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QUANTITATIVE AND QUALITATIVE FEATURES OF SOIL HUMUS IN MOUNTAIN TREELINE ECOSYSTEMS

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ABSTRACT

The ecosystems in treeline mountainous regions are considered as vulnerable to climate change, since these areas will experience stronger temperature fluctuations at regional scale than the global climate and are exposed to different intensity of land use and to land use change, both affecting their functioning and services. Forests, pastures and meadows with different land-use intensity in the treeline region of Central Balkan were studied to define the effects of the management intensity and different land uses on the quantitative and qualitative features of soil humus. Forest floor and mineral soils samples were analyzed. Land use change caused a slight decrease in organic carbon, total nitrogen contents, soil pH and the overall soil organic carbon stock 45 years after the afforestation activities as well as caused differences in the amount of humic and fulvic acids. Similar effect was observed 67 years after conversion of natural beech forest to spruce plantation. The presence or lack of management activities in the created coniferous plantations in the treeline zone were not a prerequisite for betterment humification processes and further incorporation of organic substances in the mineral soil in forest land uses. Opposite could be concluded for mountain grasslands, where relatively long term intensive grazing is hence with an increase of carbon content in superficial soil and improved soil fertility to some extents. The potential effect of land use and management intensity on carbon storage capacity in treeline ecosystems could be estimated by assessing the quantitative features of soil humus. They are important, but could not be applied as enough informative indicators for the effects of land use change and management impacts in treeline ecosystems.

Key words: treeline, land use types, management intensity, soil carbon stock, soil humic substances

INTRODUCTION

Mountains cover about one fifth of the earth's continental area but their ecological and economic importance, e.g. regarding supply of ecosystem services, reach beyond their boundaries (Beniston et al., 1997). Ecosystems in these regions are especially vulnerable to climate change, since these areas will experience stronger temperature fluctuations at regional scale than the global climate (Schröter et al., 2005). They are also exposed to different intensities of land use and to land use change, both affecting their functioning and services. In particular, human activities have altered the rate, pathways and efficiency of the movement of nutrients within and between ecosystem compartments. These changes in nutrient cycles do not only affect today's ecosystem functioning but may also result in long-term legacy effects on ecosystem processes, thereby changing the resilience of ecosystems and their adaptive capacity to sustain ecosystem services in the face of uncertainty and global change (Carpenter et al., 2009; Elmqvist et al., 2003; Folke et al., 2002).

In Central Balkan Range, the treeline ecotone is formed by three vegetation zones: broadleaved, limited areas of coniferous (plantations, natural) and sub-alpine zone. The first one is dominated by *Fagus sylvatica* L. and often forms the upper boundary of the treeline. It is situated between 1200-1700 m a.s.l. Coniferous forests are only fragmentary presented in the elevational belt from 1500 (1600) to 1850 m. Natural coniferous forests are located only in some reserve territories ('Boatin', 'Tsaritchina'). The vegetation zone formed by sub-alpine sparse, dwarf pine and juniper (*Juniperus sibirica* Burgsd.) is situated between 1500 and 1850 m. Filipovich (1976, in Dakov et al., 1980) explored the development of forest communities in Central Balkan in the last 8000 years and concluded that from 4000 to 2000 BC tree species *Picea abies* (L.) Karst. and *Abies alba* Mill. were dominant in this area. Burning was widely applied in the XVII–XIX century to clear forest sites for sub-alpine pastures (Alexandrov, 1994). In addition, in the past these mountain forests have been threatened by excessive and improper use by the local population. This has replaced naturally occurring tree species by others. Due to their overexploitation in those periods and burning, nowadays, the timberline is formed mainly by *F. sylvatica* (Dakov et al., 1980). Afforestation with coniferous tree species for elevating the treeline has been widely applied in the second half of XX century. In summary, land use and land use change were the main drivers of changes in Central Balkan, and these changes presumably affected all compartments of these ecosystems, including mountain soils (Cudlin et al., 2017).

Humus is the main accumulator of carbon in soils, which is influenced by land use and climate changes. Carbon in 0-30 cm of soils is reported to be 1500 PgC in the world (Batjes, 1996) and is referred as important potential carbon sink to mitigate the greenhouse gas effect (Bolin, Sukumar, 2000). The stock of soil organic carbon (SOC) of mountain ecosystems is among the highest in terrestrial biomes (Djukic et al., 2010; Ward et al., 2014). Soil humus is highly decomposed soil organic matter and its quality represents one of the most important soil characteristics, which creates conditions for soil functioning. Humic substances play a vital role in soil fertility and plant nutrition (Khaled, Fawi, 2011). Humus is an important component of soil and is highly vulnerable to land use and climate changes. The latter factors induce changes in humification rates (Filcheva et al., 2014). The quantitative and qualitative features of soil humus, specified as the ratio between humic acids (HA) and fulvic acids (FA), depend on specific climatic conditions (Garcia et. al, 1985) and type of vegetation (Howard et al., 1998), as well as on different silviculture and land-use management practices (Miglierina et al., 1995). Fractions of HAs and FAs are used as soil organic matter (SOM) proxies even though more than a half of the organic material in mineral soils residues is non-extractable humin (NH) (Zhiyanski, 2014). Recent interest in humin, which is believed to include the more persistent components of SOM, has increased greatly with the desire to sequester C in soils on a permanent basis. The ratio of HA to FA (HA:FA) has been used as a turnover indicator to describe the intensity of humification process of SOM (Rivero et al., 2004). Humus can be efficiently extracted from mineral soils and its fractionation continues to be widely studied (Wander, 2004).

However, not enough data are available regarding the quantitative and qualitative features of soil humus as affected by different intensities of land-use in treeline forests and grassland ecosystems. Therefore the characterization of soil humus is necessary in studying soil carbon sequestration and dynamics, change in storage and understanding of transformations occurring in the soils, which affect the atmosphere. The overall objective of this study was to determine the effects of management intensity and different land uses on the quantitative and qualitative features of soil humus in mountain treeline ecosystems.

