

Effect of plant communities on aggregate composition and organic matter stabilisation in young soils

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Received: 15 November 2013 / Accepted: 9 October 2014 / Published online: 18 October 2014
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Abstract

Objectives Carbon (C) content in pools of very young soils that developed during 45 years from loess was analysed in relation to vegetation: deciduous and coniferous forests and cropland. We hypothesised that variations in the amount of particulate organic matter (POM) can explain the C accumulation and also affects the C bound to mineral surfaces in soil under various vegetation.

Methods Soil samples were collected under three vegetation types of a 45-year-old experiment focused on initial soil development. Aggregate and density fractionations were combined to analyse C accumulation in

large and small macro- and microaggregates as well as in free and occluded POM and mineral fractions.

Results Deciduous forest soil accumulated the highest C content in the 0–5 cm layer (43 g C kg⁻¹), whereas values in coniferous forest and arable soils were lower (30 and 12 g C kg⁻¹, respectively). The highest portion of C in arable soil was accumulated in the mineral fraction (80 %), whereas 50–60 % of the C in forest soils were in POM. More C was associated with minerals in deciduous forest soil (16 g C kg⁻¹ soil) than under coniferous forest and arable land (8–10 g C kg⁻¹ soil).

Conclusions Particulate organic matter explains most of the differences in organic C accumulation in soils

Responsible Editor: Kees Jan van Groenigen.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-014-2299-y) contains supplementary material, which is available to authorized users.

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developed during 45 years under the three vegetation types on identical parent material. The C content of the mineral soil fraction was controlled by plant cover and contributed the most to differences in C accumulation in soils developed under similar vegetation type (forest).

Keywords Carbon sequestration · Initial soil formation · Carbon accumulation rates · Aggregate turnover · Organic matter stabilisation · Young soils

Introduction

There is a close positive relationship between the amount of plant residues, organic carbon (C) content in soil, and aggregate structure (Six et al. 2004). Plant residues promote soil structure formation and, in turn, aggregates protect plant residues from rapid decomposition (von Luetzow et al. 2006) and effect the C sequestration (Schneckenberger and Kuzyakov 2007).

Formation of macroaggregates (>250 μm) in soils is induced by fresh plant residues (Oades 1984), whereas microaggregates (<250 μm) are formed further inside of the macroaggregates and are released from them due to natural macroaggregate turnover (Angers et al. 1997; Six et al. 1998). Microaggregates are usually abundant in agricultural soils, whereas macroaggregates are typically present in smaller amounts. This reflects intensive macroaggregate disruption during plowing and slow formation of new ones because of an insufficient amount of fresh plant residues in agricultural ecosystems (Six et al. 2004). In natural ecosystems such as forests, more macroaggregates than microaggregates are observed due to the high amount of plant litter. Although numerous studies assume a strong effect of plant litter on aggregate formation, experimental investigations in which the plant community is the leading factor in soil aggregation – when other factors are equal—are lacking.

Beyond aggregate structure, C accumulation in aggregate size classes also depends on the plant community. Plowing leads to less C accumulation in macroaggregates and to accumulation of C depleted microaggregates, whereas restoration of natural forest or grasses vegetations promotes the opposite processes (Six et al. 2000). Most studies on the C content in aggregates were performed on soils where the management history changed several times. This makes it

difficult to draw conclusions about the level of C accumulation in aggregates formed in soils that developed under individual plant communities.

Density fractionation of aggregate size classes reveals an inhomogeneous structure, and fractions of free POM, occluded POM, and C bound to mineral surfaces can be separated (Golchin et al. 1998; Gunina and Kuzyakov 2014; Yamashita et al. 2006). The POM fractions differ from each other in density according to their association with clay (range 1.6–2.0 g cm^{-3}); in allocation – occluded POM is located within aggregates, free POM is outside; and in degree of C degradation, which increases in the order free POM – light occluded POM – heavy occluded POM (Yamashita et al. 2006). Free POM promotes the formation of macroaggregates, whereas occluded POM serves as a binding agent for mineral particles in aggregates (Golchin et al. 1998). Both occluded fractions are mainly allocated in microaggregates, although heavy occluded POM is also found in macroaggregates and thus provide additional information on the C stabilised in that aggregate size class (Golchin et al. 1998). Due to the natural turnover of aggregates – 5–30 days for macroaggregates (De Gryze et al. 2006; Plante et al. 2002), 88 days for microaggregates (De Gryze et al. 2006) – the POM fractions are periodically redistributed between inside and outside parts of aggregates. Accordingly, the C contents in free and occluded POM can be similar. Further degradation of POM fractions leads to the mineral fraction, which presents C associated with clay and silt particles. The amount of C associated with the mineral fraction is directly related to the clay and silt contents (Hassink et al. 1997). After clay surfaces have accumulated the maximum amount of C, further C accumulation is possible only in POM fractions (Hassink et al. 1997). Nonetheless, nearly all previous studies were performed with soils in which C accumulation in the mineral fraction had peaked and aggregate structure was already formed. This precluded demonstrating the sequence of C accumulation in various fractions.

