Carbon dynamics of young experimental afforestations in Thuringia

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Dipl. Geoökologe Axel Don

aus Kassel

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Dekan: Prof. Dr. Peter Grathwohl

1. Berichterstatter:	Prof. Dr. Thomas Scholten (Geographisches Institut, Eberhard Karls Universität Tübingen)
2. Berichterstatter:	Prof. Dr. Ernst-Detlef Schulze (Max-Planck Institut für Biogeochemie, Jena)
3. Berichterstatter:	Prof. Dr. Nina Buchmann (Institut für Pflanzenwissenschaften, ETH Zürich, Schweiz)

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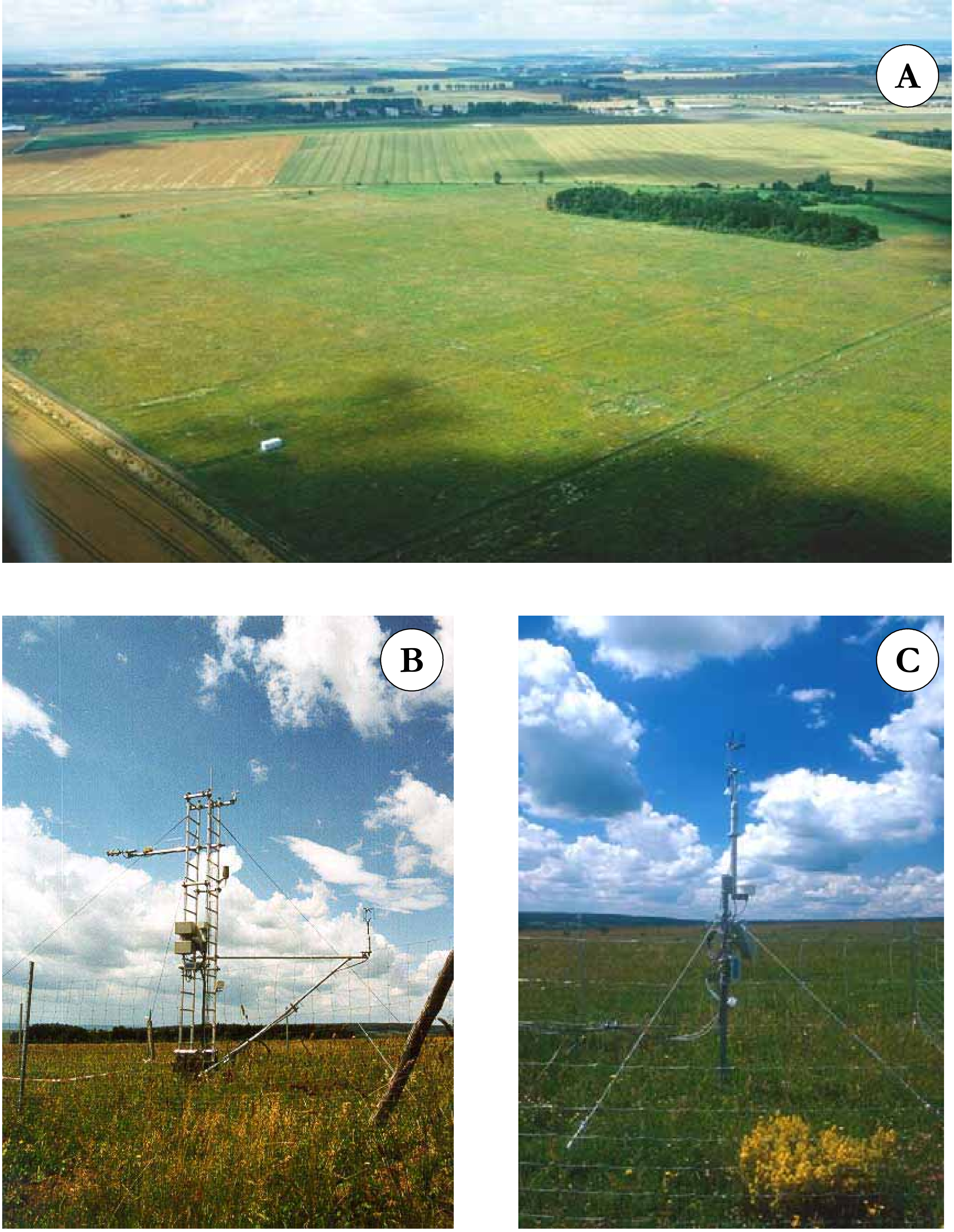

Plate I: Aerial view from NO on the BIOTREE site Mehrstedt, container with the measurement equipment of the eddy covariance tower in front (A); eddy covariance tower and meteorological station on the afforestation site Mehrstedt (B); eddy covariance tower on the grassland site at Mehrstedt.

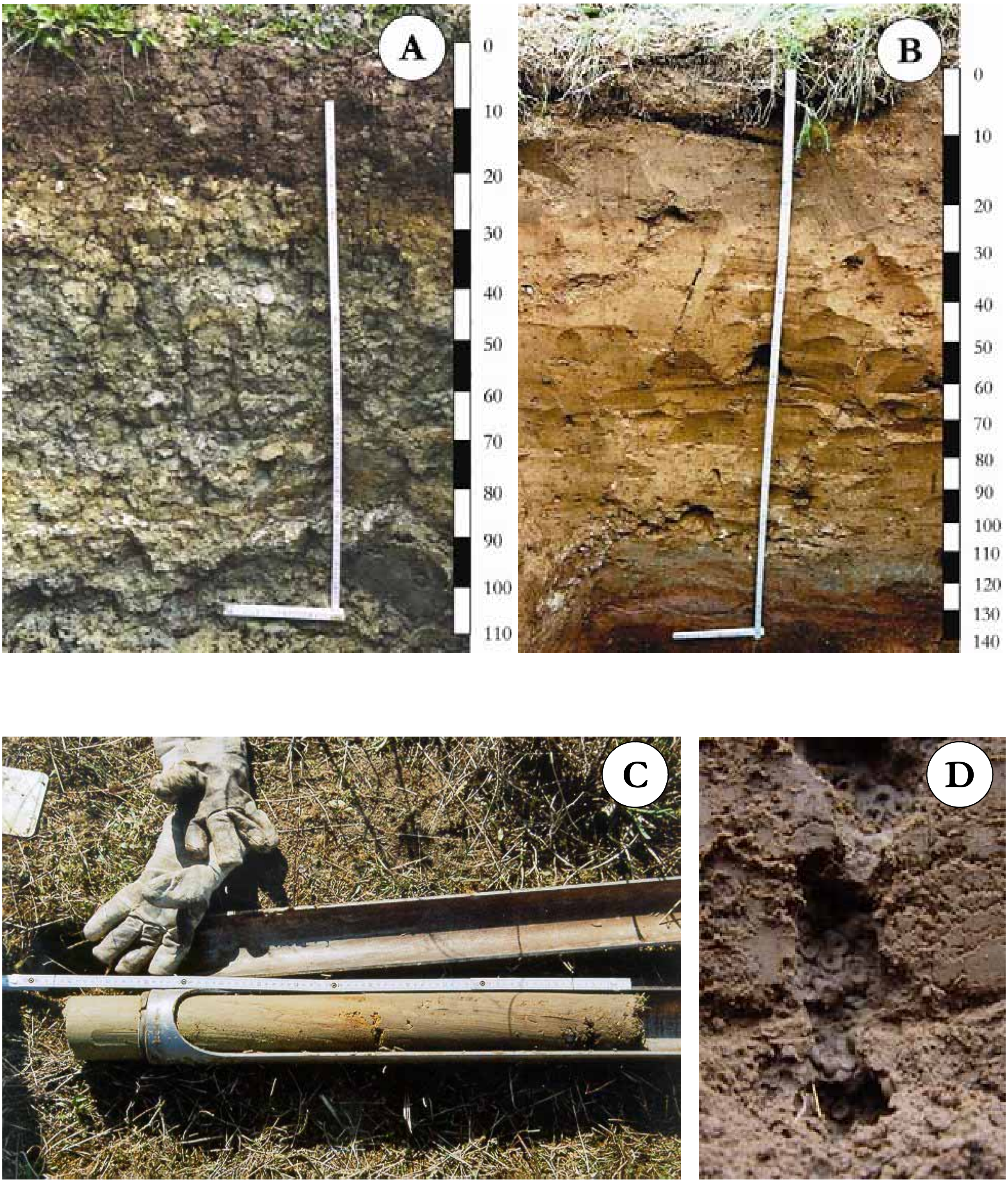

Plate II: Representative soil profiles of the Mehrstedt site with a Stagnic Vertisol (A) and the Kaltenborn site with a Ortoeutric Arenosol (B); fresh sample core (8.7 cm diameter) taken with the machine driven Cobra corer at the Mehrstedt site (C); Earthworm burrow with casts deposited on the walls in about 35 cm soil depth at the Mehrstedt site (D).

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1. Introduction and hypotheses

Land use is a key factor controlling the carbon (C) dynamics of the biosphere (Cannell et al., 1999; Guo and Gifford, 2002; IPCC, 2000). On the one hand, land use affects the net primary productivity NPP (C input) of an ecosystem by fertilisation, melioration, harvest and disturbance frequency and intensity. On the other hand, C loss by harvest and C-leaching with seepage water and C exchange with the atmosphere as CO_2 and other trace gases is directly linked to the land use. The CO_2 exchange between land surface and the atmosphere has become a major concern in relation to the observed and predicted climate change (IPCC, 2000). 15 times more C is turned over by the terrestrial biosphere than C is emitted by human activities (IPCC, 2007; Schimel, 1995).

About 50-70% of total C in European forests and more than 95% in grasslands is stored as soil organic carbon (Dixon et al., 1994; Mund and Schulze, 2006; Nöllert, 2003). If land use change, such as the conversion of grassland into forest only slightly affects soil C stocks, it can have significant effects on the total C balance of the system and its CO_2 emissions. C accumulation in soils is a rather slow process rarely exceeding 1 t C ha^{-1} a^{-1} in the long term whereas soil disturbances (e.g. ploughing, wind throw, fire) can cause a rapid soil C loss (Guo and Gifford, 2002; Jandl et al., 2007; Soussana et al., 2004). This asymmetrical dynamic was summarized as the “slow in - fast out” feature of ecosystems (Körner, 2003), pointing to the fact that soil C pools are sensible and could possibly release large amounts of CO_2 into the atmosphere without having an mechanism to re-capture this CO_2 at similar rates.

Most studies on afforestations found decreasing mineral soil C stocks during the first years to decades after forest establishment (Guo and Gifford, 2002; Paul et al., 2002; Post and Kwon, 2000; Thuille and Schulze, 2006). A literature review on the effect of afforestations on soil C reported decreasing soil C stocks with 0.63% a^{-1} for <30 cm soil depth during the first 5 years since conversion into forest (Paul et al., 2002). Afforestations on grasslands showed especially high soil C losses of 0.28 % a^{-1} or 10% decreased soil C stocks after a non-defined time period (Guo and Gifford, 2002; Paul et al., 2002). The mechanisms behind this decline are still unclear. Decreased C-input of fine roots when herbaceous vegetation is suppressed by tree shading or damaged by site preparation may be one mechanism of how afforestations lead to declining soil C stocks (Thuille and Schulze, 2006). Other studies relate C losses of young afforestations to enhanced mineralisation due to the disturbance during site preparation (Harkness and Harrison, 1989).

This thesis is part of the long-term research experiment "Biodiversity and ecosystem functioning in experimental tree stands" (BIOTREE) which was set up to investigate the influence of tree diversity on processes in ecosystems. On three sites in Thuringia a total of 70 ha were planted with more than 200 000 tree seedlings, providing the possibility to study the C dynamics of young afforestations. This thesis serves as a baseline for further studies in this unique experiment which has an expected running time of 100 years. The design and the characteristics of the three sites are presented in manuscript 1 (in press, PPEES) (Fig. 1). The establishment of experimental plots with certain mixtures out of 19 different tree species was a critical endeavour. Different factors such as summer drought and damages by voles and rabbits put a risk on the establishment success of the trees (manuscript 2, AFJZ 2007).

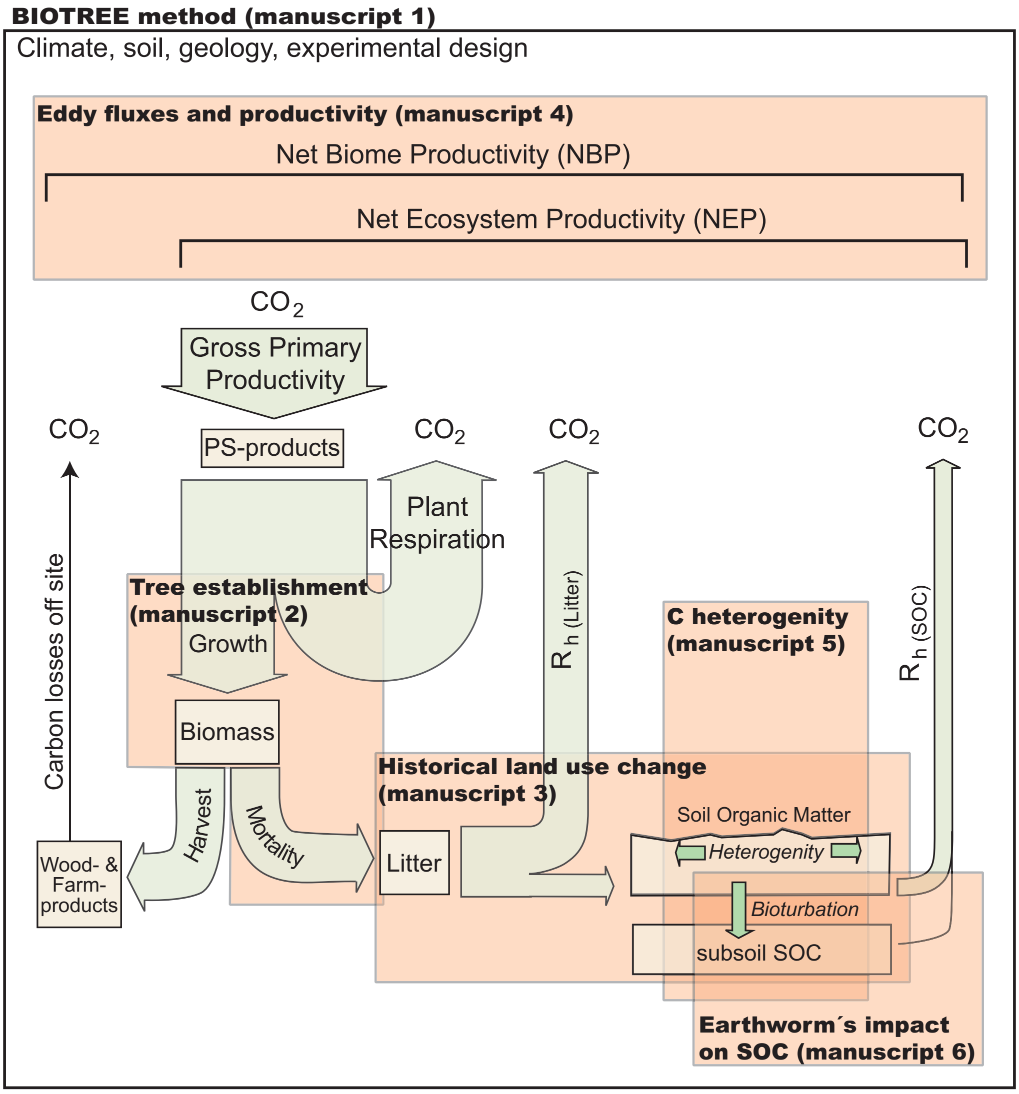

Figure 1: Schematic representation of the afforestation carbon cycle and the related manuscripts of this thesis (orange boxes). Arrows indicate fluxes; yellow boxes indicate pools. R_h: heterotrophic respiration by soil organisms, PS: photosynthesis (modified after Schulze et al., 2000).

Each setback due to browsing during tree establishment diminishes the possible C sink of the afforestation. The main additional C sink of afforestations is due to increased C stocks of the biomass because of slow C accumulation or even C loss in soils (Post and Kwon, 2000; Richter et al., 1999; Thuille and Schulze, 2006). In a long-term study on pine afforestations less than 1% of C accretion was found in the mineral soil but 80% in the trees (Richter et al., 1999). However, major native tree species are not adapted to grow on open filed sites in competition with the herbaceous vegetation. It was investigated how forest establishment success depends on the tree species and how it can be explained with a set of biotic and abiotic factors.

Land use changes can exert a long lasting effect on the soil C dynamics (Cannell et al., 1999; Guo and Gifford, 2002; Larionova et al., 2003). Mean soil C stocks in Central Europe increase from cropland (less than 45 t ha^{-1}) to grassland and forest (both nearly 70 t ha^{-1}) (Arrouays et al., 2001; estimates for France). After conversion of one land use type into another, the corresponding soil C stocks are expected to adjust within a time period of some decades. During this limited transitional time after conversion soils may sequester C when the established land use type stores more C than the former one. Beside different stabilisation mechanism the location in soils where litter C is deposited is crucial for soil C dynamics. Turnover rates of root litter were found to decrease with increasing soil depth due to more mineral surfaces available in subsoils to stabilize C with adsorptive bondages (Gill and Burke, 2002; Lorenz and Lal, 2005). Thus, in manuscript 3 (submitted to Journal of Plant nutrition and Soil science) the following hypothesis are tested: i) Land use change from cropland to grassland 21 (Mehrstedt) and 27 (Kaltenborn) years ago increased soil C stocks, ii) a reshaped C profile with more C at the soil surface soil than in deeper soil horizons influences soil C stability. Soil C dynamics as influences by this historical land use change will influence future soil C trajectories.

Current C dynamics cannot be assessed with soil- and biomass inventories only but has to be measured as C fluxes in continuous mode. The eddy covariance technique provides a powerful tool to measure CO_2 fluxes integrated over field sites of several hectares. With parallel measurements on the afforestation site Mehrstedt and adjacent grassland site, the impact of site preparation and management changes was detectable (manuscript 4, submitted to Global Change Biology). Due to the disturbance of the afforestation site during site preparation and changes in management practices both assimilation and respiration fluxes will be affected. The hypothesis was tested that site preparation with deep ploughing of the planting rows enhanced soil C mineralisation and lead to C losses on the afforestation site.

Future effects of tree diversity on soil C stocks are going to be investigated with soil C inventories. Major obstacles in detecting C stocks changes are the high spatial and vertical heterogeneity of soil C (Conant and Paustian, 2002; Ellert et al., 2001). The lack of knowledge on long-term soil C stock changes is party due to the low statistical power when soils are sampled only at single representative profiles or with insufficient sample replications. In this thesis a comprehensive soil C survey was conducted at the sites Mehrstedt and Kaltenborn (manuscript 5, Geoderma, 2007). It was hypothesised that knowledge on the spatial and vertical variability of bulk density and C concentration in soils can be used to identify underlying processes of soil C accumulation and to develop an optimized sampling design for soil C inventories.

Land use change will go along with a change in soil fauna that directly affects the C cycle (Wardle, 1995; Wolters, 2000). There is hardly any other species group with such an impact on soil C dynamics as earthworms. Earthworms consume up to 2000 kg litter ha^{-1} yr^{-1} from the soil surface, ingest, grind and digest it and transport it into the mineral soil. In particular, deep-burrowing anecic earthworms are capable of building burrows of up to 3 m depth (Hale et al., 2005), which they line with organic detritus by casting (Edwards and Bohlen, 1996). However, the long-term effect of earthworms on soil C dynamics is unclear. Whereas C turnover in earthworm casts was found to be enhanced (Tiunov and Scheu, 2000), other authors found organic carbon to be stabilised in aggregates of the casts (Bossuyt et al., 2005). Intimate mixture of minerals and organic C was expected to enhance the stabilisation of soil C. The hypotheses was challenged that the C turnover in earthworm casts deposited on burrow walls is reduced compared to the surrounding non-affected soil (manuscript 6, submitted to Soil Biology and Biochemistry).

C dynamics of the investigated afforestation sites of the BIOTREE experiment are influenced by different biotic and abiotic processes which can only be assessed using a variety of different methods. This thesis focused on the pedon to plot scale of two field sites with the aim to quantity C dynamics of the young afforestations and to provide a basis for further research in the BIOTREE experiment.

2. Overview on the six manuscripts

2.1 Background: Afforestations and the Kyoto protocol

The Kyoto Protocol from 1997 included ecosystem management practices as measures to mitigate CO_2 (IPCC, 2000). Afforestations were acknowledged by article 3.3 as a measure to sequester C and have to be reported from all Annex I countries (industrialised countries) together with the area of deforestations. The C sinks of afforestations are expected to bridge the time period until non-fossil fuel based technology will be in place. Afforestations are defined as conversion of land, which was not a forest for a period of at least 50 years prior to 1990, to forested land. For the purposes of this definition afforestation includes land containing immature forest trees which have grown as a result of a direct human induced activity, as long as it can be shown that these young trees are intended to grow to the threshold canopy cover according to the biome type. The national parties agreed in the Kyoto protocol on some basic principles like i) mandatory targets for the reductions of greenhouse gas emissions and ii) the principle of additionality. Only "additional human induced measures" to the business as usual in the base year 1990 can be accounted. Thus, only afforestations that were created or assisted by human intervention and not by pure natural succession are acknowledged as measure to mitigate CO_2. However, all afforestations since 1990 are accountable as additional measure and not only afforestations that exceed the afforestation rate of 1990.

Annually 104 000 ha are being afforested in the EU. Afforestation programs are especially successful in Mediterranean countries (Spain and Portugal) and in North-Western European countries (Denmark, UK and Ireland) with high afforestation rates. All these countries have a low forest cover due to forest clearing in historical times. The European C sequestration potential with afforestations was estimated to be 4.5 Mt C a^{-1} if all set-aside land would be converted into forest, which would offset 0.5% of the anthropogenic C emissions of Europe (Freibauer et al., 2004). Obviously, the potential effect of afforestations on the global C cycle is limited. Even a global large scale afforestation program could sequester a max. of 30% of the anthropogenic CO_2 emissions with significant impact on the C balance only 40 years after the initiation of such a program (Nilsson and Schopfhauser, 1995). Its realisation would be constrained by social, economical and political factors and could only be achieved if these new forests meet all requirements of local forestry beside the CO_2 fixation effect. However, afforestations can be used as model field experiments, in which mechanisms and processes of the C turnover in terrestrial ecosystems can be studied.

In Germany, about 9000 ha are being afforested per year, which is 1.7% of the existing forest area in a 15-years period from 1987-2002 (Schmitz et al., 2006). However, urbanisation and traffic infrastructure constructions destroyed 5500 ha per year which resulted in a net forest area growth of only 3500 ha a^{-1} or 0.7% in 15 years (Schmitz et al., 2006). This is a relatively small afforestation activity compared to other European countries. The main obstacle for conversion of agricultural land into forest is the missing economic benefit due to subsidies given to other crops such as rapeseed which are cultivated on set aside land and thus, the missing available land. Financial compensation is paid by the federal state to partly balance the loss of income for farmers that convert their arable land into forest. Additional obstacles are legal restrictions on large scale afforestations set by the environmental authorities.

2.2 The biodiversity experiment BIOTREE

(manuscript 1, Perspectives in Plant Ecology, Evolution and Systematics, in press)

This thesis is part of the long-term research experiment BIOTREE in which 70 ha were afforested on three sites in Thuringia. The establishment of these new forests was only possible on former military training areas, which where abandoned after the end of the GDR in 1990. Other, maybe more suitable areas, were not available for afforestations. About 300 000 seedlings from 19 different tree species were planted creating one of the largest coherent afforestations in Germany. Manuscript 1 gives a summary of the three sites, their environmental characteristics including the land use history and it presents in detail the design of the experiment. The long-term goal of the BIOTREE experiment is to provide a research platform to investigate the influence of tree diversity on processes in ecosystems. The effect of tree diversity on the C dynamics and sequestration is one major question to be tackled in this experiment in the future. The BIOTREE experiment is unique due to its size and the long-term perspective. It may take several years to decades until the forest plots are fully established. The BIOTREE was set up in close collaboration with the local forest authorities (*Forstamt Ebeleben, Bundesforstamt Thüringer Wald*) and the Thuringian State Institute for Forestry, Game and Fishery, Gotha. The duration of the experiment was estimated to be 100 years and its basis was set with this thesis e.g. by a comprehensive soil carbon inventory (manuscript 5). Representative soil profiles on each site were described and analysed (manuscript 1). The two largest sites of the experiment, Mehrstedt and Kaltenborn, represent complementary soil types, an Orthoeutric Arenosol on Triassic sand stone and a Stagnic Vertisol on limestone

(*Oberer Muschelkalk*). At both sites, tree diversity gradients ranging from 1 to 10 species (8 species in Kaltenborn) were created with 40 plots of 1.0 ha in Mehrstedt (Fig. 2) and 16 plots of 0.6 ha in Kaltenborn. Both sites had been used as cropland until their conversion into grassland in 1981 (Mehrstedt) and in 1975 (Kaltenborn).

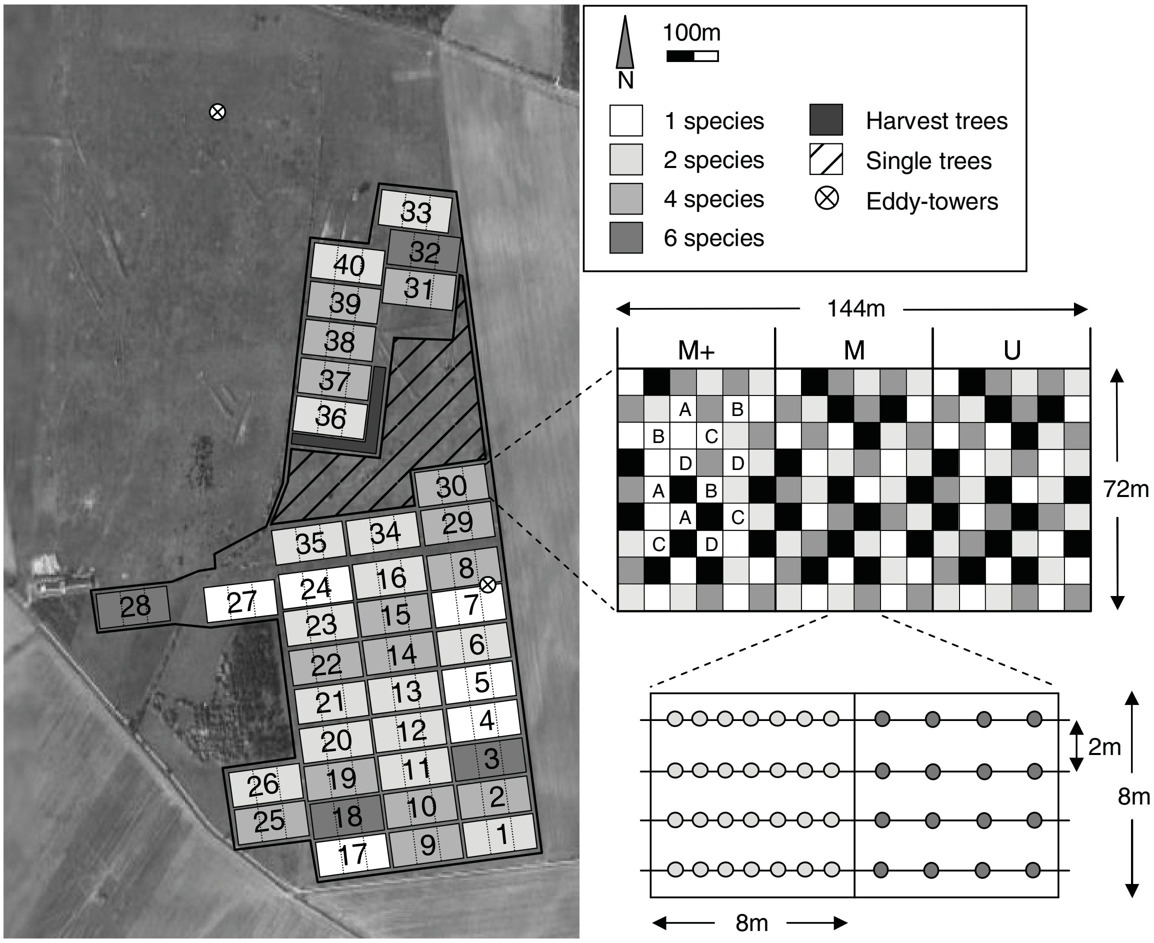

Figure 2: The experimental design of the site Mehrstedt with 40 plots of 1.0 ha with 1, 2, 4, and 6 tree species. 1/3 of the plots remain without forest use and management after the establishment phase (U), 1/3 of the plots is enriched with four subdominant tree species (M+).

A thorough investigation on the geographical features of the sites, the soil inventory and a survey on the existing herbaceous plant community provide the basis of the BIOTREE experiment together with information on the land use history and current land management (manuscript 1, 6 and 7).

2.3 Factors influencing tree survival and establishment

(manuscript 2, Allgemeine Forst und Jagd Zeitung 2007, 9/10, 164-172)

Afforestations of arable land are a challenge for forestry (Wersenger et al., 2004). However, the establishment success of the trees and the growth rate determines the C accretion in tree biomass. Manuscript 2 elucidates the factors influencing the afforestation performance such as abiotic factors like summer drought, spring frost or biotic factors like damages by rabbit and vole. More over the treatment of the seedling and their quality are major factors that determine the establishment success of the trees. 19 different tree species on 70 ha afforestations in Thuringia/Germany had been investigated during the first 3 years after planting as part the of BIOTREE biodiversity project. The tree species showed significant differences in their sensitivity towards these factors.

19-61% of the oak seedlings (*Quercus petrea*) failed to grow. For up to 1/3 of the dead seedling the damage by vole was found as death cause. Also beech (*Fagus sylvatica*) had high losses (up to 79%) among the planted seedlings. About 25% of the terminal shoots were damaged by frost or drought which led to negative growth rates during the first 2 vegetation seasons. Not only late successional deciduous trees were difficult to establish. Asp (*Populus tremula*) as pioneer species had losses up to 34% and Douglas fir (*Pseudotsuga menziesii*) as coniferous species was especially sensitive towards treatment before planting. Afforestations have to be done by professional personal to minimize the risk of failure, which is not always possible these days. Additionally, the establishment success of Douglas fir was depended on the seedling quality. The rooting success could be increased from 32 to 95% with high quality planting material. Broadleaves of high value like elm (*Ulmus glabra*), lime (*Tilia cordata*), ash (*Fraxinus excelsior*), mountain ash (*Sorbus aucuparia*) and three maple species (*Acer campestre, Acer platanoides, Acer pseudoplatanus*) had high establishment successes. These trees cover less than 6% of the forests in Germany at the moment and form a potential species pool to increase biodiversity in forests. Soil quality seems to be another important factor for the establishment success of the seedlings. A gradient of soil quality within one site did party explain the rooting success of some tree species. However, for oak and beech, the two species with highest mortality, no correlation between soil quality and establishment success could be found.

Beach, oak and cherry trees (*Prunus avium*) were particularly damaged by voles and rabbits. However, the degree of damage depended on the species number in a 1 ha plot. Plots with high biodiversity level (6 tree species) showed significant less biotic damages than plots with low biodiversity (Fig. 3). The causes of this effect may be the different susceptibility of the tree species towards vole damage. Almost no vole damage could be found for coniferous trees and lime. For insect herbivory in forest similar effects of tree diversity had been found with a strong influence of the host tree species (Vehvilainen et al., 2007).

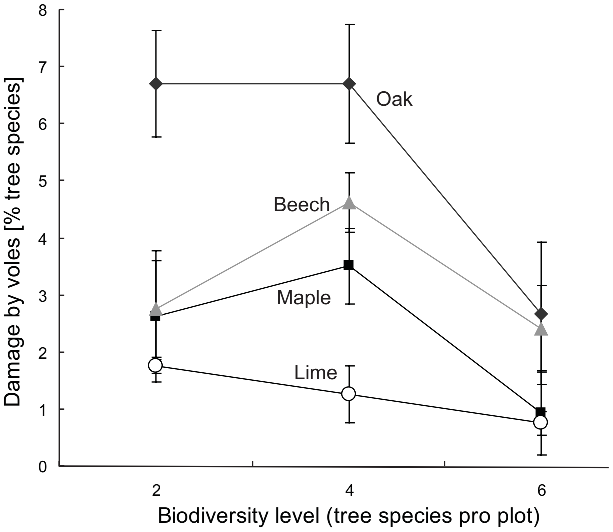

Figure 3: Tree species specific damages by voles at the site Mehrstedt in December 2005 depending on the biodiversity level of the experimental plots. High species diversity significantly decreased the damages by voles.

Forest regeneration and the establishment success of afforestations seem to be widely controlled by browsing. Major investments in material and man power are necessary for fencing of afforestations and its control and maintenance. Our study showed that the planting of species rich afforestations, including less abandoned broadleaves, may increase the establishment success of the trees and minimizes the risk of biotic damages by voles and rabbits. The week performance of some tree species has a direct feedback on the C balance of the afforestations and prolongs the time until the afforestation becomes a net C sink.