MATERIALS AND METHODS

Study area

The studied sites are located on the northern slope of Central Balkan Mountains within an altitudinal range from 1060 to 1600 m a.s.l. in Beklemeto region, i.e within the mountain climatic zone of Bulgaria (Sabev, Stanev, 1963) and are considered as representative for the land uses and changes in this mountain region. The underlying geology is formed by shale. The average annual precipitation in the studied region is 900 mm with maximum quantities in May and June and a minimum in February. Mean annual temperature varies between 4°C and 8°C with maximum in August and minimum in February (NIMH, 1972-2012). The mean summer temperatures are within 16.5 – 22.0°C, with mean value for July of 20.9°C.

The study was conducted in 2013/2014 in eight experimental plots, each of 0.1 ha (31.62 x 31.62 m) in different land uses: Forest land use (F) - two beech natural forests, two spruce plantations; and grassland land use (G) - two mountain pastures with different grazing intensities and two mountain meadows with different management intensity (Table 1). The experimental plots in grasslands (G1 to G4) were chosen in both extensively and intensively managed pasture and meadow. The intensive management was practiced for a period of ten years: in the pasture – 20 dka for one adult cow for the period from 1st of May to 1st of November; in the meadow - during the whole vegetation period one cut per month. The extensively managed grasslands were characterized by only occasional grazing and mowing during the last ten years. Forest land uses were chosen in both managed and unmanaged treatments. The unmanaged spruce plantation (F1) was created 45 years ago with planting of 2-year old samplings on the adjacent pasture in order to elevate the treeline. Since its creation no management activities were performed. The managed spruce plantation (F2) has been planted after a clear cutting of a former beech forest. The beech forests (F3 and F4) are naturally regenerated after the same clear cutting performed 67 years ago. The management activities in sites F2 and F4 included thinning with 30% intensity respectively, conducted 20 years ago.

Table 1. Characteristics of the studied plots

Site	Altitude, m a.s.l.	Aspect	Soil type (WRB 2015)	Bulk density (0-15 cm / 15-30 cm g/cm^3)	Land use – type and management intensity	Vegetation, age
F1	1490	NW	Cambisols Dystric-Eutric	0.91/0.90	Spruce plantation, unmanaged	*P. abies* 47 years old
F2	1100	NW		0.90/0.92	Spruce plantation, managed (thinning 25-30% intensity)	*P. abies* 69 years old
F3	1280	N		0.85/0.99	Beech natural forest, unmanaged	*F. sylvatica* 66 years old
F4	1300	NE		0.90/0.90	Beech natural forest, managed (thinning 30% intensity)	
G1	1490	N		0.80/0.99	Pasture, MiG	Grass – native species, *Nardus stricta* L.
G2	1290	NE		0.85/0.80	Pasture, MeG	
G3	1060	NE		1.02/1.05	Meadow, unmanaged	Grass – native species, *N. stricta, Phleum alpinum* L., *Poa ursine* Velen., *Festuca supine* Kiffmann, *Carex laevis* Kit. ex Willd., etc.
G4	1260	NE		0.85/0.91	Meadow, intensive management	

*MiG – managed intensive grazing / MeG – managed extensive grazing

Sampling and laboratory analyses

Sites were visited and sampled during the growing season between April and October in 2013 and repeated in 2014. The turf grass and forest floor samples by layers (Aol, Aof, Aoh) were collected using a plastic frame with size 25x25 cm in 3 repetitions per plot.

Soils were sampled randomly in 3 repetitions within each experimental plot by the pit method (in one representative soil profile) and by hand augers (4 cm in diameter in two additional cores) from the first two soil layers 0-15 cm and 15-30 cm. Hence, per site a total number of three replicates of soil samples was analyzed. The soils were air-dried, plant materials and roots were removed, then soil was sieved < 2 mm for further analyses. A mean sample for coarse fractions content (> 3 mm) was separated and analyzed.

Soil properties were determined in accordance with the standardized methods in the Laboratory of Forest Soil Science at the Forest Research Institute – BAS (Donov et al., 1973). Recorded data included morphological field description, bulk density, particle size distribution (wet sieving, including coarse fragments, i.e. particles with a diameter of 2-10 mm), textural classes, pH in H_2O (ISO-10390 2005), N content and organic C content. The bulk density was measured per each soil layer of undisturbed soil with cylinders with known volume in two repetitions. Fresh soil samples were individually weighed and dried for 48 h at 105°C. Each soil sample was sieved in a 2 mm mesh to remove coarse sands while soil aggregates were broken. In the next step, the sample was ground and the total organic C content was measured by the modified method of Tyurin (Arinushkina, 1970). Total N content was measured according to the method of Kjeldhal (Bradstreet, 1954). The C content, combined with bulk density, was used to estimate the amount of carbon per unit area (IPCC, 2003). We report SOC stocks based on equivalent soil masses (ESM) (Ellert, Bettany 1995; Wendt, Hauser 2013).

Humic substances were determined by the modified method of Tyurin – oxidation with $K_2Cr_2O_7/H_2SO_4$ solution in thermostat at 120°C for 45 min with presence of catalyst Ag_2SO_4 and titration with $(NH_4)_2SO_4.FeSO_4.6H_2O$, and phenylanthranilic acid as an indicator. The composition of humus in mineral soils was determined according to the methods Kononova (1966), Filcheva, Tsadilis (2002), based on the ability of sodium pyrophosphate (0.1 M solution $Na_4P_2O_7.10H_2O$) to form insoluble precipitates with calcium, iron, aluminum and other multivalent cations. The most complete extraction and replacement of Ca, Al and Fe with Na was obtained in a mixture of NaOH and $Na_4P_2O_7.10H_2O$ at pH of about 13. As a result, it was obtained the amount of soluble HAs and FAs of sodium and insoluble phosphoric acid salts of the respective cations. The advantage of this method is defined by the relatively short period of extraction of humic substances - 10-12 h. The qualitative criteria of soil humus were determined according to Orlov, Grishina (1981).