This study was therefore designed to clarify how soil organic carbon (SOC) accumulates in young soils (which are not under steady-state conditions). Under such conditions, aggregate formation occurs in parallel with the stabilisation of organic matter (OM) in clay fractions and all soil-forming factors, other than plant cover, are identical. In particular, we addressed the following research questions: i) if plant litter is directly responsible for aggregate formation, can POM fractions

explain the variation in C content in aggregate size classes and the differences in the amount of C stored in soils under different plant communities? And ii) if POM is a direct source of C, which will be stabilised on clay surfaces, can the amount of C associated with mineral fraction differ in soils that developed under various plant communities?

Two forest soils and one arable soil were examined to test these questions. The soils were developed in large lysimeters for 45 years on the C-free parent material under identical conditions except the plant cover. Physical fractionation methods (dry sieving and density fractionations) were combined to investigate the SOC accumulation in aggregate fractions of these young soils.

The specific objectives of this study were: i) to estimate the amount of OM accumulated in the soils over 45 years; ii) to analyse the distribution of SOC among the macro- and microaggregate size classes; and iii) to evaluate the contribution of C from free and occluded POM and the mineral fraction to aggregate composition and total SOC content.

Materials and methods

Experimental set-up and soil sampling

Soils from the large open lysimeters located within the Moscow State University area were used in this study. The installation of the lysimeters has been described in detail elsewhere (Vinnik and Bolyshev 1972). Briefly, the large lysimeters were installed in 1965 and included 20 plots of 3×3 m (area 9 m² each) with a depth of 1.5 m. Each lysimeter was filled with carbonate-free silty loam, which originated from the cover loams of the Valday glaciation and was collected north of Moscow from a depth of 130–280 cm. The parent material was sieved (3–4 cm) and thoroughly mixed prior to filling. The texture of the parent material was a silty loam (3.7 % sand, 65.3 % silt, and 28.6 % clay) (Vinnik and Bolyshev 1972). The mineral composition of the clay fraction was: smectite (56.1 %), illite (34.0 %), and kaolinite + chlorite (8.9 %) (Chizhikova et al. 2006b). The chemical characteristics of the parent material and the soils that developed from it are presented in Table 1.

The following plant communities were planted in lysimeters after 2 years of settling: two forests (four plots for each type of forest), arable/grassland (two plots). In the deciduous forest plots, 31 English oak

(*Quercus robur*) seedlings and 30 three-year-old seedlings of Norway maple (*Acer platanoides*) were planted. In each coniferous plot, 61 five-year-old seedlings of Norway spruce (*Picea abies*) were planted (Gerasimova et al. 1989; Vinnik and Bolyshev 1972). The arable/grassland plots consisted of agricultural nine crop rotation including potato, summer wheat, buckwheat, oat, barley, and perennial grasses. The crops were removed from the arable plot. Crop rotation was conducted for 35 years, during which soil was plowed manually (each year excluding years when perennial grasses were cultivated, namely 27 times in total) and mineral fertilizers (171 kg ga⁻¹ ammonium nitrate and 400 kg ga⁻¹ superphosphate) were applied annually. In the last 10 years, permanent grasses were grown without soil tillage and without removal of plant biomass.

The climate of the Moscow region is humid continental. The mean annual temperature in the area of Moscow State University is +5.8 °C, with July and February means of +19.2 °C and –6.7 °C, respectively. The mean precipitation is 680 mm.

Soil samples (200 g) were taken in spring 2012 from a depth of 0–5 cm (from A horizon), in four field replicates for each plant community. Each field replication was comprised of pooled samples from two random places within each lysimeter. For the arable plots, the lysimeter area was divided to two parts, which presented two additional field replications. This procedure was done because only two plots were established for arable land plant community and it was not possible to obtain four replications for these plots. The litter layers, which were developed under the forest plant communities, were omitted from the samples. The soils were sampled from the 0–5 cm layer because our and previous findings have identified this soil layer as having the greatest differences in organic C levels for the chosen plant communities (Gunina and Kuzyakov 2014; Saveliev and Vladychenskii 2001). Moreover, the development of a plant community changes the C content in the upper 0–5 cm soil layer, whereas the response of lower horizons is slower (Vesterdal et al. 2008).

