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Carbon storage in post-mining forest soil, the role of tree biomass and soil bioturbation

Jan Frouz · Václav Pižl · Emil Cienciala · Jiří Kalčík

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Abstract Carbon storage in aboveground tree biomass and soil organic matter (in depth of A layer development i.e., up to 20 cm) was studied in 22–32 year-old post-mining sites in the northwest of the Czech Republic. Four replicated sites afforested with different tree species (spruce, pine, larch, oak, lime or alder) were compared with sites left to natural regeneration which were dominated by aspen, birch and willow. No topsoil was applied at the sites; hence carbon accumulation resulted from in situ soil development on alkaline tertiary clays that were dumped on the heaps. In aboveground tree biomass, carbon storage ranged from 17.0 ± 5.9 (mean ± SEM) to 67.6 ± 5.9 t ha^{-1} and the rate of C accumulation increased from 0.60 ± 0.09 to 2.31 ± 0.23 t ha^{-1} year^{-1} (natural regeneration < pine < spruce < oak < lime < alder < larch). Carbon storage in soil organic matter varied from 4.5 ± 3.7 to 38.0 ± 7.1 t ha^{-1} and the rate of C accumulation in soil organic matter increased from 0.15 ± 0.05 to 1.28 ± 0.34 t ha^{-1} year^{-1} at sites in the order: natural regeneration < spruce < pine, oak < larch < alder < lime. Carbon storage in the soil was positively correlated with aboveground tree biomass. Soil carbon was equivalent to 98.1% of the carbon found in aboveground tree biomass at lime dominated sites, but only 21.8% at sites with natural regeneration. No significant correlation was found between C storage in soil and aboveground litter input. Total soil carbon storage was correlated positively and significantly with earthworm density, and occurrence of earthworm cast in topsoil, which indicated that bioturbation could play an important role in soil carbon storage. Hence, not only restoring of wood production, but also restoring of soil community is critical for C storage in soil and whole ecosystem.

Keywords Soil formation · Bioturbation · Carbon cycling · Reclamation · Earthworms · Carbon storage · Wood biomass · Forest

J. Frouz (✉) · V. Pižl · J. Kalčík
Institute of Soil Biology, Biology Centre ASCR, Na Sádkách 7, 37005 České Budějovice, Czech Republic
e-mail: frouz@upb.cas.cz

E. Cienciala
IFER-Institute of Forest Ecosystem Research, Ltd, Areál 1 Jílovské a.s. 1544, 25401 Jílové u Prahy, Czech Republic

J. Frouz
Institute for Environmnetal Studies, Faculty of Science, Charles University in Prague, Benatska 2, 12801 Prague 2, Czech Republic

Introduction

Carbon sequestration by changing land use has the potential to mitigate the increase in atmospheric CO_2

concentrations. Afforestation or reforestation of marginal areas are among some promising means of C sequestration. For this reason much research has focused on C sequestration in tree biomass (Niu and Duiker 2006), although afforestation may also increase soil C storage. The amount of C stored in soil depends largely on the tree species and soil conditions (Cannel and Dewar 1995; Ladegaard-Pedersen et al. 2005). Therefore, soil C storage in reforested areas is also highly variable (Akala and Lal 2000; Garten 2002; Peichl et al. 2006).

Post-mining sites are areas with a large potential for C storage (Schafer et al. 1979; Akala and Lal 2000; Skukla and Lal 2005; Ussiri et al. 2006). Beside the global impact of C storage, soil organic matter (SOM) accumulation may improve many important functions of post-mining soils. SOM is a source of energy for soil microorganisms, affects sorption capacity and the water holding capacity of soil, and supports the formation of soil aggregates and soil structure. (Allison 1973; Insam and Domsch 1988; Stewart and Scullion 1988, 1989; Frouz et al. 2006). Thus SOM accumulation plays an important role in ecosystem reconstruction at post-mining sites. This is particularly important in situations where no topsoil is applied to cover raw spoil material dumped in the heaps, which typically contain little recent organic matter (Šourková et al. 2005). SOM accumulation also brings a economic benefit in terms of ecosystem services (Sparling et al. 2006). Despite the importance of SOM for many ecosystem processes, at different scales, from local to global, the mechanisms of SOM sequestration are not adequately understood (Metting et al. 2001). Only recently has more attention been paid to the various processes leading to stabilization of SOM (Six et al. 2002), including the role of soil biota in this process (Wolters 2000).