Statistical analysis

Statistics were run using SPSS 16.0 program. An analysis of variance (ANOVA) of the studied soil properties was carried out and the differences among the means of the studied land uses were detected by soil horizon employing Tukey's test ($\alpha = 0.05$). Pearson linear correlation coefficients among studied parameters were calculated and used to reveal the direction of relationships between selected soil properties and among studied land use types (Swan, Sandilands, 1989).

RESULTS AND DISCUSSION

Soil characteristics

All studied Cambisols had strongly acid to acid pH, according to the Soil Conservation Classification of Foth, Ellis (1997). Soil pH values were significantly affected by both land use ($F=6.544$; $P\leq0.001$) and soil depth ($F=3.333$; $P\leq0.005$). The pH values of 0-15 cm soil layer varied between 4.02±0.15 and 4.31±0.20 under forest land uses and were higher for most grasslands (from 4.30±0.08 to 5.20±0.12; Fig. 1). The higher acidity in superficial soil layers under spruce and beech forests results from the chemical characteristics of the litter input material and differ in their decomposition rate (Berger et al., 2015). These results supported Toth et al. (2011) that the properties of litter in a given forest could fundamentally influence the soil pH and consequently the nutrient mobility in superficial soil. Xu et al. (2013) in their meta-analyses also

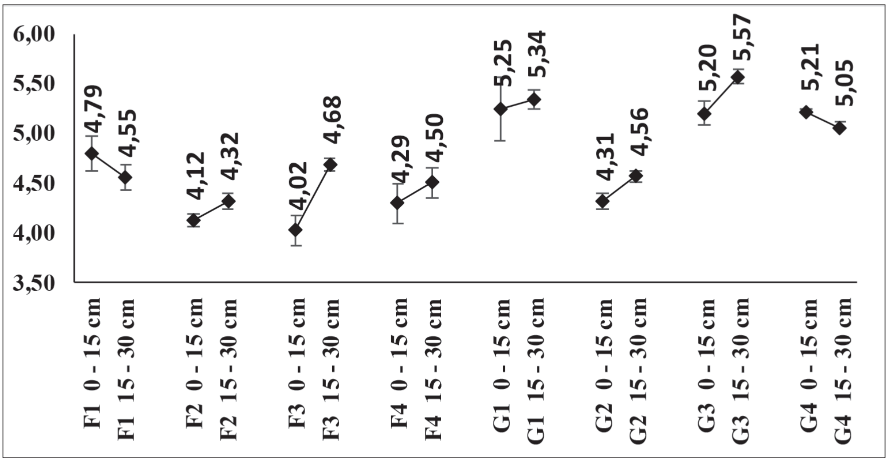

Fig. 1. Soil pH (H_2O) in studied land uses (Mean±SE)

concluded that litter inputs may change soil pH via changing the release of organic acids or the supply of exchangeable base cations during the processes of litter decomposition, but depth distribution of pH is most dependend of soil regimes under mountainous climate. The low pH of soils in the study sites indicated that many nutrients were very soluble and could be easily leached from the soil profile. Soil pH tended to increase with depths for most but two sites - in F1 and G4 the pH values were higher in the top layer and decreased with depth. This pattern might be explained by the afforestation effect and a change in the type of organic residues and by the lower intensity of management activities. For F1 the result is supported by Alfredson et al. (1998) and Davis (2001), who reported for pH reduction after afforestation up to depth of 30 cm of soil. The pH trend in G4 could be explained with previous liming in the site, but such information is not supported with any documents and is only a suggestion.

SOC content was highest in the 0-15 cm layer for all plots and varied significantly between land uses ($F=18.677$; $P \leq 0.001$). It ranged from 3.27±0.48 to 11.87±0.32% in the grasslands, and between 5.49±0.72 and 8.07±0.16% under forests (Fig. 2). SOC content decreased rapidly in deeper soil layer in all studied land uses, but most pronounced in F4 and G1. The land use change expressed by afforestation of pasture to spruce forest in order to increase the treeline in Central Balkan (G1 to F1) and further lack of management activities resulted in a decrease of C content by more than 3% for the top soil in F1.

The total nitrogen concentrations followed the tendency of the carbon – higher level in the top soil layers and a decrease with depth (Fig. 3). In 0-15 cm depth N contents varied between 0.25±0.05 and 0.82±0.07% and were significantly different between land uses ($F=4.107$; $P \leq 0.005$).

Land use change and management intensity result in differences in soil carbon and nitrogen concentrations (Zhiyanski et al., 2015). In our case, the observed decrease of soil carbon after afforestation is directly related to the prior land use and related environmental conditions (plant species composition and intrinsic edaphic properties) and human management. Hiltbrunner et al. (2013) established that stocks of SOC were only moderately affected by the afforestation: in the mineral soil, SOC stocks transiently decreased after tree establishment, reaching a minimum 40–45 years after afforestation (–25%) and increased thereafter. Among the different land uses, the soil nitrogen concentrations in the pastures and forests were higher than in the meadows. Land use change from pasture to forest in treeline zone seems to have triggered a slow decrease in soil nitrogen concentrations, while management activities in meadow and beech forest seem to have a slight effect on soil nitrogen concentration in superficial soil.