We will use the following terms for the different soils: “deciduous forest” for mixed broadleaf forest, “coniferous forest” and “arable”.

We characterised these soils as young because only the first 20 cm of initial parent material was subjected to the soil formation processes (versus soils of temperate zone developed on the same parent material). This was determined based on the color of the soil profile and

Table 1 The pH, soil organic carbon (SOC), total nitrogen (TN), and C/N ratio of soils (0–5 cm depth) in the large lysimeters after 45 years (sampled in 2012) and the silty loam parent material (initial characteristics), depending on vegetation type

	Initial soil parent material ^a	Deciduous forest	Coniferous forest	Arable land
pH	5.7 (0.1)	6.4 (0.1)	5.4 (0.1)	6.2 (0.1)
SOC, g C kg ⁻¹ soil	0.7 (ND)	43.1 (4.0)	29.6 (2.3)	11.6 (0.7)
TN, g N kg ⁻¹ soil	0.3 (ND)	3.1 (0.5)	1.2 (0.2)	0.9 (0.1)
C/N	2.3 (ND)	21.0 (1.5)	26.1 (1.3)	16.5 (1.7)
Amount of aboveground litter, kg ha ⁻¹ b	NA	730–1,300	400–1,050	5300 ^c
C/N of plant litter	NA	55.3 (ND)	42.5 (ND)	76 (ND)

ND SE was not determined due to lack of replicates, NA not applicable

^a the data on soil parent material were taken from Gerasimova et al. (1989)

^b the data were taken from Pervova and Zolotarev (2012)

^c the data were taken from Vladychenskii et al. (2000) for the grassland plot, where the same species were grown as on the arable land converted to permanent grassland. Composition of grassland species is cereals:beans = 60:40. The data on aboveground biomass during the crop history are available for wheat, which was grown 7 times and had aboveground biomass 5,100 kg ha⁻¹, which was completely removed from the plots

chemical analyses (C contents, pH, changes in proportions of clay minerals) (Chizhikova et al. 2006a; Verkhovets et al. 2006; Vinnik and Bolyshev 1972). The age of the soils around Moscow is ca. 11,000 years (i.e. soil formation in the last glaciation), making 50-year-old soils “young”.

Aggregate fractionation by size

The samples (100 g each) were air-dried at room temperature and sieved through 2,000 and 250 μm meshes on the Vibratory Sieve Shaker AS200 (Retsch, Germany) for 5 min, amplitude 1.5 mm. Large macroaggregates (>2,000 μm), small macroaggregates (250–2,000 μm) and microaggregates (<250 μm) were obtained. The mean weight diameter of aggregates was calculated according to John et al. (2005). Dry sieving was used to avoid dissolved organic carbon, fine particles and POM redistribution between the aggregate size classes. This approach minimized the mixing of SOM fractions from various aggregate size classes and helped avoid misrepresented results on C content of these fractions and their amounts.

Separation by density fractionation

Density fractions were separated for each of the three aggregate size classes obtained by dry sieving as described above. To isolate the density fractions, the method of John et al. (2005) as modified by Dorodnikov et al. (2011) was applied. Briefly, 3 g of air-dried aggregates

were placed into a centrifugation tube and 15 mL of sodium polytungstate solution with a density of 1.6 g cm⁻³ was added. The tube was then gently inverted 10 times by hand, the solution was centrifuged at 4,000 rpm for 1 h and the supernatant with floating particles was filtered (cellulose acetate filter, 0.45 μm; Sartorius, Germany) and washed with distilled water to obtain a free light fraction of OM with a $\rho < 1.6$ g cm⁻³ (fLF_{<1.6}). The remaining soil was shaken for 16 h (60 rpm) with 10 mL of sodium polytungstate solution ($\rho = 1.6$ g cm⁻³) and five glass beads ($d = 5$ mm) to crush the aggregates. The soil suspension was then centrifuged for 1 h at 4,000 rpm; the supernatant was filtered (as the first fraction) and washed with distilled water to produce an occluded light fraction of OM with a $\rho < 1.6$ g cm⁻³ (oLF_{<1.6}). The rest of the soil was dispersed with 10 mL of sodium polytungstate solution, $\rho = 2.0$ g cm⁻³, centrifuged for 1 h at 4,000 rpm, and the supernatant was filtered and washed as for the previous two steps to obtain an occluded dense fraction of OM with a $\rho = 1.6$ – 2.0 g cm⁻³ (oDF_{1.6–2.0}). The soil remaining after the separation of the light fractions was washed four times with distilled water (20 mL each time) to obtain the mineral fraction with a $\rho > 2.0$ g cm⁻³ (MF_{>2.0}).