Post-mining sites represent areas of large-scale and intensive disturbance, but at the same time they offer us a unique opportunity to study some important ecological questions, including those relating to processes of SOM accumulation and C sequestration in ecosystems (Scullion and Malik 2000; Šourková et al. 2005). Similar patches of land are replicated by similar technologies used in time and space during mining operations which makes mining sites good models for chrono-sequence studies (Frouz et al. 2001; Šourková et al. 2005). Some mining soil may be very variable; however heterogeneity is largely affected by geology way of mining and heaping. Advantage on our study sites is that dumped material was formed by lacustrine sediment which was deposited in horizontal layers which are quite homogeneous in large areas. These layers were then mined out in horizontally oriented slices about 2 m thick. Each slice was then mixed as a result of mining operation, transported in heap und dumped in horizontal layer about 2 m thick, this make substrate in our sites relatively homogeneous. The simplicity of post mining sites also makes them good experimental models with which to analyze processes that are difficult to study in more developed and more complex soils, such as the effects of soil biota on SOM translocation and accumulation (Frouz et al. 2001, 2006).

The principal questions of this study were: What is the rate of carbon accumulation in the aboveground woody biomass and in the soil organic matter of post mining sites? How does the soil C accumulation differ between various types of forest cover? What is the role of litter input and soil bioturbation in soil C accumulation?

This was achieved by inventory of carbon storage in aboveground woody biomass and soils in various types of forest growing on post mining sites and simultaneous observation of litter input and bioturbation in these sites.

Materials and methods

Study sites

The study was carried out at the Velká Podkrušnohorká spoil heap, after brown coal mining, near the town of Sokolov, Czech Republic (50°14′44″N, 12°41′4″E). The spoil heap is about 2.5 km wide and 10 km long with an elevation of 450–520 m a.s.l. It was formed during the last 40 years by dumping tertiary clay material, dominated by illite and kaolinite (Kříbek et al. 1998), with a pH of about eight (Frouz et al. 2001). Mean annual precipitation in this area is 650 mm, and mean annual temperature 6.8°C.

The heap was covered by a mosaic of patches, each covering an area of several ha, overgrown by different types of vegetation of varying age which were randomly spread over the heap. Patches covered by seven different types of forest referred here as treatments, each in four replicates, were chosen for the study, which are referred to here as lime, alder, oak, larch, pine, spruce and unreclaimed sites. Lime sites were afforested with *Tilia cordata* L., alder sites with *Alnus glutinosa* L. (with a small proportion of *Alnus incana* (L.)), oak sites with *Quercus robur* L., larch sites with *Larix decidua* Mill, pine sites with a mixture of *Pinus nigra* Arnold and *Pinus silvestris* L., and spruce sites with a mixture of *Picea omorica* Pancic and *Picea pungens* Engelm. Unreclaimed sites, i.e., those left to regenerate naturally, were dominated by *Salix caprea* L., *Populus tremula* L. and *Betula pendula* Roth.

The surface of the afforested sites was leveled before the trees were planted. No leveling was applied at unreclaimed sites, where the surface consisted of longitudinally oriented, one meter high, ridges and depressions formed by the heaping machinery. No topsoil was spread on the surface of either afforested or unreclaimed sites, so C accumulation was the result of in situ soil development. The ages of individual sites ranged from 22 to 33 years. The site age was taken as the time since the last major disturbance—heaping in the case of unreclaimed sites, and leveling by earthmoving machinery in the case of afforested sites, which was followed by tree planting within one year. In all cases four plots were selected for each forest type. Individual replicates were randomly located on the heap. To achieve this all suitable forest patches (i.e., those falling within a given age range and having an area of more than 1 ha) were mapped and numbered, then four of them were selected using a random number generator. As a consequence, the closest replicates of the same treatment were typically about 1 km apart whereas the closest plots of different treatments were usually not more than 50 m apart. There were no significant differences in the average age of sites between individual forest types (Table 1), except for the pine plantations (of which there were no older sites available).

Measurements, sampling and processing of samples

Estimation of aboveground woody biomass was conducted in April and May 2006. Circular sampling plots, with an area of 200 m^2, were established at each site to estimate the C stock in the aboveground woody vegetation, the edge of the sampling plot being located at least 5 m from the forest edge. In this way, four times seven plots were established to represent each major species type. At each plot, the individual species were identified and the diameters were measured of all the trees with a stem diameter larger than 50 mm at breast height, and of all shrubs having their largest trunk with a breast height diameter greater than 20 mm. At each plot, the height of at least five trees of each dominant species was measured. They were selected to cover the range of breast height diameters in a given plot. Heights of the remaining trees were calculated from the Chapman–Richards's equation relating tree height to stem diameter. This

Table 1 Mensurational summary for the different forest types sampled on mine-spoil sites in the Czech Republic, mean ± SEM, age mean plot age since last major disturbance which mane heaping in unreclaimed sites and levelling and tree planting in reclaimed sites