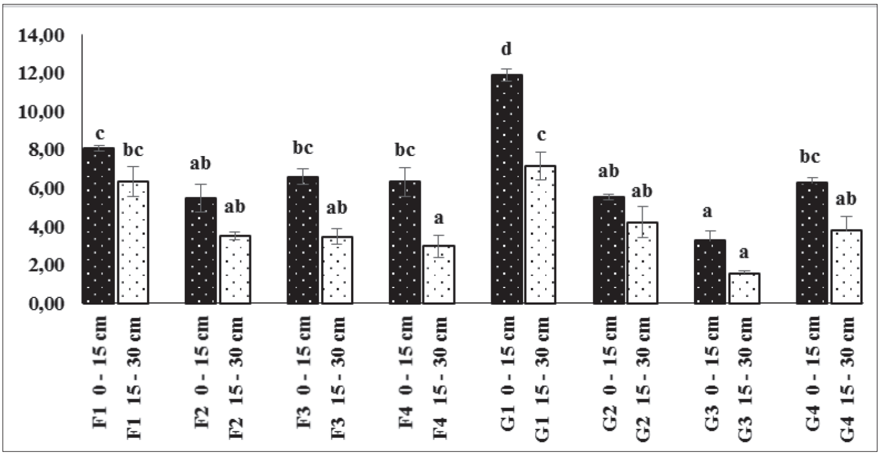

Fig. 2. Total soil organic carbon (%) in studied land uses

Note: Different letters indicate significant differences among the means after Tukey's test (P ≤ 0.05)
Error bars represent the standard error of the mean

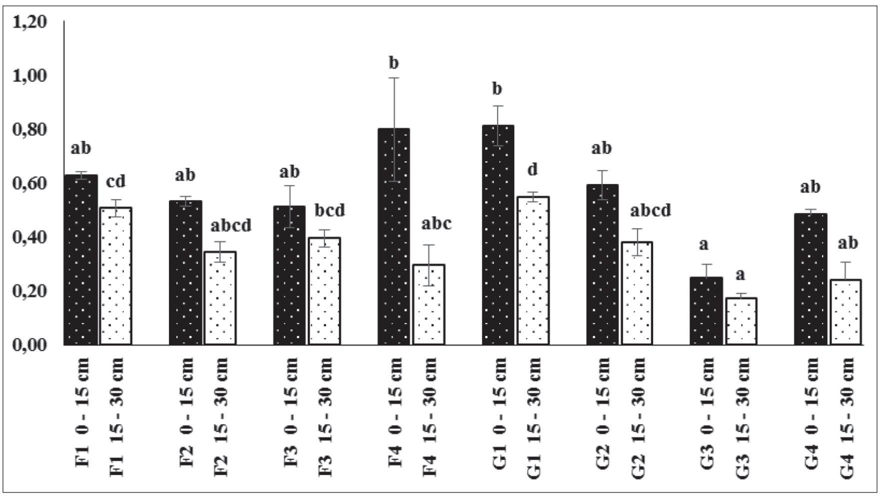

Fig. 3. Soil nitrogen content (%) in studied land uses

Note: Different letters indicate significant differences among the means after Tukey's test (P ≤ 0.05)
Error bars represent the standard error of the mean

The implemented forest management activities – thinning and change of vegetation cover distinguished managed and unmanaged forests, but did not result in significant change in SOC content. Yet, higher thinning intensity in beech forest increased slightly nitrogen contents in superficial soil. The occasional mowing practices in the studied meadow led to rapidly decrease in C content by almost 3%. The opposite trend is observed in mountain pastures, where SOC contents in the intensively grazed site (G1) was twice as high as under extensive grazing (G2). Studies on the impact of various intensity of grazing on soil C suggest that short duration and high intensity grazing leads to the greatest soil C content, while continuous grazing at high intensity resulted in the lowest soil carbon contents (Manley et al., 1995; Wood, Blackburn 1984). Conant et al. (2003) showed that total organic soil C is 22% greater under intensively managed pastures as compared to extensively managed.

Soil C/N ratios in 0-15 cm depth varied between 9.12 (F3) and 15.40 (G3) but differences were not significant (Fig. 4). C/N of soils under forests and extensively managed grasslands slightly increased in 15-30 cm soil depth. A higher C/N was found for managed grasslands – G2 and G4, but without statistically significant differences ($P \leq 0.18$). The C/N ratio showed an average nitrogen stock in the studied soils and worse conditions in terms of elements cycling processes. Afforestation and changes in tree vegetation cover result in a decrease of soil C/N ratio. Black, Harden (1995) observed that soil C/N ratio provides a signature of the influence of residues after management in forest land uses: soil C/N is initially elevated because of the incorporation of high-C/N ratio woody residues and later decreases to an equilibrium level as soil C is selectively lost. A meta-analyses of Johnson, Curtis (2001) reported that forest harvesting, on average, had little or no effect on soil C and N. In terms of the effect of thinning intensity there is currently little evidence of changes in the mineral soil with different thinning regimes (Skovsgaard et al., 2006; Vesterdal, Leifeld, 2010).

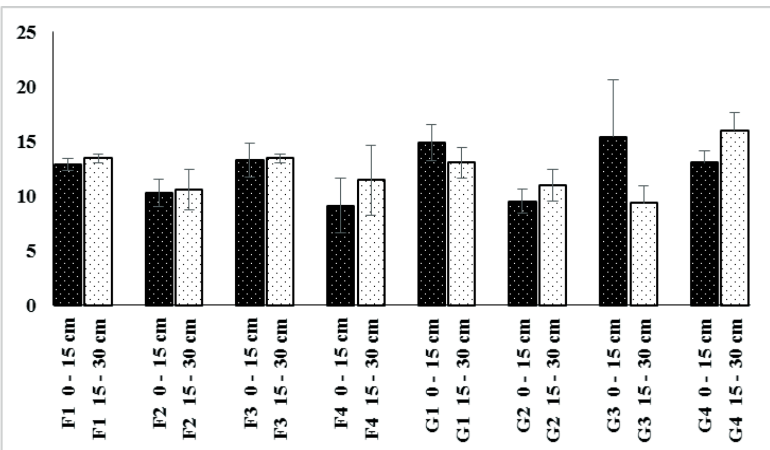

Fig. 4. C:N ratio in studied land uses
Note: Different letters indicate significant differences among the means after Tukey's test ($P \leq 0.05$)
Error bars represent the standard error of the mean