Analysis of C and N content

Fractions obtained by density fractionation were dried at 40 °C, weighed, and ground (MM2, Fa Retsch, Germany) before analysis. Aggregate size classes were also

ground (MM2, Fa Retsch, Germany) before analysis. The C and N contents of non-sieved soil samples, aggregates, and isolated density fractions were measured using an elemental analyser (Vario EL II, Germany).

Calculations and statistics

The effect of plant community on aggregate composition was tested by one-way ANOVA followed by the Tukey HSD post-hoc test (significance level $p < 0.05$). SOC and total nitrogen (N_{total}) distributions in aggregate size classes within and between the plant communities were tested by two-way ANOVA followed by the Tukey HSD post-hoc test (significance level $p < 0.05$). The effect of aggregate size on the distribution of SOC in density fractions within and between the plant communities was tested with two-way ANOVA. The statistical significance was evaluated at the $p < 0.05$ level. All data are presented as means of four replications \pm standard error.

Results

Effect of vegetation on total C and N contents and aggregate size classes

The SOC and N_{total} contents in the 0–5 cm layer increased in the following order: arable – coniferous forest – deciduous forest (Table 1). The C/N ratios of soil organic matter did not directly reflect the litter quality of the different ecosystems (Table 1).

Dry-sieving showed that the distribution of aggregate size classes, especially of the small macroaggregates, was strongly affected by the vegetation community (Fig. 1). Large macroaggregates dominated in soils of all vegetation types, but decreased in the order: arable – deciduous forest – coniferous forest. Correspondingly, the portions of small macro- and microaggregates increased in the same sequence. The mean weight diameter of aggregates significantly decreased ($p < 0.05$) in the order: arable (2.64 ± 0.02 mm) – deciduous forest (2.49 ± 0.07 mm) – coniferous forest (2.22 ± 0.06 mm).

The C content of aggregates showed no clear trend with aggregate size within an ecosystem in all investigated plots (Fig. 2). In contrast, the SOC content in small macro- and microaggregates significantly increased ($p < 0.05$) between the soils in the following

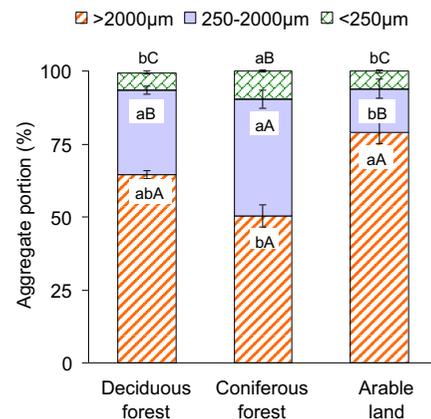


Fig. 1 The relative distribution of aggregate size classes, depending on vegetation type. Error bars represent standard error. Small letters reflect significant differences between plant communities. Capital letters show significant differences between aggregate size classes within the soil

order: arable—coniferous forest – deciduous forest. The N content followed that of C content in all soils and aggregate size classes.

Effect of aggregation on free, occluded, and mineral-bound organic C

The analysis of density fractions separated from dry-sieved aggregates showed that the C content of the POM (considering all POM fractions: $fLF_{<1.6}$, $oLF_{<1.6}$, $oDF_{1.6-2.0}$) differed between the vegetation types (Fig. 3). The C content in POM ranged from 150 to 300 g C kg^{-1} fraction for the forest soils, but only from 80 to 180 g C kg^{-1} fraction for the arable plot. The mineral fraction had the lowest C content (10–30 g C kg^{-1} fraction) and was significantly different only for the large macroaggregates and microaggregates between plant communities.

The C content of each density fraction in the aggregate composition (Suppl. Fig. 1) was calculated according to the weight of each fraction and its C content (Fig. 3). The C content of the POM fractions did not differ between aggregate size classes in arable soil (except $fLF_{<1.6}$) (Suppl. Fig. 1). However, it increased in the forest soils with decreasing aggregate size for the most of these fractions (Suppl. Fig. 1). Forest soils contained a higher portion of C in the $fLF_{<1.6}$ in each aggregate size class compared with that in arable soil. The heavy mineral fractions dominated the SOC content of aggregate size classes in all soils (Suppl. Fig. 1).