Site type	Age (years)	Tree no trees (ha^{-1})	Diameter (cm)	Height (m)	Biomass (t ha^{-1})
Alder	28.0 ± 2.2a	2000 ± 191ab	9.5 ± 0.9	9.7 ± 1.2bc	91.2 ± 17.2b
Lime	31.0 ± 0.5a	2787 ± 391bc	9.8 ± 0.9	11.3 ± 1.2c	90.0 ± 15.2b
Oak	28.0 ± 0.0a	1881 ± 85a	7.9 ± 0.5	6.9 ± 0.7ab	65.2 ± 7.9ab
Unreclaimed	27.8 ± 1.3a	3943 ± 1018c	8.2 ± 2.5	7.3 ± 1.6ab	34.0 ± 6.3a
Larch	29.5 ± 1.3a	2162 ± 342ab	14.5 ± 1.3	15.4 ± 0.6c	135.2 ± 11.9c
Spruce	27.0 ± 2.1a	1050 ± 125ab	14.5 ± 2.27	8.9 ± 1.4abc	59.0 ± 11.5ab
Pine	22.0 ± 0.9b	4193 ± 627c	8.6 ± 1.67	5.2 ± 0.5a	35.5 ± 6.6a
ANOVA *F*; *P*	2.95; 0.0298	3.90; 0.0089	2.38; NS	6.89; 0.0003	7.08; 0.0003

Bottom line contains *F* and *P* values of one way ANOVA comparing individual types of sites, $n = 28$ in all cases; *NS* means not significant. Statistically homogeneous groups of sites are marked by the same letter in individual columns (LSD post hoc test, $P < 0.05$)

equation is commonly used for calculating tree heights in tree-based forest statistical forest inventories (e.g., Zhao-Gang and Feng-Ri 2003; Nieuwenhuis et al. 2007). The equation was parameterized on the basis of the measured tree samples in a plot. The coefficients of determination (r^2) for the fitted tree height on individual plots ranged from 0.47 to 0.89 for alder, 0.53 to 0.97 for lime, 0.68 to 0.97 for oak, 0.58 to 0.98 for larch, 0.49 to 0.96 for spruce and 0.73 to 0.98 for pine. To estimate the biomass of smaller trees, three circles with an area of 4.19 m^2 were randomly selected in each sampling plot and breast height diameters were measured for all woody plants taller than 1.3 m with a breast-height diameter less than 50 mm. The diameter was measured using an electronic caliper Mantax (Haglöf, Inc., Sweden), and height with a laser rangefinder (Forest Pro, Laser technology Inc., USA); the data were stored in the field in a PC Hammerhead (WalkAbout Computers, Inc., USA). The total aboveground biomass of individual trees was calculated from height and breast diameter using the following allometric equations: Johansson (2000) for alder, Ter-Mikaelian and Korzukhin (1997) for lime, Hochbichler (2002) for oak, Ker (1980) for larch, Wirth et al. (2004) for spruce and Chroust (1985) for pine. In the unreclaimed sites the equations of Marklund (1988), Johansson (1999) and Utkin et al. (1996) were used for birch and aspen, respectively.

No suitable allometric equation was found in the literature for shrubs of *Salix caprea* that were abundant on the plots left to natural regeneration, frequently growing as polycormon formations. For the purpose of this study, we therefore developed an allometric equation based on estimation of biomass of individual stems in polycormons. To calibrate this equation, 30 stems (including branches) covering the range of observed diameters were destructively sampled. They were cut, weighed and their diameters and heights were recorded, together with the number of stems in a given shrub. Samples, each about 50 cm long, were taken from each sampled stem to determine dry matter content in the laboratory (by drying samples at 80°C to constant weight), which was used to convert fresh weight to dry weight. The equation that gave the best fit of sampled biomass ($r^2 = 0.839$, $P < 0.0001$) had a form $1.72{*}D$^$2.694/N$, where D represented the stem diameter and N was the number of stems in the polycormon. This equation was used to estimate the biomass of polycormon shrubs of *Salix caprea*. To convert tree biomass to carbon, a coefficient of 0.5 was used (IPCC 2003).

To measure the litter input from woody vegetation, the litter was collected using nylon sacks of 0.5 mm mesh size, fixed on an iron frame of 0.5 × 0.5 m about 0.5 m above the soil surface. Three collectors were placed close to the centre of each sampling plot, at equal distances between two neighboring shrubs or trees. The collectors were exposed for a whole year and the litter collected at the end of December 2005 and 2006. Six quadrates (0.25 × 0.25 m) were harvested in each plot to estimate aboveground herb biomass in the period of maximal development of the herb vegetation (August 2005 and 2006). The litter and aboveground herb samples were dried at 35°C for 7 days, weighed and converted to 1 m^2. The total annual litter input was estimated as the sum of woody litter input and the herb layer biomass in the period of maximum vegetation development.