2.4 Land use changes and soil C

(manuscript 3, submitted to Journal of Plant nutrition and Soil science)

Central European ecosystems have been exploited till the first half of the 20th century, especially during and after wartimes. Since the 19th century, soil degradation with declining soil C stocks occurred on agricultural land due to the mechanisation of the agriculture (e.g. development of tractor driven reversible steel ploughs) (Jenkinson and Rayner, 1977;

Küster, 1999). Also erosion may have significantly contributed to these C losses. However, with the introduction of chemical fertilizers in the second half of the 20th century, NPP was increased considerably and C transfer from grassland to cropland with manure additionally helped to maintain or even re-increase cropland soil C stocks (Körschens et al., 2005; Schmidt, 2004). One of the few large scale long-term soil C stocks observations demonstrated for Belgian soils C stocks increasing by 25% since the 1960s within 40 years (VanMeirvenne et al., 1996). Farming practice aimed to maintain or increase C levels in cropland soils with C input from manure to improve soil fertility (soil structure, water holding capacity, nutrient status etc.) (Körschens et al., 2005). Increasing mean temperatures, in particular during the last decades, are discussed to decrease soil C stocks. C stocks in British grassland soil were found to slightly decrease by 0.3 g kg DW^{-1} a^{-1} and in cropland soils by 0.2 g kg DW^{-1} a^{-1}, which is a C loss of around 0.5% a^{-1} (Bellamy et al., 2005). Whether these finding can be generalised to other regions is under discussion.

Land use change adds an additional dynamic on soil C stocks and has the highest potential among the human induced measures to increase the C sequestration capacity of the biosphere or to release additional C into the atmosphere (Gebhart et al., 1994; Guo and Gifford, 2002; Lal, 2004). Interestingly, long-term soil C accumulation on afforestations was similar to C accumulation after cropland conversion into grasslands with 33 g C m^{-2} a^{-1} (Post and Kwon, 2000). In this thesis the effect of conversion from cropland into grassland at the Mehrstedt site 23 years ago and at the Kaltenborn site 29 years ago was investigated, to understand the background of past land use changes and management (manuscript 3).

The grassland at the Mehrstedt site contained 14% higher C stocks than the adjacent cropland if only 0-30 cm soil depth was sampled. When the whole soil profile (0-60 cm depth) was considered no significant difference in soil C stocks were found between cropland and grassland at the two BIOTREE sites Kaltenborn and Mehrstedt. C profiles where reshaped due to land use change with e.g. 34% of soil C stocks stored in the upper 10 cm of the grassland at the Mehrstedt site and only 20% in the cropland surface soil (Fig. 4). Many former studies on the effect of land use change and land use management did not include the subsoil but only sampled the upper 10-20 cm of the soil which may lead to biased conclusions (Baker et al., 2007). High cropland C stocks at the two investigated sites were reached by active C management with manure application (Kaltenborn) and crop residuals left on the site (Mehrstedt). Higher NPP on the croplands (e.g. due to fertilisation) compared to the extensively managed grassland may have adjusted cropland C stocks to grassland level.

Ploughing is expected to decrease the soil C content due to disruption of aggregates and exposure of occluded protected C (Mann, 1986; Vellinga et al., 2004). However, mainly the light C fraction is affected by grassland cultivation. The light fraction comprises the young detritus, partly decomposed roots and litter that is not physically stabilised (Christensen, 2001; John et al., 2005). In this thesis the C stability was assessed with physical density fractionation with a 1.6 g cm^{-3} sodium polytungstate solution. The free light fraction (f-LF), which is not part of aggregates, and the occluded light fraction (o-LF), which is incorporated into aggregates, can be separated during density fractionation by using different degrees of mechanical disruptions to the aggregates. Spatial separation between organic C and microorganisms or enzymes can prevent mineralization and thus, stabilise C. Inaccessibility of C is caused by occlusion in aggregates or micropores or by intercalation within phyllosilicates (von Lützow et al., 2006). There is a positive influence of aggregation on the C accumulation in soils, even though the lifespan of the aggregates may restrict the stabilisation effect of aggregates (Golchin et al., 1994; Six et al., 2002). A lower C/N ratio in the occluded light fraction was found in this thesis, which indicated a higher degree of degradation and stabilisation of C in aggregates compared to the free light fraction. 16% (Mehrstedt) and 25% (Kaltenborn) of soil carbon in the surface soil was isolated as free light fraction and 7% (Mehrstedt) and 5% (Kaltenborn) as occluded light fraction.

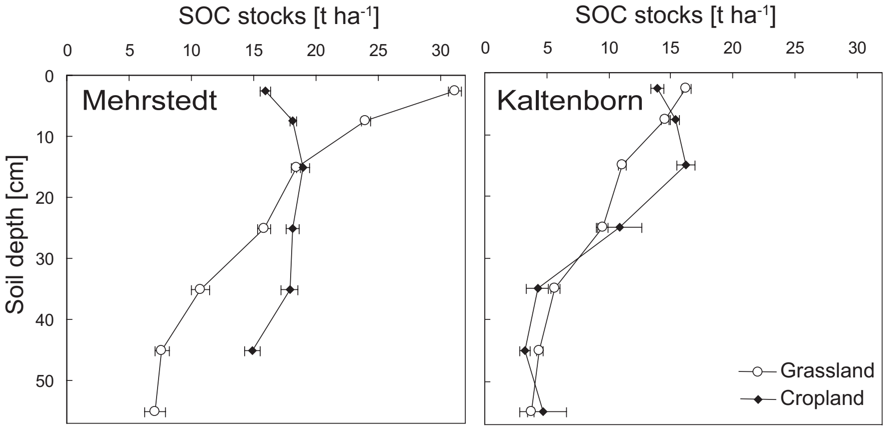

Figure 4: Profiles of the soil organic carbon stocks of the grassland and the cropland at the sites Mehrstedt and Kaltenborn. High C stocks were found in the subsoil of the Mehrstedt cropland.

77% (Mehrstedt) and 70% (Kaltenborn) of soil C was stabilised by adsorption on mineral surfaces and was separated as heavy fraction. The interaction of organic matter with mineral surfaces may decrease its turnover time and is one mechanism of physical stabilisation. The interactions includes ligand exchange, polyvalent cation bridges, weak interactions and interaction with metal ions (complexations) (von Lützow et al., 2006). These mechanisms are summarised as `absorption´ that is assumed to sterically prohibit enzyme attacks. Both light fractions have high turnover rates and are expected to be mostly affected by ploughing, e.g. during site preparation of the planting rows.

The subsoil provides a huge capacity of mineral surfaces to stabilise soil C by adsorption. Turnover times of roots were found to be decreased by 50% in 1.0 m depth compared to 0.1 m depth (Gill and Burke, 2002). If C transfer into the subsoil can be enhanced with a certain management option, e.g. by cultivating deep rooting plants, soil C stocks can be increased (Lorenz and Lal, 2005). Surprisingly high C stocks were found at the Mehrstedt cropland site and could be attributed to an effective translocation of C into the mineral soil below the ploughing horizon. Clay mineralogy of the Mehrstedt site was dominated by the 2:1 minerals illite and smectite with a clay content of up to 70%. Swelling and shrinking dynamics of these minerals could enhance the C translocation into the subsoil.

2.5 Effect of afforestations on net biome productivity (NBP) compared to non-afforested grassland

(manuscript 4, submitted to Global change biology)

Land use change from grassland to forest carries the risk to mobilise soil C. However, in a meta-analysis no effect of different site preparation intensities on soil C stocks changes was found (Paul et al., 2002). After 3 to 35 years of soil C loss, increasing soil C stocks were observed in most studies. In the long term, soil C accumulation on afforestations depended on former soil C stocks levels, climatic conditions, the planted tree species and soil characteristics like texture and pH (Deckmyn et al., 2004; DeGryze et al., 2004; Jug et al., 1999; Paul et al., 2002; Vesterdal et al., 2002). On some sites former soil C stocks of the native forests were never reached again with afforestations (Paul et al., 2002).

To assess the CO_2 exchange of the afforestation site with the atmosphere, eddy covariance measurements provide a powerful tool since computational power allowed to run these measurements in continuous mode (Baldocchi et al., 1988; Schulze, 2006). There is no reliable method other than eddy covariance with which C fluxes can be determined directly on the ecosystem (plot) scale. Continental and global networks of eddy towers covering different land use types and biomes across the globe were created since the 1990s with projects like CarboEurope or Fluxnet (Aubinet et al., 2000; Baldocchi et al., 2001). In Europe, more than

80 eddy tower sites belong to the CarboEurope project, the BIOTREE site Mehrstedt being one of these. Data formats and measurement protocols are partly homogenised and all data are collected in a common data base (Aubinet et al., 2000).

Fluxes of CO_2, water vapour, heat and energy are determined by measuring the covariance of fluctuations in these scalars in a certain distance above the vegetation. Thus, this method integrates over a source area of several hectares (foot print). In fact, eddy covariance systems measure the net ecosystem exchange (NEE) which is the C-flux between land surface (including the rooting zone of the soil) and atmosphere (Chapin et al., 2006; Schulze et al., 2000). Net ecosystem productivity (NEP) is obtained when NEE is integrated over a time (one year). Net biome productivity (NBP) accounts also for non respiratory C losses such as C-leaching with seepage water, harvest or grazed biomass, volatile organic carbon, methane, soot emissions during fire and erosion thus, NBP = NEP – non-respiratory C loss (Schulze et al., 2000) (Fig. 1). NBP is equal to the fraction of C remaining in soils in ecosystems without biomass C accumulation. Measured C loss with seepage water as dissolved organic carbon (DOC) at the site Mehrstedt and Kaltenborn was very small with <4 g C $m^{-2}a^{-1}$ (Don et al., in prep.) other C losses can be assumes to be negligible. Only at the investigated grassland site, biomass export with sheep grazing has to be taken into account. Thus, eddy covariance fluxes allow tracing the soil C stock dynamics on a yearly time scale when biomass C stocks are assumed to remain constant (Ammann et al., 2007).

For this thesis, two eddy towers were operated in parallel, one on the afforestation site and one on the grassland next to the afforestation. Most flux tower sites are equipped with only one tower. However, climatic variability with high seasonal and interannual fluctuation in the C fluxes makes it difficult to assess the impact of a certain land use management on C dynamics. Using the difference between the two fluxes or their ratios allowed detecting the effect of management on the C fluxes (manuscript 4). Additionally, the international flux tower networks lack sites which were recently disturbed by natural hazards or human intervention (Knohl et al., 2002). The Mehrstedt site has got the only existing eddy covariance tower in Europe measuring on young afforestations.

Yearly sums of NEE are prone to various errors including gap filling of night time data and missing data, advective fluxes during non-turbulent conditions on sloping terrain and inhomogeneous source areas due to large foot prints (Baldocchi, 2003; Falge et al., 2001; Schmid, 2002). Relatively homogeneous source areas topography was ensured by selecting appropriate tower locations while the low vegetation height allowed a shallow measurement height of 3 m which reduces advective C losses.

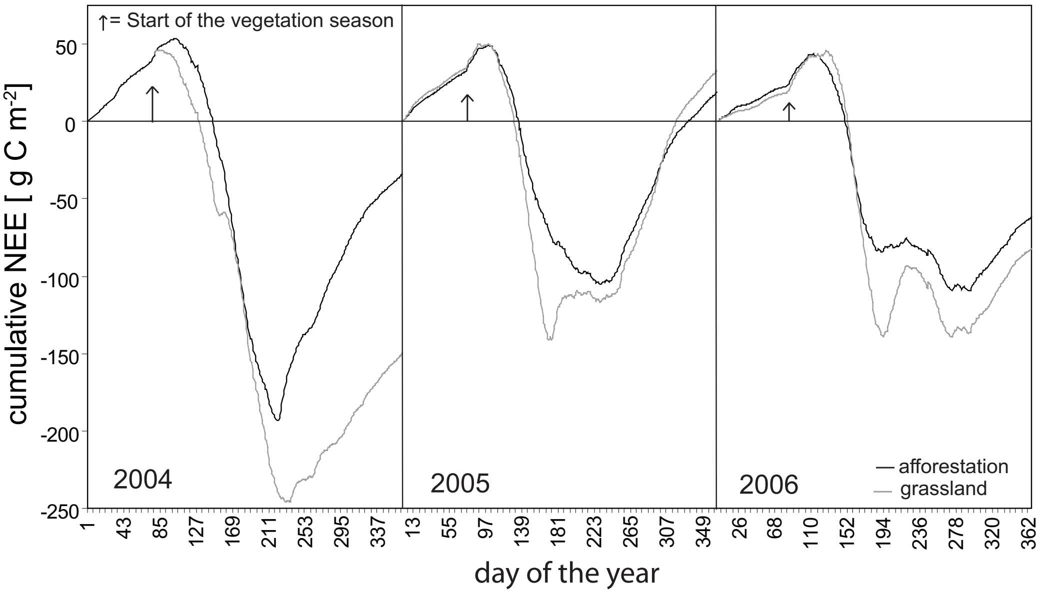

Figure 5: Time series of cumulative NEE at the afforestation and the grassland at the Mehrstedt site. NEE was decreased on the afforestation in 2004 compared to the grassland due to the disturbance during site preparation.

Results of a 3-years measurement period are presented in manuscript 4. Enhanced soil C mineralisation was found after site preparation with ploughing of the planting rows which resulted in a net C loss of 1.2 t C during the first year after planting (Fig. 5). In the second and third year no enhanced C mineralisation was observed but there was still a lower gross primary productivity GPP compared to the grassland site. Lower GPP can be explained by the damage to the vegetation during site preparation and possible mulching effects due to no mowing on the planting rows. The planted trees accounted for less than 2% of the standing biomass of the afforestation site. Thus, C fluxes were dominated by herbaceous vegetation on both sites. Water limitation during summer largely determined the NEE of both sites. Deep roots of trees may access in the future new water recourses which are presently not available for plant growth. A forest micro-climate may reduce the drying of the soils during summer. However, water shortage is a problem of the Thuringian basin region with only 500-550 mm annual precipitation. Climatic trends during the last decades showed for this region a decreased summer precipitation by 30% (Franke and Köstner, in press).

2.6 The detectability of the C flux balances with soil C inventories

(manuscript 5, Geoderma 2007, 141 272-282)

Annual eddy flux sums may be biased by systematic errors and eddy measurements can not resolve the diversity of the experimental plots with different tree diversity but they rather integrate over them. To verify eddy flux results and to set the basis for investigations on soil C sequestrations as influenced by tree diversity, soil C inventories were conducted at the sites Mehrstedt and Kaltenborn. It was one of the biggest soil sampling campaigns reported in literature for single plots. 480 soil cores divided into more than 3100 soil samples were collected, processed and analysed (manuscript 3). The data set was used for geostatistical analysis and as input for a simulation model to assess the variability and the error of different sampling designs. The basis of geostatistical analysis is the intrinsic hypothesis, which assumes a weak form of stationarity for a random function to describe similarities of properties depending on their position. Soil parameters are influenced by various factors which may confound the spatial correlation. However, sample numbers and sample design with minimal distances of only 16 (Kaltenborn) and 20 m (Mehrstedt) between sample cores enabled a geostatistical analysis for this thesis. Higher small scale variability was found with a shorter range of autocorrelation (39-51 m) for the bulk density of the soil than for soil C concentration (range 47-131 m). Variability of the bulk density decreased with increasing soil depth whereas for soil C concentrations increasing variability was found in the clay rich soil at the Mehrstedt site with increasing soil depth. The variability of both parameters, bulk density and C concentration, determine the variability of the soil C stocks. The information on the plot scale variability were used to derive an improved sampling design with 19% (Mehrstedt) and 12% (Kaltenborn) less sample numbers needed to achieve the same statistical power (Fig. 6). As the variability of the bulk density was 1 to 2 magnitudes lower than the C concentration variability, less bulk density samples would be needed than C concentration samples (manuscript 3).

The sampling design distributed 18 (Mehrstedt) and 15 (Kaltenborn) sample cores per plot (tree diversity treatment). Based on the variability analysis minimum detectable differences in soil C stocks were calculated using power analysis (Conant et al., 2003). The heterogeneity of a field site determines the number of samples needed to detect a minimum C stocks change with a certain statistical power.

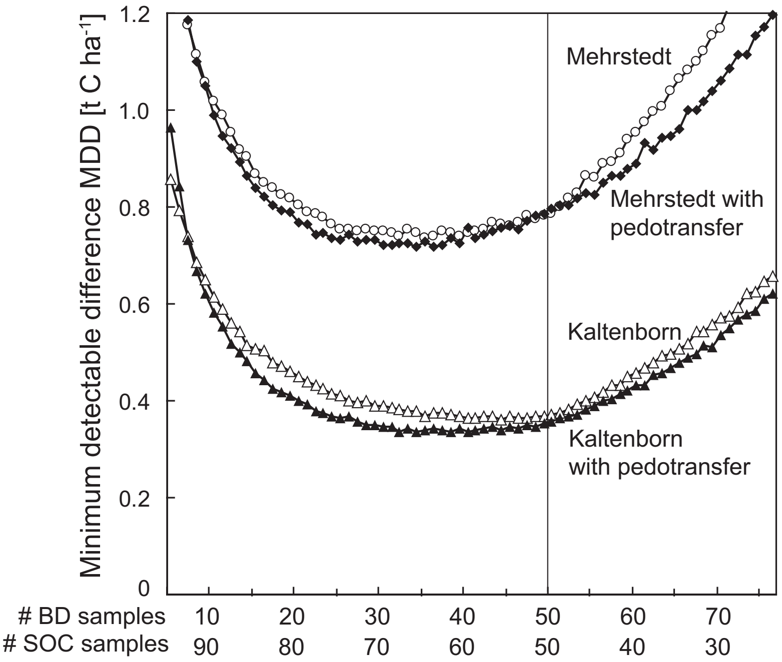

Figure 6: Minimum detectable difference of soil C stocks at the sites Mehrstedt and Kaltenborn depending on the sample ratio bulk density (BD) and C concentration (SOC): An optimized sampling design would consist of x and bulk density samples and x C concentration samples.

In the BIOTREE experiment C stocks changes on the different tree diversity treatments shall be detected in future studies. Minimum detectable C stock changes were found to be between 1.0 and 2.6 t C ha^{-1} for both BIOTREE sites using 15 samples (Kaltenborn) and 18 samples (Mehrstedt) per diversity treatment plot. Taking into account the reported average soil C stock changes in young afforestations (see chapter 1) significant C stock changes on the plot scale could be detectable with a second soil C inventory after 4 – 8 years.

2.7 The impact of earthworms on soil carbon turnover rates

(manuscript 6, submitted to Soil Biology and Biochemistry)

Earthworm abundance was found to be at a maximum in grassland systems and will decline when organic layers build up during forest succession (Grossi and Brun, 1997; Thuille and Schulze, 2006). The long-term impact of earthworms is their influence on the C distribution in the soil profile by actively mixing organic layer material with mineral soil (bioturbation). Mixing of beech litter into the mineral soil was found to decrease C decomposition rates (Scheu and Wolters, 1991). Earthworms were given their own category as `ecosystem engineers´ (Lavelle, 1988) among the soil fauna because they are able to actively change their environment, e.g. by preventing organic layer accumulation to some extent. Also the conversion of cropland into grassland affects earthworm abundance, with higher abundances in grasslands (Wardle, 1995). However, soil conditions like texture and pH are the main factors determining the abundance of different earthworm species. In fact, high numbers of earthworm burrows were found at the BIOTREE site Mehrstedt with a high abundance of earthworms (manuscript 6) but almost none at the sandy acidic site Kaltenborn.

Deep-burrowing anecic earthworms, in particular, remove plant litter from the soil surface, ingest, grind it and transport it into the mineral soil were burrow linings were found to be C enriched (Fig. 7) (Edwards and Bohlen, 1996; Lee, 1985). This C enrichment of the burrows added up to a 3.1 t ha^{-1} increase in the soil C stocks at the Mehrstedt site. Earthworms enhance the binding of mineral particles and organic carbon by mixing both components in their gut and they produce aggregates by excretion of water and mucus which are suspect to protect organic material from degradation (Rasmussen et al., 2005). However, a literature survey revealed that the long-term effect of earthworms on C storage has never been quantified. Most studies have focused on short-term effects of earthworms, which are strongly linked to the accelerated decomposition of mucus and detritus, the release of nutrients and the formation of soil aggregates (Martin, 1991; Scheu and Wolters, 1991). The lifecycle of aggregates is controlled by short-lived C sources (gluing agents) like the earthworm mucus (Guggenberger et al., 1996; Six et al., 2004). Thus, long-term stabilisation of organic carbon due to earthworms should involve adsorption on mineral surfaces, a physically C stabilisation mechanism. Clay minerals play a major role for C stabilisation because they comprise most mineral surfaces in temperate soils, whereas interactions with oxides may be less reversible and thus, more effective in C stabilisation (Kaiser et al., 2002; Kaiser and Guggenberger, 2000).

Fe^{3+} in close vicinity to organic molecules influence the cross polarisation between ^{1}H and ^{13}C by shortening the relaxation time at ^{13}C solid state nuclear magnetic resonance (NMR) relaxation time experiments (Mehring, 1983; Smernik and Oades, 1999). At the same time NMR spectroscopy revealed an insight into bondages of the C-atoms from which a quantification of the compound classes of natural organic carbon can be derived. Thus during the last decade, NMR spectroscopy became a major tool for characterisation of soil organic carbon (Kiem et al., 2000; Kögel-Knabner, 2000). However, with a NMR relaxation time experiment no enhanced C adsorption on iron oxides was found in earthworm casts compared to the non-ingested surrounding soil.

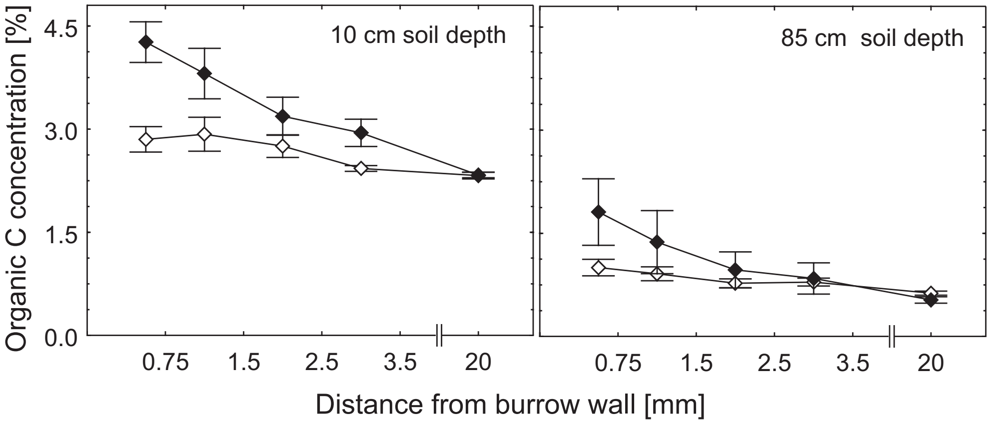

Figure 7: Gradient of organic C concentrations in inhabited (closed symbol) and abandoned (open symbols) earthworm burrows from the inner burrow wall to the surrounding soil (20 mm distance from the burrow) in 10 and 85 cm soil depth. Average C concentrations in abandoned burrows dropped to a level only slightly higher than in the surrounding soil.

C inventories of inhabited and abandoned earthworm burrows revealed that C accumulated in burrow linings are mineralised quickly with turnover times of 3-5 years (Fig. 7). Also measurements of the enzyme activity within and outside the burrow linings could not confirm the original hypothesis of earthworms decreasing C turnover. Microorganisms excrete large amounts of extracellular enzymes to break up organic molecules that are to big to be incorporated by microorganisms (Ekschmitt et al., 2005). Among the different extracellular enzymes six common oxidative enzymes were selected and quantified using methylumbelliferon (MU) as a fluorescent marker in microplate assays (Marx et al., 2001). Enzyme activity decreased in the order fresh earthworm casts>inner part of burrows>outer part of burrows>surrounding soil.

The hypothesis of soil C stabilisation due to earthworm gut passage and deposition in subsoil burrows could not be confirmed with the various methods used. However, the incorporation of fresh organic C into the soil was found to be fast and could possibly account for 44% of the C accumulation after conversion of cropland with low earthworm abundance into grassland with high earthworm abundance. Earthworm burrows as persistent structures may also indirectly affect the soil C dynamics by facilitating deeper root growth into the clay rich subsoil horizons where roots may access additional water and nutrient sources. Thus, earthworm burrows may enhance the establishment success of the trees.

3. Conclusions

Establishment of the afforestations

- BIOTREE is an ambitious experiment with the aim to establish 19 different tree species in a complex design on three sites with a total of 70 ha. The establishment success of the trees was found to be highly species dependant. For some tree species, which were found to be especially sensible to the open field conditions of afforestations, the critical phase of establishment will last several more years.
- Even though all three afforestation sites were still dominated by herbaceous vegetation incipient differences between plots of different levels of biodiversity were detected. Thus, the biodiversity of the plots already influences the establishment success of the trees.

C balance of the first 3 years

- Afforestation is a relatively slow transitional process from agricultural land to forest with minor impact of trees on the ecosystem C dynamics during the first 3 - 4 years. C fluxes as measured with eddy covariance technique showed strong seasonal patterns of growth and senescence of the herbaceous vegetation. A break-down of the vegetation under water stress decreased the C sink capacity of the sites. Future climate scenarios with decreased summer precipitation in Central Europe may turn grasslands on water limited locations such as limestone bedrock into C sources.
- Site preparation caused a net C loss of 1.2 t ha^{-1} in the first 14 months. However, in the second and third years no enhanced C loss was detected compared to the adjacent grassland. Site preparation with deep ploughing on only the planting rows successfully reduced the C losses reported from other studies on afforestations or cultivation of grasslands.
- The ratio between gross primary productivity GPP and total ecosystem respiration TER was a proxy for the current C sink of the ecosystem. On weekly time scales this ratio was relatively constant but was increased by disturbances like mowing and sheep grazing. On yearly time scales it was variable and influenced by climatic factors. Grass dominated system are much more prone to climatic impacts than forests which can maintain their own microclimate. The precipitation pattern during the summer was a critical factor which determined the GPP/TER ratio and the C balance of the afforestation and the grassland.

Soil C inventories

- The quantification of soil C stock changes due to afforestations is possible with a work intensive sampling campaign (100 sampling cores per site) with a minimum detectable difference of 0.4 t C ha^{-1} (Kaltenborn) and 0.8 t C ha^{-1} (Mehrstedt). The results of a simulation model show that the sampling design can be optimized with a reduced number of bulk density samples compared to soil C concentration samples. However, if very few bulk density data and regional or national scale soil C concentration data are used to calculate soil C stocks – as it is frequently done in other studies – the statistical power to detect C stock changes decreases drastically.

Land use history: Conversion from cropland into grassland

- The conversion of the BIOTREE sites Kaltenborn and Mehrstedt from cropland into grassland some decades ago was accompanied by extensification of the management. High C input due to fertilisation of the cropland and active C management with crop residuals left on the site (Mehrstedt) and manure application (Kaltenborn) maintained soil C stocks as high as in the grassland. Thus, soil C stocks can only be maintained by a continuously high C input.
- Low C saturation of mineral surfaces and high ^{14}C ages of organic C in the subsoil of the investigated sites showed the high potential of the subsoil to store additional C. C translocation into deeper soil horizons was found to be very effective in the clay rich cropland site. However, subsoil horizons with a clay content >60% could also hamper the vertical C transport. The cultivation of deep rooting plants could probably increase subsoil C stocks.

The impact of earthworms on soil C dynamics

- C translocation by deep burrowing earthworms was found to be very effective but restricted to the burrow linings. The expected C stabilisation of C in earthworm casts could not be confirmed, but rather high turnover rates were found. The concept of C stabilisation in aggregates of earthworm casts has to be revised. Measurements of extracellular enzymes showed that high enzyme concentrations were correlated with high soil C concentrations. Thus, the spatial separation between soil C and enzymes/microorganisms as a C stabilisation mechanism will be most successful in C poor subsoils.

4. Outlook

4.1 Projected C sequestration depending on tree species and diversity

While the afforestation sites of the BIOTREE experiment are aging, differences in system behaviour, like C cycling, between the plots with different tree diversity will develop. Litter quality is intimately linked with the tree species, thus, litter decomposition and built up of an organic layer will be determined by the planted tree species. Shallow rooting conifers tend to accumulate C in the organic layer, but less in the deep mineral soil as compared to deciduous trees (Jandl et al., 2007). However, in a decomposition study with three deciduous and two coniferous litter types, beech leaves as deciduous litter, had the slowest decomposition rates (Don and Kalbitz, 2005). The effect of mixing of different litter species and the biodiversity effect with different amounts of litter from different tree species on the decomposition rate are an open question to be investigated in the BIOTREE experiment in the future. Up to now, little is known on the effect of tree diversity on C sequestration in soils (Gleixner et al., 2005). Not only chemical properties of the litter but also its structural and physical properties (e.g. contact to mineral soil, moisture content, aeration) play an important role for litter decomposition. Existing studies about the litter mixing effect show inconsistent results ranging on a continuum from antagonistic to synergistic effects but mostly with no mixing effect (reviewed by Rothe and Binkley, 2001).

C sequestration in forest soils is directly influenced by the C-input from leaf and needle litter and root litter. Thus, the productivity of the forest may be directly related to soil C sequestration. In experimental grassland biodiversity studies, the predecessor of the BIOTREE experiment, positive relations were found between the above ground productivity and the diversity of the herbaceous species (Hector et al., 1999; Tilman et al., 2001). Such a positive correlation was found also for forests by analysing North American forest inventory data (Caspersen and Pacala, 2001). However, these results are always endangered by the confounding influence of different climatic and soil conditions on tree diversity when different sites of the inventory are compared. Thus, at the moment it can only be anticipated that the species rich plots sequester more C than the less species rich plots and the monocultures. The BIOTREE experiment with the soil inventory published in this thesis provides the possibility to overcome the problem of confounding environmental factors because different biodiversity levels are planted at one site.

4.2 Changes in soil fauna along with forest development

Decreasing pH values of litter during transition of grassland to forest and during forest development are a well known phenomena causing a decreasing soil pH (Ritter et al., 2003; Thuille and Schulze, 2006). Together with micro climate changes and changes in litter substrate quality the microbial community may shift from bacteria dominance to fungi dominance (Henriksen and Breland, 1999). Mainly fungi are able to decompose lignin derived compounds (Rabinovich et al., 2004). Additionally, grasslands are dominated by arbuscular myccorrhiza whereas trees mostly contain ectomycorrhiza which is supposed to be more aggressive towards soil organic C. Mycorrhiza fungi are effective catalysts and carriers of nutrients but their role in the terrestrial C cycle is poorly understood. Mycorrhiza formation is influenced by soil properties and micro climate, thus, land use change from grassland to forest impact on myccorrhiza abundance (Kahle et al., 2005). Ectomycorrhiza hyphae were found to be more chemically calcitrant that arbuscular myccorrhiza, which would affect the C input quality along with the afforestation (Langley and Hungate, 2003). Initial myccorrhiza community introduced with the planted seedling was sampled and it will be a major research task to follow up the take over of ectomycorrhiza and its possible impact on soil C dynamics at the BIOTREE sites (Anderson and Cairney, 2007).

Not only will microbial community change along with forest development. Earthworms were found to be highly abundant in grasslands but less in forests (Edwards and Bohlen, 1996). This thesis showed the importance of earthworms for translocation of fresh C into the subsoil (manuscript 6). The impact of soil fauna changes on soil C turnover will be important to study to understand future C dynamics in the experimental forests of the BIOTREE experiment.

4.3 Soil C sequestration mechanism in the subsoil

With the common mechanism of chemical and physical C stabilisation soil C dynamics can be explained to some extend (von Lützow et al., 2006). The actual mechanism how adsorption inhibit enzyme attack or how mineral particle prevent the access for microorganism to the substrate by exclusion in micro pores or aggregates is still under discussion and needs further research. A neglected factor for C stabilisation is the position of C in the soil as determined either by direct placing (root litter into mineral soil, foliar litter in the organic layer) or transport as particulate or dissolved organic matter. Root litter has been found to contribute much more effectively to soil C accumulation than foliar litter due to its placing directly into the mineral soil (Rasse et al., 2005). The same litter substrate may have very different turnover times depending only on its placing in or on the soil. There are different possible explanations for the low C turnover times in the subsoil as determined with ^{14}C measurements (manuscript 3). Only in semiterrestrial soils oxygen limitation can explain differences between C turnover in the soil and the surface soil. No oxygen limitation has been found for decomposition processes down to 90% decreased oxygen concentrations. Thus, the energy balance of the microbial community and the special conditions in soils which prevent that substrate and enzyme will meet should be taken into account. Only 2-3% of the soil surfaces are inhabited by microorganism (Foster, 1988) and subsoils (>50 cm depth) at the sites Mehrstedt and Kaltenborn contained only 0.2-0.4% organic C (manuscript 5). Microorganism and the extracellular enzymes released by microorganisms have to meet a substrate molecule to start the mineralisation process. Mineralisation of large organic molecules is only possible after cleaving it into molecules <600 Dalton by extracellular enzymes and subsequent incorporation into cells or microorganisms (White, 2000). Extracellular enzymes are excreted by microorganism and diffuse from there into all possible direction. The likelihood for these enzymes to bump into a substrate molecule decreases exponentially with increasing distance between substrate molecules (Ekschmitt et al., 2005). Thus, energy loss of microorganisms by enzyme excretion may be too high to survive in C poor substrates. In forests less root-C input was measured than in grasslands and indirect impacts on earthworm abundance will additionally affect the amount of C incorporated into the subsoil.