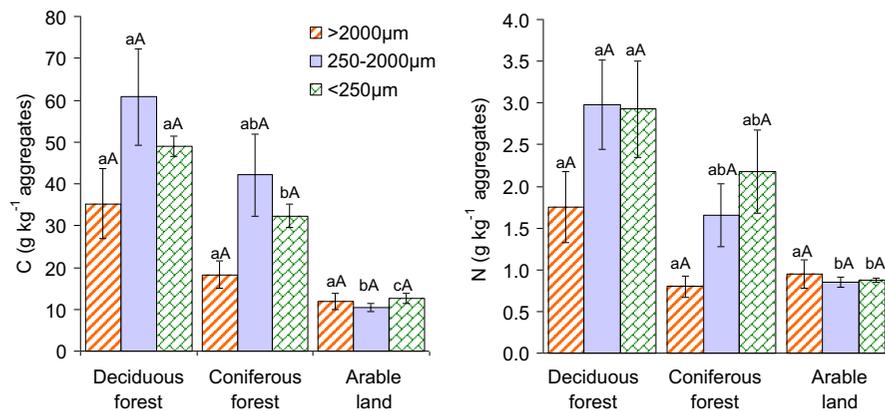


Fig. 2 Organic C and N_{total} contents (g kg⁻¹ aggregates) in the aggregate size classes, depending on vegetation type. Error bars represent standard error. Small letters reflect significant differences

between plant communities. Capital letters show significant differences between aggregate size classes within the soil

Effect of vegetation type on the C/N ratio and C accumulation in density fractions

The oLF_{<1.6} contributed only 3–8 % to the total SOC in each ecosystem type.

The C/N ratio, which reflects organic matter quality and the degree of its decomposition, was lower for the mineral than for the POM fractions within the coniferous forest and arable soils (Fig. 4).

Discussion

Effect of vegetation on total C and N contents and aggregate size classes

A comparison of the C contents in density fractions showed that most of the C in arable soil (82 %) accumulated in the mineral fraction (Fig. 5). In contrast, in forest soils, this fraction constituted 38–52 % of the SOC. Within the POM, fLF_{<1.6} contributed most to the SOC contents in forest soils (27–34 %); in arable soil it contributed only 5 %. The second highest contribution was from oDF_{1.6-2.0}, which ranged from 10 to 13 % in arable and deciduous forest soils, and increased up to 25 % in coniferous forest soil.

Effect of vegetation on total C and N contents

Our results on the C contents in forest soils (Table 1) agree with data reported for similar experiments, namely for 30-year common garden (Vesterdal et al. 2008) and 41-year large lysimeter experiments (Ulery et al. 1995). C contents in forest soils for broadleaf species (pedunculate oak, scrub oak, sycamore maple) ranged from 33 to 43 g kg⁻¹,

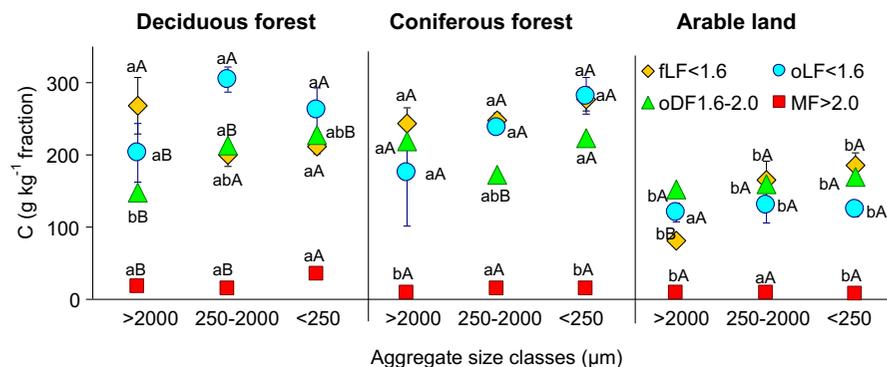


Fig. 3 The organic C contents (g C kg⁻¹ fraction) of four density fractions separated from aggregates of three size classes, depending on vegetation type. Error bars represent standard error. In case error bars are not visible, they are smaller than sign size. Small letters reflect significant differences between plant communities;

capital letters show significant differences between aggregate size classes within the soil. The letters for the rhombuses are always above the sign; for circles are above or on the right side from the sign, for triangles are below or on the left side from the sign; for squares are always above the sign

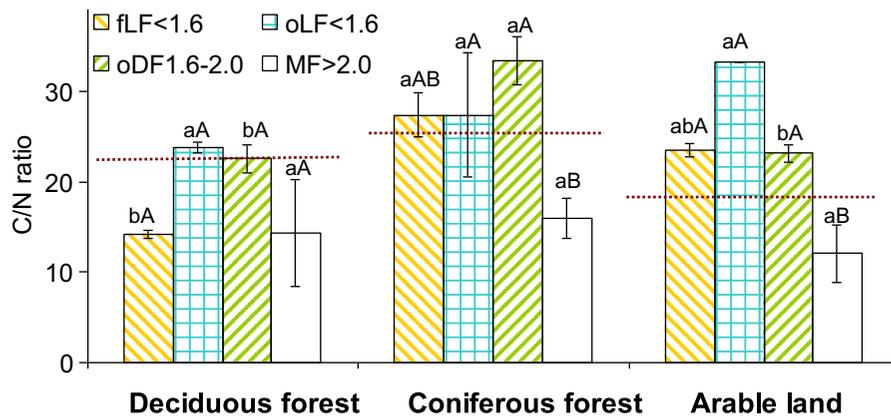


Fig. 4 The C/N ratio of density fractions, depending on vegetation type. Error bars represent standard error. Small letters reflect significant differences between plant communities; capital letters