Soil sampling was carried out in September 2005. To estimate the amount of carbon in the fermentation layer, Oe + Oa (partly decomposed remainder of leaf litter branches and other organic residues on the soil surface), six samples of that layer (each 625 cm^2 in area) were taken from each sampling plot. Fresh litter, however, was not sampled. The samples were dried, weighed and homogenized. A sub-sample of about 10 g was taken from each sample, ground up and used for C analysis. To estimate the amount of carbon in the topsoil—layer A (that part of the soil profile enriched by organic matter, typically darker than the yellowish spoil material), a soil profile (2 m long and 0.75 m deep) was dug in the middle of each sampling plot. The thickness of the A layer was measured at 20 points distributed randomly along the soil profile. Four samples were randomly taken from the A layer with an iron corer with a surface area of 20 cm^2 and height of 5 cm. The samples were air dried. After the removal of visible roots, they were weighed and homogenized. The 10 g sub-samples were then ground and used for C analysis. Additionally, four samples from the bottom of the soil profile (mineral substrate at a depth of 75 cm, C horizon) were sampled and processed as above. These samples were assumed to represent the original amount of fossil C in the heap material. This assumption is based on a previous study which showed that layers below the A horizon did not show any significant increase in C content over 40 years of succession (Šourková et al. 2005). The carbon content of the soil samples was determined using the method of wet acidified dichromate oxidation (Jackson 1958). The C stock in the fermentation layer was calculated as the average weight of the fermentation layer per 1 ha multiplied by the C ccentration. To estimate C accumulated in the A layer, the weight of the layer was calculated from the average thickness of the layer and its bulk density; the weight of the layer was multiplied by the C content of the A layer which was subtracted for the C content in the deepest layer (75 cm depth). This was used to correct data for the fossil organic C present in the dumped spoil material, which consists mostly of kerogen of algal origin (Kříbek et al. 1998). In this text, if not mentioned otherwise, soil carbon means C that accumulated in recent geological time, already corrected for fossil C as described above. Despite some variations, no significant differences were found in the fossil organic matter content between individual forest types (Table 2).

Two composite samples, each consisting of five separate samples (area of each 125 cm^2, depth 7 cm), were taken at each sampling plot in March 2005 and 2006 to assess the density of earthworms. These samples were transported to the laboratory where the earthworms were extracted using the modified Kempson apparatus (Kempson et al. 1963; Pižl 2001). Thin soil sections were used to asses proportion of earthworm casts in top 10 cm of soil (Frouz et al. 2007b; 2008). In each site one soil monolith 5 × 5 × 10 cm was taken in to depth 10 cm. Soil was dried, saturated in epoxi-resin and after hardening vertical thin section was prepared. Proportion of earthworm cast in soil volume was quantified using the cross intercept method. Regular grit forming 1,000 crosses was projected on each thin soil section. The intercepts of earthworm casts with the crosses projected were recorded under stereomicroscope at 40 magnification. The proportion of intercepts of a particular structure from all fields observed on a given slide was used as an estimate for the proportion of volume of a given structure in the investigated part of the soil (Kooistra 1991).

Statistical analysis

Parameters measured in individual forest types were compared using one way ANOVA with the LSD post-hoc test. Linear regression and linear correlation coefficients were used to test statistical relationships between individual parameters investigated. A general linear model was used to compare the effects of trees species and tree biomass, using tree species as the categorical, and biomass as the continual, predictor. All statistical tests were computed using SPSS 10.0 software. Annual increase in the amount of C stored in individual compartments (rate of C accumulation) was calculated as an amount of C in each compartment divided by plot age and expressed in t ha^{-1} year^{-1}. Numbers before the ± symbol

Table 2 Selected soil properties and litter characteristics for the different forest types sampled on mine-spoil sites in the Czech Republic

Site type	$O_e + O_a$ layer		A layer			C layer
	Mass (kg m^{-2})	C content (%)	Thickness (cm)	Bulk density (kg m^{-3})	C content (%)	C content (%)
Alder	0.34 ± 0.10ab	29.0 ± 1.1bc	9.8 ± 2.1c	780 ± 3	7.1 ± 0.5c	2.5 ± 0.5
Lime	0.03 ± 0.03a	23.6 ± 1.3ab	7.1 ± 1.2bc	728 ± 3	9.5 ± 0.2d	1.9 ± 0.5
Oak	0.31 ± 0.09ab	21.1 ± 2.3a	3.8 ± 1.8ab	766 ± 2	6.7 ± 0.8bc	1.9 ± 0.6
Unreclaimed	1.06 ± 0.39bc	19.1 ± 1.2a	2.3 ± 1.7ab	766 ± 4	6.5 ± 0.5bc	1.4 ± 0.2
Larch	1.36 ± 0.15c	30.8 ± 1.1c	5.1 ± 0.7ab	718 ± 4	4.2 ± 0.2a	3.7 ± 0.3
Spruce	1.66 ± 0.40c	29.9 ± 3.3c	0.2 ± 0.3a	638 ± 5	4.8 ± 0.2ab	2.4 ± 0.2
Pine	1.03 ± 0.17bc	30.5 ± 0.2c	2.2 ± 2.1ab	653 ± 4	4.6 ± 0.4a	3.0 ± 0.4
ANOVA *F*; *P*	5.58; 0.001	5.58; 0.001	2.97; 0.029	1.86; NS	10.08; >0.0001	2.25; NS