During the 1960s and 1970s a deepening of the ploughing layer from < 25 to > 35 cm depth was promoted in Central and East Europe. This results in a short term decrease of the C concentrations in the ploughing layer (Rauhe and Koepke, 1964). However, resampling after 7 – 30 years indicated a significant increase in total C-stocks for all soil types (Nieder and Richter, 2000). Dilution of C within a thicker ploughing horizon may be one explanation for decreased C turnover rates and increased soil C stocks. Mechanisms of C stabilisation in the subsoil need further research activity beyond the BIOTREE project.

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Summary

Afforestations are acknowledged as C sinks under the Kyoto protocol article 3.3. However, young afforestations may be considerable C sources. Losses of soil C may offset the C sink of the tree biomass. The aim of this thesis was to i) investigate the factors that affect the establishment success of the new forests, ii) quantify the impact of site preparation and management changes along with the afforestation on the C balance of the system, iii) understand how soil C dynamics are influenced by historical land use changes and activity of the soil fauna (earthworms), and iv) to explore soil C variability to set up an optimized sampling scheme for future soil C studies at the two afforestation sites.

The essence of this research is presented in the form of six manuscripts. This thesis sets the basis for the long-term experiment BIOTREE which was started at three sites in Thuringia with a total of 70 ha. The future aim of this experiment is to investigate the influence of tree diversity on ecosystem processes. Therefore, 300 000 seedlings from 19 different tree species were planted. The design of the experiment is outlined in manuscript 1 together with a description of the three study sites.

Manuscript 2 explores the differences between the establishment success of the tree species and the influencing factors. Establishment failure of the species up to 79% extends the time before afforestations become net C sinks. Experimental plots with higher tree diversity were found to be more resistant against damages by voles and rabbits than plots with less tree species.

Parts of the sites Kaltenborn and Mehrstedt were converted from cropland to grassland, 23 and 29 years ago, respectively. The impact of this historical land use change on soil C stocks and C fractions was investigated (manuscript 3). Surprisingly, there was no significant difference in soil C stocks between both land use types but a different vertical C distribution was observed. High C stocks at the clay rich Mehrstedt site were found below the ploughing horizon. The swelling and shrinking dynamic of the clayey soil was expected to enhance the C transport into the subsoil. Measurements of the ^{14}C age of this subsoil C confirmed this hypothesis. In the uppermost horizon of the sandy soil in the Kaltenborn grassland mineral surfaces were found to be C-saturated, thus, this horizon cannot physically stabilise additional C. The large area of unsaturated mineral surfaces in the subsoil provides an unused capacity to stabilise and store additional C at of both sites.

Net C exchange fluxes between land surface and atmosphere were measured with two eddy covariance towers at the afforestation site Mehrstedt and an adjacent grassland site (manuscript 4). Gross primary productivity of the afforestation was reduced by 41% (first

two years) to 14% (third year) compared to the grassland. Site preparation of the afforestation with deep ploughing damaged parts of the herbaceous vegetation that dominated the C fluxes. Enhanced C mineralisation was detected at the afforestation only during the first year, causing a net C loss of 1.2 t ha^{-1}. Seasonal C dynamics were determined by climatic factors (mainly precipitation during summer) and disturbances by site management (grazing on grassland site, mowing on the afforestation site).

The probability to detect expected soil C stock changes depends on the vertical and spatial heterogeneity of the C stocks. The variability of the soil C concentration was found to be one to two magnitudes higher than the variability of the bulk density. Both parameters directly affect the calculated soil C stocks. A simulation model revealed the possibility to improve the sampling design for soil C stocks with sample numbers reduced by 12-19% but unchanged statistical power. This is of major importance because high sample numbers are usually needed to make soil C stock changes detectable.

The effect of earthworms on soil C translocation and stabilisation was investigated to understand how afforestations may influence the C cycling indirectly by reducing the earthworm abundance (manuscript 6). Deep burrowing earthworms were found to be effective in translocating recently assimilated C into the subsoil by depositing it along the burrow walls. Contrary to the original hypothesis of C stabilisation due to earthworm gut passage, organic C in earthworm burrows was lost rapidly with half life times of only 3-5 years. Nuclear magnetic resonance (NMR) relaxation experiments and enzyme activity measurements showed no enhanced C stabilisation by earthworms.

The C dynamics of the investigated afforestation sites were found to be influenced by different factors. Some of them, such as earthworm abundance and seasonal soil moisture pattern, change along with the forest development feeding back on the C cycle and the C sequestration.

Zusammenfassung

Nach Artikel 3.3. des Kyoto-Protokolls können Aufforstungen als Kohlenstoff (C)-Senken angerechnet werden. Junge Aufforstungen können jedoch signifikante C-Quellen darstellen, wenn der C-Austrag durch Bodenatmung die C-Speicherung durch den Biomassezuwachs der Bäume übersteigt. Das Ziel dieser Arbeit war, i) die Faktoren zu untersuchen, die den Anwuchserfolg der Bäume bei Freiflächenaufforstungen bestimmen, ii) den Einfluss der Flächenvorbereitungsmaßnahmen und Veränderungen im Flächenmanagement auf die C-Bilanz des Systems zu quantifizieren, iii) den Einfluss historischer Landnutzungswechsel und der Bodenfauna (Regenwürmer) auf die C-Dynamik zu untersuchen und iv) die Heterogenität der C-Vorräte im Boden auf zwei Aufforstungsflächen zu analysieren, um ein optimiertes Beprobungsdesign für zukünftige Untersuchungen auf diesen Flächen zu entwickeln.

Die Ergebnisse dieser Studien sind in sechs Publikationen zusammengefasst. Sie bilden die Grundlage für das Langzeitexperiment BIOTREE, in dessen Rahmen diese Arbeit angefertigt wurde. Das Experiment umfasst drei Flächen von insgesamt 70 ha in Thüringen. Das Ziel dieses Experimentes ist es in Zukunft den Einfluss der Baumartenvielfalt auf ökosystemare Prozesse zu untersuchen. Dazu wurden 300 000 Setzlinge 19 verschiedener Baumarten gepflanzt. Das Versuchsdesign des BIOTRE-Experiments ist im Manuskript 1 beschrieben zusammen mit der Charakterisierung der Versuchsstandorte.

Der Anwuchserfolg der 19 verschiedenen Baumarten, sowie die Einflussfaktoren, die den Anwuchserfolg bestimmen, wurde untersucht (Manuskript 2). Ausfälle von bis zu 79% pro Baumart verlängern die Zeit bis die Aufforstungsfläche zu einer Netto-C-Senke wird. Untersuchungsflächen mit hoher Baumartenvielfalt waren resistenter gegenüber Verbissschäden durch Schermäuse und Hasen als Flächen mit geringer Baumartenvielfalt.

Die Flächen Mehrstedt und Kaltenborn wurden vor 23 und 29 Jahren teilweise von Acker zu Grünland umgewandelt. Diese vergangenen Landnutzungsänderungen ergaben keine signifikanten Veränderungen der C-Vorräte im Boden aber eine veränderte vertikale C-Verteilung (Manuskript 3). Hohe C-Vorräte wurden unterhalb der Pflugsohle auf den tonreichen Böden der Ackerfläche Mehrstedt gefunden. Die Quell- und Schrumpfdynamik der Tonminerale führte zu einem beschleunigten C-Transport in den Unterboden. ^{14}C Altersbestimmungen des organischen Kohlenstoffs bestätigten diese Hypothese. Nur im obersten Bodenhorizont auf der Grünlandfläche Kaltenborn sind die mineralischen Oberflächen C-gesättigt und können deshalb keinen zusätzlichen Kohlenstoff physikalisch stabilisieren. Die großen ungesättigten mineralischen Oberflächen der Unterböden stellen

ein ungenutztes Potenzial zur Stabilisierung und Speicherung von zusätzlichem Kohlenstoff dar.

Der Netto-C-Fluss zwischen der Landoberfläche und der Atmosphäre wurde auf der Aufforstungsfläche Mehrstedt und einem angrenzendem Grünland mit zwei Eddy-Kovarianz-Türmen gemessen (Manuskript 4) Die Bruttoprimärproduktion der Aufforstungsfläche war um 41% (erstes Jahr) bis 14% (drittes Jahr) geringer als die der benachbarten Grünlandfläche. Die Flächenvorbereitung der Aufforstung mit Tieffräsen der Pflanzreihen zerstörte 30% der nicht-verholzten Vegetation, die die C-Flüsse der Fläche bestimmten. Eine beschleunigte Mineralisierung von Bodenkohlenstoff auf der Aufforstungsfläche führte im ersten Jahr zu einem Netto-C-Verlust von 1.2 t ha^{-1}. Dahingegen war die saisonale C-Dynamik durch klimatische Faktoren (hauptsächlich Sommerniederschlag) bestimmt und durch Störungen durch das Flächenmanagement (Beweidung auf dem Grünland und Mahd auf der Aufforstung).

Die Detektierbarkeit von Veränderungen der C-Vorräte im Boden wird durch deren räumliche und vertikale Heterogenität bestimmt. Die Variabilität der Bodenkohlenstoffkonzentration war ein bis zwei Größenordnungen größer als die der Feinbodendichte. Aus diesen beiden Parametern werden die C-Vorräte im Boden errechnet. Mit einem Simulationsmodel konnte gezeigt werden wie diese Information genutzt werden kann, um das Beprobungsdesign zu optimieren mit 12 - 19% weniger Proben aber unveränderter statistischer Genauigkeit.

Der Einfluss von Regenwürmern auf den C-Transport und die C-Stabilisierung wurde untersucht, um den Effekt von verringerter Regenwurmabundanz in Wäldern auf die C-Dynamik im Boden abschätzen zu können (Manuskript 6). Tiefgrabende Regenwürmer haben frischen Detritus schnell und effektive in den Unterboden transportiert und dort an den Gangwänden abgelagert. Entgegen der Hypothese, dass Regenwürmer zur C-Stabilisierung beitragen wurde ein schneller C-Abbau in den Regenwurmgängen gemessen mit Umsatzzeiten von 3 bis 5 Jahren. Ein NMR (*nuclear magnetic resonance*) Relexationszeitexperiment und Messungen zur Enzymaktivität in den Regenwurmgängen ergaben keine Hinweise auf eine C-Stabilisierung durch Regenwürmer.

Die C-Dynamik der untersuchten Aufforstungsflächen wird durch verschiedene Faktoren bestimmt, von denen sich einige kontinuierlich mit Heranwachsen des Waldes ändern werden, wie z.B. die Regenwurmabundanz oder die Bodenfeuchtedynamik. Dies wird zu Rückkopplungen auf den C-Kreislauf und auf die C-Speicherfunktion der Aufforstung führen.

Manuscript 1

Exploring the functional significance of forest diversity: A new long-term experiment with temperate tree species (BIOTREE)

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Michael Scherer-Lorenzen[1,2], Ernst-Detlef Schulze[2], Axel Don[2], Jens Schumacher[2,a], Eberhard Weller[3,b]

1) Institute of Plant Sciences, ETH Zürich, Universitätsstr. 2, 8092 Zürich, Switzerland
2) Max Planck Institute for Biogeochemistry, Hans-Knöll Strasse 10, 07745 Jena, Germany
3) Thuringian State Institute for Forestry, Game and Fishery (Thüringer Landesanstalt für Wald, Jagd und Fischerei), Jägerstr. 1, 99867 Gotha, Germany
a) Present address: Institute for Probability, Friedrich-Schiller-University Jena, Ernst-Abbe-Platz 2, 07743 Jena, Germany
b) Present address: Rudolf-Alander-Str. 36, 07570 Weida, Germany

Abstract

Effects of biodiversity on ecosystem functioning have been mainly studied in experiments that artificially create gradients in grassland plant diversity. Woody species were largely excluded from these early experiments, despite the ecological and socioeconomic importance of forest ecosystems. We discuss conceptual aspects of mechanistically driven research on the biodiversity – ecosystem functioning relationship in forests, including the comparison of scientific approaches like `observational studies´, `removal experiments´, and `synthetic assemblage experiments´. We give a short overview on the differences between herbaceous and forest ecosystems, focusing on canopy characteristics, and the possibilities for individual versus population based investigations.

We present detailed information about the first large-scale, multisite and long-term biodiversity – ecosystem functioning experiment with tree species of temperate forests (BIOTREE - BIOdiversity and ecosystem processes in experimental TREE stands). At three sites of differing geology and local climate, we planted 200,000 saplings on a total area of

70ha. At two sites, diversity gradients were established by varying the number of tree species (BIOTREE-SPECIES). At a third site, only functional diversity at a constant level of tree species richness was manipulated by selecting mixtures that differ in the functional trait values of the corresponding species (BIOTREE-FD). Additional experimental treatments at the subplot level include silvicultural management options, the addition of subdominant species, and the reduction of genetic diversity. Response variables focus on productivity, biogeochemical cycles and carbon sequestration, and resource use complementarity.

We explore the use of different measures of functional diversity for *a posteriori* classifications of functional richness and their use in the analysis of our tree diversity experiment. The experiment is thought to provide a long-term research platform for a variety of scientific questions related to forest biodiversity and ecosystem processes.

Keywords: afforestation; biodiversity; ecosystem processes; experimental design; functional diversity; temperate forests.

1. Introduction

Alarmed by the increasing loss of biodiversity, since the 1990's, scientists started to systematically seek an answer to one central question of ecology: 'Does biodiversity matter for the functioning of ecosystems?' The search for such biodiversity – ecosystem functioning relationships has become a major challenge in community and ecosystem ecology, and has resulted in a steadily growing number of publications (Balvanera et al., 2006). While biodiversity has historically been seen as a response variable that is affected by climate, nutrient availability and disturbance, functional biodiversity research has increasingly used experiments manipulating diversity as a treatment variable and monitoring ecosystem processes such as productivity or nutrient cycling as the response variables. These approaches have elucidated the importance of biodiversity and the functional traits of the organisms involved for ecosystem functioning, thus underlining the active role of biota in governing environmental conditions (Loreau et al., 2001; Hooper et al., 2005).

Almost all manipulative biodiversity experiments carried out so far have used fast-growing and small model systems, mostly semi-natural or early successional grasslands, and aquatic or terrestrial microcosms (for reviews see Schläpfer and Schmid, 1999; Loreau et al., 2001; Mikola et al., 2002a; Petchey et al., 2002; Schmid et al., 2002a; Wardle and van der Putten, 2002; Hooper et al., 2005; Balvanera et al., 2006; Cardinale et al., 2006). In most cases, a positive relationship between plant diversity and average biomass production was found. Diversity effects on other parameters such as nutrient retention, decomposition, changes in trophic interactions or invasibility by external species have also been documented. The mechanisms causing increases in productivity and related ecosystem properties with species richness have, up to now, been grouped into two main classes: (i) those due to the inclusion of species with specific traits in more diverse communities (sampling/selection effect) and/or (ii) those due to species interactions and physiological adaptations leading to increased resource use (complementarity effects and facilitation) (Loreau et al., 2001; Hooper et al., 2005). These mechanisms are not mutually exclusive and transition between sampling and complementarity effects can occur over longer time spans (Pacala and Tilman, 2002).

Not surprisingly, woody species were largely excluded from these early experiments, despite the ecological and socioeconomic importance of forest ecosystems. It therefore remains fairly open whether causal relationships between biodiversity and ecological processes and their underlying mechanisms exist in forests in a manner similar to those found in other ecosystems. Nevertheless, a large body of knowledge has accumulated in forestry sciences about effects of mixing species, starting with first trials in the 18th century (Pretzsch, 2005). Recent reviews suggest that niche partitioning and complementary resource use or facilitation do occur in certain mixed forest types where species' functional traits cause interspecific competition to be less than intraspecific competition (Cannell et al., 1992; Kelty et al., 1992; Pretzsch, 2005). For example, stratified mixtures of sun-adapted species in the overstorey and shade-adapted species in the understorey, or the combination of species with different rooting patterns may fall under this category. Positive mixture effects have been reported for stemwood production (Pretzsch, 2005), nutrient cycling (Rothe and Binkley, 2001), decomposition (Hättenschwiler, 2005), and damage by insect pests (Jactel et al., 2005) or fungal pathogens (Pautasso et al., 2005). In the tropics, positive mixture effects on productivity or nutrient cycling have also been reported from agroforestry or afforestation experiments, for example in Costa Rica (Haggar and Ewel, 1997; Montagnini, 2000) or Australia (Erskine et al., 2006). However, no or even negative mixing effects through competitive interactions, the importance of specific functional traits, and the context dependency of any mixture effects have been observed by foresters (Scherer-Lorenzen et al. 2005a). Additionally, in his Unified Neutral Theory of Biodiversity and Biogeography Hubbell (2001, 2006) challenged the classical niche paradigm, which focuses on competitive niche differentiation in the exploitation of limiting resources. He argued that ecological equivalence or functional redundancy of plant species in terms of specific population parameters (vital rates, birth, death, dispersal, and speciation) may have evolved easily and naturally in communities of strongly dispersal- and recruitment-limited species, such as sessile plants. Thus, plants with similar or broadly overlapping niches are able to coexist, and communities differing in diversity may thus not show positive diversity–productivity relationships, as demonstrated in a neotropical forest (Hubbell, 2006).

Existing mixture experiments established in the context of forest management usually lack intermediate and highly diverse treatments, because forestry is mainly interested in one- or two-species assemblages of economically important species. Furthermore, most plantations promoted by foresters are planted with varieties that have been genetically selected to have high production (Vilà et al., 2005). This limits our possibilities to investigate ecosystem consequences of changes in tree diversity, either through species loss or species additions. There is a need to complement those approaches by establishing longer gradients in tree species diversity, ranging from monocultures to intermediate and highly diverse mixtures.

In this article, we first discuss some conceptual aspects of mechanistically driven research regarding biodiversity – ecosystem functioning relationships in forest ecosystems. This includes the comparison of scientific approaches that can be adopted, and a short overview on the differences between herbaceous ecosystems (where most functional biodiversity research has been carried out) and forest ecosystems. Finally, we present detailed information about a new biodiversity – ecosystem functioning experiment with tree species (BIOTREE - BIOdiversity and ecosystem processes in experimental TREE stands) in which communities differing in producer-level diversity were established by planting. This project complements other recently established forest diversity experiments in Finland, Panama, and Borneo (Scherer-Lorenzen et al. 2005b).

2. The conceptual background

2.1 Experimental approaches to the study of forest biodiversity – ecosystem functioning relationships

Exploring the functional significance of biodiversity in complex ecosystems such as forests certainly requires a multitude of complementary scientific approaches. In \`observational studies´, biodiversity and ecosystem processes are compared in different existing forest stands (e.g. Schulze et al., 1996; Caspersen and Pacala, 2001; Vilà et al., 2007). These studies have not found strong evidence for consistent mixture effects on productivity or nutrient cycling. However, the interpretation of such across-habitat or across-locality comparisons must be done carefully because co-varying factors such as environmental conditions, land use history, or management may obscure potential within-habitat effects of biodiversity on ecosystem processes unless site conditions are extremely similar (Mund and Schulze, 2005; Vilà et al., 2005). Additionally, these environmental factors themselves determine the diversity of the system. For example, Caspersen and Pacala (2001), using forest inventory data from the United States of America, found an asymptotical increase of growth with increasing tree species. One conclusion following the niche complementarity concept could be that higher diversity of trees enhances productivity due to functional differences between species, leading to higher resource exploitation and, hence, higher growth. However, causality could also run the opposite way: more productive stands may simply permit the coexistence of more species.

Thus, although such surveys or comparative studies are important to document correlations between diversity and ecosystem processes, they have limitations to detect causality or underlying mechanisms of relationships. Therefore, manipulative experiments that artificially create gradients in forest tree diversity are needed to complement observational studies. One promising way to do so is a `removal experiment´, where certain aspects of forest biodiversity – genotypes, species, functional groups, or structural characteristics – are removed from an existing, multi-species stand. Thinning trials may fall under this category. It might also be possible to increase diversity by adding certain components to low diverse stands (`addition experiments´) and compare those plots with suitable controls. Especially removal experiments have been used widely in plant community and population ecology to detect species interactions (for a review see Aarssen, 1990). Despite having some drawbacks (e.g. large disturbance, change of densities, spatial segregation, Díaz et al., 2003), they are increasingly performed in functional biodiversity studies (e.g. Wardle et al., 1999). In an alternative approach, `synthetic-assemblage experiments´ establish forest stands differing in diversity by planting while keeping environmental conditions as constant as possible, like done in the majority of the above mentioned manipulative studies in grasslands. The interpretation of results from such experiments, however, has been debated controversially concerning the role of keystone and dominant species or functional groups, experimental design, data analysis, and applicability to non-experimental situations or to other ecosystems than grasslands or microcosms (Aarssen, 1997; Huston, 1997; Wardle, 1999; Mikola et al., 2002; Lepš, 2004; Srivastava and Vellend, 2005). However, only those studies can unambiguously demonstrate within-habitat effects of biodiversity on ecosystem functioning, minimizing the influence of confounding variables (Schmid et al., 2002b; Schmid and Hector, 2004).

2.2 Forest biodiversity – ecosystem functioning experiments: what's the difference?

Most empirical work on the biodiversity – ecosystem functioning relationship has been done at fast growing grassland model systems. It is thus interesting to consider some basic differences between herbaceous and woody systems, focusing on complementarity in resource use as a main potential mechanism responsible for positive biodiversity effects. We do not discuss practical considerations concerning the establishment and maintenance of tree diversity experiments, which can be found elsewhere (Scherer-Lorenzen et al. 2005b, Don et al. 2007).

A major difference between herbaceous and woody systems is, of course, the long time needed for growth before trees reach a certain size. The occurrence of direct interactions between individuals will therefore certainly take longer than in grasslands, where diversity effects can be observed during the first growing season after establishment from seed. This is so even if perennial species are considered (e.g. Dimitrakopoulos and Schmid, 2004). The establishment phase of woody systems may be important for the development of biodiversity – ecosystem functioning relationships, yet it has rarely been studied due to the shortness of this phase in grassland experiments. For example, tree seedlings may benefit from growing in mixtures due to Janzen-Connell effects with lower pathogen load or herbivory than in pure stands (Jactel et al., 2005; Pautasso et al., 2005). Such effects might be responsible for the lower damage by voles in mixtures found in both the BIOTREE experiment presented below (see also Don et al. 2007) and the tree diversity experiment in Finland (Vehvilainen and Koricheva, 2006).

Related to these different temporal scales is the fact that woody species invest a large fraction of their biomass in long-lasting and non-reversible structures, implying a "memory effect" in crown architecture, which is in sharp contrast to grasses and herbs that renew their biomass almost completely each year (Scherer-Lorenzen et al., 2005a). This opens the possibility to react more flexibly to changes and fluctuations in resource levels (Zobel and

Eek, 2002) and can lead to complementarity in these herbaceous systems. Canopy architecture is a major determinant of light use and growth, influencing aboveground complementarity. Tree diversity experiments offer the possibility to study architectural traits such as branch demography at a level of resolution that is very difficult if not impossible in grassland systems. For instance, quantification of canopy structure or leaf angles of grasses and herbs is very sensible to disturbance induced by the measurement itself (e.g. due to destructive harvest by the stratified clipping method or due to the difficulty to access plants within a dense canopy), and it may be highly variable in time, e.g. due to the influence of wind.

A fundamental difference of using trees rather than perennial grassland species – where individuals often cannot be identified due to clonal growth or dense intermingling of neighbours – is the ability to focus on individuals rather than populations. This focus on individuals allows addressing new questions about the role of demographic processes and biotic interactions as underlying mechanisms of the diversity – functioning relationship. For example, Potvin and Gotelli (in prep.) developed a model to assess plot productivity based on individual tree growth and mortality. Such approaches based on demography are not available when measurements are taken on the plot level. Results from a tree diversity experiment in Panama, for instance, suggest that the net biodiversity effect observed at the community level may result from direct complementarity and enhanced per capita performance (Potvin and Gotelli, in prep.; C. Potvin and P. Dutilleul, pers. comm.). In their modelling exercise, Pacala and Deutschmann (1995) have also shown that the spatial distribution of individual trees have large effects on system-level properties such as standing crop and on community-level properties such as successional species turnover.

Further, an individual-based perspective could help to elucidate the role of intraspecific variability and plasticity for complementarity effects. A species with a high variability and plasticity in certain traits may have similar or even higher levels of functional diversity than a diverse mixture of less plastic species, potentially leading to increased ecosystem functioning in monocultures. In contrast, plastic species with overlapping fundamental resource niches may be constrained to less overlapping realized niches when growing in mixtures, which could result in higher ecosystem functioning in mixtures compared to monocultures (H. Bruelheide and B. Schmid, pers. comm.). The importance of such intraspecific variation has yet not been adequately assessed in biodiversity experiments, and measurements of traits of individual plants are required.

Finally, a focus on individuals in tree diversity experiments opens the possibility to further clarify the underlying mechanisms of the diversity – stability hypothesis, which states that measures of ecosystem stability should increase with increasing species richness (see McCann 2002 for a review). Field observations, laboratory and field experiments, and models of multispecies competition have shown that increasing biodiversity is associated with higher ecosystem stability, while individual species show greater fluctuations (Tilman et al., 2006 and references herein). This difference between ecosystem and species stability may result from interspecific competition (Tilman, 1996): Ecosystem biomass production is stabilized because a decrease of abundance or growth of one species allows its competitors to increase, partially compensating the initial decrease. The individual species' biomass is destabilized with increasing diversity because a higher number of interactions between species and feedback loops may cause change in abundance of one species to impact on many more other species in a competitive network. How stability at the level of plant individuals may fit within this concept of the diversity – stability relationship remains unknown. Because competition essentially is an interaction between plant individuals, by analogy, one could speculate that the stability of individual plants could also be lower with increasing diversity. Because it is possible to collect data on growth stability of individual trees – even in retrospective by analyzing tree rings – the establishment of tree diversity experiments offers new ways to approach this highly debated concept in ecology.

3. The BIOTREE experiment

BIOTREE is not a classical, scientific cultivation test trial for silvicultural purposes related to mixed forests, although forestry aspects and operational experience in silviculture have been considered in the design. Rather, the project directly addresses the above mentioned need to extent functional biodiversity research to forest situations. BIOTREE has been planned since 2000 in close co-operation between the Max-Planck-Institute for Biogeochemistry Jena, Germany, the Thuringian State Institute for Forestry, Game and Fishery, Germany, and the Federal Forestry Office Thüringer Wald. Planting of almost 200,000 saplings covering approx. 70 hectare at three different sites has been finished in spring 2004.

3.1 Sites

The three BIOTREE sites are located in Thuringia, Germany, within a radius of 70 km (Fig. 1, Tab. 1), on calcareous (sites `Mehrstedt´ and `Bechstedt´) and acidic bedrock (site `Kaltenborn´).

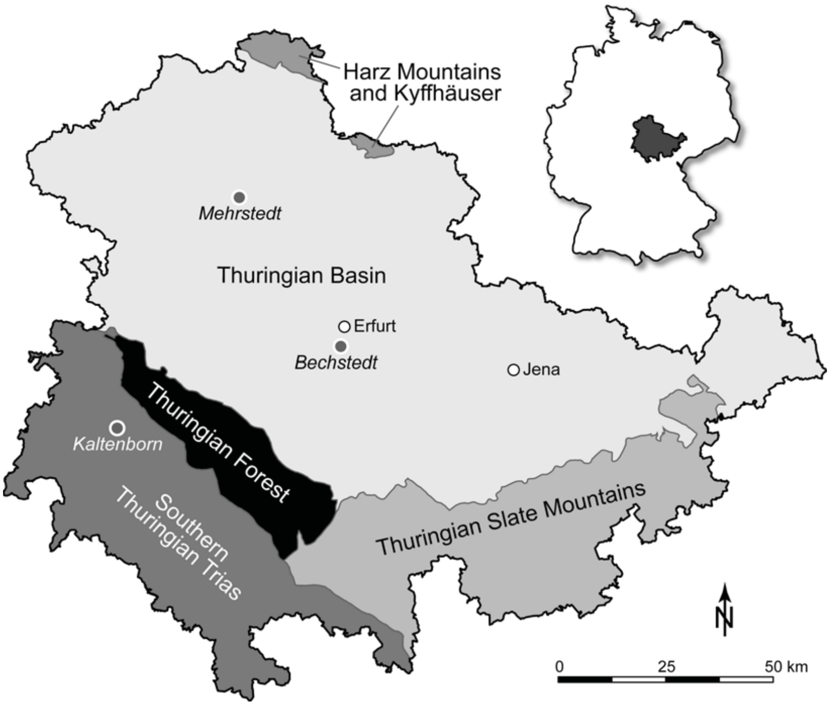


Figure 1: Main landscape units within Thuringia, Germany, and location of the three BIOTREE experimental plantations `Mehrstedt´, `Kaltenborn´, and `Bechstedt´.

The natural vegetation at all sites would be beech dominated deciduous forests with a somewhat higher proportion of oak at the site `Mehrstedt´. Conifers would not naturally occur, but have been introduced into the region by forestry since more than 200 years and constitute an important element in recent forest communities.

Table 1: Location and climatic characteristics of the BIOTREE sites. Meteorological data are from the nearest stations of the German Meteorological Service (DWD); thirty year means (1961-1990).

Site	**Coordinates**	**Elevation** [m.a.s.l.]	**Bedrock**	**Mean annual temperature** [°C]	**Annual precipitation** [mm]
`Mehrstedt´	10°39’E, 51°16’N	270-315	limestone	8.0	547
`Kaltenborn´	10°13’ E, 50,47’N	320-350	sandstone	7.8	650
`Bechstedt´	11°05 E, 50°54’N	400-415	limestone	7.9	553

The site `Mehrstedt´ is located at the north-western border of the Thuringian Basin, a relatively flat area, which is mainly used for agricultural production. `Mehrstedt´ is situated at the transition between subatlantic climate of the mountainous region of Hainich and Dün in the South-West and North, respectively, and subcontinental climate of the Thuringian Basin. The bedrock consists of limestone, marl and clay beds of the Upper Muschelkalk formation (Ceratites layer, "Letten-Grenzschichten"), which belongs to the German Trias. They are partly covered with loess of varying depth on which stagnic Vertisols (WRB-Classification) developed (Tab. 2).

Table 2: Main soil properties of a representative soil profile from the site `Mehrstedt´.

Depth [cm]	**Horizon**	**Sand** [%]	**Silt** [%]	**Clay** [%]	**pH** (KCl)	**C $_{anorg}$** [%]	**C $_{org}$** [%]	**N $_{tot}$** [%]	**C/N**	**CEC** [cmolc/kg]	**BS** [%]
0-9	Ah	2.9	61.8	35.2	7.0	0.08	2.51	0.25	10.2	27.6	100
9-17	Ap	2.4	63.4	34.1	6.8	0.08	1.39	0.15	9.3	27.0	99
17-36	Ap-Bi	3.4	60.5	36.1	6.9	0.20	0.99	0.11	9.0	30.8	100
36-46	Bdi1	5.2	42.7	52.1	7.0	0.53	0.44	0.06	7.4	45.8	100
46-61	Bdgi1	1.6	28.0	70.4	7.2	2.78	0.33	0.05	6.5	49.9	100
61-100	Bdgi3	2.0	30.9	67.2	7.4	4.54	0.20	<0.01	n.d.	45.2	100

At some parts of the site, cropland use led to the development of Cumulic Anthrosol with an M-horizon up to 120 cm depth. The site `Mehrstedt´ has a total size of 50 ha of which 41 ha were afforested (Fig. 2). The site has been used for agriculture as long as there are historical records, dating back to the 12th century. Archaeological records from 2,500 b.c. (Middle Bronze Age) found at the site, however, indicate that the area has been populated for much longer times. Since 1981 the site has been used by the Soviet Army as training area, which caused the conversion of the former agricultural fields into grassland. Some disturbances to soils and vegetation mainly occurred at the northern part of the site. After abandonment of the military training camp some shrub species established in the grassland. The pasture was grazed by sheep and partly mowed once a year until plantation of the experimental plots in 2003.