The total SOC stocks differed significantly between soil depth increments (F= 21.856; P≤0.001) and land use (F=10.660; P≤0.005). The highest SOC stock was observed in the intensively grazed pasture G1– 142.46±3.82 t/ha and 106.15±10.58 t/ha in 0-15 and 15-30 cm, respectively (Fig. 5). In comparison, the extensively grazed grassland G2 stored only half of that amount – 70.39±2.02 t/ha and 49.80±9.65 t/ha, respectively. Hence, management activities in mountainous pasture uses resulted in a significant decrease of SOC stock in both soil layers. The lower SOC stock in meadows is defined by the lower carbon contents in soils despite of the relatively higher bulk density (0.85-1.05 g/cm^3) and the low content of coarse fractions in the studied Cambisols. The soil layers of the managed meadow stored 80.03±3.42 t/ha and 51.74±9.67 t/ha in 0-15 and 15-30 cm, respectively. This is more than in the unmanaged one (49.99±7.32 t/ha and 24.56±1.89 t/ha). In the intensively managed meadow mowing resulted in a regular export of aboveground organic material and, hence, reduced C inputs to soil. At the same time, however, there might have been increased root litter production as well as a more efficient humification owing to the improved micro-climatic conditions. Management activities expressed by intensive grazing in G1 resulted in the highest soil carbon stock compared with the extensively managed treatment and forest land uses. The higher grazing disturbance in G1 might have led to an increase of root biomass in patch upper soil related with increase of soil humus content. This could be explained with the fact that the higher amount of root litter input with decreased recalcitrance to decomposition provoked the increase of organic carbon content and in changes in its composition under intensive grazing.

The land use change from pasture G1 to forest F1 in the mountainous treeline zone resulted in a significant decrease of SOC stocks in both soil layers. Study of Hiltbrunner et al. (2013) supported this results that afforestation with Norway spruce on a subalpine pasture alters carbon dynamics but only moderately affects soil carbon stock. That change is presumably related to the presence of higher organic matter inputs in the intensively managed grassland than in the forest. No significant differences were observed in the deeper layers, which confirmed the low speed of the processes of carbon accumulation toward deeper soil. This could be explained by the fact that in the spruce forests low-lying branches stay alive for a relatively longer period of time even in dense plantations (Suits et al., 2005), which resulted in formation of a different microclimate – with higher air and soil moisture, and lower temperatures comparing to the pasture conditions. The latter affects the rate of decomposition of the forest floor and reduces the humification rate.

Differences in SOC stock in the top layer were visible between natural beech forests F3 and spruce managed plantation F2, whereas in 15-30 cm depth the differences were not clearly pronounced. Despite the improved microclimate conditions that occurred after intensive thinning activities in F2, that allow faster decomposition of the coniferous forest floor, the conversion from natural beech forest to spruce plantation induces a decrease of the SOC stock in the upper 15 cm of soil. Our data showed that the decrease of SOC stock after forest conversion of natural beech forest to spruce plantation cannot be compensated by performed thinning activities even 67 years after the conversion. The

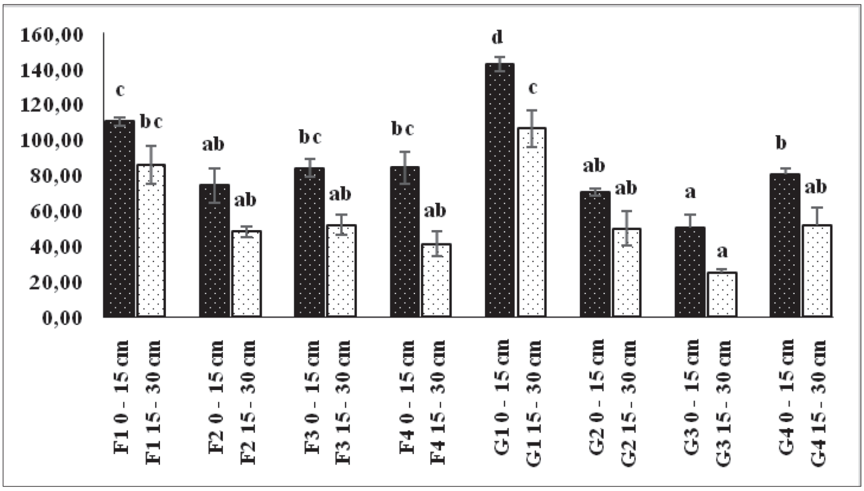

Fig. 5. Soil carbon stocks (t/ha) in studied land uses
Note: Different letters indicate significant differences among the means after Tukey's test ($P \leq 0.05$).
Error bars represent the standard error of the mean

thinning activities have no significant effect on the soil carbon stock in the superficial soil of the natural beech forests, despite of the improved micro-climatic conditions and a higher quantity of forest floor measured in unmanaged F3 (2438 kg/ha) than in managed F4 (751 kg/ha) (unpublished data).

Soil humus quality

The parameters describing soil humus qualitative features as C in HA, C in FA and NH, differed significantly among studied land uses and depths (Table 2). HA:FA ratio is one of the main parameters, which describes the type of soil humus and its quality. The humus type in studied Cambisols is generally referred to fulvic-humic type (Giurov, Artinova, 2001), and only in the first soil layer under the intensively managed pasture G1 it was classified as humic (HA:FA>2) (Fig. 6). The difference was not statistically significant, which supposed that land use change has not affected the humus type for the period of 47 years after afforestation. Concerning the percentile distribution of acids in the soil humus the highest amounts of HAs and FAs were determined in G1 (from 2.98±0.32 to 1.99±0.55% for HA in both soil depths respectively and from 1.46±0.22 to 1.14±0.07%, for FA). The lowest values were determined in the unmanaged meadow (G3).