$O_e + O_a$, fermentation layer, *A*, organo-mineral layer, *C*, pedogenetic substrate (tertiary clay). Bottom line contains *F* and *P* values of one way ANOVA comparing individual types of sites, $n = 28$ in all cases; *NS* means not significant. Statistically homogeneous groups of sites are marked by the same letter in individual columns (LSD post hoc test, $P < 0.05$)

represent means, numbers after that symbol are the standard error of the mean.

Results

Carbon storage in aboveground tree biomass varied from 17.0 ± 5.9 to 67.6 ± 5.9 t ha^{-1} among all the individual sites (Fig. 1a). The highest biomass of woody vegetation and, consequently, the highest amount of associated C was found at larch sites, while the lowest values were measured at the unreclaimed sites (Table 1; Fig. 1a). The biomass of woody vegetation was closely positively correlated with the height of the trees ($r = 0.835$; $P < 0.0001$), while a negative correlation was found between aboveground tree biomass and tree density ($r = -0.531$; $P = 0.0036$).

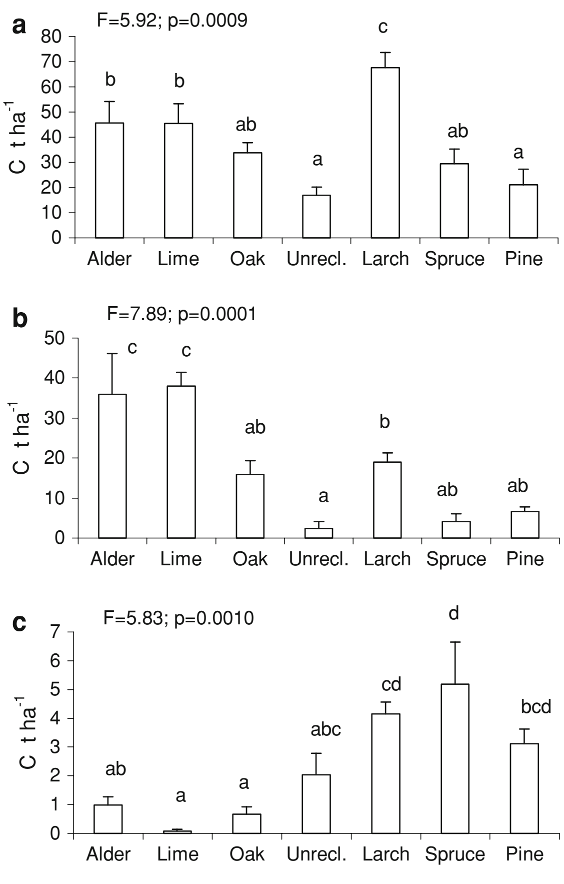

Fig. 1 Carbon storage in post-mining sites afforested with different tree species: **a** in aboveground woody biomass; **b** in the organo-mineral A layer of the soil; **c** in the fermentation layer of the soil. *Bars* represent SEM, $n = 4$ for each forest type, individual parameters were compared by ANOVA among forest types, F, and P values are inserted in each chart, statistically homogeneous group are marked by the same letter (LSD post hoc test, $P < 0.05$)

The mass of the Oe + Oa layer was significantly higher at sites afforested with coniferous trees than at sites planted with deciduous trees, while the values measured at unreclaimed sites lay in between (Table 2). The amount of C stored in the Oe layer ranged from 0.07 ± 0.06 to 5.2 ± 1.4 t ha^{-1}, tending to be higher under coniferous than under deciduous trees (Fig. 1c). The amount of C stored in the A layer of soil was much higher than that in the Oe + Oa layer, ranging from 2.3 ± 1.8 to 37.9 ± 3.5 t ha^{-1} (Fig. 1b, c). The highest amount of C was stored in the A layer of lime and alder plantations and the lowest in that of the unreclaimed sites. In general, the amount of C in the A layer was negatively correlated with C in the Oe + Oa layer of the soil ($r = -0.401$; $P = 0.0430$).