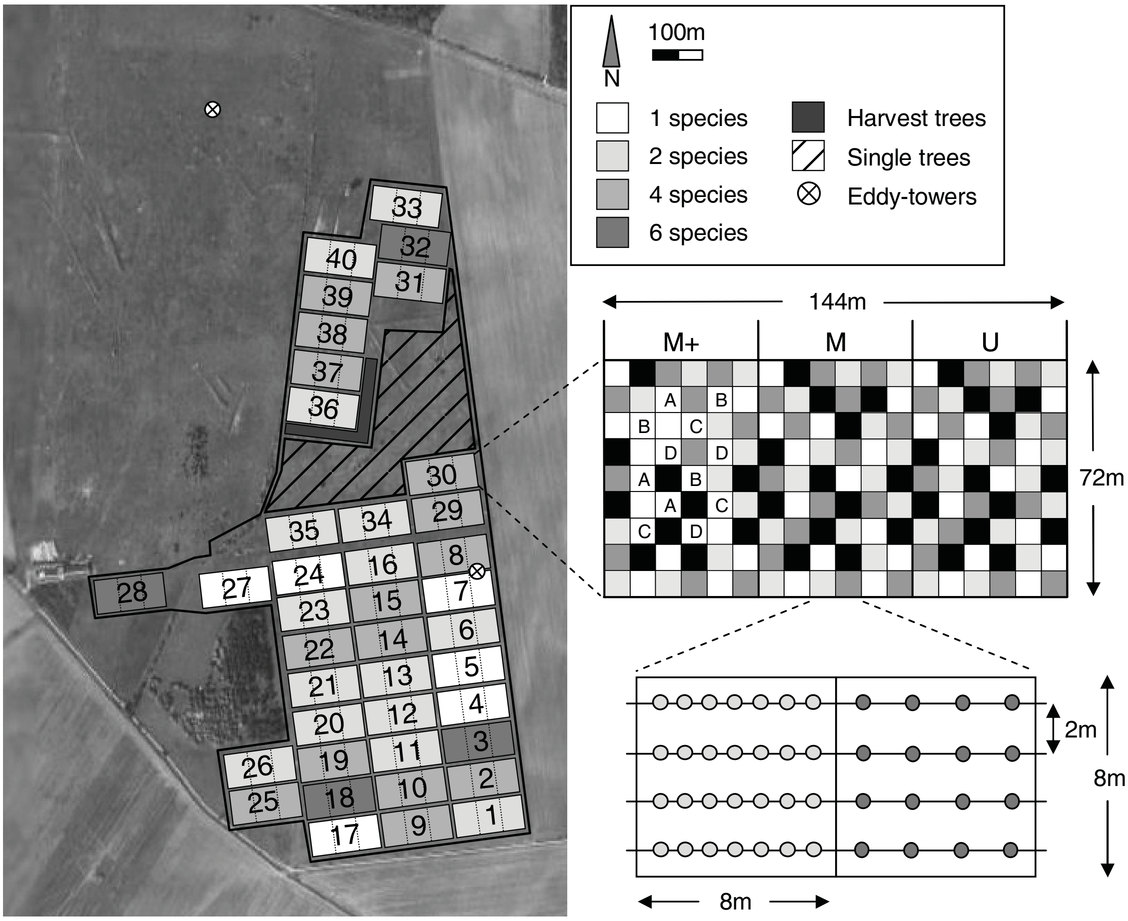


Figure 2: Experimental layout of the BIOTREE-SPECIES site `Mehrstedt´ with plot numbers. Within-plot design is shown for a 4-species mixture as an example. Different grey scales of patches within this plot represent different main species; different letters represent different additional species. M+: managed with additional species, M: managed, U: unmanaged. Planting patterns are shown for two adjacent species patches, representing one with small and one with large tree-to-tree distances, respectively (see also tab. 6). "Harvest trees" and "Single trees" refer to areas planted with trees for destructive harvest, and to areas planted with trees without direct neighbours (25m planting distance) to determine potential growth. Background photography: © by Google™

The site `Kaltenborn´ is located in the Southern Thuringian Trias between the mountain regions of Thuringian Forest and Rhön. The climate is sub-atlantic, influenced by the nearby Werra river valley and a luv effect of the Rhön mountains. The bedrock in `Kaltenborn´ consists of sandstone disrupted by some schist clay layers. It belongs to the Lower Buntsandstein, the oldest part of the German Trias. Arenosols with low cation exchange capacity developed on this sandy bedrock (Tab. 3). The experimental afforestation site is adjacent to an existing mixed pine-beech-forest and has a size of 20 ha (Fig. 3).

Table 3: Main soil properties of a representative soil profile from the site `Kaltenborn´.

Depth [cm]	Horizon	Sand [%]	Silt [%]	Clay [%]	pH (KCl)	C_{org} [%]	N_{tot} [%]	C/N	CEC [cmolc/kg]	BS [%]
0 - 8	Ah	80.5	10.8	8.7	5.0	2.14	0.19	11.3	3.6	90
8 - 27	Ap	80.7	10.3	9.0	4.5	0.71	0.07	10.5	5.8	94
27 - 38	Bw1	78.3	13.8	8.1	4.7	0.23	0.02	9.3	3.6	91
38 - 75	Bw2	76.6	16.9	7.3	4.3	0.21	0.02	9.2	2.2	79
75 - 105	2Bw	79.2	13.8	7.1	4.3	0.15	0.02	8.4	2.8	86
105 - 135	3C	78.9	13.5	8.0	4.5	0.08	0.01	6.9	3.6	96

Until 1975 the site was used as crop land and than converted to grassland. The grassland was managed by mowing once a year and some cattle and sheep grazing.

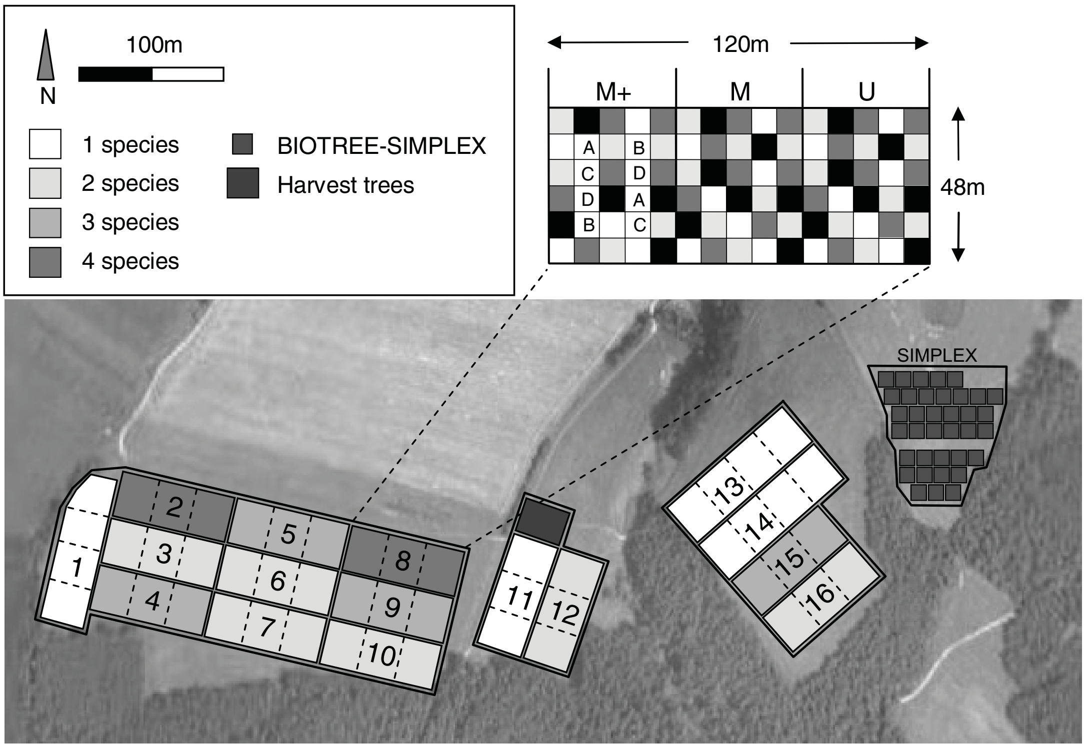


Figure 3: Experimental layout of the BIOTREE-SPECIES site `Kaltenborn´ with plot numbers. Within-plot design is shown for a 4-species mixture as an example. Different grey scales of patches within this plot represent different main species; different letters represent different additional species. M+: managed with additional species, M: managed, U: unmanaged. BIOTREE-SIMPLEX is an additional experiment to study intra- and interspecific competition using the same species as in the main experiment. It adopts a simplex design, which is an extension of the replacement series design (Ramseier et al., 2005), manipulating both total tree density and dominance patterns. Plot size is 16 x 16 m. Background photography: © by GoogleTM

The site \`Bechstedt´, also located within the Thuringian Basin, represents the smallest of the three BIOTREE sites (9 ha, Fig. 4).

Table 4: Main soil properties of a representative soil profile from the site \`Bechstedt´.

Depth [cm]	**Horizon**	**Sand** [%]	**Silt** [%]	**Clay** [%]	**pH** (KCl)	**C $_{anorg}$** [%]	**C $_{org}$** [%]	**N $_{tot}$** [%]	**C/N**	**CEC** [cmolc/kg]	**BS** [%]
0-17	Ah	1.6	42.8	55.6	6.8	0.08	2.62	0.25	10.9	80.0	100
17-28	Ap	1.4	43.7	54.9	6.9	0.10	2.27	0.22	10.9	39.2	100
28-40	Bdi	0.4	31.4	68.2	6.9	0.62	0.90	0.11	8.4	39.2	100
40-55	Bdgi1	0.4	28.6	71.0	7.0	2.09	0.33	0.04	7.3	39.1	100
55-71	Bdgi2	0.4	34.8	64.8	7.1	2.07	0.34	0.05	7.2	36.3	100

The climatic conditions are more sub-continental than those of \`Mehrstedt´ and \`Kaltenborn´. The bedrock consists of limestone, which belongs to the same geological formation as at the site \`Mehrstedt´. Stagnic Vertisols (WRB) have been developed since pre-pleistocene times. They lead to periodic water logging mainly during winter and spring (Tab. 4). The site was used as pasture before.

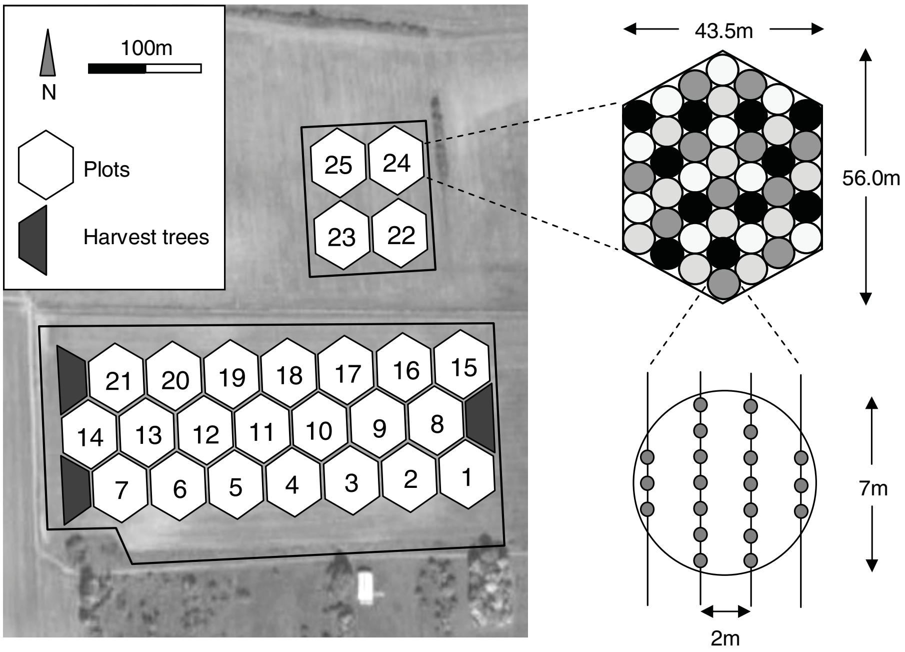


Figure 4: Experimental layout of the BIOTREE-FD site \`Bechstedt´ with plot numbers. Within-plot design is the same for all plots. Different grey scales of patches within this plot represent different species. Planting pattern is identical in all patches. Background photography: © by GoogleTM

3.2 Site preparation and planting

At the sites `Mehrstedt´ and `Kaltenborn´, planting rows of 30 cm width and 60 cm depth were prepared with a deep-spade moulding cutter. In `Bechstedt´, the entire field site was ploughed to a depth of 40 cm. Planting was done in March/April and October/November 2003 and in March/April 2004, using plants of regional provenances. Trees had an age between three to four years grown in a seed bed and an approximate height of 20 – 60 cm, depending on the species. Due to the extremely dry and warm summer 2003, mortality had reached very high levels (mean values between 20 and 40% depending on sites) with a high variability between species. For example, mortality reached up to 79% for *Fagus sylvatica* L., a shade-tolerant species, and 61% for *Quercus petrea* Liebl., which was also highly damaged by voles. In contrast, broadleaves of high economic value like *Ulmus glabra* Huds., *Tilia cordata* Mill., *Fraxinus excelsior* L., *Sorbus aucuparia* L. and three *Acer* species had low mortality rates, mostly <20% (Don et al., 2007). Replanting of species was carried out in November 2004 and March 2005 to ensure an average establishment success of at least 85% per plot.

3.3 Experimental design

We divided the experiment into two distinct approaches to manipulate diversity: At two sites (`Mehrstedt´ and `Kaltenborn´), we established diversity gradients by manipulating the number of tree species (BIOTREE-SPECIES), thus varying taxonomic diversity. At the site `Bechstedt´, in contrast, we varied only functional diversity at a constant level of species richness (BIOTREE-FD).

3.3.1 BIOTREE-SPECIES

LEVELS OF DIVERSITY AND SPECIES POOL

We established a gradient in tree species richness of 1, 2, 4 and 6 species at the calcareous site `Mehrstedt, and of 1, 2, 3 and 4 species at the acidic site `Kaltenborn´ (Tab. 5). Plots differing in diversity were randomly allocated within the sites. We replicated each species-diversity level with different mixtures, thus avoiding confusion between species identity and effects of species number *per se* (Schmid et al., 2002b). Specific mixtures were not replicated. Thus, the main factor of interest – species richness – is adequately replicated, whereas this design does not allow for a statistical test of composition-effects, i.e. the comparison of assemblages with particular species composition.

Table 5: Number of mixtures at each diversity level for BIOTREE-SPECIES. Note that replicates at the highest diversity level have identical species composition.

Calcareous site `Mehrstedt´					Number of plots
Species number	1	2	4	6	
Replicates	6	15	15	4	40
Acidic site `Kaltenborn´					
Species number	1	2	3	4	
Replicates	4	6	4	4	16

Only the highest diversity level has been replicated with several identical species combinations so that an estimate of variability within mixtures is possible. Within each level of species richness, we planted all possible combinations from a species pool of six (`Mehrstedt´) and four (`Kaltenborn´). Growing all possible polycultures, as well as all monocultures, helps to partition selection from complementarity effects (sensu Loreau and Hector, 2001) and to identify possible physiological effects related to mixtures. Some species combinations are not of particular relevance for forestry as they are not considered in current planting plans or stand type recommendations. However, we also want to ensure the possibility of "surprises" in terms of unexpected performance of certain mixtures that normally wouldn't be part of silvicultural planting schemes in mid-Europe, but may be of importance under climate change.

Selection of tree species planted was based on site condition requirements of the species, based on long-term experience from silviculture. All species are common in surrounding forests at each site, representing economically important timber species, and are thus of practical relevance for forestry (Tab. 6). The species pools therefore do not represent the naturally occurring forest communities (which would be low diverse beech stands without conifers) but include trees of managed forests of central Europe. One exotic species (*Pseudotsuga menziesii* (Mirb.) Franco – Douglas fir) has also been used because it is regarded as an "ecologically reconcilable" species of high economic importance by local forestry.

Table 6: Species pool of BIOTREE-SPECIES. Planting distance refers to the distance between individuals within rows. Distance between rows is always 2m. Planting density is the number of individuals per hectare.

Calcareous site \`Mehrstedt´			**Acidic site \`Kaltenborn´**		
Main species	Planting distance	Planting density	**Main species**	Planting distance	Planting density
Fagus sylvatica L.	1 m	5,000	*Fagus sylvatica* L.	1 m	5,000
Quercus petraea Liebl.	1 m	5,000	*Quercus petraea* Liebl.	1 m	5,000
Acer pseudoplatanus L.	1.5 m	3,300	*Picea abies* (L.) H. Karst.	2 m	2,500
Tilia cordata Mill.	1.5 m	3,300	*Pseudotsuga menziesii* (Mirb.) Franco	2 m	2,500
Larix decidua Mill.	2 m	2,500			
Pseudotsuga menziesii (Mirb.) Franco	2 m	2,500			
Additional species			**Additional species**		
Fraxinus excelsior L.	1.5 m	3,300	*Fraxinus excelsior* L.	1.5 m	3,300
Acer platanoides L.	1.5 m	3,300	*Acer pseudoplatanus* L.	1.5 m	3,300
Prunus avium L.	2 m	2,500	*Sorbus aucuparia* L.	2 m	2,500
Carpinus betulus L.	1.5 m	3,300	*Abies alba* Mill.	2 m	2,500

PLOT SIZE AND WITHIN-PLOT DESIGN

Ideally, each plot should have a certain minimum size so that microclimate resembles that of closed forest stands. However, restrictions of space availability limited plot sizes to 1.04 ha (144 x 72 m) on the site \`Mehrstedt´ (Fig. 2) and 0.58 ha (120 x 48 m) at the site \`Kaltenborn´ (Fig. 3). Because plots are adjacent to each other with only small access tracks in between, each experimental site as a whole (41 and 20 ha, respectively) should develop microclimatic conditions similar to closed forests.

A major difference to most large-scale manipulative experiments in grasslands is the necessity to determine the within-plot planting design. In principal, tree individuals could be spatially arranged (i) at random, (ii) in clumped patches, or (iii) in a regular pattern. For practical reasons, we adopted option (iii) and trees were planted in rows of 2 m distance. In order to prevent early outcompetition of slow growing species by fast growing ones and thus to allow for complete establishment of all species, each species was planted in monospecific rectangular patches of 64 m² each (Figs. 2 and 3). The size of this area is adopted from canopy properties of adult trees (mean crown diameter). Triggered through the processes of self-thinning in the unmanaged subplots (see "Additional treatments"), and through silvicultural thinnings in the managed subplots, we therefore expect that each patch will be occupied by only one single individual in the long-term. The monospecific patches were randomly distributed within each subplot, and the same spatial distribution was replicated in all subplots (Figs. 2 and 3). However, it has been assured for all mixtures that (i) no species is adjacent to itself except at the corners between two patches, and that (ii) each species has approximately the same number of neighbouring species. The within-row distance of trees was approximated to common planting practice and therefore differs depending on species (Fig. 2), resulting in three groups of planting density (Tab. 6). However, in a mid-term time frame, similar overall densities will be achieved through self-thinning and management.

ADDITIONAL TREATMENTS

In order to study influences of other factors apart from varying tree species richness, we applied a split-plot design with random allocation of additional treatments to several subplots (Figs. 2 and 3). The size of these subplots is 0.35 ha (48 x 72 m) at `Mehrstedt´, or 0.19 ha (40 x 48 m) at `Kaltenborn´.

a) *Management effects* ("U"/"M"): Silvicultural interventions may mask potential diversity effects because of changes in stand structure (e.g. stands are kept at a constant density or basal area), or because of direct influences on the processes of interest (e.g. soil carbon dynamics, Mund and Schulze, 2005). One subplot will thus be kept without any management (labelled "U" for "unmanaged"), except replanting of dead individuals in the first three years, and mowing of the herbaceous layer in order to ensure successful establishment. The other subplots will be thinned according to regional state-of-the-art management rules of close-to-nature forestry (labelled "M" for "managed").

b) *Additional species* and *reduced genetic diversity* ("M+"/"M-"): Close-to-nature forestry aims to increase tree species richness by promoting sub-dominant or minor species. Therefore, a third subplot includes four additional species at lower abundances than the main species ("M+", Tab. 6). This allows to test the effect of including sub-dominant species on ecosystem processes at different levels of background tree diversity It also increases the gradient in species richness up to eight (site `Kaltenborn´) and ten species (site `Mehrstedt´), respectively. At the site `Kaltenborn´, another subplot has been added to all monocultures to observe influences of genetic diversity on the performance of single-species stands ("M-"). This subplot was planted with saplings originating from seeds of a single mother tree per species, which have a lower genetic variability than those of the adjacent subplots that were raised from many mother trees. These subplots will also be managed.

3.3.2 BIOTREE-FD

LEVELS OF DIVERSITY AND SPECIES POOL

In contrast to the BIOTREE-SPECIES approach, the diversity gradient in the BIOTREE-FD experiment has not been established by manipulating the number of species (taxonomic diversity), but by mixing species with different functional attributes. We adopted this approach to account for the importance of functional traits in explaining diversity effects on ecosystem functioning (Díaz and Cabido, 2001; Hooper et al., 2002). Several other experiments have incorporated a gradient of functional trait diversity into their designs by manipulating the number of *a priori* defined functional groups, in addition to the manipulation of taxonomic diversity, e.g. experiments at Cedar Creek (Tilman et al., 2002), BIODEPTH (Spehn et al., 2005), or The Jena Experiment (Roscher et al., 2004). However, due to the co-linearity of species richness and functional group richness involved in these approaches, it is not possible to fully separate both factors (Schmid et al., 2002b). We therefore kept the number of tree species at a constant level of four.

We used the method by Petchey and Gaston (2002) to calculate a quantitative measure of functional diversity (FD) of mixed assemblages. In a first step, we performed a cluster analysis (Ward's method) of nine relevant traits from a total pool of 16 species, grouping species according to functional similarity. As nominal, ordinal and numeric traits are mixed in our trait matrix we used the daisy algorithm (Kaufman and Rosseeuw, 1990), specifically designed for such situations and implemented in S-Plus 6.1. (Insightful Corp.), to calculate dissimilarities between species. The traits selected represent attributes which are indicative for productivity, resource use and nutrient cycling, representing the main functions of interest in this experiment (Tab. 7). Out of the functional dendrogram obtained from the cluster analysis (Fig. 5), which groups species according to their similarity in functional traits, the FD-values of all 1,820 possible four-species mixtures were calculated as the total branch length of a subtree connecting all species within a mixture (including basal branches common to all species, see Petchey and Gaston (2002) for details of the method). Finally, the full range of FD of all 1,820 mixtures was divided into four even-sized groups. Out of each group, six mixtures were randomly selected so that the whole range of possible values of functional diversity was covered. The plots differing in functional diversity were randomly allocated within the site. All species used are common in natural forest communities surrounding the experimental plantation and no exotic species have been used (Tab. 8).

Table 7: List of species traits used to calculate an index of functional diversity. Data were obtained from expert estimates, local growth tables and various sources (Lyr et al., 1967; Köstler et al. 1968; Thomasius, 1978; Bergmann, 1986; Kramer, 1988; Röhrig and Ulrich, 1991; Dengler, 1992; Ellenberg, 1996; Burschel and Huss, 1997; Puhe and Ulrich, 2001).

Traits	Data type	Scale
Leaf type	categorical	(0) evergreen, (1) deciduous
Light requirements as adults 1)	ordinal	(1) low, (2) medium, (3) high, (4) very high
Height growth vigour 2)	ordinal	(1) very low, (2) low, (3) medium, (4) high, (5) very high
Mean annual increment growth	ordinal	(1) < 4 m^3 ha^{-1} a^{-1}, (2) 4-8 m^3 ha^{-1} a^{-1}, (3) 8-12 m^3 ha^{-1} a^{-1}, (4) 12-20 m^3 ha^{-1} a^{-1}, (5) > 20 m^3 ha^{-1} a^{-1}
Rooting vigour 3)	ordinal	(1) very low, (2) low, (3) medium, (4) high, (5) very high
Crown architecture	categorical	(1) monopodial, (2) sympodial-narrow, (3) sympodial-broad
Root architecture	categorical	(1) shallow-rooted, (2) heart-rooted, (3) tap-rooted
Leaf N concentration	metric	standardized to mean value of 0 and variance 1
C/N ratio of litter	metric	standardized to mean value of 0 and variance 1

1) "Light requirements as adults" encompasses the position of a species within the shade tolerant – shade intolerant continuum.

2) "Height growth vigour" is the capacity of a species to become dominant within mixed stands, according to Puhe and Ulrich (2001).

3) "Rooting vigour" describes the capacity to intensively penetrate the soil volume (Puhe and Ulrich, 2001).

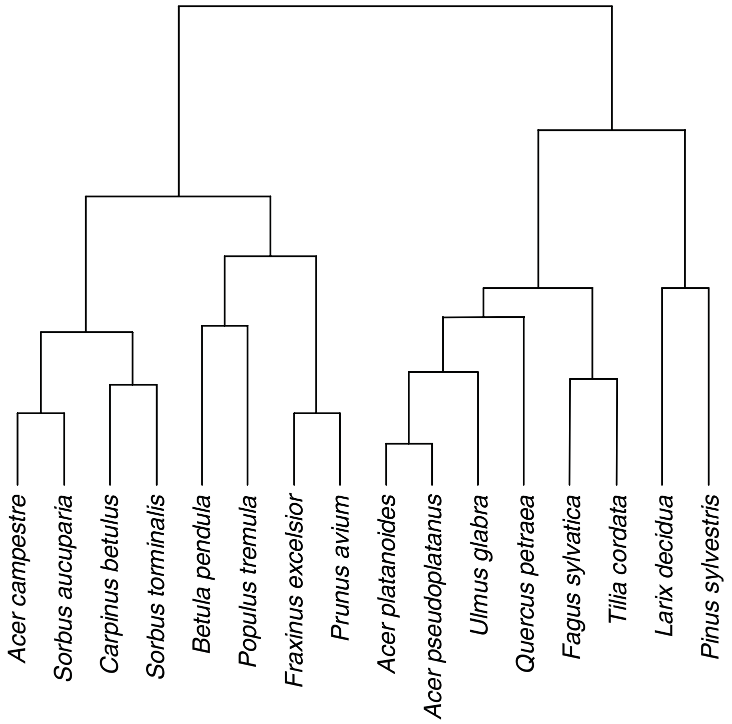
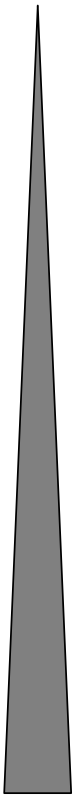


Figure 5: Functional dendrogram obtained from cluster analysis of nine traits from all 16 species used in the BIOTREE-FD experiment, grouping species according to functional similarity.

PLOT SIZE AND WITHIN PLOT DESIGN

Plots of the BIOTREE-FD experiment have a size of 1,700m² with a hexagonal shape (Fig. 4). We expect that microclimatic conditions similar to closed forests should develop within the experimental site of nine hectares, because plots will be adjacent to each other with only small alleys in between. Within each plot, species have been planted in rows with a plant-to-plant distance of 1m and a row-to-row distance of 2m. Different species were arranged in circular groups of 20 individuals, covering an area of 38.5m² each, thus preventing early outcompetition of slow growing species by fast growing ones. Each species is represented by 11 groups per plot (Fig. 4).

Table 8: Species mixtures of BIOTREE-FD. The ordering of mixtures along the gradient of functional diversity might change depending on the index used for calculating functional diversity, and on the selection of traits and their values (see text for details).

Plot No.	Functional diversity	Species 1	Species 2	Species 3	Species 4
1		*Acer platanoides*	*Quercus pertraea*	*Tilia cordata*	*Ulmus glabra*
2		*Acer campestre*	*Acer platanoides*	*Acer pseudoplatanus*	*Sorbus aucuparia*
3		*Acer platanoides*	*Acer pseudoplatanus*	*Betula pendula*	*Quercus pertraea*
4		*Acer campestre*	*Sorbus aucuparia*	*Sorbus torminalis*	*Ulmus glabra*
5		*Populus tremula*	*Prunus avium*	*Sorbus aucuparia*	*Sorbus torminalis*
6		*Carpinus betulus*	*Fraxinus excelsior*	*Sorbus aucuparia*	*Sorbus torminalis*
7		*Acer platanoides*	*Carpinus betulus*	*Tilia cordata*	*Ulmus glabra*
8		*Acer platanoides*	*Fagus sylvatica*	*Sorbus aucuparia*	*Ulmus glabra*
9		*Betula pendula*	*Fagus sylvatica*	*Quercus pertraea*	*Ulmus glabra*
10		*Quercus pertraea*	*Sorbus aucuparia*	*Tilia cordata*	*Ulmus glabra*
11		*Betula pendula*	*Pinus sylvestris*	*Populus tremula*	*Prunus avium*
12		*Acer platanoides*	*Acer pseudoplatanus*	*Populus tremula*	*Sorbus aucuparia*
13		*Acer platanoides*	*Fraxinus excelsior*	*Prunus avium*	*Sorbus torminalis*
14		*Betula pendula*	*Fagus sylvatica*	*Sorbus aucuparia*	*Sorbus torminalis*
15		*Acer platanoides*	*Carpinus betulus.*	*Populus tremula*	*Ulmus glabra*
16		*Acer campestre*	*Fraxinus excelsior*	*Pinus sylvestris*	*Populus tremula*
17		*Betula pendula*	*Carpinus betulus*	*Prunus avium*	*Tilia cordata*
18		*Acer campestre*	*Acer platanoides*	*Fraxinus excelsior*	*Tilia cordata*
19		*Acer campestre*	*Fraxinus excelsior*	*Larix deciduas*	*Tilia cordata*
20		*Acer pseudoplatanus*	*Betula pendula*	*Pinus sylvestris*	*Sorbus torminalis*
21		*Acer pseudoplatanus*	*Carpinus betulus*	*Larix deciduas*	*Prunus avium*
22		*Betula pendula*	*Carpinus betulus*	*Larix deciduas*	*Ulmus glabra*
23		*Betula pendula*	*Larix deciduas*	*Quercus pertraea*	*Sorbus torminalis*
24		*Carpinus betulus*	*Fraxinus excelsior*	*Pinus sylvestris*	*Quercus pertraea*

4. Baseline data and first results

4.1 Soil parameters

At the time of planting, an intensive sampling campaign was carried out to collect baseline data for soil physical and chemical parameters, with special emphasis on soil carbon pools. In addition to several soil profiles, soil cores were analysed to a depth of max. 80 cm in 5 and 10 cm increments. To account for the high heterogeneity of soil carbon stocks we took 18 and 15 cores per plot in \`Mehrstedt´ and \`Kaltenborn´, respectively. All samples have been archived for future analyses. The sites \`Mehrstedt´ and \`Kaltenborn´ represent two major polarities of soil types. At \`Mehrstedt´, high clay content and a loess layer supply high nutrient availability with high cation exchange capacity (CEC) and almost 100% base saturation of these stagnic Vertisols (Tab. 2). Arenosols at \`Kaltenborn´, in contrast, have a sandy texture with only <10% clay content, and low CEC at pH around 5 (Tab. 3), which is typical for forest soils developed on silicate bedrock. Soil characteristics of the site \`Bechstedt´ are similar to that at \`Mehrstedt´. The subsoil is rich in clay ensuring good nutrient status of the site (Tab. 4). Carbon concentrations were almost twice as high at \`Mehrstedt´ compared to \`Kaltenborn´ (Tables 2 and 3). Mean C-stocks (0-50 cm depth) were 86 t C ha^{-1} at \`Mehrstedt´ and 48 t C ha$^{-1}$ at \`Kaltenborn´.

4.2 Ground layer vegetation

A total of 584 permanent quadrates of 1 and 4 m^2 (BIOTREE-SPECIES and BIOTREE-FD, respectively) were marked in the experimental plots for the assessment of successional changes of ground vegetation. Initial species richness in these quadrates ranged from 9 to 30 species (\`Mehrstedt´), 8 to 20 species (\`Kaltenborn´), and 6 to 20 species (\`Bechstedt´, note different quadrate size). In total, 173 species have been found in \`Mehrstedt´, 69 species in \`Kaltenborn´, and 91 species in \`Bechstedt´. At the time of planting, vegetation at \`Mehrstedt´ could be characterised as an extensively grazed and irregularly mown mesophytic grassland, with *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl and *Dactylis glomerata* L. as the most abundant species. Species composition was patchy with areas of drought resistant species and lower productivity due to soil depth (mesoxerophytic type). At \`Kaltenborn´, the regularly mown mesophytic grassland was dominated by *Holcus lanatus* L. and *Bromus hordeaceus* L.. At \`Bechstedt´, a ruderal community with many agricultural weed species has developed shortly after ploughing, with *Fumaria vaillantii* Loisel. and *Cirsium arvense* (L.) Scop. as the most abundant species.

4.3 Response variables

Current measurements focus on changes in carbon pools and fluxes at the transition from grassland to forest, which are driven by the disturbance prior to planting (deep cultivation of planting rows), and the introduction of tree saplings. At the site `Mehrstedt´, two eddy covariance towers have been installed in the plantation and in an adjacent grassland, continuously measuring CO_2- and water vapour fluxes between atmosphere and biosphere. Although the spatial resolution of these measurements is not high enough to separate the different diversity treatments, it will be possible to quantify the C-sink strength of this afforestation on a former grassland.

Other measurements currently in progress include tree survival and growth, tree canopy structure and leaf area index, stand productivity and carbon sequestration in woody biomass (via allometric relationships obtained from harvested trees from additional orchard plots), development and productivity of ground vegetation, soil water chemistry (leaching of dissolved organic carbon and nitrate), carbon sequestration in soil and soil respiration. Additionally, species-specific responses of trees to shading are investigated with experimental shading of buds, branches and whole trees. Carbon allocation within tree individuals is quantified in order to understand competitive performance during the phase when crowns of neighbouring trees start to interact. These data shall provide the basis for a tree individual based model to simulate growing patterns for different tree species involved in the BIOTREE experiment. Future measurements will include nutrient retention in plants and soil, litter fall, decomposition and mineralisation. It would be very interesting to perform regular inventories of herbivores, pathogens and soil fauna to obtain information on associated biodiversity. Additional manipulations of certain components of forest biodiversity (e.g. herbivores), or of certain processes (e.g. nitrification) could provide deeper insights into underlying mechanisms of diversity-functioning relationships.