In the managed meadow G4 the effect of the practices applied were visible up to 30 cm depth, while in unmanaged one the typical distribution of soil humus was observed. According to the HA:FA ratio in meadows' profile soil humus is referred to humic-fulvic type and HAs are 'free' and R_2O_3 bonded. In G3 soil humus in deeper soil layer was determined as fulvic-humic type, with predomination of HAs totally bonded with Ca. The total organic carbon extracted by 0.1 M $Na_4P_2O_7$ + 0.1 M NaOH (HA+FA) showed similar values to those obtained with extraction with 0.1 N NaOH and only in a deeper layers small differences were observed. In the soils under meadows the total organic carbon was twice higher in the managed one than in unmanaged, which could be explained by higher content of organic residues which are deposited on superficial soil due to intensity of mowing activities performed at least once per months during the vegetation period. Meanwhile the humic substances in soils of both treatments did not differ, which is mostly related with the same type of grass coverage.

Among grassland land uses the studied pastures were also characterized by well-expressed differences in quality of humic substances. The soil humus type under extensive pasture G2 could be referred as humic-fulvic, while management with intensive grazing appears to be related with more favourable soil conditions, where humic substances were characterized by predomination of HAs and fulvic-humic type of soil humus. In both pastures the organic carbon is 'free' and bonded with R_2O_3 and there were no Ca-bonded forms. The total organic carbon extracted by 0.1 M $Na_4P_2O_7$ + 0.1 M NaOH (HA+FA) showed similar values as those extracted by 0.1 N NaOH. The organic carbon extracted by 0.1 N H_2SO_4 showed similar values in the both studied pastures. NH was higher in G1 compared with G2.

Thinning activities performed in beech forests in treeline area has changed the soil humus type in superficial soil. While the unmanaged beech forest F3 is characterized with humic-fulvic type of soil humus (HA:FA = 0.5÷1.0) with prevalence of FAs, in the top layer of the managed beech forest F4 an opposite trend with predominance of the HAs and fulvic-humic type of humus (HA:FA = 1.0÷2.0) is observed.

The change of tree species composition due to conversion of beech forest to spruce forest (F2) has also reflected in improved humus quality with HA:FA ratio between 1.46 and 1.59 in 0-30 cm of soil. The change of tree composition combined with thinning caused change in the humus type with higher HA:FA ratio, which is clearly pronounced in F2. Management is related with slightly expressed prevalence of HA and could be related with better temperature regime and soil aeration in the in this site, and the input of litter substances, which affected the oxidation processes.

Concerning the studied experimental plots, especially in terms of their management intensity, the most important influence on the qualitative parameters of soil humus and on its amount had the kind and the quantity of post-management residues.

The amount of NH was over 50% in all studied sites, which underlined the high capacity of mountain soils in treeline ecosystems to store carbon. The highest amount was measured in F2 (72.4±0.73%) and differ significantly from the value obtained for F1 (56.6±4.46%), suggesting the effect of previous land-use or the management activities on this parameter. Same tendency is observed in comparing managed and unmanaged land uses for both superficial and deeper soil layers.

For all the studied land uses the content of free or R_2O_3 bonded HAs was very high (>80%), which has led to the maintenance of an acid soil pH (Fig. *7*). Moreover studied forest soils are poor of basic cations and the new-formed HAs remain free (Sprengel, 1826). The Ca-bonded HAs can increase the soil pH and their higher amount in the deeper soil layer correlates with the observed increased of soil pH in deeper layer in most of the studied sites.

Table 2. Content (%) of C-HAs, C-FAs and C-NH (Mean±SE)

Object	Soil layer	
	0-15 cm	15-30 cm
	C-HAs	
F1	2.15^{ab}±0.36	1.49^{a}±0.29
F2	1.13^{ab}±0.21	0.75^{a}±0.04
F3	1.16^{ab}±0.18	0.49^{a}±0.09
F4	1.34^{ab}±0.32	0.42^{a}±0.10
G1	2.98^{c}±0.32	1.99^{b}±0.55
G2	1.24^{ab}±0.18	0.66^{a}± 0.15
G3	0.62^{a}±0.14	0.30^{a}±0.07
G4	1.27^{ab}±0.20	0.70^{a}±0.21
	C-FAs	
F1	1.33^{a}±0.19	0.84^{ab}±0.15
F2	1.05^{a}±0.17	0.71^{ab}±0.16
F3	1.37^{a}±0.13	0.93^{b}±0.13
F4	0.95^{a}±0.34	0.58^{ab}±0.13
G1	0.61^{a}±0.09	0.28^{a}±0.04
G2	1.11^{a}±0.13	0.75^{ab}±0.14
G3	1.46^{a}±0.22	1.14^{b}±0.07
G4	0.85^{a}±0.08	0.93^{b}±0.15
	C-NH	
F1	63.79^{ab}±2.37	70.33^{a}±3.04
F2	69.33^{ab}±2.79	71.07^{a}±2.64
F3	56.58^{a}±4.46	62.19^{a}±1.90
F4	72.40^{b}±0.73	73.00^{a}±5.60
G1	61.54^{ab}±3.41	64.17^{a}±3.00
G2	64.64^{ab}±2.18	70.74^{a}±6.24
G3	62.73^{ab}±3.05	46.40^{a}±13.08
G4	61.85^{ab}±0.97	73.59^{a}±5.13

Note: Different letters indicate significant differences among the means after Tukey's test ($P \le 0.05$)

The quantity of 0.1 N H_2SO_4 extracted carbon was lower than 10% in all studied land uses and depths and varied between 2.34±0.36% in G1 and 5.21±0.91% in G2 for superficial soil layer (Fig. 8). A slight increase was observed in deeper layer with variations between 3.24±0.44% and 7.91±1.28%, but the differences were not statistically significant. The amounts of carbon extracted with NaON were higher than extracted with H_2SO_4 and varied within the range of 19.89±2.10% and 35.68±0.44% in superficial soils. The low amount of acid extractable carbon mitigated the mobility and aggressiveness of FAs, therefore the quality of soil humus in these land uses is more favourable for the aboveground vegetation.