show significant differences between the density fractions within the soil. Dashed line is C/N ratio for the unfractionated soil

whereas for coniferous species (Norway spruce and Coulter pine) the values ranged from 12 to 43 g kg⁻¹ soil (Ulery et al. 1995; Vesterdal et al. 2008). The differences between our results and those of others can be attributed to variations in parent material, climate, and net primary production of the forest ecosystems. The lower C contents in arable soils vs. forest soils reflect 1) above-ground biomass removal during agricultural history and 2) better decomposition of arable crop residues compared with forest litter (Cadisch and Giller 1996). Even the high amount of above-ground biomass on the arable plot in the last 10 years did not promote the C accumulation to the same degree with forest soils.

Considering the C contents, the accumulation rates were 0.2, 0.6 and 0.9 g C kg⁻¹ soil year⁻¹ for the arable

plot, coniferous and deciduous forest soils, respectively (assuming a linear increase over 45 years). The initial C accumulation rates are within the range previously reported for young soils: 0.8–1.1 g C kg⁻¹ soil year⁻¹ for soils developed under deciduous forests and 0.3–1.5 g C kg⁻¹ soil year⁻¹ under coniferous forests (Vesterdal et al. 2008). Our results (for the C accumulation in forest soils) were higher than the C accumulation rate in arable soils that were converted to abandoned meadow, where 0.2 g C kg⁻¹ soil year⁻¹ accumulated over 25 years (Kurganova et al. 2007).

The N contents we recorded in the forest soils corresponded with the results of a common garden experiment, where 2.5–2.9 g N kg⁻¹ for the soils under oak, maple, and coniferous forests were reported, with

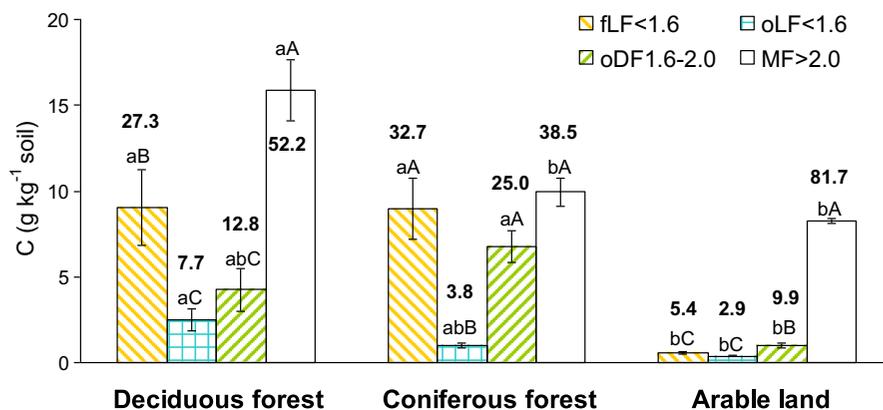


Fig. 5 Carbon content (g kg⁻¹ soil) in density fractions. Error bars represent standard error. Small letters reflect significant differences between plant communities; capital letters show significant differences between the density fractions within the soil.

Numbers above the columns (or inside of the column in case of mineral fraction for deciduous forest) present the portion (%) of C in density fractions from the total soil C

no clear effect of vegetation communities (Vesterdal et al. 2008). In our study, however, more N was accumulated in deciduous forest soil compare coniferous forest, even though deciduous forest litter contained less N than coniferous litter. This can be attributed to the fact, that deciduous leaf litter is decomposed faster than spruce litter (Berg 2008) and thus, more N can be accumulated in the soil. The relatively high N content of arable soil can be related more to agricultural management and mineral fertilizer application.

Thus, a clear effect of vegetation on SOC accumulation was observed for the young soils. Variations in the amount of litter input and its quality led to less SOC accumulation in arable vs. forest soils and, consequently, to slower C accumulation rates.