4.4 Including functional trait diversity into the design

In the BIOTREE-SPECIES approach we have not manipulated the number and identity of functional types *a priori*. However, by calculating an index of functional diversity for each community, functional richness can be determined *a posteriori*. As described before, we used the FD approach of Petchey and Gaston (2002) to estimate functional diversity of tree species mixtures for our BIOTREE-FD experiment, but other indices have been developed recently (Petchey and Gaston, 2006). Because each index is based on different inherent assumptions about quantifying distance in trait space or algorithms used to cluster species, the use of several indices will give different `values´ for functional diversity (Petchey and Gaston, 2006; Schumacher et al., in prep.). Functional diversity will also change if the trait matrix will be refined, e.g. by replacing literature values of species traits with values measured on site, or by including more traits or excluding traits that are not relevant in the specific context. In addition, the relative importance of functional traits for certain target ecosystem processes can be assessed by estimating optimal weights of traits from observed data (Schumacher, in prep.). The use of functional diversity is thus a much more flexible measure of tree diversity than species richness or other indices based on species numbers and abundances only. This flexibility clearly is an advantage for testing for more specific hypotheses about the functional significance of tree diversity. Finally, in most cases different mixtures used in biodiversity experiments will continuously cover a wide range of functional diversity-values, which enhances the possibility to analyze the functional form of diversity effects using regression approaches in contrast to the ANOVA-analyses restricted to comparison between species richness levels.

To demonstrate some of these aspects, we calculated functional diversity of our experimental communities using both the FD index of Petchey and Gaston (2002) and the quadratic diversity Q index (Rao, 1982; Ricotta, 2005; Schumacher et al., in prep.). We hypothesized that functional diversity of the planted mixtures will span a continuous range, and that some low-diversity mixtures composed of functional very dissimilar species may reach higher values than certain mixtures with more, but more similar species. Furthermore, we expect a fairly good correlation between both measures of functional diversity.

Q is an extension of the Gini-Simpson index of diversity and incorporates functional dissimilarities between species as well as relative abundances into one measure of functional diversity. Q is calculated as $Q = \sum_{i,j=1}^{S} p_i p_j d_{ij}$, where S is the number of species, p_i with $i=1, \dots, S$ are the relative abundances and d_{ij} describe the distance between species i and j in the community, i.e. their dissimilarity. Basis for the calculation of both FD and Q is a matrix of functional traits of all species which is used to determine the functional dissimilarities between species in a multidimensional trait space. The minimal value is of Q is zero and occurs if all species are identical with respect to the functional traits considered (i.e. $d_{ij} = 0$ for all i,j). The maximal value depends on the number of traits and their distribution and it is often not easy to calculate this value. Generally the maximum is not achieved for an even species composition.

As shown in figure 6, *Q* generally increases with increasing tree species richness, although this increase is not linear but levels off at higher species richness. *Q* may even decrease slightly with increasing species richness, e.g. if certain 4-species mixtures are compared with the 6-species level at the site \`Mehrstedt´. Additionally, there is some overlap among the levels of species richness.

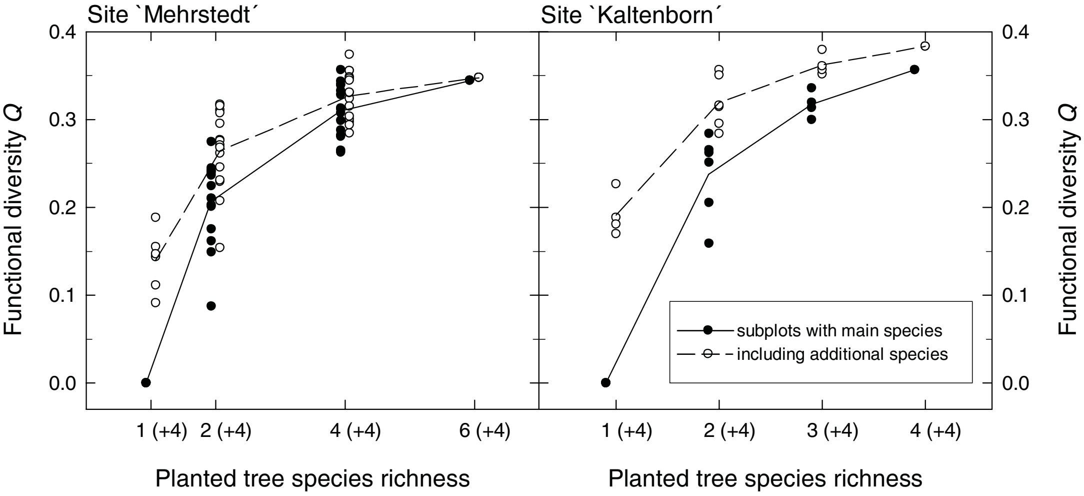


Figure 6: Functional diversity Q by planted tree species richness for the experimental communities in \`Mehrstedt´ (calcareous site) and \`Kaltenborn´ (acidic site). Q was calculated based on nine species traits (Tab. 7). Black symbols represent subplots with main species only, white symbols represent subplots with four additional species (see section "Experimental Design" for details). Lines connect mean values of Q per level of species richness. Symbols are slightly staggered for better visibility.

Thus, there are some mixtures with a lower functional diversity despite having a higher number of species. For example, the highest level of *Q* at the calcareous site \`Mehrstedt´ is attained by a 4-species mixture (*Larix decidua* Mill., *Pseudotsuga menziesii*, *Fagus sylvatica*, *Quercus petraea*), and adding two more species (*Acer pseudoplatanus* L. and *Tilia cordata*) actually decreases functional diversity slightly. This remarkable finding can be explained by associated changes in abundance, which *Q* takes into account: if the former four species are functionally very distinct from each other, then addition of species similar to some of those is comparable to an increase of abundance of the functional trait values associated with the "similar" species leading to a more uneven distribution of trait values. The overall functional diversity of the community will therefore decrease. In an analogous way, evenness of a community can also decrease while adding more species if the abundance of certain species disproportionally increases. This is also consistent with the perception that a mixture of four shade-intolerant species is functionally less diverse than a mixture of three species including one shade-intolerant, one shade-tolerant, and one intermediate species, for instance (Schumacher et al., in prep.). Figure 6 also shows that the addition of less abundant species in the "M+" subplots strongly increases *Q* at low species numbers, whereas it has only marginal effects at higher numbers. Finally, experimental communities are spread over a wide range of *Q*-values which opens the possibility for regression analyses for diversity – functioning relationships.

As shown in figure 7, the two indices for functional diversity are positively correlated, although with substantial scatter. Thus, it is possible to deliberately select sequences of mixtures where both measures actually show a negative correlation, even within one site. Selecting such subsets of mixtures might be interesting for testing the explanatory power of different indices for ecosystem processes. The inclusion of nine additional traits (successional status, longevity, light requirements as juveniles, litter decomposition rates, drought resistance, susceptibility against late frost, susceptibility against strong winds, frequency of fructification, and mode of seed dispersal) does not change the value of the index *Q* very much (Pearson correlation between $Q_{9\ \text{traits}}$ and $Q_{18\ \text{traits}}$: r = 0.92). In large part, this is due to intercorrelations between the traits used, e.g. between C/N ratio of leaf litter and decomposition rate. Thus, by selecting key traits, or traits that represent rather a syndrome than specific species characteristics (Körner, 2005), it is possible to represent a large part of functional variation between species.

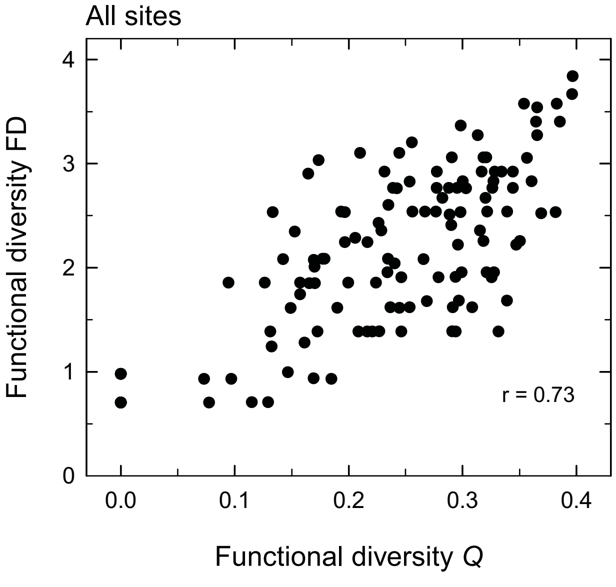


Figure 7: Correlation between two measures of functional diversity, Q and FD, calculated for all experimental mixtures of BIOTREE-SPECIES and BIOTREE-FD. Q and FD are based on nine species traits (Tab. 7). Pearson correlation coefficient is given.

5. Outlook

As life-cycles of mid-European tree species are in the order of hundreds of years, experiments to elucidate the importance of tree diversity for ecosystem functioning have to be planned with a long-term perspective. It is clear that every ecosystem is undergoing long-term changes of both species composition and ecological processes and that those changes can only be studied with long-term experiments and measurements. In addition, there is growing evidence that species with similar effects on ecosystem functioning (functional effect groups) do not respond similarly to changes in the environment. Conversely, species responding similarly to such changes (functional response group) often vary in their effects on ecological processes (Hooper et al., 2002; Symstad et al., 2003). The relationship between biodiversity and ecosystem functioning will thus not stay the same over time and the strength of biotic vs. abiotic control on ecosystem processes might change. We already observe large species effects towards climatic extremes and towards pests during the phase of establishment. The question whether biodiversity can affect stability of ecosystem properties needs to allow for the occurrence of extreme weather conditions or disturbances, for example. In addition, the importance of underlying mechanisms of the biodiversity – ecosystem functioning relationship will change too, as shown in grassland experiments with a transition of sampling to complementarity effects through time (Pacala and Tilman, 2002). We therefore hope that the BIOTREE project with its long-term perspective will help to shed light on some of these aspects.

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Manuscript 2

Anwuchserfolg von 19 verschiedenen Baumarten bei Erstaufforstungen– Ergebnisse eines Biodiversitätsexperiments

Establishment success of 19 different tree species on afforestations – results of a biodiversity experiment

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Axel Don1), Wolfgang Arenhövel2), Roland Jacob3), Michael Scherer-Lorenzen1)4), Ernst-Detlef Schulze1)

1) Max Planck Institute for Biogeochemistry, Hans-Knöll Strasse 10, 07745 Jena, Germany
2) Thuringian State Institute for Forestry, Game and Fishery (Thüringer Landesanstalt für Wald, Jagd und Fischerei), Jägerstr. 1, 99867 Gotha, Germany
3) Bundesforst Hauptstelle Thüringer Wald, Am See 25, 36433 Bad Salzungen, Germany
4) current address: Institute of Plant Sciences, ETH Zürich, Universitätsstr. 2, 8092 Zürich, Switzerland

Zusammenfassung

Erstaufforstungen von landwirtschaftlichen Flächen sind eine forstliche Herausforderung, deren Erfolg durch eine Reihe von Faktoren bedingt wird. Sowohl abiotische Faktoren wie Sommertrockenheit und Spätfröste als auch biotische Faktoren wie Hasen- und Mäuseschäden spielen neben der Pflanzenqualität und der Behandlung des Pflanzmaterials incl. Pflanzung eine zentrale Rolle für den Anwuchserfolg der Bäume. Die 19 untersuchten Baumarten an drei Erstaufforstungsstandorten in Thüringen waren unterschiedlich empfindlich gegenüber diesen Faktoren. Besonders Eichen, Buchen und Douglasien waren schwer auf den Flächen zu etablieren, während Edellaubhölzer wie Ulme, Linde, Esche und verschiedene Ahornarten gute Anwuchserfolge aufwiesen. Diese

Erkenntnis stimmt überein mit den forstpraktischen Erfahrungen auf mehr als 2000 ha Erstaufforstungsfläche in Thüringen seit 1991.

Ein Faktor, der sehr artspezifisch besonders Eichen, Buchen und Vogelkirschen gefährdet, sind Schermäuse. Die Schäden durch Schermäuse und auch Hasenverbiss waren jedoch in den hochdiversen Beständen (6 Baumartenmischung) signifikant niedriger als in den Beständen mit geringerer Baumartenvielfalt. Diese Studie zeigt die Möglichkeit der Risikominimierung von Erstaufforstungen durch Pflanzung artenreicher Bestände auf.

Summary

Afforestations of arable land are a challenge for forestry. Their success may be jeopardised by abiotic factors like summer drought, spring frost or biotic factors like damages by rabbit and vole. More over the treatment of the seedling and their quality are major factors that determine the establishment success of the trees. 19 different tree species on 70 ha afforestations in Thuringia/Germany had been investigated during the first 3 years after planting as part the of BIOTREE biodiversity experiment. The tree species showed significant differences in their sensitivity towards these factors. Especially oak (*Quercus petrea*), beech (*Fagus sylvatica*) and Douglas fir (*Pseudotsuga menziesii*) were difficult to establish on the former grassland. Broadleaves of high value like elm (*Ulmus glabra*), lime (*Tilia cordata*), ash (*Fraxinus excelsior*), mountain ash (*Sorbus aucuparia*) and three maple species (*Acer campestre, Acer platanoides, Acer pseudoplatanus*) had high establishment successes. Our results are in line with experience on afforestations of 2000 ha afforested land in Thuringia since 1991. The damages by voles were particular tree species dependant. However, the degree of damage depended on the species number in a 1 ha plot. Plots with high biodiversity level (6 tree species) showed significant less biotic damages than plots with low biodiversity. The causes of this effect may be the different endangerment of the tree species towards vole damage. Almost no vole damage could be found for coniferous trees and lime. Our study showed that the planting of species rich afforestations, including less abandoned broadleaves, may increase the establishment success of the trees and minimizes the risk of biotic damages by voles and rabbits.

Schlagwörter – keywords: Erstaufforstung, Anwuchserfolg, Baumarten, biotische Schädlinge, Wühlmäuse, Biodiversität. *Afforestation, establishment success, tree species, biotic damages, voles, biodiversity.*

1. Einleitung

Erstaufforstungen stellen eine wichtige Maßnahme zur Verbesserung der Landschaftsstruktur in waldarmen Regionen und zur Nutzbarmachung von Brachflächen dar. Sie werden seit 1992 von der EU im Rahmen der EU-Agrarreform (GAP) zur Verringerung der landwirtschaftlichen Überproduktion gefördert (VO EG 1257/99) und cofinanziert aus Mitteln des Bundes und der Länder (Dohrenbusch, 1996). In Deutschland werden jährlich etwa 5000 ha erstaufgeforstet (BMU, 2002). Durch das Kyotoprotokoll zum Klimaschutz von 1997 bekommen Aufforstungen außerdem die Funktion von mittelfristigen zusätzlichen Kohlenstoffspeichern. Treibhauswirksames CO_2 soll in der Biomasse und als Bodenkohlenstoff gespeichert werden. Der Aufbau von artenreichen Waldbeständen gehört zu den Zielen der modernen Forstwirtschaft. Auf internationaler Ebene fordert dies u.a. die Biodiversitäts-Konvention 1992 von Rio de Janeiro. Erstaufforstungen bieten die Chance, Wälder mit entsprechender Baumartenvielfalt zu begründen.

Die Erstaufforstung vormals landwirtschaftlich genutzter Flächen ist oft nicht ohne Schwierigkeiten und erfordert den Einsatz großer Mengen Pflanzenmaterials sowie aufwendiger Vor- und Pflegearbeiten (Wersenger et al., 2004). Besonders auf extremen Standorten wie landwirtschaftlichen Grenzertragsflächen ist bei der Pflanzung von Setzlingen mit hohen Ausfällen zu rechnen (MLUM Brandenburg, 2000) – allerdings verfügen selbst vergleichsweise schlechte landwirtschaftliche Standorte über meist erheblich bessere Nährstoffvorräte als Waldstandorte (Dohrenbusch, 1996). Der Pflanzschock, Forstschädlinge und die Konkurrenz der unverholzten Vegetation sind nur einige Gründe, die zu diesen Ausfällen führen können (Jacobs et al., 2004). Der Anwuchserfolg der ersten Jahre entscheidet maßgeblich über den Gesamterfolg einer Aufforstungsmaßnahme. Mit Ausnahme von Sturmwurfschäden nimmt Resistenz der Waldneuanlagen gegenüber abiotischen und biotischen Stressoren mit zunehmendem Alter zu.

Die heimischen Baumarten unterscheiden sich signifikant in ihrer Eignung für Erstaufforstungen (Leder, 1996), doch bisher liegen wenig vergleichende Studien zum Anwuchserfolg verschiedener Arten vor. Dies liegt zum einen in der Beschränkung vieler Aufforstungen der Vergangenheit auf wenige Baumarten, zum anderen sind kausale Rückschlüsse auf Arteigenschaften nur bei Aufforstung am gleichen Standort möglich. Beim Vergleich unterschiedlicher Aufforstungsstandorte sind immer die unterschiedlichen

Standortverhältnisse wie Bodeneigenschaften und Klima als zusätzliche Faktoren zu berücksichtigen. Im Rahmen des BIOTREE-Experiments zu Biodiversität und ökosystemaren Funktionen in experimentellen Waldbeständen (Scherer-Lorenzen et al., 2005) wurde an drei Standorten in Thüringen der Etablierungserfolg von 19 verschiedenen Baumarten untersucht. Der Forschungsansatz des BIOTREE-Experiments erfordert die Einbeziehung einer hohen Anzahl von Baumarten. Insofern wurden auch Baumarten gepflanzt, die für Erstaufforstungen auf großer Fläche eher kritisch zu beurteilen sind (z. B. Douglasie, Buche, Weißtanne) oder die die Natur häufig kostenfrei beisteuert (Aspe, Birke).

In den ersten zwei Jahren nach Pflanzung der Bäume wurde der Anwuchserfolg von etwa 200.000 Setzlingen kontrolliert, deren Vitalität bestimmt und mögliche Ausfallursachen analysiert. In dem Experiment sind Versuchsparzellen mit je ein bis 10 Baumarten angelegt. Ein Paradigma der Biodiversitätsforschung ist, dass artenreichere Bestände stabiler sind als artenarme. Als Stabilität wird hierbei die Resilienz und Resistenz gegenüber Störungen bezeichnet. Auch bei jungen Aufforstungen sollten demnach einige Schadfaktoren, wie Schermaus- und Hasenfraß weniger Schäden in artenreichen Beständen anrichten als in Reinbeständen. Ziele der Studie waren:

i) Vergleich von 19 verschiedenen Baumarten hinsichtlich ihrer Eignung für Erstaufforstungen.
ii) Untersuchung der räumlichen Variabilität des Anwuchserfolgs und dessen Ursachen.
iii) Untersuchung des Einflusses der Baumartenvielfalt auf das Schadensmaß durch Schermäuse und Hasen.

In vielen Ländern ist die staatliche Förderung von Erstaufforstungen nicht nur an den Etablierungserfolg gekoppelt, sondern in ihrer Höhe auch abhängig von der angepflanzten Baumartenmischung bzw. vom Erstaufforstungstyp. Um nicht nur ökonomischen sondern auch ökologischen und sozial-kulturellen Zielen des Waldbaus gerecht zu werden, wird die Förderung von artenreichen Waldbeständen in Zukunft weiter forciert werden müssen.

Für die ungleich schwierigere Pflanzung von artenreichen Mischwaldbeständen im Vergleich zu bspw. Nadelreinbeständen sind umfangreiche Kenntnisse über die Eignung verschiedener Baumarten für die Erstaufforstung nötig. Im Folgenden sollen daher der Anwuchserfolg von 19 standortgerechten Baumarten auf Erstaufforstungsflächen gezeigt und soweit als möglich Ursachen für den Ausfall der Pflanzen analysiert werden.

2. Material und Methoden

Die Untersuchungen fanden im Rahmen des BIOTREE-Experiments (Scherer-Lorenzen et al., akzeptiert) an drei Standorten auf insgesamt 70 ha statt. Die Standorte liegen in einem Umkreis von 60 km und wurden zwischen Frühjahr 2003 und Frühjahr 2004 mit Pflanzmaterial aus derselben Baumschule aufgeforstet. Die Pflanzenqualität war aber an den drei Standorten teilweise unterschiedlich. Die Pflanzung erfolgte manuell mit Spaten nach streifenweiser maschineller Tiefspatenfräsung.

Die Standorte unterscheiden sich in ihrem Ausgangssubstrat. Da nur standortgerechte Arten gepflanzt werden sollten, variiert die Artenzusammensetzung der drei Standorte (Tab. 1). Der Standort „Mehrstedt" bei Schlotheim am Nordrand des Thüringer Beckens ist mit 40 ha der Größte der drei Standorte. Die Fläche ist seit Anfang der 1980er Jahre als Grasland genutzt, bis 1991 als Teil eines russischen Truppenübungsplatzes. Das Ausgangssubstrat besteht aus Ton-Kalkstein-Schichten des Oberen Muschelkalks mit Lößbeimengungen, auf dem sich Terra fusca-Pseudogleye entwickelt haben, die teilweise kolluvial verlagert wurden. Hier wurden 10 verschiedene Baumarten in 40 Versuchsparzellen à 1 ha gepflanzt (Tab. 1).

Dabei wurde ein Baumartenzahlgradient angelegt von Flächen mit 1, 2, 4 und 6 Hauptbaumarten. Die Baumarten wurden mit unterschiedlichem Pflanzverband (Tab. 1) in Trupps von 8x8 m gepflanzt. Auf 1/3 der Flächen sind zusätzlich 4 Nebenbaumarten in Trupps eingemischt. 4 ha der Fläche wurden im Frühjahr 2003 etabliert und mit einer ersten Bauminventur im Juli/August 2003 kartiert. Der Hauptteil der Fläche (36 ha) wurde zwischen November 2003 und Mai 2004 gepflanzt und im August 2004 kartiert. Nach Nachbesserungen im Herbst 2004 und Frühjahr 2005 erfolgte im Dezember 2005 eine weitere Inventur der Pflanzung. Die Pflanzung erfolgte im Frühjahr 2003 durch Fachpersonal des Forstamtes, die anderen Pflanzungen aber größtenteils durch angelernte Hilfsarbeiter der privaten Baumschule. Der Standort wird betreut vom Forstamt Sondershausen (ehemals Ebeleben) der Thüringer Landesforstverwaltung. Die Frühjahrsaufforstung 2004 und die Nachbesserung im Frühjahr 2005 konnten aus organisatorischen Gründen jeweils erst sehr spät (eigentlich zu spät) durchgeführt werden und wurden erst in der ersten Maiwoche abgeschlossen.

Tabelle 1: Anzahl der gepflanzten Baumarten auf den drei Standorten sowie Pflanzdichte und Pflanzverband für die Standorte Kaltenborn und Mehrstedt. Die Pflanzdichte in Bechstedt war für alle Baumarten einheitlich 1 x 2 m mit 3468 Stk. ha^{-1}.

Number of planted seedlings at the three sites and planting density for site Mehrstedt and Kaltenborn. At the site Bechstedt the planting density for all species was 1 x 2 m with 3468 plants ha^{-1}.

Standorte		*Mehrstedt*		*Kaltenborn*		*Mehrst. & Kaltb.*		*Bechstedt*	
		Anzahl	**Sortiment**	**Anzahl**	**Sortiment**	**Pflanzverband**	**Pflanzzahl ha^{-1}**	**Anzahl**	**Sortiment**
Abies alba	WTA	-	-	512	2/2	2 x 2	2400	-	-
Acer campestre	FAH	-	-	-	-	-	-	1200	1/2
Acer platanoides	SAH	2400	1/1*	-	-	-	-	2080	1/2
Acer pseudoplatanus	BAH	20080	1/1	640	1/1	1,5 x 2	3000	1200	1/2
Betula pendula	GBI	-	-	-	-	-		1860	1/1
Carpinus betulus	HBU	2400	2/0	-	-	1,5 x 2	3000	1860	1/2
Fagus sylvatica	BU	28224	2/0	9100	2/0	1 x 2	4200	760	1/2
Fraxinus excelsior	ES	2400	1/1	640	1/2	1,5 x 2	3000	1640	1/2
Larix decidua	ELA	15952	1/1	-	-	2 x 2	2400	980	1/1
Picea abies	FI	-	-	5296	2/2	2 x 2	2400	-	-
Pinus sylvestris	KI	-	-	-	-	-	-	1200	1/1
Populus tremula	AS	-	-	-	-	-	-	1200	1/1
Prunus avium	VKI	1920	1/1	-	-	2 x 2	2400	1200	1/1
Pseudotsuga menziesii	DGL	15968	2/0	5296	2/0	2 x 2	2400	-	-
Quercus petraea	TEI	28168	2/0	9100	2/0	1 x 2	4200	1640	1/1
Sorbus aucuparia	EB	-	-	512	1/1	2 x 2	2400	1860	1/1
Sorbus torminalis	ELS	-	-	-	-	-	-	1640	1/2
Tilia cordata	WLI	20100	2/0	-	-	1,5 x 2	3000	1420	2/0
Ulmus glabra	BUL	-	-	-	-	-	-	1860	1/1
Baumartenzahl		*10*		*8*				*16*	

*) 1/1 zweijährig, 1 Jahr Saatbeet, 1 Jahr Pflanzbeet. *Two years old, 1 year nursery, 1 year nursery bed.*

2/0 zweijährig, 2 Jahre Saatbeet. *Two years old, 2 years nursery.*

1/2 dreijährig, 2 Jahre Saatbeet, 1 Jahr Pflanzbeet. *Three years old, 1 year nursery, 2 years nursery bed.*

2/2 vierjährig, 2 Jahre Saatbeet, 2 Jahre Pflanzbeet. *Four years old, 2 years nursery, 2 years nursery bed.*

Der Standort „Bechstedt“ liegt 15 km südlich von Erfurt. 9 ha vormals als Acker genutzte Fläche wurden dort im Frühjahr 2003 mit 16 verschiedenen Baumarten aufgeforstet (Tab. 1). Das geologische Ausgangssubstrat ist vergleichbar mit dem am Standort Mehrstedt. Es fehlen nennenswerte Lößbeimengungen. Als Hauptbodentyp hat sich ein Terra fusca-Pseudogley entwickelt. Vor der Pflanzung erfolgte auch hier ein Tiefumbruch der Fläche. Nachgebessert wurde im Frühjahr 2005. Bauminventuren wurden im Juni-August 2003 und im September 2005 durchgeführt. Zusätzlich erfolgte eine Ausfallanalyse durch das Bundesforstamt im September 2004.

Der Standort „Kaltenborn“ liegt am Osthang des Werratals bei Bad Salzungen. 8 verschiedene Baumarten wurden im Frühjahr 2004 in Reihen gepflanzt (Tab. 1). Die Fläche wurde vorher als extensives Grünland genutzt. Das Ausgangssubstrat ist Unterer Buntsandstein, auf dem sich Braunerden entwickelt haben. Die 23 ha große Fläche wurde im Frühjahr 2005 nachgebessert, Bauminventuren wurden im September 2004 und Februar 2006 durchgeführt. Eine 5 ha große Teilfläche wurde schon im November 2003 mit den gleichen Hauptbaumarten aber von einer anderen Baumschule aufgeforstet. Diese Fläche wurde mit einer Inventur im September 2005 separat kartiert.

Die Pflanzung und Betreuung der Flächen „Bechstedt“ und „Kaltenborn“ fand und findet durch den Bundesforst Hauptstelle Thüringer Wald statt. Nur gelernte Waldarbeiter kamen bei der Pflanzung zum Einsatz.

Durch das Pflanzdesign sind die Positionen der Bäume auf der Fläche festgelegt und lassen sich anhand von Pflanzplänen im Gelände wiederfinden. Bei den Bauminventuren wurden folgende Parameter erfasst:

- Baumhöhe (lotrecht von der höchsten lebenden Knospe mit Zollstock gemessen)
- Vitalität (Ansprache visuell und mit Saftprobe durch anritzen, Unterscheidung in „abgestorben“ und „lebend“)
- Ausfallursache
- Verbissschäden durch Hasen (identifiziert durch Abbissspuren)
- Dürreschäden am Terminaltrieb (ja/nein)
- Schäden durch Schermäuse (*Arvicola terrestris*). Schermausgeschädigte Bäume besitzen keine Wurzel mehr und ließen sich so durch Ziehen an den Pflanzen identifizieren.

Für eine räumliche Analyse wurden die Ergebnisse des Standortes Mehrstedt auf Versuchsparzellenebene (Subplots) ausgewertet. Die prozentualen Anteile p der Schermaus- und Hasenschäden wurden transformiert mit

$$p_{trans} = \sin^{-1}\sqrt{0.01 \cdot p}$$

und eine ANOVA mit den Faktoren Biodiversitätsstufe (Artenzahl) und Baumart für die vier Laub-Hauptbaumarten durchgeführt (S-PLUS 6.1).

3. Ergebnisse und Diskussion

3.1 Anwuchserfolg der Baumarten

Der Anwuchserfolg der Bäume war neben Pflanzenqualität und Behandlung bei der Pflanzung sowie biotischen und abiotischen Schadfaktoren im Wesentlichen abhängig von der Baumart, was vor allem in deren spezifischen Eigenschaften begründet liegt. Trotz großer Unterschiede in den Ausfallraten zwischen den drei Standorten und den drei untersuchten Jahren ergaben sich ähnliche Muster für die Ausfallraten der einzelnen Baumarten (Tab. 2).

Tabelle 2: Anteil der ausgefallenen Bäume [%] auf den drei BIOTREE-Standorten. Pro Baumart n>110. ***Seedling mortality percentage at the three BIOTREE sites. Per tree species n>110.***

	Bechstedt			*Mehrstedt*			*Kaltenborn*		
Baumart	**2003**	**2004 4)**	**2005 1)**	**2003 5)**	**2004 6)**	**2005 1)**	**2004**	**2005 3)**	**2006 1)**
AS	34	58	22						
BAH	8	25	1	10	26	21	9		17
BU	71	79	20	65	33	25	24	4	15
BUL	3	8	1						
DGL					37	18	68	4	11
EB	6	19	3				15		20
ELA	17 2)	15	1		33	15			
ELS	41		5						
ES	9	12	0	2	56	15	10		7
FAH	2	8	0						
FI							16	2	3
GBI	2	13	8						
HBU	6 2)	32	3	18	51	22			
KI	93	100	34						
SAH	8	7	2	12	37	23			
TEI	30	61	22	53	39	43	27	19	20
VKI	41	58	33	56	32	33			
WLI	2	7	0	6	40	14			
WTA							45		47
Mittel	19.2	28.0	8.3	39.7	37.6	23.1	30.8	7.6	13.9

1) Fläche wurde zwischenzeitlich nachgebessert (siehe Text) Auffallrate umfasst alle gepflanzten Bäume zu diesem Zeitpunkt. *Replanting was done meanwhile. Tree mortality rates refer to all planted tree at the time of the inventory.*

2) n=66

3) Zusatzexperiment 5 ha mit Pflanzmaterial einer anderen Baumschule. *Additional experiment 5 ha with plants from a different nursery*

4) Inventur der Ausfälle wurde durch Forstamt durchgeführt. *Inventory was carried out by the forestry office.*

5) Im April 2003 aufgeforstete 4 ha. *In April 2003 planted 4 ha.*

6) Im November 2003 bis Mai 2004 aufgeforstete 36 ha. *In November 2003 till Mai 2004 afforested 36 ha.*

An allen drei Standorten waren Traubeneichen mit Ausfallraten von 19-61 % am stärksten betroffen (Tab. 2). Obwohl die Traubeneiche eine Lichtbaumart mit subkontinentalem Verbreitungsareal und schnellem Jugendwachstum ist, traten Schwierigkeiten bei ihrer Etablierung auf. In Kaltenborn waren 2005 75 % der ausgefallen Pflanzen Eichen. Bei 6% der Jungpflanzen konnte 2005 in Mehrstedt Verbiss durch Schermäuse als Todesursache festgestellt werden (Abb. 1). Die Verdämmung der kleinen Eichenpflanzen durch die Konkurrenzvegetation ist eine weitere wesentliche Ursache für die anhaltend hohen Ausfallraten. Die Eichen litten auch unter dem Trockenjahr 2003. Ohne ihre Fähigkeit zur Regeneration durch Stockausschläge wären die Ausfälle aber noch höher ausgefallen. Die Rotbuche ist als Schattbaumart schwer auf Freiflächen zu etablieren (Röhrig und Bartsch, 1992). Jungpflanzen sind besonders durch Spätfrost gefährdet, der auf den Aufforstungsflächen teilweise zu erheblichen Schäden an den Terminaltrieben führte und Ausfälle von bis zu 79 % aller Buchensetzlinge an einem Standort mit verursachte. Ein Viertel aller Rotbuchen in Bechstedt wiesen 2 Jahre nach der Pflanzung abgetrocknete Terminaltriebe auf. Keine andere Baumart hatte so hohe Schäden an den Terminaltrieben. Dies führte zu einer Verringerung der Höhe um durchschnittlich 4 cm im Vergleich zur Höhe bei der Pflanzung (Höhe der höchsten lebenden Terminalknospe, Abb. 2). Vergleichsweise geringe Ausfälle (24 %) hatte die Buche auf dem etwas feuchteren Standort Kaltenborn, der mehr dem atlantischen Klimatyp entspricht, der das Rotbuchenoptimum darstellt (Tab. 2).