The land use change affected slightly the amount of acid and basis extracted carbon (G1 vs. F1). Management activities in forest land uses (F2 and F4) did not resulted in any changes in this parameter.

HAs in the studied soils under grasslands have high optical density with E4/E6 between 3.5 and 4.5, which indicated high molecular weight and high degree of aromaticity (Fig. 9). The optical characteristics showed more condensed HAs in G1 and

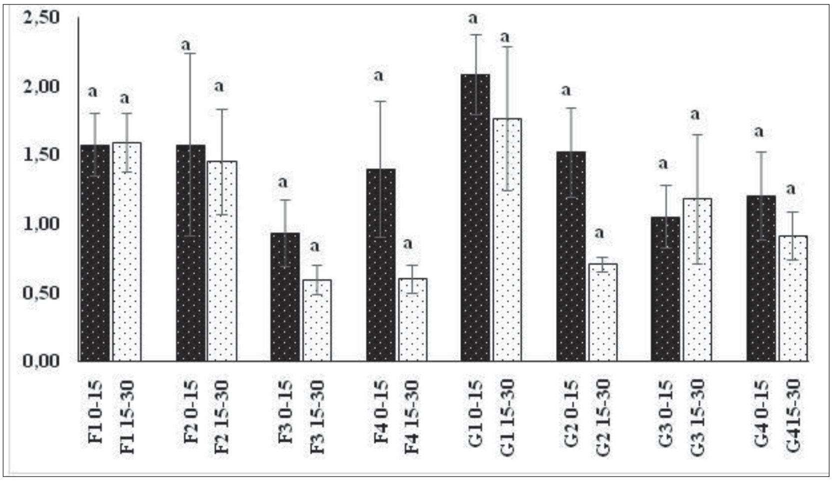

Fig. 6. HA:FA ratio in studied land uses (Mean±SE)
Note: Different letters indicate significant differences among the means after Tukey's test (P ≤ 0.05).
Error bars represent the standard error of the mean

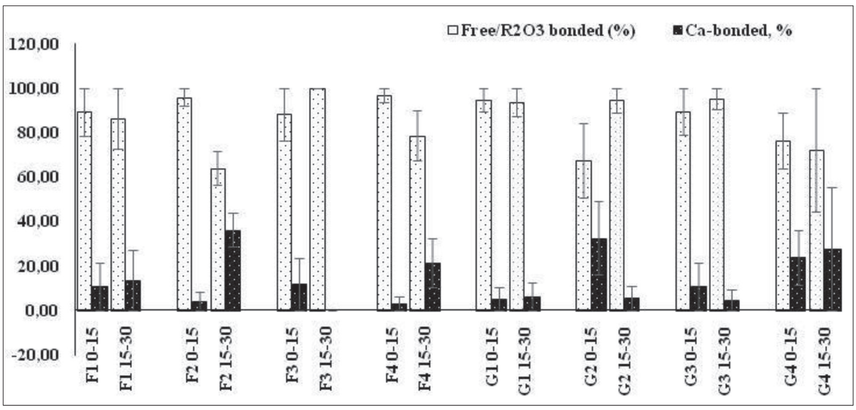

Fig. 7. Free or R_2O_3 bonded, and Ca-bonded HAs, % from the HAs (Mean with SE-bars)

G2, as well as in F1, which referred to better physical properties of these soils. Humic substances in soils under long-term forest land use are characterized with lower optical density (E4/E6=5-6), lower molecular weight and degree of aromaticity, which is typical for Dystric Cambisols (Artinova, 2014).

The results from Pearson correlation test showed that nitrogen content, HA and FA content and HA/FA ratio had high significant and positive correlations with carbon content (Table 3). Statistically significant and positive correlations were found also for organic C extracted by base and pH (r=0.413**), for HA and FA content and nitrogen content (r=0.613**, 0.581**), HA content and C stock (r=0.413**) and between most of the studied quantitative traits. HA content was negatively and significantly correlated with NH and C extracted by acid (r=-0.498**, r=-0.406**, respectively). High statistically

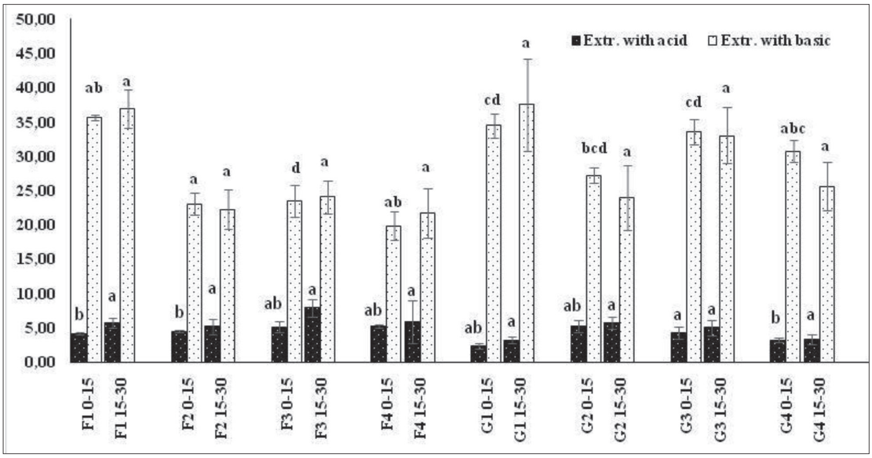

Fig. 8. Soil organic carbon (%) extracted by H_2SO_4 and by NaOH

Note: Different letters indicate significant differences among the means after Tukey's test ($P \leq 0.05$). Error bars represent the standard error of the mean