Carbon storage in the Oe + Oa and A layers pooled varied from 38.0 ± 7.1 to 4.5 ± 3.7 t ha^{-1}, decreasing in the following order: lime > alder > larch > oak > pine > spruce > unreclaimed sites. The amount of C stored in the A layer closely correlate with of total C stock (Oe + Oa + A layer ($r = 0.991$; $P < 0.001$). However, there was a large variability among sites in the proportion of total soil C stock (A + Oe + Oa) found in the A layer. For example, 99.8 ± 0.1% of the organic soil C was stored in the A layer at lime sites, but only 21.8 ± 16.5% at unreclaimed sites. The soils with greater C storage on the A + Oe + Oa layer also stored relatively more C in the A layer. Moreover, correlation between the relative proportion of C stored in the A layer and total carbon stored in the soil (A + Oe + Oa) was highly significant ($r = 0.650$; $P = 0.0001$). The amount of C stored in the A layer (as well as the soil carbon in the Oe + Oa + A layer), was significantly correlated with earthworm density ($r = 0.576$; $P = 0.0034$ and 0.548; $P = 0.0049$, respectively). There was also strong positive correlation between proportion of earthworm casts in topsoil layer (0–10 cm depth) and C storage in soil (Fig. 2; $r = 0.611$; $P = 0.0001$). Thus there is strong positive correlation between C storage in soil and two various indicator of earthworm bioturbation (Fig. 2).

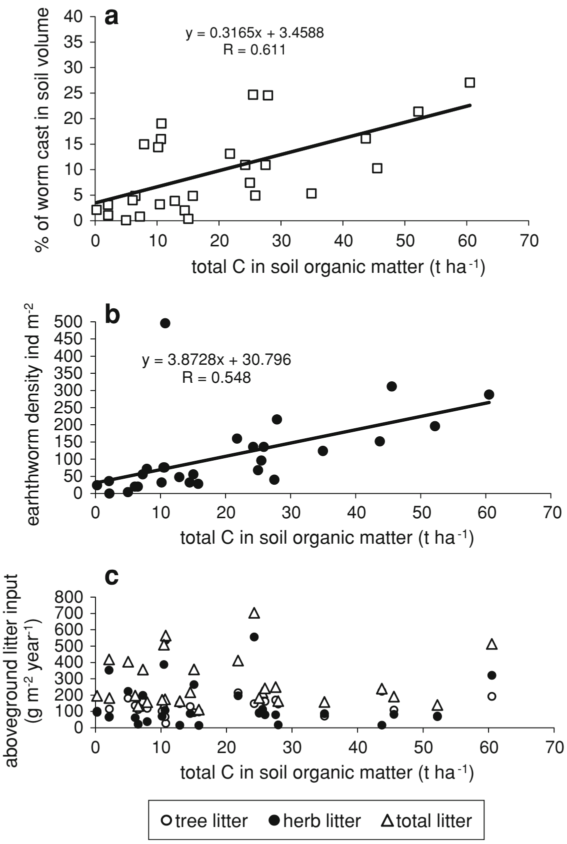

Fig. 2 Correlation between total C stock in soil (Oe + Oa + A layer) and proportion of earthworm cast in topsoil (0–10 cm depth, volume percentage) (**a**); Correlation between total C stock in soil (Oe + Oa + A layer) and earthworm density (**b**); and correlations between total C stock in soil (Oe + Oa + A layer) and litter inputs (**c**). Line equations and *r* values are presented only for significant correlations

No significant correlation was found between C storage in the Oe + Oa and A layers of the soil and the input of litter ($r = 0.264$, -0.063 and 0.017 for tree litter, herb litter and total litter amount, respectively) (Fig. 2). The total C stored in the Oe + Oa + A layers of the soil did, however, show a significant positive correlation with the C stored in the aboveground woody plant biomass (Fig. 3a). Considering all sites, carbon in the Oe + Oa and A layers of the soil was equivalent to 53.9 ± 7.3% of the C in the aboveground woody biomass. However, individual sites differed substantially in the ratio between C stored in the soil (Oe + Oa + A layer) and in aboveground woody biomass. Soil C was equivalent to 98.1 ± 21.5% of the C in the aboveground woody biomass at lime sites, but only 21.8 ± 7.3% at unreclaimed sites (Fig. 4).