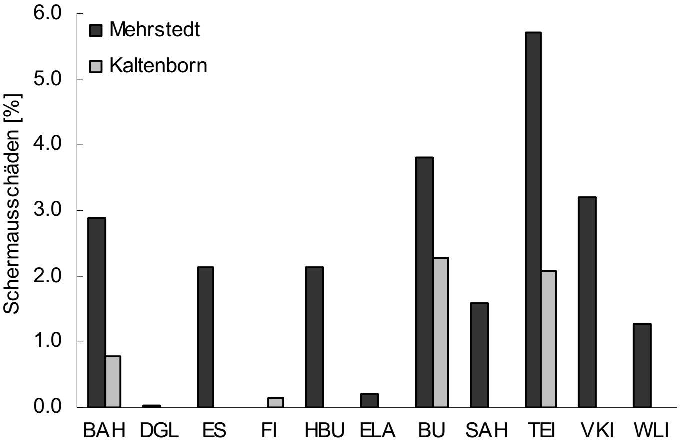

Abbildung 1: Anteil der Schermausschäden der verschiedenen Baumarten in Mehrstedt (2005) und Kaltenborn (2006). Bei Eberesche und Weißtanne wurden in Kaltenborn keine Schermausschäden festgestellt.

Proportion of vole damages of different tree species at the sites Mehrstedt (2005) and Kaltenborn (2006). For mountain ash and white fir no vole damages were detected at the Kaltenborn site.

Nicht nur spätsukzessionale Arten, sondern auch Pionierbaumarten wie die Aspe zeigten Etablierungsschwierigkeiten auf den Freiflächen. Die Aspe hatte 2003 nach der Anpflanzung 34% und auch nach einer Nachbesserung im Frühjahr 2005 mit 22% immer noch überdurchschnittlich hohe Ausfälle auf der Versuchsfläche Bechstedt. Aspen sind auf eine gute Wasserversorgung angewiesen (Burschel und Huss, 1997; Röhrig und Bartsch, 1992) und waren deshalb von der Trockenheit im Jahr 2003 besonders betroffen. Die vitalen Pflanzen hatten aber Zuwächse von über 40 cm in zwei Vegetationsperioden (Abb. 2).

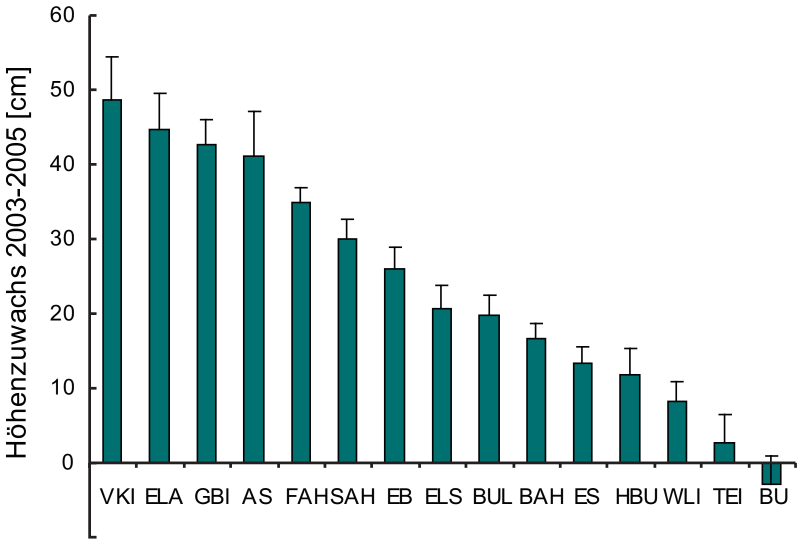

Abbildung 2: Höhenzuwachs zwei Vegetationsperioden nach Pflanzung der Bäume für 15 Baumarten am Standort Bechstedt. Mittelwert + Standardfehler.

Two years of height growth of seedlings of 15 different tree species after planting at the site Bechstedt. Mean + Standarderror.

Sehr gute Anwuchsergebnisse konnten bei den Edellaubhölzern Ulme, Bergahorn, Spitzahorn und Esche sowie Feldahorn und Eberesche erzielt werden. Deren Ausfälle lagen meistens unter 10% mit durchschnittlichen Zuwächsen von 14-35 cm in zwei Vegetationsperioden nach der Pflanzung. Auch Winterlindenbestände ließen sich problemlos auf den Freiflächen anlegen, obwohl sie in den ersten zwei Jahren nur geringe Zuwächse von 8 cm hatten. Die Vogelkirsche hatte überdurchschnittlich hohe Ausfälle. Dieser Befund überrascht, da die Vogelkirsche an sich für Erstaufforstungen geeignet ist (Lindner, 1992). Vogelkirschen benötigen eine gute Nährstoffversorgung, die an den BIOTREE-Standorten gegeben ist, können aber nur bedingt Trockenstress ertragen und sind besonders gefährdet durch Wildverbiss und Mäuse (Röhrig und Bartsch, 1992; Ammer und Preen, 1997).

An 10 % der lebenden Vogelkirschen konnte Hasenfraß festgestellt werden, was im Vergleich zu den anderen Baumarten ein hoher Anteil ist. Auch die Ausfälle durch Schermausschäden waren bei Vogelkirsche überdurchschnittlich hoch. Dennoch zeigte die Vogelkirsche bei erfolgreichem Anwuchs starkes Höhenwachstum mit durchschnittlich 48 cm Zuwachs in den ersten zwei Jahren nach Pflanzung (Abb. 2). Neben Eiche und Rotbuche ist die Vogelkirsche die durch Wildverbiss und Mäuseschäden am stärksten gefährdete Baumart.

Insgesamt hatten Nadelbaumarten keine besseren Anwuchsergebnisse als Laubbaumarten. Die Douglasie hatte mit 37 bzw. 68 % hohe Ausfälle im ersten Jahr nach der Pflanzung. Douglasien gehören bei der Pflanzung zu den empfindlichsten Baumarten (Wenk, 1990); ganz entscheidend für hohe Anwuchsprozente ist die Frische der Pflanzen. Schwer tun sie sich auch mit der Graskonkurrenz (Burschel und Huss, 1997). Die Kiefer als weitere Nadelbaumart hatte einen Totalausfall in Bechstedt. Dies lässt sich teilweise mit der Qualität des Pflanzgutes erklären. Die Kiefernsämlinge mit durchschnittlich 16 cm Höhe waren nicht vital genug, um die Sommertrockenheit 2003 zu überleben. Fichte und Lärche waren mit überdurchschnittlich hohem Anwuchserfolg von bis zu 85% im ersten Jahr nach der Pflanzung relativ einfach etablierbar. Dies lag auch an deren geringeren Anfälligkeit gegenüber Schermausschäden, die bisher an fast keinem der Nadelbäume nachgewiesen werden konnte (Abb. 1).

3.2 Einflussfaktoren für den Etablierungserfolg

Erstaufforstungen stellen in vieler Hinsicht eine forstliche Herausforderung dar (Wersenger et al., 2004). Um bestimmte Waldgesellschaften etablieren zu können, sind verschiedene vorbereitende und begleitende Maßnahmen ratsam bzw. nötig. Dazu gehört die Flächenvorbereitung mit Tiefenlockerung der Pflanzreihen. Die Begleitvegetation ist eine Wasser-, Licht- und Nährstoffkonkurrenz für die Bäume (Cogliastro et al., 1993). Sie wurde zumindest in den ersten Jahren nach der Pflanzung durch die Tiefenlockerung zurückgedrängt. Der Nachteil der reihenweisen Bodenbearbeitung ist, dass der lockere Boden auch die Lebensbedingungen für Schermäuse verbessert und ihnen den Zugang zu den Forstpflanzen deutlich erleichtert wenn nicht gar vorgibt. Auch verschlechtert die Bodenbearbeitung durch Unterbrechung des Kapillarwassers die Wasserversorgung der jungen Bäume. Die Begleitvegetation wurde zumindest zwischen den Pflanzreihen (in

Bechstedt und Kaltenborn auch um die gepflanzten Bäume herum) gemäht, um die Wasserkonkurrenz zu verringern und die Lebensraumbedingungen für Mäuse zu verschlechtern. In Mehrstedt wurde nur zwischen den Reihen gemäht, was zu teilweise erheblicher Verdämmung der Pflanzen führte. Trotz der getroffenen Kulturschutzmaßnahmen auf allen drei Flächen gab es große Unterschiede im Anwuchserfolg. Vier Faktorengruppen sollen im Folgenden beleuchtet werden, die wesentlichen Einfluss auf den Anwuchserfolg der Bäume hatten und teilweise sehr artspezifisch wirksam waren.

3.2.1 Schermaus- und Hasenschäden

Auf verkrauteten und vergrasten Standorten wie Erstaufforstungsflächen finden sich günstige Lebens-bedingungen für Mäuse (Ammer und Preen, 1997, Pusenius und Ostfeld 2002). Besonders Schermäuse sind eine erhebliche Gefahr für Erstaufforstungen speziell von Laubhölzern (Thiel, 2003). Ab 2003 kam es zu einer Gradation der Schermauspopulationen in Thüringen, die sich auch auf die untersuchten Standorte niedergeschlagen hat. 2003 wurden an allen drei Standorten kaum Schäden durch Schermäuse beobachtet. Erst ab 2004 kam es trotz Bekämpfungsmaßnahmen zu einer Ausbreitung der Population Eine chemische und mechanische Bekämpfung der Schermäuse erfolgte 2004 und 2005 auf den Flächen Kaltenborn und Mehrstedt. Am erfolgreichsten war diese in Kaltenborn, wo durch das Einbringen von Schermausriegeln mit einem Schermauspflug in den Boden, die Gesamtschäden durch Schermäuse auf unter 1 % der Pflanzen gedrückt werden konnten. Insgesamt sind das Aufsuchen der Schermausgänge und die Bekämpfung sehr aufwendig, da die Tiere unterirdisch ein weit verzweigtes Gangsystem anlegen.

Bis zu 20% der Ausfälle einer Baumart konnten auf Schermausschäden zurückgeführt werden, wobei das Ausmaß der Schäden für die verschiedenen Baumarten unterschiedlich stark ausfiel (Abb. 1). Die größten Schäden durch Schermäuse wurden sowohl in Mehrstedt als auch in Kaltenborn bei Traubeneichen festgestellt. Offensichtlich sind Eichen gefährdeter durch Wildverbiss und Mäuseschäden als andere Baumarten (Wenk, 1990; Thiel, 2003). In kanadischen Studien konnte ein Zusammenhang zwischen der Konzentrationen bestimmter Monoterpene und Phenole in verschiedenen Nadelbaumarten und dem Schadausmaß durch Schermäuse nachgewiesen werden (Bucyanayandi et al., 1990; Roy und Bergeron, 1990). Bei hohen Populationsdichten der Mäuse ließ dieser Einfluss jedoch nach.

Durch die truppweise Pflanzung der Baumarten stehen kleinflächig gemischt Arten mit hoher Anfälligkeit gegenüber Verbiss- und Schermausschäden neben weniger anfälligen, „unschmackhaften“ Baumarten. Sowohl die Schermaus- als auch die Hasenschäden waren nicht zufällig über alle Versuchsparzellen verteilt, sondern signifikant abhängig von der Baumartenzahl in der Versuchsparzelle. Die Flächen mit einer Mischung aus 6 Baumarten hatten geringere Schäden pro Baumart als die Flächen mit geringerer Baumartenzahl (Abb. 3). Dieser Befund ist umso erstaunlicher, wenn man die vielen Einflussfaktoren bedenkt, die den Anwuchserfolg der Bäume bestimmen. Die Einmischung von gegenüber Schermausschäden weniger anfälligen Baumarten, wie bspw. Winterlinde, kann folglich die Ausbreitung der Schermauspopulation eindämmen. Durch die Pflanzung von artenreichen Beständen kann deshalb durch Risikostreuung einem Totalausfall vorgebeugt werden und zusätzlich der Verlust durch Verbissschäden reduziert werden.

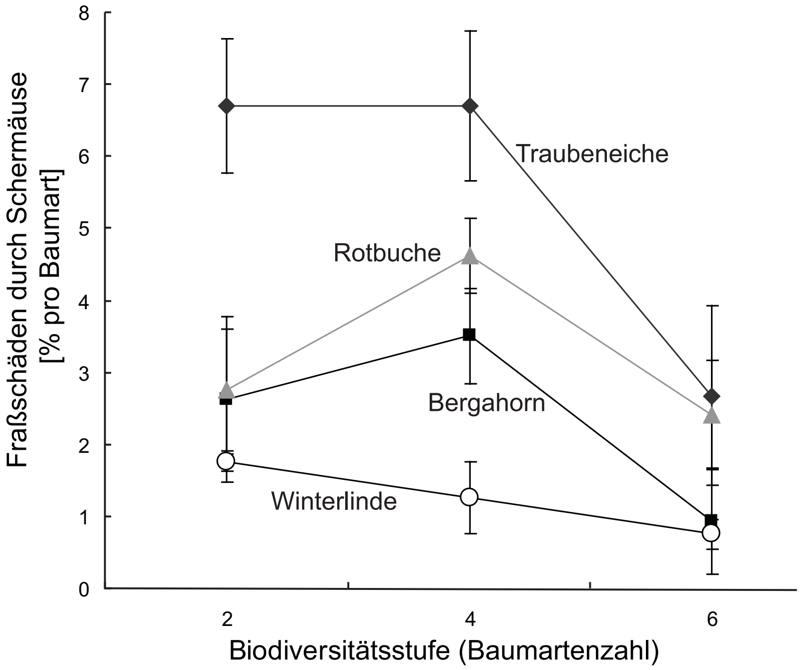

Abbildung 3: Anteil der Schermausschäden der vier Laub-Hauptbaumarten [%] pro Teilfläche der BIOTREE-Fläche Mehrstedt im Dezember 2005 in Abhängigkeit von der Artenzahl der jeweiligen Versuchsfläche. Die Reinbestände wurden wegen fehlender Wiederholungen nicht in die Untersuchung eingeschlossen.

Percentage of vole damages on the four deciduous major tree species per subplot at the BIOTREE site Mehrstedt in December 2005 vs. biodiversity level (number of tree species) of the plot. Single tree species stands were not included due to lacking repetitions.

In Kaltenborn wurden nur in den Beständen mit hohem Laubbaumanteil nennenswerte Schäden nachgewiesen. Der Schermausfraß trat oft lokal gehäuft auf, nicht selten einer Pflanzreihe folgend. Die Schermausschäden wurden also durch die linienhafte auflockernde Bodenbearbeitung begünstigt.

Alle Flächen wurden gezäunt und waren rehwildfrei. In Mehrstedt kam es aber zu Hasenschäden, da die 40 ha große Fläche nicht vollständig hasenfrei zu halten war. Bei 0,3% der gepflanzten Bäume konnte Hasenverbiss als Todesursache festgestellt werden, verbissen waren aber 10-mal mehr Bäume. Besonders Rotbuche, Spitzahorn und Vogelkirsche waren von Hasenverbiss betroffen. Auch der Anteil von Hasenverbiss an den einzelnen Baumarten war in den Flächen mit höherer Baumartenzahl niedriger als in den artenärmeren Mischungen.

3.2.2 Sommertrockenheit und Frost

Die drei Standorte sind für Erstaufforstungen typisch und stellen unterschiedliche Extremsituationen für die jungen Bäume dar. Die meisten Aufforstungsstandorte in Deutschland sind Grenzertragsflächen mit Bodenzahlen von durchschnittlich 38, auf denen bei der Etablierung von gemischten Waldbeständen mit standortbedingten Problemen zu rechnen ist (Ammer und Preen, 1997). Auf den Flächen Bechstedt und Mehrstedt stehen Schichten des Oberen Muschelkalks an. Die Kalkverwitterungsböden neigen mit ihrem geringen Wasserhaltevermögen zu starker Austrocknung in den Sommermonaten, während im Frühjahr stellenweise Staunässe auftritt. Die exponierte Lage der Standorte mit permanent hoher Windbelastung, begünstigt die Austrocknung der Flächen zusätzlich. Außerdem war an allen drei Standorten mit Spätfrost zu rechnen.

Das Jahr 2003 war bekanntermaßen besonders trocken und warm (Ciais et al., 2005). In den Monaten Juli bis August fiel kaum Niederschlag. Die Niederschlagssumme der 30 km entfernt gelegenen meteorologischen Station Gebesee lag 2003 mit 388 mm um 25% niedriger als im darauf folgenden Jahr und deutlich unter dem langjährigen Jahresmittel. Dies führte zu besonders hohen Ausfällen bei den im Frühjahr 2003 erfolgten Pflanzungen. Die Ausfälle von Eiche, Buche und Vogelkirsche in Mehrstedt waren 2003 um 38 bis 98% höher als im darauf folgenden Jahr mit als durchschnittlich zu bezeichnender Witterung (Tab. 2). Selbst die als anspruchslos gelten Pionierbaumarten hatten unter den Bedingungen von

2003 hohe Ausfälle. Insgesamt waren durch Trockenheit besonders die Baumarten betroffen, die ohnehin schon schwer auf Freiflächen zu etablieren sind.

Die erhobenen Daten lassen keine Quantifizierung der Schäden durch Spätfrost zu. An den Standorten Bechstedt und Kaltenborn sind durch deren Lage im Kaltluftstrom angrenzender Höhenzüge Spätfrostschäden als Ausfallursache allerdings sehr wahrscheinlich.

3.2.3 Behandlung vor der Pflanzung und Pflanzmaterial

Die Pflanzenqualität und die Behandlung des Pflanzmaterials von der Entnahme im Saat- oder Verschulbeet bis zur Verwendung am Pflanzort nimmt eine zentrale Stellung für den Anwuchserfolg ein (Wersenger et al., 2004). Gleichermaßen wichtig sind der Einsatz von geschultem Personal und die gute Aufsicht der Arbeiten durch den Revierleiter. Die höheren Ausfälle von Eschen, Hainbuchen und Winterlinden bei den 2004 gepflanzten Bäumen im Vergleich zu den 2003 gepflanzten auf der Fläche Mehrstedt verdeutlichen dies: Während die Pflanzung 2003 von Fachkräften des Forstamtes durchgeführt wurde, waren 2004 angelernte und Aushilfskräfte der Baumschule mit der Pflanzung betraut. Die bei der wesentlich umfangreicheren Pflanzung 2004 erforderliche längere Zwischenlagerung der Pflanzen im Pflanzeinschlag, ein höheres Austrocknungsrisiko durch längere Wege auf der 36 ha großen Fläche, die 2004 gepflanzt wurde im Vergleich zu der 4 ha großen Fläche von 2003, der späte Pflanztermin sowie teilweise unsachgemäßes Pflanzen der Bäume erklären die teilweise erheblich höheren Ausfälle der Pflanzung von 2004 auf der Fläche Mehrstedt (Tab. 2).

Baumarten wie Douglasie bedürfen bei der Pflanzung besonderer Sorgfalt. Ihre Etablierung ist ungleich schwieriger als bspw. bei Fichte und verlangt ausgesprochene Fachkenntnis und besondere Vorsichtsmaßnahmen gegen die Austrocknung der Wurzeln. Auch die Zwischenlagerung des Pflanzmaterials nach dem Aushub aus dem Verschulbeet in Kühlkammern ist für Douglasien besonders kritisch zu sehen. Auf der Fläche Kaltenborn wurde zusätzlich zu dem Hauptexperiment eine Teilfläche mit Bäumen aus einer anderen lokalen Baumschule im Herbst 2003 aufgeforstet. Die Qualität des Pflanzmaterials aus dieser Baumschule war deutlich besser. Beide Pflanzungen erfolgten durch Fachkräfte aber mit erheblichen Unterschieden im Anwuchserfolg. Die Ausfälle der Douglasie auf der zusätzlichen Teilfläche lagen mit 4% deutlich unter denen der Gesamtfläche mit 68% Ausfall. Auch Buchen und Fichten hatten 80-90% höheren Anwuchserfolg auf der Teilfläche mit dem besseren Pflanzmaterial im Vergleich zu der Hauptfläche.

3.2.4 Bodeneigenschaften

Die Bodeneigenschaften sind bei Erstaufforstungen ein wichtiger Faktor für den Anwuchserfolg (Cogliastro et al., 2005). Der Standort Mehrstedt ist mit 40 ha und über 130.000 Pflanzen groß genug, um den Einfluss kleinräumiger Unterschiede der Bodeneigenschaften auf den Anwuchserfolg untersuchen zu können. Ausfallraten wurden für alle 10 Baumarten für 120 Teilflächen à 0,34 ha getrennt berechnet. Es ergaben sich große räumliche Unterschiede mit Ausfallanteilen aller Baumarten zwischen 1 und 33% (Abb. 4).

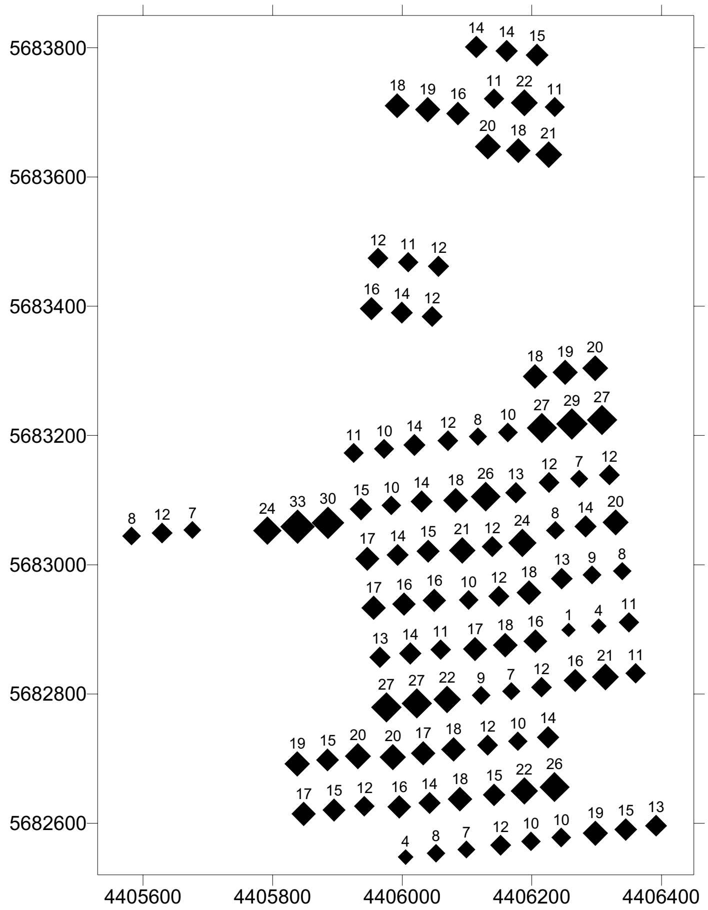

Abbildung 4: Ausfälle [%] zwei Jahre nach Pflanzung und Nachbesserung auf den Teilflächen (je 0,34 ha) der BIOTREE-Versuchsfläche Mehrstedt. Gauß-Krüger Koordinatensystem.
Seedling mortality [%] for each subplot (0.34 ha) of the BIOTREE site Mehrstedt two years after planting. Gauß-Kruger coordinate system.

Diese Unterschiede sind nicht allein mit der Verteilung der Baumarten, die diskontinuierlich über die Fläche erfolgte, zu erklären. Die Muster der Ausfälle, die sich für die verschiedenen Baumarten ergaben, ähnelten sich, und ließen sich neben nicht erfassten Faktoren wie Qualität der Pflanzen und der Pflanzung (s.o.) auf Bodeneigenschaften zurückführen. Für vier der sechs Hauptbaumarten ergaben sich signifikante Korrelationen zwischen der Bodenzahl der Teilflächen und dem Anwuchserfolg bzw. den Ausfallraten. Die Bodenkartierung erfolgte 1951 im Rahmen der Bodenschätzung und gibt die bis heute bestehenden Unterschiede in Bodentextur und Verwitterungszustand wieder.

Es wurden Bodenzahlen zwischen 38 und 78 auf der heutigen Aufforstungsfläche Mehrstedt kartiert. Flächen mit niedrigen Bodenzahlen hatten hoch anstehende Tonschichten, die zu Stauwasser im Frühjahr und rissigem Austrocknen im Sommer neigen. Die schwere Durchwurzelbarkeit und die geringe nutzbare Feldkapazität sind in diesen tonreichen Böden für die jungen Bäume problematisch. Die Quell- und Schrumpfdynamik der Tonböden kann auch zu Schäden an den Feinwurzeln führen. Während für Winterlinde, Douglasie, Bergahorn und Lärche ein signifikanter Zusammenhang zwischen Anwuchserfolg und Bodenqualität nachweisbar war, ergab sich für Rotbuche und Traubeneiche dieser Zusammenhang nicht. Bei letzteren beiden Baumarten, die besonders schwer auf den Freiflächen zu etablieren waren, sind andere Faktoren wie oben diskutiert entscheidender für den Anwuchserfolg als die Bodenqualität.

4. Folgerungen

Neben saisonalen Extremwitterungsbedingungen entscheiden vor allem die Auswahl der Baumarten und die Qualität des Pflanzgutes und der Pflanzung über den Erfolg der Aufforstungsmaßnahme. Sehr gute Anwuchserfolge vieler Edellaubhölzer bieten die Chance, diese Baumarten bei Aufforstungen stärker zu berücksichtigen. Die unterschiedliche Reaktion der verschiedenen Baumarten auf Belastungen, die im Zusammenhang mit Erstaufforstungen auftreten, verdeutlicht den Wert von artenreichen Pflanzungen zur Risikostreuung und Erhöhung der Erfolgschancen von Erstaufforstungen. Zusätzlich können Verbissschäden durch Wühlmäuse und Hasen durch die Pflanzung artenreicher Bestände im Vergleich zu artenarmen Beständen verringert werden.

Insgesamt konnten maximal 20 % der Schäden einer Baumart auf Wurzelfraßschäden durch Wühlmäuse und Verbiss durch Hasen zurückgeführt werden. Für die verbleibenden 80% und damit die übergroße Mehrheit der Ausfälle spielen die Pflanzenqualität, die Behandlung der Pflanzen nach Verlassen der Verschulbeete sowie die Pflanzung an sich eine wesentliche Rolle. Ohne fachkundige Aufsicht und Durchführung der Arbeiten bei der Anlage von Erstaufforstungen ist deren Erfolg ungewiss.

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Conversion of cropland into grassland – implications for soil organic carbon stocks in two soils with different texture

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Axel Don1), Thomas Scholten2), Ernst-Detlef Schulze1)

1) Max Planck Institute for Biogeochemisty, Hans-Knöll Strasse 10, 07745 Jena, Germany

2) University of Tübingen, Institute of Geography, Rümelinstraße 19-23, 72070 Tübingen, Germany

Abstract

Soil organic carbon stocks are expected to increase after conversion of cropland into grassland. Two adjacent cropland and grassland sites – one with a Vertisol with 23 years after conversion and one with an Arenosol 29 years after conversion - were sampled down to 60 cm depth. Concentrations of soil organic carbon (SOC) and total nitrogen (N_{tot}) were measured before and after density fractionation in two light fractions and a heavy fraction with mineral associated C. For the soil profiles SOC stocks and radiocarbon (^{14}C) concentrations of the mineral associated C were determined.

C stocks and mineral associated SOC concentrations were increased in the upper 10 cm of the grassland soil compared to the cropland. This corresponded to the root biomass distribution, with 59% and 86% of the total root biomass in 0-5 cm soil depth of the grasslands. However, at the Arenosol site, in 10-20 cm depth, C in the mineral associated fraction was lost 29 years after the conversion into grassland. Over all, SOC stocks were not significantly different between grassland and cropland at both sites when the whole profile was taken into account. At the Arenosol site the impact of land use conversion on SOC accumulation was limited by low total clay surface area available for C stabilisation. Subsoil C (30-50 cm) at cropland of the Vertisol site comprised 32% of the total SOC stocks with high ^{14}C concentrations below the ploughing horizon. We concluded that fresh C was effectively translocated into the subsoil. Thus, subsoil C has to be taken into account when land use change effects on SOC are assessed.

Keywords: Land use change, soil organic carbon stabilisation, ^{14}C age, subsoil, clay mineralogy.

1. Introduction

Soil organic carbon (SOC) stocks are mainly controlled by: i) land use and management history, ii) geological bedrock and the pedogenetic history, and iii) climate conditions (temperature and humidity). For example, the conversion of native grassland to cropland in Midwestern USA caused a rapid decline of SOC stocks by 30-50% which was assumed to be released into the atmosphere as CO_2 (Lal, 2002). The reverse process of SOC accumulation due to land use changes is slower and rarely reaches the former level of SOC stocks (Janzen et al., 1998; Soussana et al., 2004). In Europe 7.3 Mha are set-aside land with the potential for a permanent conversion from cropland into grassland and a C sequestration potential of 9-12 Mt C yr^{-1} for a 20 years time period after land use change (Freibauer et al., 2004). Conversion from cropland into permanent grassland is one of the few types of land use change that is acknowledged as a measure to mitigate additional atmospheric CO_2 as SOC in soils (Guo and Gifford, 2002). SOC stocks may increase by 18% due to the establishment of grassland on cropland and therefore sequester on average 332 kg C ha^{-1} yr^{-1} for a limited time period of a few decades (Guo and Gifford, 2002; Post and Kwon, 2000). The mean difference between arable land and grassland was estimated to be 25 t C ha^{-1} for 0-30 cm soil depth with higher stocks in grasslands in France (Soussana et al., 2004).

The differences in SOC stocks between the two land use types are used to calculate the net effect of land use changes on C stocks which can be accounted as C sinks under article 3.4 of the Kyoto protocol. The differences in SOC stocks between plots under new land use relative to that of unchanged management are used as a basis to quantify the effect of land use change after a certain time period. It does not necessarily provide the absolute SOC stock change, but the land-use-change-induced effect on SOC stocks (Ellert et al., 2001). One problem of former assessments of land use change was the insufficient soil sampling depth (Baker et al. 2007). Many studies focused on the upper 10-20 cm of the soil and did not include the whole ploughing horizon and the subsoil. Especially when tilled cropland and grassland are compared, the full accounting of SOC stocks in grasslands for a depth corresponding to at least the ploughing horizon in the cropland is obviously a precondition. C accumulation in grasslands is mainly restricted to the surface soil, whereas C input in cropland is dispersed within the whole ploughing horizon. Differences between grassland and cropland SOC stocks may be caused by a different C distribution within the profiles and an inappropriate sampling depth. Reeder et al. (1998) estimated that 40-60% of the decreased surface soil C in arable soils compared to grassland can be attributed to the mixing with subsurface soil in croplands. Additionally, C translocation processes within the profile play an important role for the C turnover and SOC stocks (Paul et al., 1997). The effect of land use changes on translocation and C placing – the location of C deposition in and on the soil - has hardly been recognized; e.g. earthworm activity is often reduced in croplands compared to grasslands, which may reduce the biotic C translocation into the subsoil (Kladivko, 2001).

In the past, radiocarbon (^{14}C) data have been used to estimate the C translocation and input of modern C into different soil horizons (Dörr and Münnich, 1989; Trumbore et al., 1989). It has been observed that the ^{14}C age of the bulk soil increases sharply with increasing soil depth which indicates decreasing turnover rates in the subsoil but it also shows that C translocation of recently assimilated C into the subsoil is relatively low (Römkens et al., 1998). Land use changes can not only affect total SOC stocks but also influence the contribution of different SOC fractions to C storage. With density fractionation, soils can be separated into light fractions (LF), which contain mainly recent C, and a heavy fraction (HF) which contains stabilised C due to adsorption on mineral surfaces (von Lützow et al., 2006). Among different stabilisation mechanisms, adsorption of C to minerals is considered to be most effective and persistent (von Lutzow et al., 2006). The LF was found to change fast upon land use changes and thus was proposed as an early indicator of SOC stock changes (Hassink et al., 1997). However, on medium- and long-term scale, land use changes can also result in changes in mineral bound SOC (John et al., 2005). The aim of this study was

i) to quantify the impact of cropland conversion into grassland on soil carbon stocks in different soil density fractions in two differently textured soils.

ii) to investigate the role of vertical C translocation and adsorption on mineral surfaces for SOC stocks in relation to land use change.

2. Material and Methods

2.1 Study sites

Two study sites with similar land use history but different geological substrate were chosen: Mehrstedt, a limestone site with stagnic Vertisols, and Kaltenborn, a sandstone site with Arenosols (Tab. 1). Both sites belong to the BIOTREE project and are located in Thuringia/Germany within 70 km distance (Scherer-Lorenzen, in press).

Table 1: Characteristics of the two investigated sites "Mehrstedt" and "Kaltenborn", each with a long term cropland and an adjacent grassland that was established on former cropland.