Table 3. Pearson's correlation matrix for soil quantitative and qualitative features

	pH	C	N	C/N	SOC stock	HA	FA	HA/FA	NH	org. C extr. by acid	org. C extr. by base
pH	1										
C	-,017	1									
N	-,174	,724**	1								
C/N	,146	,237	-,310*	1							
SOC stock	,104	,303*	,101	,231	1						
Ha	,074	,927**	,613**	,242	,413**	1					
FA	-,234	,785**	,581**	,242	,291*	,618**	1				
HA/FA	,127	,519**	,334*	,044	,231	,711**	-,019	1			
NH	-,348*	-,342*	-,183	-,113	-,303*	-,498**	-,216	-,338*	1		
org. C extracted by acid	-,265	-,391**	-,230	-,011	-,250	-,406**	-,278	-,297*	,105	1	
org. C extracted by base	,413**	,335*	,025	,301*	,286*	,483**	,077	,450**	-,725	-,025	1

Note: *Correlation is significant at the 0.05 level (2-tailed); ** - correlation is significant at the 0.01 level (2-tailed)

significant and negative correlation was found also for NH and C extracted by base (r=-0.725**). However, these results are in good agreement with findings of Kilic et al. (2012) and Gebeyaw (2015), indicating significantly high correlations between some physical and chemical soil properties under different land uses in Turkey and Ethiopia.

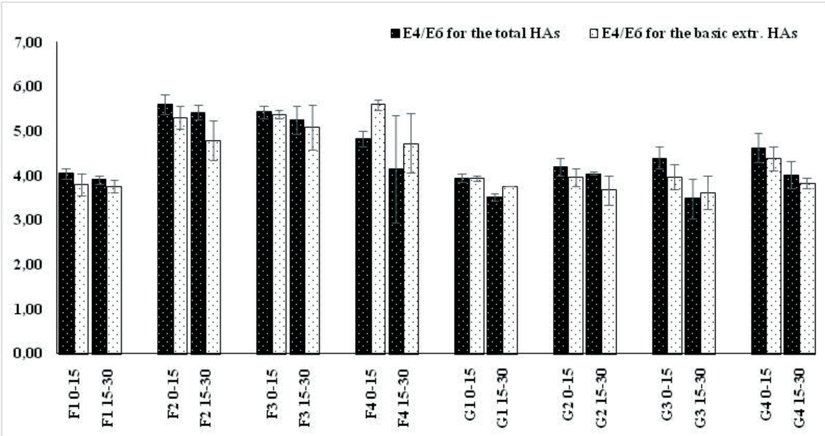

Fig. 9. Optical index E4/E6 of the HAs

CONCLUSIONS

This study improves our understanding in changes of quantitative and qualitative features of soil humus in mountain treeline ecosystems with different types of land use and management intensity. Regarding land use change, high-mountain intensive pasture to coniferous forest, caused differences in quantitative characteristics of soil humus expressed with a slight decrease in organic carbon and total nitrogen contents, decrease in soil pH and in the overall SOC stock in 0-30 cm of soils 47 years after the afforestation. Moreover land use change caused differences in the amount of HA and FA defined by the different character of organic substances from both the aboveground and root litter inputs. Similar effect on superficial 0-15 cm of soil is observed after conversion of natural beech forest to spruce plantation 67 years after the change. The change of tree coverage combined with thinning is related with slightly expressed prevalence of HA and could be related with better temperature regime and soil aeration in the in this site, and the input of litter substances, which affected the oxidation processes. The presence or lack of management activities in the created coniferous plantations in the treeline zone were not a prerequisite for betterment of humification processes and further incorporation of organic substances in the mineral soil in forest land uses. This conclusion is supported by the fact that thinning in forest land uses had no clearly expressed effect on both qualitative and quantitative features of soil humus in studied mountain Cambisols. Opposite could be concluded for mountain grasslands, where intensively managed pastures and meadows were characterized by different quantitative and qualitative features of soil humus. Relatively long term intensive grazing of mountain grasslands is hence with an increase of carbon content is superficial soil and improves the soil fertility to some extent in our system. Moreover, the amount of humic substances as HA and FA could be used as an informative indicator for changes in land use intensity in mountain grasslands. Concerning the studied experimental plots, especially in terms of their management intensity, the most important influence on the qualitative parameters of soil humus and on its amount had the kind and the quantity of post-management residues.

The amount of NH in studied Cambisols in forest and grassland land uses varied between 46.4% and 73.07% in superficial 0-30 cm soil layer. The high amount of NH is due to previous land use history of the region combined with the specifics of the humification process in studied soils. In terms of sustainable carbon storage mountain soils has relatively strong potential to preserve carbon under its stable form – humin.

It has been generally assumed that soil humus with a higher value of HA:FA ratio is feature of improved CEC and more fertile soils as was comparatively established for the intensively grazed high mountain pasture.

It could be summarized that in terms of sustainable supply of regulating ecosystem services in treeline regions and sustainable carbon storage in the soil one of the main elements of preserving soil fertility is the supply of organic matter which improves physical, chemical and biological soil properties to be ensured.

The potential effect of land use type and management intensity on carbon storage capacity in treeline ecosystems could be estimated by assessing the quantitative features of soil humus. Qualitative features of soil humus could give additional information, but could not be applied as enough informative indicators for land use change and management impacts.

Finally, this study provides much needed information on the role of soils in assessing the sustainable supply of ecosystem services by different ecosystems in treeline regions.

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