Effect of vegetation on aggregate size classes

The main differences in soil structure were observed in the macroaggregates (Fig. 1), which can be explained by biotic and abiotic factors (Six et al. 2002, 2004). Several biotic factors can affect the accumulation of macroaggregates: amount of above- (Table 1) (Pervova and Zolotarev 2012; Vladychenskii et al. 2000) and below-ground litter (Gerasimova et al. 1989); high amount of fine roots and therefore a high amount of root exudates (Six et al. 2002; Tian et al. 2013); microbial biomass content and activity (Blagodatskaya et al. 2009; Liao et al. 2006; McCulley et al. 2004); activity of earthworms (Bethlenfalvay et al. 1999) and mycorrhizal fungi (Bedini et al. 2009).

Among the abiotic factors, tillage and water regime can affect aggregate composition. Tillage leads to macroaggregate disruption and relative accumulation of microaggregates. Apparently, however, the effect of plowing was already negligible 10 years after plowing operations stopped in the arable plots, because macroaggregates were more abundant in the aggregate composition of arable soil. Moreover, the varying root architecture of trees and arable crops and the effect of shading of the forest vs. arable plots caused differences in water regimes between treatments in our experiment (Gerasimova et al. 1989). These factors can lead to intensive drying and rewetting cycles in arable as opposed to forest soil. These cycles strongly contribute to macroaggregate stabilisation (Denef et al. 2001) and can result in a higher proportion of large macroaggregates in arable compared with forest soils (Fig. 1).

SOC fractions in aggregate size classes

The sequential density fractionation to aggregates of various size classes enabled estimating the composition of SOC fractions. There were no significant differences in C content in the POM fractions between small macro and microaggregate size classes (Fig. 3) in the investigated soils. Furthermore, the C content of free and both occluded POM fractions within the classes of small macro- and microaggregates was similar (Fig. 3). These observed similarities in the C content of POM fractions can be due to identical conditions for decomposition inside and outside the aggregates (Besnard et al. 1996). This can occur when the formation and disruption of aggregates (their turnover) is faster than the C accumulation in density fractions. Proving this statement based solely on the C contents in SOC fractions is not possible; this would require parallel measurement of aggregate turnover using non-organic tracers such as rare-earth metal oxides (Zhang et al. 2001).

The C content in the aggregate size classes differed only between the soils, not between the various aggregate size classes within a single soil (Fig. 2). Note, however, that the C content of all POM fractions was identical in all aggregate size classes in arable soil, but increased with decreasing aggregate size in forest soils (Suppl. Fig. 1). These differences were due to the variations in C content in the POM fractions (Fig. 3) and the relative proportions of the POM fractions in aggregate composition (data not shown). Thus, POM fractions can explain most of the differences in aggregate C content between the soils.

The lowest C content was in the $MF_{>2.0}$ in all soils (Fig. 3). C contents in microaggregates and large macroaggregates in deciduous forest soil were significantly higher than for other plant communities (Fig. 3). This supports the assumption that the amount of C associated with clay is dependent on the amount of POM. According to literature data, the C content of the mineral fraction in soils that developed on similar parent material in arable and forest communities, but during a longer period, ranged from 11 to 21 g C kg⁻¹ for the various aggregate size classes (John et al. 2005). A comparison of the C content of mineral fractions in the soils developed in lysimeters showed that the mineral fraction accumulated less C.

The C/N ratios of the free POM fractions differed between vegetation types (Fig. 4), reflecting the various

initial quality of plant litter and also their different biodegradability. In contrast, the C/N ratio in MF_{>2.0} was the lowest (in coniferous forest and arable land) and similar for studied ecosystems (Fig. 4). This shows that this fraction had been processed by microorganisms and that part of the C was lost as CO₂, independent of the initial source of plant residues (Sollins et al. 2009).

Effect of vegetation on SOC contents in density fractions

Density fractionation showed that fLF_{<1.6} contributed 30 % to the total SOC contents in forest soils, but only 5 % in arable soil (Fig. 5). The reported portion of fLF_{<1.6} in the total SOC for older forest soils (formed during 80–200 years) ranged from 18 to 40 % (Besnard et al. 1996; Gruenewald et al. 2006; Grueteneberg et al. 2013; John et al. 2005; Yamashita et al. 2006). The high proportion of this fraction in the forest soils is due to: 1) high litter input in combination with a high C/N ratio of litter in forest soils (Besnard et al. 1996); 2) enrichment of plant residues in aromatic, alkyl-C, and phenolic compounds (for the coniferous forest litter) (Helfrich et al. 2006) and, consequently, a low microbial decomposability of litter (Yamashita et al. 2006); 3) protection in total aggregates (stable and non-stable) (Besnard et al. 1996). However, the low contribution of fLF_{<1.6} in arable soil (Fig. 5) was due to the different quality of C inputs relative to forest litter (John et al. 2005) and the previous long cultivation history of the plot, during which plant residues were removed from the soil.