Site	Mehrstedt	Kaltenborn
Coordinates	10°39' E, 51°17' N	10°14' E, 50°57' N
Elevation [m.a.s.l.]	270-315	320-350
Mean annual temperature [°C]	8.0	7.8
Annual precipitation [mm]	550	650
Bedrock material	limestone/loess	sandstone
Soil type [WRB 1998]	Stagnic Vertisol	Orthieutric Arenosol
Current land use	a) cropland b) extensive grassland since 1981	a) cropland b) extensive grassland since 1975

Both sites comprise an arable land, which is managed conventionally by local farmers, and an adjacent grassland which had been used as arable land until 1981 (Mehrstedt) and 1976 (Kaltenborn) and was converted to extensively used grassland with sheep grazing and occasional mowing as part of a military training area. The tillage depth at the cropland was 25-30 cm. At the Mehrstedt cropland ploughing was reduced during the last years and replaced by 15 cm depth tillage at maximum. At the Kaltenborn site annual ploughing down to 25 cm depth was conducted. No organic fertilizers have been applied on both grasslands and the Mehrstedt cropland at least for the last 40 years. At the Kaltenborn cropland 40 t ha^{-1} cow manure was applied on the field every third year and annually 20 t ha^{-1} liquid manure which equals about 1700 kg C ha^{-1} a^{-1}.

At the Mehrstedt cropland barley, wheat and occasionally fodder beet, peas and field bean was cultivated. Crop residuals were left on the field since 1990. Additional C input at the Mehrstedt cropland due to left crop residuals can be estimated as between 600 and 700 kg C ha^{-1} a^{-1} (Klimanek, 1997). In Kaltenborn two years of triticale is cultivated with one year corn as rotation.

2.2 Soil Sampling

Soil sampling was conducted in a paired plots design in the grassland and the cropland following the border between both land use types for about 180 m. The distances between the paired plots of grasslands and arable lands were 25 m in Mehrstedt and 45 m in Kaltenborn. Soils were sampled separately for density fractionation (0-10, 10-20 cm depth) and for quantification of SOC stocks in profiles (0-60 cm depth):

Surface soil was sampled in May 2004 from 0-20 cm depth in the croplands and 0-10 and 10-20 cm depth in the grasslands. To account for the differences in heterogeneity of the soils (Don et al. accepted), Mehrstedt was sampled with 10 paired replicates, whereas Kaltenborn was sampled with 5 paired replicates at both land use types.

Soil profiles were sampled to a depth of 60 cm using a machine driven core (Cobra, Eijkelkamp) with 87 mm diameter. Sampling was conducted as well in paired plots 450 m along the cropland-grassland border .Compaction of the soil core during the sampling procedure was accounted by measuring the length of the soil core and the depth of the corresponding soil pit. The soil cores were divided into the following depth increments: 0-5, 5-10, 10-20, 20-30, 30-40, 40-50 and 50-60 cm. In Mehrstedt, the cropland was sampled with 12 cores, the grassland with 36 cores. The Kaltenborn cropland was sampled with 7 cores and the grassland with 30 cores to account for different heterogeneity of the sites.

2.3 Sample preparation and C- and N-analysis

Roots, plant and animal residuals were picked out by hand from the fresh samples. The soil samples were air dried, weighed and sieved to 2 mm with a sieving machine that forces also very hard clayish clumps through a sieve (Jehmlich GmbH, Nossen). The sieved sample (< 2 mm) was weighed again to determine the mass of the stones. Mass of the finesoil Mass$_{finesoil,i}$ [mass] was calculated as follows:

$$Mass_{finesoil} = \frac{\left(mass_{sample} - mass_{stones}\right) \cdot volume_{sample}}{volume_{sample} - \dfrac{mass_{stones}}{\rho_{stones}}} \qquad \text{equ. (1)}$$

ϱ=density of stones [mass volume^{-1}] approximated by 2.6 g cm^{-3}

Volume $_{sample}$ was 297.2 cm³ for 0-10 soil depth and 594.5 cm³ for >10 cm soil depth.

A sub-sample was dried until constant weight at 40°C and bulk density was corrected for residual water in the air dried samples. SOC concentration of the ground samples was determined by measuring total carbon concentration (TC measured with VarioMax, Elementar) subtracting the soil inorganic carbon (SIC) which represents the carbonate-C. SIC was determined after ignition of the ground soil sample at 450°C for 16 h. Total nitrogen concentration (N_{tot}) was measured simultaneously with TC and both concentrations were corrected for free water of the air dried sample. SOC stocks [t ha^{-1}] of a certain soil depth interval were calculated as follows:

$$SOC_{stocks} = \sum_{i=1}^{i=n} Mass_{finesoil,i} \cdot SOC_{conc,i} \qquad \text{equ. (2)}$$

where *n* is the number of sampled soil depth intervals i and $SOC_{conc\ i}$ the SOC concentration of the fine soil [mass C mass <2 mm soil^{-1}] in the soil depth interval *i.*

2.4 Below ground biomass

Below ground biomass (root biomass) was estimated from the content of visible roots in the extracted soil core samples. Roots were picked from the fresh soil core samples by hand. Separation between roots and other organic and mineral particles was done manually and the samples were washed from minerals particles and dried at 70°C for at least 48 h. A minimum of 7 replicates per sampling depth and treatment was used.

2.5 Density fractionation

Bulk soil samples were density fractionated before and after aggregate disruption to obtain the following three SOC fractions: free light fraction (f-LF), light fraction occluded in aggregates (o-LF) and mineral associated organic carbon in a heavy fraction (HF). 15 g air dried soil (< 2mm) was added to 100 g fresh sodium polytungstate solution (Sometu, Berlin, Germany) of a density of 1.6 g cm^{-3}. The suspension was treated with an ultra sonic beak (60 J/ml) to crack unstable macro aggregates (Schmidt et al., 1999). Then, the samples were centrifuged for 30 min with 3500 U min^{-1}. The floating organic particles (f-LF) were thoroughly transferred to a glass fibre filter on a vacuum bottle with a pipette. The f-LF fraction was washed with about 1000 ml distilled water to remove the polytungstate salt until the conductivity of the washing water was <10 µS cm^{-1}. The remaining sample was stirred with a glass stick and the sodium polytungstate solution was added again to the sample. In the next step ultra sonic treatment (450 J ml^{-1}) as proposed by (Schmidt et al., 1999) was used again to destroy the aggregates and the sample suspension was centrifuged. Floating particles (o-LF) were removed from the solution and treated like described above. Both LF-fractions, f-LF and o-LF, were dried at 40°C, quantified gravimetrically and ground with a mortar for further analysis. The supernatant of the remaining sample (heavy fraction: HF) was discharged and the sample was washed with distilled water and freeze dried. The LF samples contained only 0.5 +/- 1% polytungstate salt after the washing procedure. The mean mass recovery rate was 97% total dry matter; it was higher in the sandy soil than in the clayey soil.

2.6 General soil analysis and radiocarbon analysis

Soil pH was determined in a 0.1 M $CaCl_2$ solution (soil:solution ratio 1:2.5). Effective cation exchange capacity (CEC_{eff}) was determined by NH_4Cl percolation for acidic soils (Kaltenborn) and by NH_4Ac for carbonatic soil (Mehrstedt). Texture was determined with a combined sedimentation and sieving method after Köhn. Dithionite extractable pedogenic iron (Fe_d) was extracted with tri-sodiumcitrat-dihydrate and sodium dithionite for 16 h. Fe content was measured with Inductively-Coupled Plasma Atomic Emission Spectrometer (ICP-AES).

Radiocarbon concentrations (^{14}C) of the HF-fractions were determined with accelerator mass spectroscopy, 3MV AMS (Steinhof et al., 2004) (High Voltage Engineering Europe). If necessary, samples were decalcified with HCl and freeze dried. CO_2 evolved during dry combustion of 60-200 mg sample was reduced to graphite by heating a mixture of H_2 and CO_2 with iron powder at 650°C. The graphite was pressed into targets and measured with the AMS facility. All values were corrected for fractionation using $\delta^{13}C$ values. The average error was 0.25 pmC. The ^{14}C content was expressed in percent modern carbon (pMC).

Clay mineralogy was determined semi-quantitatively with X-Ray technique according to (Niederbudde et al., 2002).

2.7 Statistics

All measured variates were first characterised by classical descriptive statistics (means and standard error of the mean). Throughout the paper, error bars and error of mean values (± X) indicate the standard error of the mean (Webster, 2001). Significance of difference between both sites and both treatments were tested with *t*-tests. Statistical analyses were performed with Statistica 5.5 software.

3. Results

3.1 Soil properties and productivity of the study sites

The two sampling sites represent extreme ends of soil texture gradients for temperate agricultural soils. The soil at Mehrstedt is dominated by clay and silt which derived from loess deposits and the limestone bedrock (Tab. 2). Surface soil pH was in the range of the carbonate buffer (7.0-7.5 in $CaCl_2$) and surface soil CEC was high (163-266 $mmol_{c+}$ kg^{-1}). In Kaltenborn sand was the main particle size fraction (77-84%) mostly belonging to the fine sand fraction (63-200 µm). The pH was lower (4.8-5.6) than in Mehrstedt and the CEC was low as well (69-94 $mmol_{c+}$ kg^{-1}) (Tab. 2). There was no significant difference in any surface soil parameter between cropland and grassland given in the table 2, except for a slightly higher clay ($p < 0.001$) and silt content ($p = 0.02$) in the Kaltenborn cropland compared to the Kaltenborn grassland.

Table 2: Surface soil properties of the grassland and the adjacent cropland at Mehrstedt and Kaltenborn; n=5 (Kaltenborn), n=10 (Mehrstedt). Standard errors in brackets.

	Mehrstedt			Kaltenborn		
	cropland	grassland		cropland	grassland	
depth [cm]	0-20	0-10	10-20	0-20	0-10	10-20
pH (CaCl)	7.3 (0.1)	7.2 (<0.1)	7.3 (0.1)	5.3 (0.1)	5.1 (0.1)	5.2 (0.1)
CEC_{eff} [$mmol_{c+}$ kg^{-1}]	185.1 (4.1)	215.9 (7.6)	191.1 (7.9)	60.2 (1.7)	48.5 (1.3)	44.4 (2.1)
clay [%]	30.0 (0.8)	30.2 (1.5)	29.6 (1.6)	7.0 (0.16)	5.8 (0.4)	5.1 (0.2)
f+mU silt [%]	30.3 (0.5)	31.2 (0.8)	31.4 (0.9)	7.8 (0.3)	5.8 (0.5)	7.4 (0.5)
gU silt [%]	34.4 (1.3)	32.5 (1.1)	33.9 (1.5)	7.7 (0.1)	6.3 (0.6)	6.6 (0.4)
sand [%]	5.3 (0.9)	6.0 (0.4)	5.0 (0.7)	77.4 (0.2)	82.0 (0.8)	80.9 (0.9)
Fe_d [g kg^{-1}]	12.3 (0.4)	12.5 (0.5)	13.1 (0.5)	2.3 (>0.1)	2.2 (0.1)	2.2 (<0.1)

The Mehrstedt soil had higher contents of smectite and vermiculite (Tab. 3). This resulted in a more than doubled specific surface area of the clay minerals in Mehrstedt compared to Kaltenborn. The additionally higher clay content in Mehrstedt resulted in 15 times larger total clay surfaces compared to Kaltenborn. Total root biomass in 0-30 cm depth was 250 g DW m^{-2} at the Mehrstedt grassland and 154 g DW m^{-2} at the Kaltenborn grassland (Fig. 1). The major part (59 % at Mehrstedt and 86% at Kaltenborn) of root biomass in the grassland was located in the uppermost 5 cm of the soil. In the arable field, roots derived from annual crops and did not show such distinct concentration maxima at the surface soil as in the permanent grassland.

Table 3: Dominant clay minerals [% of total clay content], specific surface area of clay*) [m^2 g^{-1} DW], and total clay surfaces [m^2 clay surface m^{-2} soil surface] for both sites for 0-60 cm depth accounting for different clay contents (data from Don et al., in press).

	Mehrstedt clay fraction	**Kaltenborn** clay fraction
Illite [% of total clay]	60	60
Smectite	2	0
Illite/Semectite Mix.	5	10
Smectite/Illite Mix.	30	0
Vermiculite	3	0
Kaolinite	2	2
Chlorite	0	28
Specific surface clay [m^2 g^{-1} DW]	247 ± 39	104 ±17
Total clay surface area [m^2 clay surface m^{-2} soil surface]	104.1 10^6	6.8 10^6

*calculated after data from (Hintermaier-Erhard and Zech, 1997)

Both sites had similar productivity capacities with an above ground peak biomass of 652 ± 39 g DW m^{-2} at the Mehrstedt grassland and 693 ± 64 g DW m^{-2} at the Kaltenborn grassland ($p = 0.76$) determined in July 2004. Above ground biomass was harvested by clipping within a 50 x 50 cm² sampling frame close to the soil pits for surface soil sampling and dried at 70°C for at least 48 h (n=10 in Mehrstedt and n=5 in Kaltenborn).

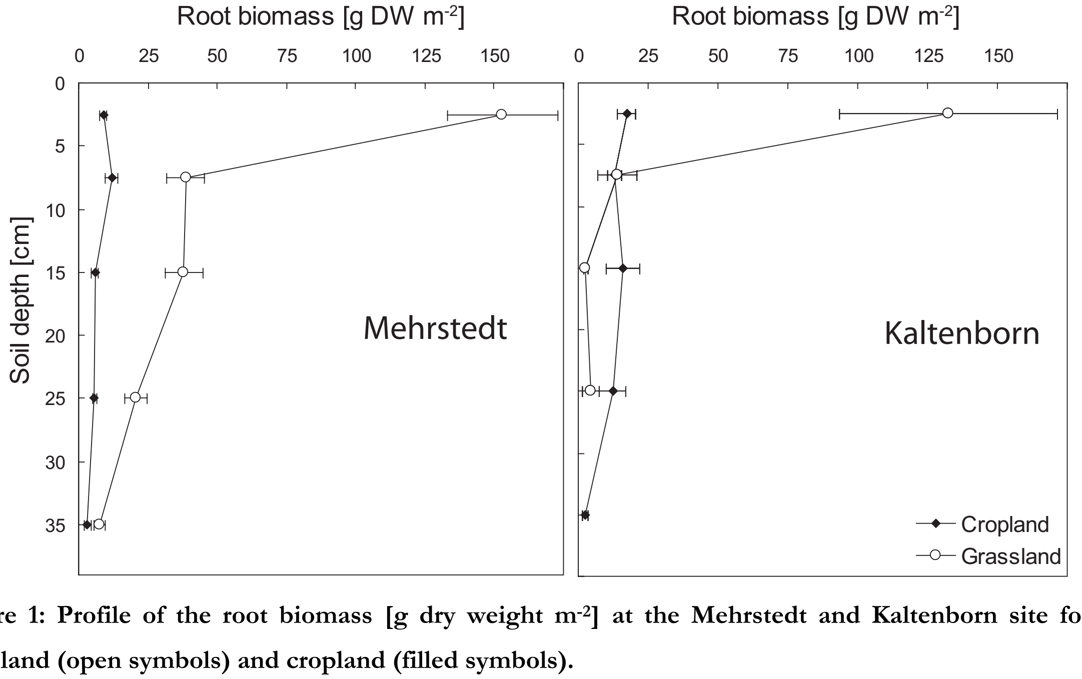

Figure 1: Profile of the root biomass [g dry weight m^{-2}] at the Mehrstedt and Kaltenborn site for grassland (open symbols) and cropland (filled symbols).

3.2 Organic carbon in soil density fractions

With density fractionation we separated the mineral associated C (heavy fraction, HF) from the free light particulate organic carbon located between aggregates (f-LF) and the light particulate organic carbon released upon aggregate disruption (o-LF). For both land use types the main proportion (57-84%) of total SOC was found in the mineral associated form (HF fraction) in 0-20 cm depth. 4-15% of SOC was found in the o-LF fraction and the f-LF fraction comprised 12-37% of SOC. The LF content was highly variable with an average coefficient of variation (CV) of 55% for f-LF and 67% for o-LF. Mean CV of the mineral associated HF-fraction was in contrast only 17%. There was no significant difference between cropland and grassland in the LF fractions (Fig. 2). Sampling had been carried out in early summer when roots of the winter crops may contribute already considerably to the f-LF fraction.

In Mehrstedt, the total amount of SOC in the heavy fraction (HF) was 22% higher in grassland than in the cropland. The difference was even higher (37%) if the upper 10 cm were compared (Fig. 2). In Kaltenborn, the higher content of SOC stored in the HF fraction in 0-10 cm depth at the grassland was compensated by a lower SOC content in 10-20 cm depth. Over 0-20 cm depth, there was no significant difference between grassland and cropland in the HF fraction in Kaltenborn.

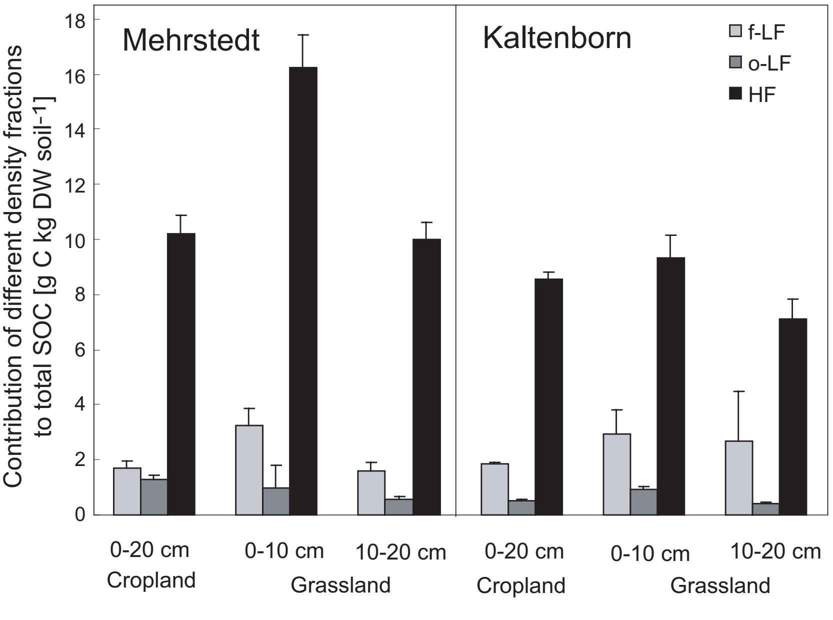

Figure 2: Contribution of three density fractions (f-LF, o-LF, HF) to total SOC stored in 1 kg dry soil [g C kg^{-1} DW soil] for grassland and cropland of the sites Mehrstedt and Kaltenborn.

We found significant differences in the C/N ratio of the three density fractions which always followed the order f-LF>oLF>HF (Fig. 3). C/N ratio is a measure for the level of degradation of the organic matter (Stemmer et al., 1998). Young, less decomposed organic matter with a high C/N ratio (16.0 ± 0.3) was found in the f-LF fraction.

Organic matter associated with mineral particles showed lower C/N ratios (7.9 ± 0.2). The C/N ratio of the o-LF fraction was intermediate with 13.5 ± 0.3. Even though C in the HF has a high degree of degradation, the effect of land use change on soil C was found most explicit in this fraction. There was no significant effect of land use on the C/N ratio of the analysed density fraction in Mehrstedt. At the Kaltenborn site the C/N ratio of the f-LF faction (p=0.01) and the o-LF fraction (p=0.03) was significantly higher in the grassland than in the cropland.

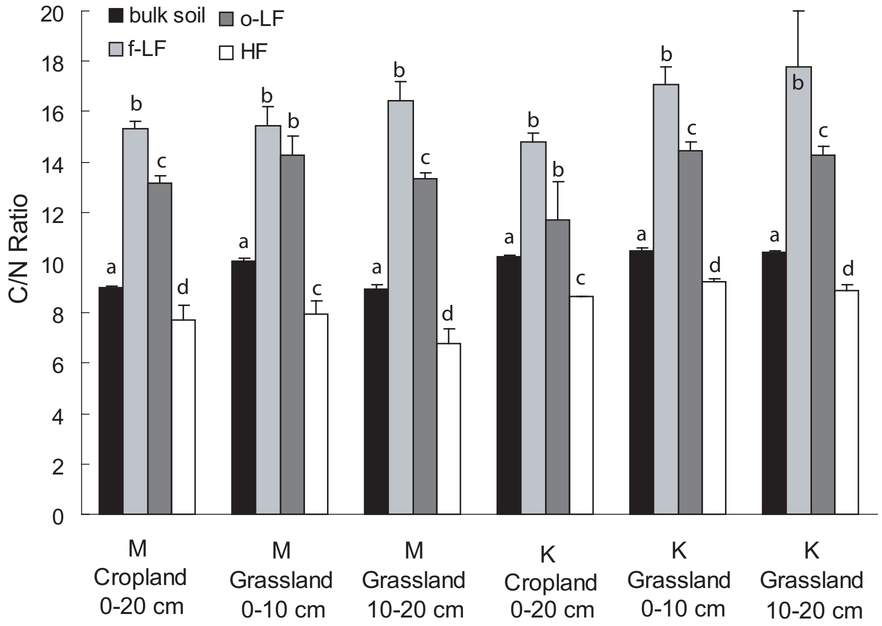

Figure 3: C/N ratio of the bulk soil and three density fractions (f-LF, o-LF, HF) for grassland and cropland of the sites Mehrstedt (M) and Kaltenborn (K). Significant differences between fractions of the same site and depth are indicated with different letters.

3.3 Stocks of total and mineral associated SOC

Total SOC stocks down to a depth of 50 cm were 77.9 ± 2.2 t C ha^{-1} at the Mehrstedt grassland and 79.4 ±4.8 t C ha^{-1} at the Mehrstedt cropland. In Kaltenborn 46.1 ±1.2 t C ha^{-1} were stored in the grassland and 49.2 ± 4.0 t C ha^{-1} in the cropland. SOC stocks at both sites were not significantly different between cropland and grassland when the whole soil profile up to 50 cm depth was considered (Fig. 4). When only 0-30 cm depth was considered, grassland in Mehrstedt stored significantly (p=0.006) more C than the cropland. Total SOC stocks in the Mehrstedt grassland were 74% higher than at the Kaltenborn grassland, the difference between the cropland soils was 77%. This was in line with a higher clay content at the Mehrstedt soils compared to the Kaltenborn soils. However, the difference between the total clay surface areas of the two sites was much larger (Tab. 3) than the difference in SOC. The ratio of mineral associated SOC to clay content (specific C load) was considerably higher in the surface horizons (0-20 cm depth) in Kaltenborn than in Mehrstedt. This ratio is an indicator for the saturation of mineral surfaces with organic matter. Specific C load was the highest in the Kaltenborn grassland A horizon (1.67 ± 0.25 g C kg^{-1} per % clay) followed by the Kaltenborn cropland A horizon (1.24 ± 0.11).

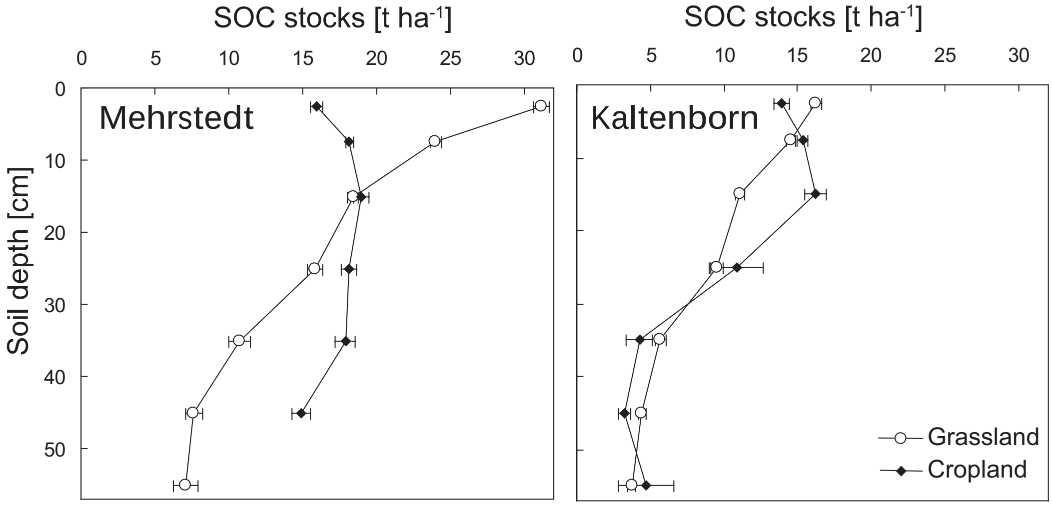

Figure 4: SOC stocks [t C ha^{-1} per 10 cm sampling depth interval] at the grassland and the cropland of the sites Mehrstedt and Kaltenborn.

In Mehrstedt the specific C load was only 0.56 ± 0.04 g C kg^{-1} per % clay at the grassland and 0.35 ± 0.03 g C kg^{-1} per % clay at the cropland. Specific C loads in 50-60 cm depth were even smaller with 0.05 g C kg^{-1} per % clay in Mehrstedt and 0.25 g C kg^{-1} per % clay in Kaltenborn. This indicates the high potential of subsoils for physical C stabilisation and storage.

3.4 C and radiocarbon distribution in profiles

SOC profiles indicate that land use significantly influences the C translocation within the soil profile. As it is typical for grassland soils, SOC gradients were steeper than in the cropland soils (Fig. 4). 34 % and 33% of SOC was stored in the uppermost 10 cm of the grassland im Mehrstedt and Kaltenborn. At the croplands only 20% was stored in 0-10 cm depth in Mehrstedt and 30% SOC in Kaltenborn. Besides the apparent mixing of SOC within the ploughing horizon we found at the clay rich Mehrstedt soil unusual high SOC stocks in the cropland below the ploughing horizons compared to the grassland (Fig. 4). In 40-50 cm depth, C stocks were twice as high as in the cropland compared to the grassland. This additional carbon was mainly found in the HF fraction with twice as high C concentrations in the cropland compared to the grassland ($p=0.039$). In the sandy soil in Kaltenborn no C-enrichment of the horizon below the ploughing horizon could be observed as in Mehrstedt. SOC stocks below the ploughing horizon were similar or even smaller in the cropland compared to the grassland (Fig. 4).

Radiocarbon (^{14}C) concentrations can be used to trace the origin and mean age of SOC in different horizons. ^{14}C concentrations of >105 pMC correspond to recent, young C which is typical for the LF fractions (Römkens et al., 1998; Trumbore et al., 1989). To eliminate the confounding influence of varying proportions of young LF in bulk soil, we measured ^{14}C concentrations of the mineral associated SOC (HF fraction). Conversion from cropland into grassland changed the ^{14}C distribution in the soil in Mehrstedt but less in Kaltenborn (Fig. 5).

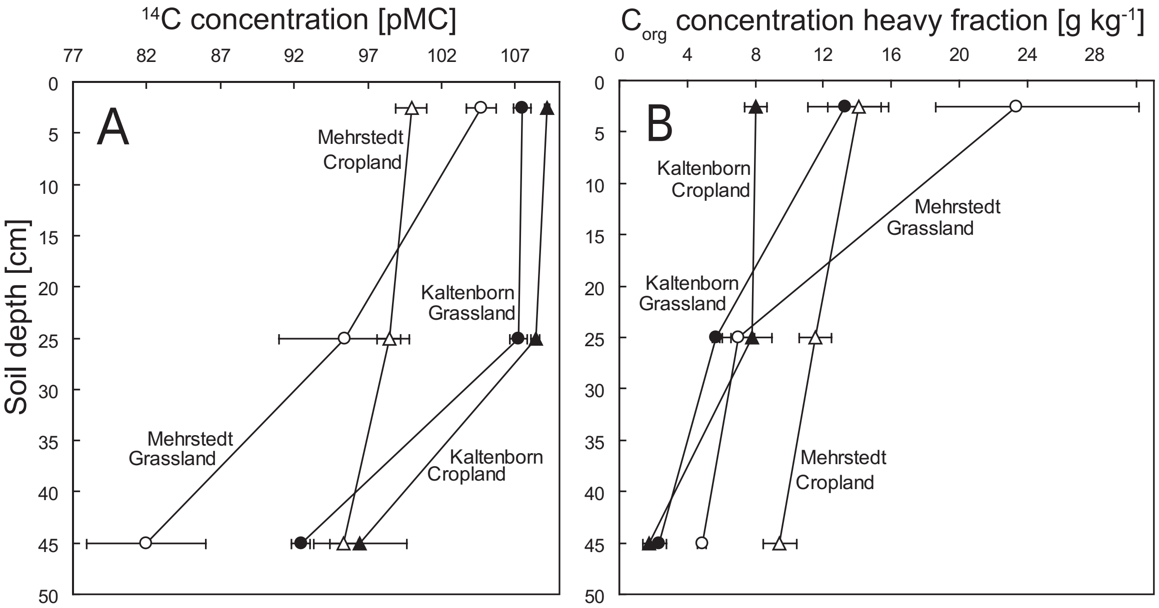

Figure 5: ^{14}C concentration [pMC] of mineral associated SOC (A) and SOC concentrations (B) of the mineral associated C (HF) for grassland and cropland at the sites Mehrstedt and Kaltenborn for 3 different sampling depths; n=3.

Mean ^{14}C concentrations of the mineral associated SOC decreased with increasing soil depth in both grasslands and both croplands. Mean ^{14}C concentrations of 82 pMC in the Mehrstedt grassland in 40-50 cm depth correspond to a mean ^{14}C age (turnover time) of around 2000 years. Lower ^{14}C ages of mineral associated SOC were determined for all horizons at the cropland and grassland plots in Kaltenborn (92–109 pMC) compared to Mehrstedt (82-105 pMC). An exception was SOC in the subsoil horizon (40-50 cm) of the Mehrstedt cropland. There, we found significantly higher ^{14}C concentrations compared to the Mehrstedt grassland (p=0.032). This is in line with high SOC stocks in this horizon and indicates an effective translocation of young C into the subsoil of the cropland in Mehrstedt. In Kaltenborn, there was no clear difference in ^{14}C concentrations between cropland and grassland (p=0.65). There was also only a slight decrease in the ^{14}C content within the upper 30 cm of the soil (Fig. 5).

4. Discussion

4.1 SOC stocks and density fractions

Light fractions of SOC are known to respond faster to land use changes than the HF fraction (Hassink et al., 1997) and were found to be higher in pastures compared to croplands (Chan, 2001). However, the LF fraction is very variable in space (plant- and inter-plant-space) and time (seasonal, annual) and this limits its use as indicator for land use changes (Leifeld and Kogel-Knabner, 2005). f-LF comprises young detritus and fine roots and is considered to be mostly determined by C input levels and quality of the C input (John et al., 2005; Six et al., 1998). We found land use change 23 and 29 years ago to influence the stabilized C in the HF fractions, but not the LF fraction. The light fraction of the croplands may be directly related to the amount of manure application (Kaltenborn) or crop residuals left on the site (Mehrstedt). Also, different soil texture at the two sites did not significantly influence the f-LF and o-LF fractions like reported by other studies (Franzluebbers and Arshad, 1997; Kolbl and Kogel-Knabner, 2004). In biologically active soils, the HF fraction comprises medium- till long-term-storage SOC and is built up simultaneously with the LF fraction (Jastrow, 1996). SOC is converted from LF to HF as indicated by decreasing C/N ratios from f-LF>o-LF>HF.

The differences between SOC stocks of croplands and grassland depended on the sampling depth. Many studies on the effect of no tillage or conversion of cropland into grassland did not include soil depth below 10-20 cm depth (Baker 2007). Guo and Giffort (2002) found in a meta analysis of 76 studies an increase of SOC by 19 ± 4% after conversion of cropland into pasture. However, the sampling depth influenced the magnitude of the SOC change: the deeper the sampling depth, the smaller the effect of the conversion. No difference in SOC stocks in grassland compared to arable land has been found by other studies (John et al., 2005; Merino et al., 2004). Lower bulk densities in grasslands compared to cropland partly offset higher C concentrations, resulting in unchanged SOC stocks.

Land use management may as well affect the C input into the soil. Medium-term experiments of a few months showed that grasses led to a 47% higher below ground C input than cereals (Kuzyakov and Domanski, 2000). However, 84% lower root biomass in the cropland in Mehrstedt and 62% in the Kaltenborn cropland compared to the grassland was not mirrored in the SOC stocks. At the cropland sites, crop residuals mixed into the ploughing horizon with tillage and manure may contribute considerably to the formation of SOC. In the Mehrstedt cropland crop residuals and straw were not harvested but left on the site for SOC built up.

At both site, an intensively managed cropland with high productivity, e.g. due to fertilisation, was compared with and extensively managed grassland with relatively low productivity. We found the productivity of the sites to exert a strong influence via the C input on the SOC stocks.

4.2 C stabilisation on mineral surfaces

Clay surfaces provide the major mineral surface area in temperate soils which effectively stabilize SOC by decreasing C turnover rates (von Lützow et al., 2006). Lower mean ^{14}C concentrations throughout the profile at the clay rich soils in Mehrstedt indicate a higher fraction of stabilized, old SOC and a lower fraction of recent SOC compared to the sandy Kaltenborn soils (Fig. 5). However, the question remains whether surfaces capacity for C adsoption is exhausted (Guggenberger and Kaiser, 2003). The C storage capacity was mainly determined by the clay mineral surface area available for C binding and stabilisation; other mineral surfaces like iron oxides only play a minor role in these soils due to their low abundance. In Mehrstedt, stocks of SOC and mineral associated SOC were increased in the surface soil due to conversion from cropland into grassland. The high C input by root biomass in 0-10 cm depth at the grassland led to twice as high SOC stocks in the grassland compared to the cropland. In contrast, at the Kaltenborn grassland the high C input in the surface soil (0-10 cm) did not facilitate higher SOC stocks than in the cropland. The storage capacity of the surface soil seems to be limited. The more than 10 times smaller estimated total surface area of clay minerals in Kaltenborn contained a three times as high C load compared to Mehrstedt. C load per % clay found in Kaltenborn was 3-4 times as high as reported for a range of Swiss and Dutch agricultural soils (Hassink and Whitmore, 1997; Leifeld et al., 2005). Even though the specific C loads of clay minerals could be increased, low clay content of soils like in Kaltenborn restricts the capacity to accumulate additional SOC after conversion from cropland into grassland.