As individual sites differed in aboveground woody biomass, the forest type appeared to be a more important predictor for soil carbon storage than aboveground biomass per se when compared by general linear models (Fig. 3a). The same was true

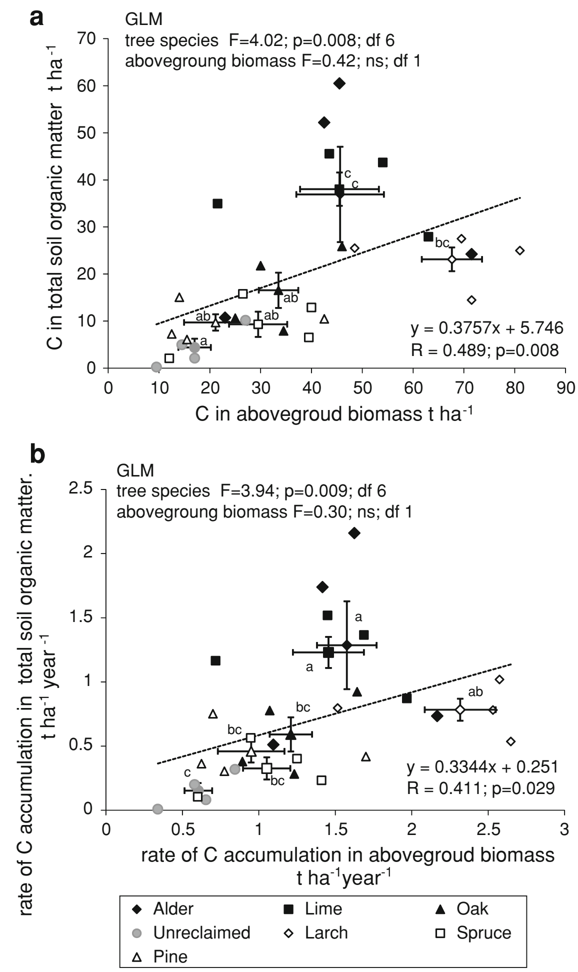

Fig. 3 Carbon accumulation in soil (fermentation and humus layer pooled) as a function of forest type and of carbon accumulation in aboveground biomass (**a**) and rate of carbon accumulation in soil as a function of forest type and of the rate of C accumulation in aboveground biomass (**b**). The effect of forest type and amount C in the aboveground biomass, or rate of aboveground biomass accumulation, was tested by General linear models. Symbols with *bars* represent mean values for the given forest type; *bars* represent SEM. Statistically homogeneous groups of various forest types are indicated by different letters (GLM, LSD post hoc test, $P < 0.05$). Line, function, *R* and *P* values are given for linear regressions between aboveground and soil C and their rate of their increase

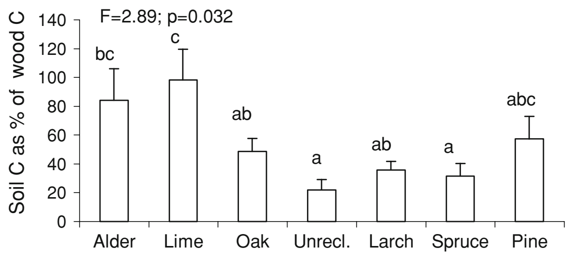

Fig. 4 Overall soil carbon stock in different types of forest as a proportion of C stock in aboveground woody biomass, *Bars* represent SEM, $n = 4$ for each forest type, individual parameters were compared by ANOVA among forest types, *F*, and *P* values are shown and statistically homogeneous groups are marked by the same letter (LSD post hoc test, $P < 0.05$)

when the rate of soil and woody biomass C accumulation was used (Fig. 3b). The rate of C accumulation in aboveground plant biomass varied from 0.60 $\pm$ 0.09 t ha^{-1} year^{-1} at unreclaimed sites to 2.31 $\pm$ 0.23 t ha^{-1} year^{-1} at larch sites. The rate of C accumulation in the soil ranged from 0.15 $\pm$ 0.05 at unreclaimed sites to 1.28 $\pm$ 0.34 t ha^{-1} year^{-1} at lime sites. The rate of C accumulation in the soil showed a significant positive correlation with the rate of C accumulation in aboveground woody biomass (Fig. 3b).

Discussion

Carbon storage can be used as one of the indication of ecosystem recovery in post mining sites. Carbon storage at the post-mining sites studied was comparable to C storage measured in another recently restored forest ecosystem (Akala and Lal 2000; Šourková et al. 2005; Peichl et al. 2006) but the temporal dynamics of C accumulation may have affected the comparison of individual sites. For example, earlier studies have shown that the accumulation of C in soil was a little bit faster during the first 15–20 years after heap afforestation with alder and then slowed down, while the opposite was true for unreclaimed sites (Šourková et al. 2005; Frouz and Kalčík 2006). Apparently this was due to the slower initial development of vegetation at unreclaimed sites (Frouz and Kalčík 2006). However, these differences were not statistically significant and both trends can best be described by linear regression (Frouz and Kalčík 2006; Šourková et al. 2005), so linear approximation can be assumed to be adequate for our sites.

