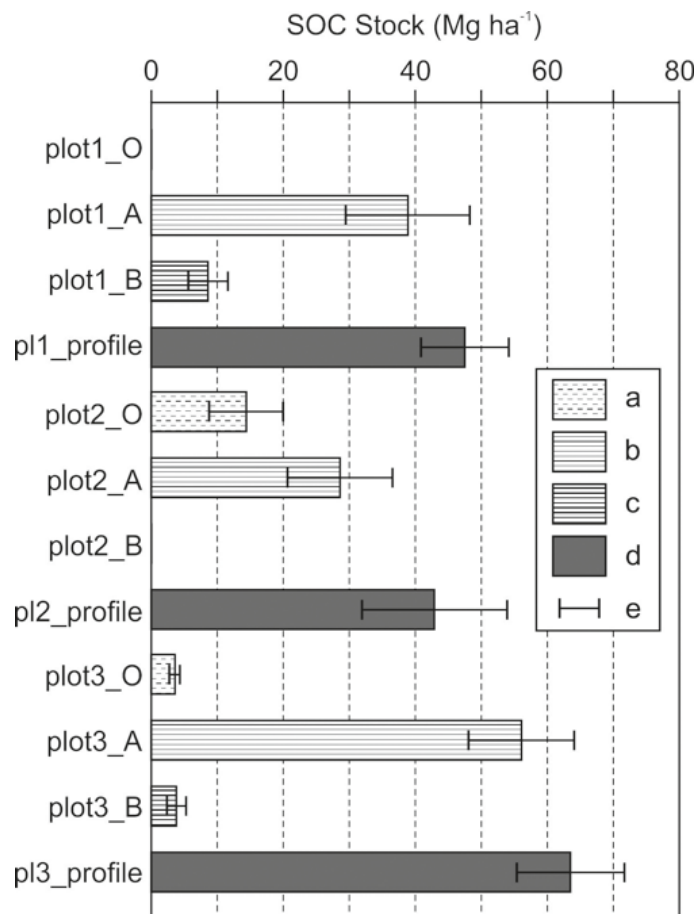


Figure 5. SOC stock for particular plots (data from 27 soil profiles): a – SOC stock in organic O-horizons, b – SOC stock in humus A-horizons, c – SOC stock in mineral B-horizons, d – SOC stock in the entire soil profile, e – SD.