All investigated soils were characterised by a high contribution of oDF_{1.6–2.0} to the total SOC contents (Fig. 5). The oDF_{1.6–2.0} fraction allocates mainly in microaggregates, but is also present in the macroaggregates (Golchin et al. 1998). For that reason, this fraction is thought to be a binding agent for microaggregates inside macroaggregates; accordingly its content should be correlated with the portion of macroaggregates (Golchin et al. 1998). Similar results were obtained by John et al. (2005) for agricultural, grassland, and coniferous forest soils. The contribution of oDF_{1.6–2.0} to total SOC contents was independent of the proportion of macroaggregates (Fig. 1). Its contribution was higher in the coniferous forest soil than in the deciduous forest and arable soils (Fig. 5), although the proportion of large macroaggregates in the former was lower than in other two soil types (Fig. 1). These results did not confirm those of Golchin et al. (1998), who stated that the

contribution of oDF_{1.6–2.0} to SOC content depends on the amount of macroaggregates (Golchin et al. 1998). In arable and deciduous forest soils, this contribution was similar (10–12 %; Fig. 5). Taking into account the higher bioavailability of leaf and grass litter vs. spruce-needle litter, we can conclude that the contribution of oDF_{1.6–2.0} to SOC contents is regulated by initial plant residue quality and not by aggregate structure. However, also the low biodegradability of belowground litter of spruce vegetation compare to broadleaf forest and grasses can explain the differences in oDF_{1.6–2.0} accumulation between the investigated soils. Accordingly, POM fractions explain the differences in SOC contents in the soils developed under contrasting land use (forest vs. arable).

The highest amount of C in all soils was associated with MF_{>2.0} (Fig. 5). This observation agrees with that of other studies (Dorodnikov et al. 2011; John et al. 2005; Yamashita et al. 2006) and mainly reflects the high total weight of this fraction. The amount of C accumulated in this fraction differed between the investigated soils, peaking in deciduous forest soil (Fig. 5). The corresponding C contents in this fraction were higher for older soils that developed in similar conditions (Hassink et al. 1997). For example, the C contents in the clay fraction of soils with a predominance of smectites and similar clay concentration, located in various geographic areas, ranged from 19 to 31 g C kg⁻¹ soil (Hassink et al. 1997; Helfrich et al. 2006). This, together with the fact that the amount of C in clay fractions differed for the investigated soils (Fig. 3), leads us to conclude that the maximum level of C accumulation had not yet been reached in our soils. Thus, C accumulation in clays is an ongoing process that lasts for at least 45 years and appears to be affected by vegetation type. As the C contents in the mineral fractions differed for our forest soils (Fig. 5), this fraction should be considered when comparing soils developed under similar plant communities.

Conclusions

Analysing the C distribution in young soils that developed on identical parent material (initially free of C), during the same period and in the same climate, enabled estimating the effect of vegetation cover on SOC accumulation when aggregate formation occurred in parallel to the C accumulation.

Variations in the quality and quantity of plant litter between arable crops and forests led to differences in the amounts and rates of accumulated C.

We established that the pronounced differences in C content in aggregate size classes between the soils under the three vegetation types were mainly due to the accumulated C in the POM fractions.

The contribution of various density fractions to total SOC contents differed between forest and arable soils. For arable crops, most of the C accumulated in the heavy mineral fraction, whereas for forests, half of the C was in the POM fractions. Thus, the POM fractions can explain most of the differences in the amount of C stored in soils under different vegetation types of contrasting land use that developed on identical parent material. Nonetheless, between similar land use (forest ecosystems), differences in the mineral-associated OM fraction should be considered.

The proportion of fLF_{<1.6} in total SOC contents in forest soils in this study was similar to that reported for mature soils. We therefore assume that C accumulation in this pool initially peaked by protection in aggregates and the primary recalcitrance of plant residues.

The C content of the mineral fraction was higher in large macro- and microaggregate size classes under deciduous forest soil than under coniferous and arable soils. This positively answered our research question whether the differences in the amount of POM determine the amount of C accumulated in the clay fraction. Moreover, the C contents in the mineral fraction were lower in these young soils than in mature ones that developed on similar parent material and vegetation. Hence, we assume that the maximal level of C accumulation on clays had not been reached after 45 years of soil formation.

Acknowledgments Thanks are extended to the members of the Faculty of Soil Science of the Moscow Lomonosov State University for the installation and maintenance of lysimeters during the past five decades. The authors are grateful to the staff of the Dept. of Soil Science of Temperate Ecosystems of Goettingen University for their help in C and N analyses. This study was supported by a DAAD fellowship for A.G.

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