Ecosystem C storage has certain limits, because of limitation in tree growth and also because soil may get C saturated (Six et al. 2002). Based on previous studies of C dynamic in alder plantation and unreclaimed sites, however, we believe that these limits were not reached yet and that there is still some potential for addional C storage. As mentioned above, post mining sites under study were quite favorable for plant growth with no substrate toxicity, adequate supply of nutrients (Šourková et al. 2005), this may not be universal condition valid for all post mining sites in general (Bradshaw 1993).

Our results showed that soil C represented an important part of the total C accumulated at post mining sites. The rate of soil C accumulation was similar to that reported from other restored forests (Akala and Lal 2000; Garten 2002; Peichl et al. 2006) and, in order of magnitude, comparable with the rate of C accumulation in aboveground woody biomass. It should be emphasized that roots, which may represent an important contribution to total C storage (IPCC 2003), were not taken into account in our study. Hence, in some of the sites included in this study it is easily possible that belowground C storage, including that in soil and roots, may be more important than aboveground C storage. This agree with findings of Walle et al. (2001) and Jia and Akiyama (2005) who found more C below then above ground in some mature forest ecosystems. The average content of C in soil of 30 years old temperate forests varies widely around 134 t ha^{-1} (Brady and Weil 2002). Soil at our sites gained much less C than other temperate sites, which may indicate that there is still potential for future growth of C stock. This underlines the importance of belowground C storage which clearly requires more attention in future research and restoration efforts.

In our study soil C varied widely around one half of the amount of C stored in aboveground woody biomass but there were large differences between sites afforested with different trees. The largest proportion of C stored in soil organic matter relative to C in the aboveground biomass, and the largest soil C storage, was found at sites afforested with lime and alder. This is in agreement with the conclusion by

Cannel and Dewar (1995) that deciduous trees support soil carbon storage more than conifers.

In this study, no significant correlation was found between the input of plant litter and C storage in the soil. On the other hand, a significant positive correlation was found between C accumulation in soil and the density of earthworms and occurrence of earthworm casts in soil profile. In sites with greater storage of C in the soil (A + Oe + Oa layer pooled) a relatively larger proportion was stored in the deeper A layer. This pattern suggests that C storage in soil could be supported by bioturbation activity of soil invertebrates, and earthworms in particular. This seems to be contrasting with the results of previous studies based on the exposure of litter bags accessible or non-accessible to macrofauna, which indicated that decomposition was about 5–40% higher in the presence of macrofauna (Irmer 1995). However, the term decomposition is frequently used for any loss of litter matter from an enclosure when interpreting the results of litterbag experiments. Nevertheless, the matter loss may be caused by various processes, such as mineralization resulting in volatile CO_2, leaching of water soluble substances or by fragmentation of the litter and its deposition in the soil in the form of soil fauna excrements. Our earlier enclosure experiments, that included both litter and the underlying mineral layer, showed that soil macrofauna did not significantly increase mineralization of organic matter, but accelerated soil mixing, resulting in the translocation of organic matter from the litter to the mineral soil (Frouz 2002; Frouz et al. 2006, 2007a). Similar conclusions were also reached by Wachendorf et al. (1997).

The processing of litter by soil invertebrates may increase the stability of soil organic matter in various ways (Wolters 2000). Earthworms in particular may support stabilization of soil organic matter by its incorporation into soil aggregates and by physical binding (Lavelle and Martin 1992; Guggenberger et al. 1996; Zhang et al. 2003; Bossuyt et al. 2005). On the other hand, the supply of easily decomposable litter supports earthworm populations (Lavelle et al. 1997; Ponge 2003). Hence, the abundant earthworm populations that occurred at heap sites afforested with alder and lime (Pižl 2001), may support soil mixing and SOM stabilization, which increased the amount of C stored in the A layer. This idea is consistent with the fact that in our study both C soil storage in the A layer, and also soil C storage in the Oe + Oa + A layer pooled, positively correlated with earthworm density (Fig. 2). There are also some literature data that support this idea. For example, Zou and Bashkin (1998) showed that higher earthworm density correlated with higher soil C content, and Wironen and Moore (2006) indicated that massive soil mixing caused by earthworm invasion into North American forests might have increased C storage in deeper soil layers and consequently overall soil C storage. Interestingly, most of the available literature data about the impact of earthworms on ecosystem processes come from areas where earthworms are non-native species (McInerney and Bolger 2000; Bohlen et al. 2004). European post-mining sites give us an interesting opportunity to study large scale effects of earthworm colonization on soil and ecosystem processes in the area of their natural geographic distribution.

This study indicate that soil pool of carbon from important contribution to overall C storage in restored ecosystems and that belowground processes that determine decomposition and or stabilization of organic matter may play more important role in C soil C storage than litter input. Thus reclamation practices should consider not only trees that support fast aboveground growth but also accumulation of soil C and should consider factors that may enhance soil biota migration to post mining sites such as habitat connectivity and communication with surrounding landscape.

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