4.3 C allocation and translocation

SOC placing and allocation plays an important role for C accumulation due to increased C stabilisation with increased soil depth (Lorenz and Lal, 2005; Post and Kwon, 2000). First of all, land use conversion from cropland into grassland changed the C placing in the profile. 60 and 86% of the root biomass as major C input source was restricted to the upper 5 cm of the grassland soil. In 10-30 cm depth, rooting of the grassland could not compensate the former C input of crop residuals via ploughing: SOC and mineral associated C were lost after conversion from cropland in these horizons. In this sense, ploughing results not only in a high input of organic matter into this soil depth, it also facilitates the contact between mineral surfaces and SOC and thus increases SOC stocks. Since the 1950^{th} the deepening of the ploughing depth from <20 cm to >35 cm soil depth has been propagated to increase soil fertility in Europe. Nieder and Richter (2000) found increasing SOC stocks after increasing of the ploughing depth over a period of a few decades. Below 10 cm depth at both grassland sites and in the whole profile of the croplands, SOC storage did not seem to be limited by the quantity of mineral surfaces but by the accessibility for organic matter which has to reach these surfaces within the soil profile in order to be stabilized. On the other hand, tillage has been shown to decrease C stocks when grassland was converted to cropland (Guo and Gifford, 2002). During the first years, mainly SOC from the LF fractions was lost due to disruptions by ploughing of the native sod (Cambardella and Elliott, 1992).

Unusual high SOC content with young ^{14}C ages in the subsoil of the Mehrstedt cropland suggests that SOC stocks are increased due to effective C translocation mechanisms which transport C into mineral surface rich subsoils. Soil texture analysis showed that the two times higher SOC content under cropland than grassland could not be explained by differences in clay content. In general, different factors control the C translocation in the soil profile: i) Soil fauna´s activity is promoted in clay rich soils with high pH as compared to sandy acidic soils. Effective C translocation is accomplished by the macro fauna, like earthworms, which cause a more even distribution of SOC in the profile (Lorenz and Lal, 2005). Soil macro fauna is more present in grasslands than in highly disturbed systems like croplands (Wardle, 1995). ii) C allocation by root and mixing of crop residuals in the ploughing horizon determines the initial position of C in the soil profile. iii) Transport as dissolved organic carbon (DOC) with seepage may be discontinuous in preferential flow paths. Leaching of DOC is promoted under conventional tillage compared to non tilled grasslands (Kisselle et al. 2001). In general, DOC is of minor quantitative importance in grassland and cropland systems as compared to forests (Chantigny, 2003). Thus, the observed SOC differences in the subsoil may not be explained by different DOC fluxes. iv) The high content of swelling clay minerals in Mehrstedt caused a shrinking and swelling dynamic and supported the formation of cracks, which serve as preferential flow paths. Translocation of particulate or minerals associated C in preferential flow paths (self mulching) may be very effective. Plant residuals incorporated with tillage into the lower ploughing horizons may be easier translocated further down than surface soil C. Translocation may be discontinuous and dependent on storm events (Kaiser and Guggenberger, 2005). An increased clay content up to 70% in 60 cm depth in Mehrstedt caused enhanced formation of cracks as macro pores in the subsoil. The relative variability of SOC concentration increased with increasing soil depth in the Mehrstedt grassland but not in the Kaltenborn grassland (Don et al., in press). Preferential flow paths contain higher amounts of C than the soil matrix and increase the heterogeneity of SOC in soils (Bundt et al., 2001). Different pedogenesis of the cropland and grassland soils in Mehrstedt is unlikely to cause the differences in SOC stocks in the subsoils, because the paired sampling followed the cropland-grassland border over 450 m orthogonal to the isohypses. However, the quantification of these transport processes could not been achieved in this study. The data on SOC stocks and ^{14}C concentrations indicate that the conversion of cropland into grassland in Mehrstedt, more than in Kaltenborn, changed the C distribution and translocation in the profile.

5. Conclusions

- The conversion of cropland into grassland 23 and 29 years ago did not change total SOC stocks. However, it reshaped the SOC distribution with a steeper C gradient from the surface soil to the subsoil in the grassland. Sampling depth for SOC stocks have to take this gradient into account when comparing grassland and cropland.

- Clay mineral surfaces provide the capacity to increase SOC stocks after conversion from cropland into grassland. Even a high C input like in the surface soil of the Arenosol grassland in Kaltenborn did not considerably increase SOC stocks. Only if enough mineral surfaces are available, land use management changes may lead to increased SOC stocks.

- C stability in different soils measured as mean ^{14}C age of SOC was mainly influenced by soil texture and less by land use.

- The LF fractions were no appropriate indicators for the medium-term C dynamics after land use change. The conversion from cropland into grassland affected the quantity and quality (e.g. ^{14}C concentration) of the HF fraction indicating that in biologically active soils, parts of the mineral-associated C are under continuous change and involved in the medium-term C cycling.

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Manuscript 4

Impact of afforestation of an extensively managed grassland on C fluxes

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Axel Don[1], Corinna Rebmann[1], Olaf Kolle[1], Michael Scherer-Lorenzen[1,a], Ernst-Detlef Schulze[1]

1) Max Planck Institute for Biogeochemistry, Hans-Knöll Strasse 10, 07745 Jena, Germany

a) Present address: Institute of Plant Sciences, ETH Zürich, Universitätsstr. 2, 8092 Zürich, Switzerland

Abstract

Young afforestations can be considerable C sources due to C losses from the mineral soil after tree planting or decreased primary productivity. Short-term changes in soil C stocks can barely be detected with soil carbon inventories but only assessed with continuous flux measurements using eddy covariance technique. In this study, the transition of grassland to afforestation was investigated using two eddy flux towers, which were operated in parallel for three years, one on a young afforestation and one on an adjacent grassland. Differences between the fluxes at the two sites can be attributed to the management of the sites without confounding influences from weather conditions and seasonal cycles. Site preparation with deep ploughing of the planting rows destroyed 30% of the grassland vegetation at the afforestation site and reduced gross primary productivity GPP deduced from eddy fluxes by 41% in the first year. Periods of enhanced total ecosystem respiration TER/GPP ratios at the afforestation revealed a C loss of 1.1 t C ha^{-1} during the first year. Soil respiration measurements on the disturbed planting rows indicated that C loss derived from enhanced mineralisation on the afforestation site. However, during the following two years GPP was still reduced on the afforestation site compared to the grassland but there was no significant difference in the C balance of the sites. Both sites were small C sources in 2005 (67 g C m^{-2} a^{-1} at the grassland and 19 g C g^{-1} a^{-1} at the afforestation) and small C sinks in 2006 (-7 g C m^{-2} a^{-1} at the grassland and -61 g C g^{-1} a^{-1} at the afforestation). Sheep grazing and mowing shifted the TER/GPP for a few weeks to higher values. On an annual timescale, this ratio was strongly affected by climate variability, mainly precipitation pattern during summer, but it did not differ between the two sites. Uncertainties of the C-export during sheep grazing are a major obstacle in deriving reliable C-balances of grazed grassland sites.

***Keywords*:** Afforestations, eddy covariance, carbon fluxes, grassland, soil respiration, net primary productivity.

1. Introduction

Afforestations are acknowledged under the Kyoto Protocol article 3.3 as a measure to mitigate additional atmospheric CO_2 (IPCC, 2000). However, during early stages of afforestations C loss from soils may offset the C sink of the trees (Guo and Gifford, 2002) with 3.4% yr^{-1} decrease of soil C stocks in the upper 10 cm of the soil for the first 5 years after planting and 0.64% a^{-1} when soil horizons down to 30 cm depth are included (Paul et al., 2002). C loss was especially high below 10 cm soil depth whereas a organic layer only starts to build up 8 years after tree planting to compensate this C loss (Vesterdal et al., 2002). Thuille and Schulze (2006) estimated that between 60 to 80 years are necessary to regain former stock levels in the mineral soil after afforestation. These observations cumulated in the hypothesis of fast soil C loss after disturbances, similar to afforestations, and a slow soil organic carbon (SOC) accumulation during non-disturbance periods (Covington, 1981; Korner, 2003). Thus, long term C sequestration of afforestations depends on the soil C loss during the forest establishment phase. Site preparation for tree planting may lead to an enhanced C mineralisation and reduced litter C input (Jandl et al., 2007). Site preparation on grassland sites involves deep ploughing of planting rows or the whole site for seedbed preparation and to reduce the competition between the non-wood vegetation and the planted trees. Site preparation aims to improve the soil water and nutrient availability to increase the tree establishment success (Querejeta et al., 2001). Grasslands already contain high C stocks and high fractions of labile C which are especially sensitive to disturbances (Chan, 2001; John et al., 2005). 30% of soil C was lost from native grassland while being cultivated as arable land (Mann, 1986).

Short term effects of land use changes can hardly be assessed with C stock inventories. Soil C stock changes <2 t ha^{-1} cannot be detected with a reasonable number of samples against a high background soil C stock and a high spatial and vertical heterogeneity (e.g. Don et al., 2007). However, the eddy covariance technique provides a tool to measure CO_2 exchange of a surface with the atmosphere in continuous mode integrated over a source area of a few hectares, depending on the measurement height, atmospheric stability, wind speed and surface roughness of the measurement site (Baldocchi et al., 1988; Stull, 1984). Thus, short-term C-losses during the first years of forest establishment should be detected with the eddy covariance technique. However, seasonal and inter annual climate variability may obscure the effect of afforestations on the C fluxes. Grasslands are especially sensitive to such climatic variability, with net ecosystem exchange (NEE) spanning from C sinks of 8 t ha^{-1} a^{-1} till C sources of 4 t ha^{-1} a^{-1} depending on climate and land use management (Novick et al., 2004; Suyker et al., 2003).

In this study we measured in a paired design using two flux towers, one on an afforestation site and one on an adjacent grassland site used for sheep grazing. Differences in fluxes between the two towers should reflect the management of the sites independent of climatic factors. Thus, the aim of this study was to investigate the impact of a young afforestation on the CO_2 fluxes. The main effects of site preparation and management changes on the herbaceous vegetation on the young afforestation should be quantified.

2. Material and Methods

2.1 Site description

The Mehrstedt study site is located in Germany at the border of the Thuringian basin, a region dominated by agricultural cropland (10°39 E, 51°17 N). Climate with relatively low precipitation (547 mm mean annual precipitation; 1961-90, German weather service DWD) and relatively high mean air temperature (8.5°C) represent the extreme ends of climate conditions for forest growth. The site is relatively flat with a slope of 1.0 - 2.8°. The 80 ha site was used as cropland since historical times and was converted to an extensively managed grassland in 1981 when it became part of a Soviet military training area. In 2003, 40 ha of this site was afforested as part of the BIOTREE experiment on biodiversity of tree stands (Scherer-Lorenzen, in press) with 10 different broadleaf and coniferous tree species. This part of the Mehrstedt site is named "afforestation" throughout the manuscript even though it has been still dominated by herbaceous vegetation. Trees had an average height of 0.2 - 1.0 m and were planted in a density of 2600 - 4400 trees per ha depending on the tree species (Don et al., 2007). Above ground tree biomass only accounted for 2% of the peak above ground biomass. Planting rows were ploughed in parallel lines with 2.0 m distance down to 60 cm soil depth. Ploughing removed or destroyed 30% of the initial grassland vegetation. The grass stripes between the planting rows were cut once a year (twice in 2005) and the cut biomass was left on site.

The non-afforested part of the Mehrstedt site was continuously managed with sheep grazing and is named `grassland site´. The management of the grassland site was adjusted to the sites low productivity using sheep grazing for a few days three times per year (twice in 2005) (Tab. 1). The flock comprised of approximately 500 adult and 100 young sheep. There was some shrub succession on the grassland.

Table 1: Time periods of management at the two sites with mowing between the planting rows at the afforestation site and sheep grazing at the grassland site.

Mowing on the afforestation site		
from	to	
08.08.2004	12.08.2004	
05.06.2005	08.06.2005	
05.09.2005	10.09.2005	
19.06.2006	21.06.2006	
Sheep grazing on the grassland site		
from	to	Export [g C m^{-2}]
05.06.2004	06.06.2004	37
22.08.2004	23.08.2004	30*
23.11.2004	24.11.2004	10*
20.06.2005	24.06.2005	24
24.11.2005	25.11.2005	10*
01.05.2006	03.05.2006	20*
14.07.2006	15.07.2006	36
11.11.2006	12.11.2006	10*

*) estimated from GPP and LAI data

Both sites were characterised as mesophilic grasslands with a high coverage of *Trisetum flavescens* and partial transition to semi-dry calcarious grassland. High abundances of *Odontites vulgaris* and *Cirsium arvense* are indicators for the loamy soil texture. The vegetation composition was similar at both sites with mean species numbers of 17.8 species per m^2 at the afforestation before site preparation and tree planting and 14.3 species per m^2 at the grassland site. Similar vegetation composition indicates similar environmental conditions at both sites. Thus, differences in measured fluxes can be assumed to be independent from environmental site conditions but related to different land use management. Vegetation disturbances caused while preparing the planting rows, increased the proportion of rudural species in the first year from 28 to 40%. Above ground biomass was dominated by grass species (40%) with less contribution from legumes (31%) and herbs (29%). On the disturbed planting rows herbs were promoted (62% of above ground biomass), whereas grass species (30%) and especially legumes (8%) were suppressed compared to undisturbed grass stripes. The recovery of non-woody vegetation on the planting rows was rather slow. Above ground biomass on the planting rows comprised only 51% (2004), 78% (2005) and 90% (2006) of the biomass of the non-disturbed stripes.

The soils in the footprint area of both towers were investigated using 30 (afforestation) and 12 (grassland) soil cores. A representative soil profile was analyzed at each site (Don et al., in press). Soil at both sites was characterised as Stagnic Vertisols with variable influence of loess in the surface soil. A field capacity of 42%Vol. can be approximated for the silty-clayey texture (Ad-hoc AG Boden, 2005). Soil organic carbon stocks were 75.5 ±1.5 t ha^{-1} in 0-60 cm depth at the afforestation and 78.5 ± 2.5 t ha^{-1} at the grassland site.

2.2 Eddy covariance flux measurements

From September 2003 till December 2006 a comparative study was conducted on the grassland site (10°38'56" E, 51°17'04" N, 318 m a.s.l.) and the young afforestation site (10°39'26" E, 51°16'34" N, 293 m a.s.l.) using two eddy flux towers to investigate the effect of afforestation on carbon dioxide (CO_2) and water vapour fluxes (Fig. 1). The distance between both flux towers was 1080 m. CO_2, H_2O, heat and momentum fluxes were measured at 3 m height using two closed path systems in compliance with CarboEurope-IP recommendations (Aubinet et al., 2000). Both sites were equipped with a three-dimensional sonic anemometer (Gill Solent R3, Gill instruments, UK) and a fast response CO_2/H_2O infrared gas analyser IRGA (LiCor 7000, LiCor Inc., USA). Air was drawn into the gas analyser with a pump through a Decabon tube (25 m length and 80 mm inner diameter at the afforestation site and 5 m length and 64 mm diameter at the grassland site). The IRGAs were operated in absolute mode with CO_2- and H_2O-free nitrogen gas continuously passing through the IRGA reference cell with $8 \cdot 10^{-4}$ L min^{-1}. An aerosol filter (Acro 50 vent, Pall Gelman, USA) prevented contamination of the tubing and of the IRGA. The flow rate was controlled at between 8 and 10 L min^{-1} providing turbulent flow in the tubing. Sampling frequency was 20 Hz for the sonic anemometer and 10 Hz for CO_2 and H_2O. All data were stored on a laptop using the acquisition software EddyMeas (Kolle and Rebmann, 2007). The infrared gas analyser was calibrated with two gas standards (CO_2) and a dew point generator (H_2O) biweekly. Power supply was via public power supply system. Thus, data gaps due to power failure or other technical problems were only 0.4% of the measurement time at the afforestation site and 4.2% at the grassland site.

The fluxes were post-processed with the calculation software EddyFlux (Kolle and Rebmann, 2007) using covariances of 30 min time series of vertical wind velocity, temperature, CO_2 concentration. Net ecosystem exchange (CO_2 flux) was determined as $NEE = \rho\overline{w'c'}$, where w is the vertical wind speed, ρ is the density of dry air and c the CO_2 concentration. Overbars denote time averages and primes denote departures therefrom. The temperature (T) is deduced from the sonic temperate (T_s) and corrected for crosswind and humidity effects (Schotanus et al., 1983). We adopt the micrometeorological convention in which fluxes from the biosphere to the atmosphere are positive. The time lag between measurement of the vertical wind and CO_2 concentrations owing to transport in the tubes was estimated by cross correlation between both times series separately for each half hour time interval. Depending on tubes' surface properties and length of the tubing, it was between 2.6 and 3.9 sec at the afforestation tower and between 1.1 and 3.2 at the grassland tower for CO_2. The data were corrected by shifting the time series by the estimated time-lag.

Frequency losses in the data due to damping in the tube and the analyser were corrected using the approach by Eugster and Senn (1995). The correction parameters were determined from the cospectra analysis of the vertical wind velocity, temperature T_s and CO_2 time series. Water vapour dilution and air pressure differences in the sampling cell and in the atmosphere were automatically corrected via internal software of LiCor 7000. A 2D coordinate rotation was applied to account for the gentle slope in the terrain. The data quality was checked thoroughly and any data that did not mach the following criteria was excluded: Change rate for w-component exceeded 5 ms^{-1} >50 times per 30 min; change rate for CO_2-component exceeded 10μmol mol^{-1} >100 times per 30 min; CO_2-component <300 or >600μmol mol^{-1} for >100 times per 30 min; variance of CO_2 or H_20 concentrations >5 and $\neq$ 0 μmol mol^{-1}; no sheep in close vicinity to the tower at the grassland site; friction velocity u* >0.1 ms^{-1}. The u* threshold was deduced from relationship between the annual C balances and the u* threshold and compared with u* threshold derived from the automated gab filling tool (Reichstein et al., 2005). There was no significant influence of different u* thresholds >0.1 ms^{-1} on the NEE balances for both towers. The effect of the different filter criteria on the remaining data coverage is presented in Table 2.

Table 2: Data coverage after stepwise exclusion of data that did not match the quality criteria (see text), u* <0.1 [m s^{-1}] and wind direction 0°<WD<160°.

Site	Year	Remaining data [%] Quality criteria	u*	WD<160°
Afforestation	2004	76	65	49
Afforestation	2005	71	63	45
Afforestation	2006	76	64	46
Grassland	2004	69	59	44
Grassland	2005	76	65	44
Grassland	2006	76	62	43

Foot print analysis of the representative periods 09/2003, 04-05/2004 and 01/2004 were performed with a Lagrangian model (Göckede et al., 2006). According to this analysis, 87% of fluxes derived from the area of interest (afforestation), 12% from the arable land and 1 % from grassland at the afforestation site (Fig. 1A). The tower had a 450 m fetch in the main wind direction on afforestation land. To eliminate the influence of the nearby arable land, fluxes were excluded for wind direction from between 0 and 160°, although fluxes from the excluded wind directions may still contain high fractions of afforestation as source area. This was a conservative measure to restrict the source area to the afforested land. The land use of the source area for the grassland flux tower was homogeneous with 400 m of extensive grassland in the main wind direction from the flux tower (Fig. 1B). All fluxes of both towers from wind directions from 0-160° were excluded to assure comparability in fluxes from both sites.

Data gaps were filled using the look up table approach from Reichstein et al. (2005) which is available as an online-tool. Intercomparison of different gap filling techniques showed that this method together with neural networks, is the most successful method (Moffat et al., in press). For flux partitioning into total ecosystem respiration (TER) and gross primary production (GPP), original night time date was only selected (global radiation <20 Wm^{-2}). An exponential model after Lloyd and Taylor (1994) was used to fit the relation between TER and air temperature. The activation energy parameter E_o was kept variable over the seasons to account for different temperature sensibility of the ecosystem as modified by e.g. different soil moisture or grazing and cutting (for more details see Reichstein et al., 2005). Air temperature at 2 m height was used as a short-term driver to calculate the TER dynamic with the model equations.

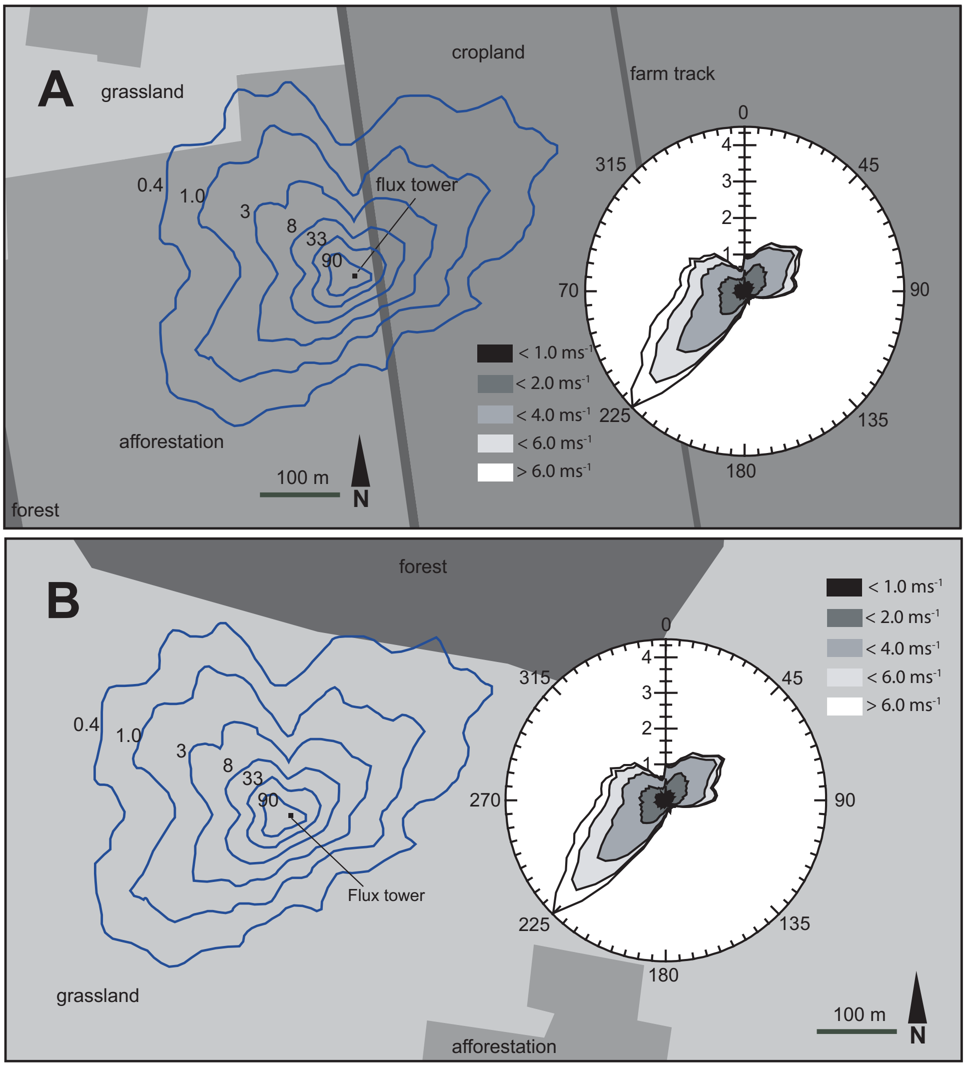

Figure 1: Maps of the measurement sites ´afforestation´ (A) and ´grassland´ (B) with the source area of the fluxes (footprint); frequency distribution of wind directions and wind speed classes (grey scale legend) for 2004-2006.

Energy balance between latent LE and sensible heat flux H and the available energy R_a (available energy) determined by net radiation R_n – soil heat flux was not closed, which is familiar in most eddy flux measurements (Wilson et al., 2002). The slope between LE-H and R_a was 0.68 at the afforestation and 0.69 at the grassland site.

2.3 Soil respiration measurements

CO_2 efflux from the soil (soil respiration) was measured every 1-4 week in the source area of the two towers during each growing season. Five measurement locations were installed permanently along transects in the main wind direction (SW) of each tower (foot print) in 80-120 m distance from each other. At each location at the afforestation site, three open collars (diameter 10 cm) were installed on the soil surface in the disturbed planting rows and three in the undisturbed grass stripe between the planting rows. The five measurement locations on the grassland site were equipped with four collars each; thus, soil efflux was determined with 20 replications (collars) at the grassland site and 30 at the afforestation site. Measurements were performed using a closed manual chamber system with an infrared gas analyser (Licor 6400, Li-Cor, USA). The chamber was placed on the collars, and CO_2 was scrubbed to below ambient concentration and then allowed to rise above ambient. At least three measurement cycles were carried out at each location following the measurement protocol of Soe and Buchmann (2005). Collars were kept free of vegetation by clipping; however, roots could enter the soil below the collars to insure similar soil conditions to those outside the collars.

Mixed linear models were used to test the significance between soil respiration on the disturbed planting rows and the non-disturbed grass stripes (treatment). This treatment was set as fixed effect and time was set as random effect. Mixed linear models are a robust method to assess differences between different time series with certain data gaps.

2.4 Above ground biomass sampling

Above ground biomass was determined with 12 to 24 replicates by separately clipping of 80 cm x 80 cm squares (in 2004: 50 cm x 50 cm) in the planting rows and the non-disrupted grass stripes at the afforestation and the grassland site. Harvested biomass was dried for at least 48 h and then weighed. Biomass removal caused by main grazing events during summer was measured with biomass sampling before and after grazing plots and on a non-grazed, fenced reference plot.

2.5 Additional meteorological measurements

The Mehrstedt site is equipped with a complete meteorological station at the afforestation tower. Incoming and upward short and long wave radiation (CNR1, Kipp &

Zonen, Delft, Netherlands) and downward and upward photosynthetic photon flux density (PAR Lite, Kipp & Zonen) were measured from the tower on a southward pointing arm at a height of 5 m. At the grassland site, the surface temperature was measured next to the tower (Infrared radiation pyrometer, KT15, Heitronics, Wiebaden, Germany). Surface temperature at the afforestation site was calculated from upward global radiation measurements. Sensors were installed on the afforestation tower at 2 m and 4 m heights and additionally on the grassland tower at 2 m, to measure humidity, temperature (HMP, Vaisala, Finland) and air pressure (PTB1001B, Vaisala, Finland). The start of the vegetation period was defined as time when the mean daily air temperature in 2 m height exceeded 5°C for at least 5 consecutive days.

Precipitation (heated tipping bucket rain gauge 5.4032.30.008, Thies, Göttingen, Germany) was collected at a height of 1 m.

Soil temperature (PT100, Jumo, Germany) was measured with two replications in the following soil depths: 2, 4, 8, 16, 32, and 64 cm. Volumetric soil water content was measured with a TDR system in 8, 16, 32, and 64 cm depths (ML-2x, DeltaT) using two additional sensors as replications in 8 cm depth. Soil heat flux was measured close to the soil surface using five heat flux plates (HP3/CN3, McVan Instruments, Australia). All data were collected every 10 s and stored as 10 min average values with data loggers (CR23X, Campbell, Canada at the afforestation and CR10 at the grassland). 100% data coverage was achieved for all meteorological data except soil water content, and allows for gap filling of flux data according to meteorological conditions (see chapter 2.2).

3. Results

3.1 Climate drivers and their inter-annual variability

There was interannual and seasonal variability of the main climate drivers of the C fluxes during the three years of measurements (Fig. 2). Mean annual precipitation was 547 mm as long-term average and during the three investigated years (2004, 2005, 2006) 549, 503 and 513 mm, respectively, with less interannual than seasonal variability (Fig. 2). The monthly precipitation measured in June 2004 (93 mm) was almost twice as high when compared to the same month in 2005 and 2006 (55, 43 mm). In 2006 two to three times as much precipitation was measured in August (107 mm) compared to 2004 (53 mm) and 2005 (31 mm).

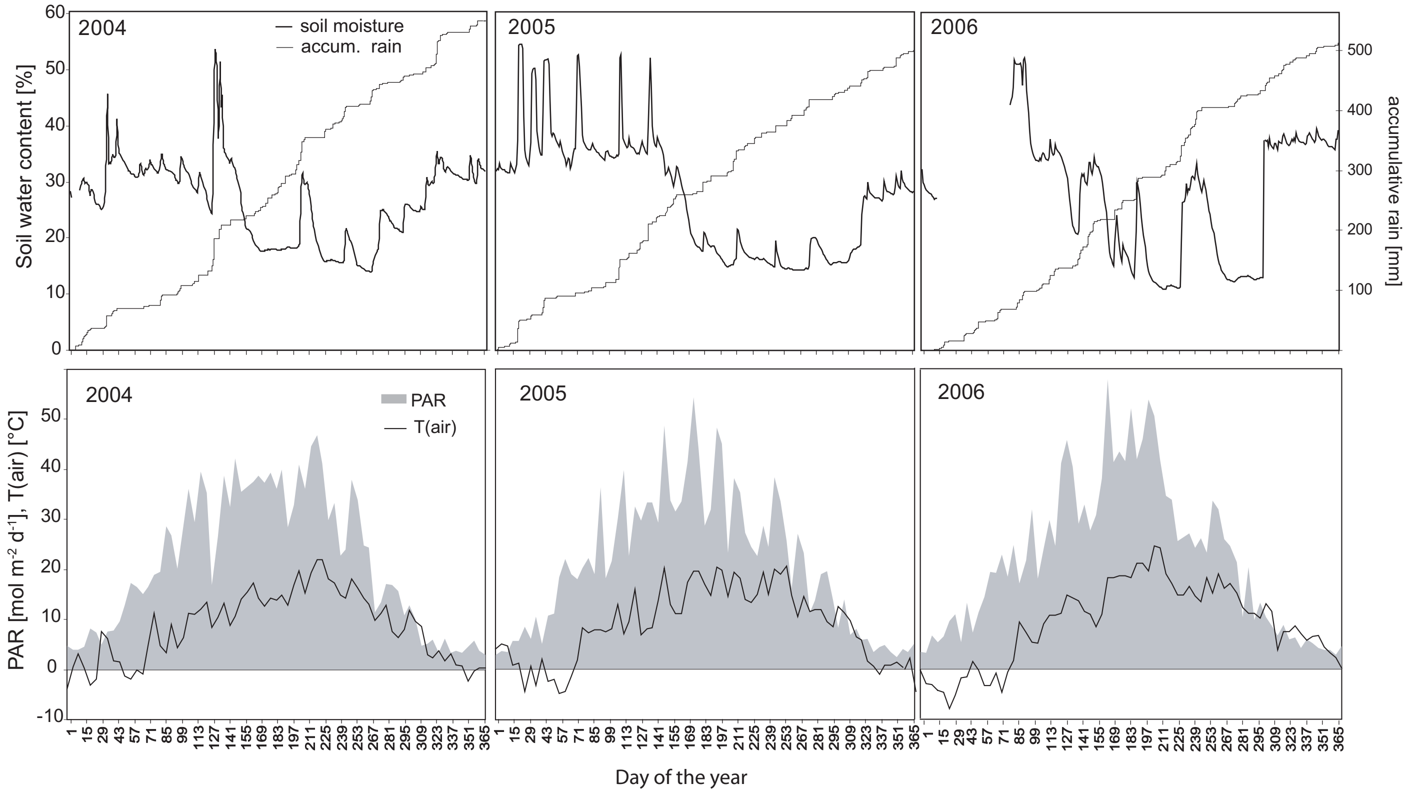

Figure 2: : Meteorological conditions over the 3-year measurement period: a) Soil water content [%], accumulative rain [mm] b) photosynthetic active down radiation (PAR) [mol m^{-2} d^{-1}] and air temperature at 2 m height (T (air)) [°C].

Soil moisture continuously decreased in April, May and remained on a low level during the summer till October, November (Fig. 2). The dry period during which soil moisture dropped to below half the fields capacity of 21%, lasted 113 days in 2004 starting on June 8th, 149 days in 2005 starting on June 20th and 161 days in 2006, from June 12th on. Rain fall events during summer rarely influenced the soil moisture as measured with the soil moisture sensor in 8 cm depth. Maximum soil moisture content of about 50% was reached again in February. This seasonality could hardly be changed by unusual precipitation patterns. High precipitation in July 2004 and August 2006 increased soil water content for just a few weeks. However this could not change seasonal soil drying during summer. The year with the highest cumulative precipitation (2004) had the lowest mean soil moisture content.

Mean air temperature during the growing season (April-September) was highest in 2006 (15.1 °C) compared to 2004 (13.9 °C) and 2005 (14.4 °C). High incoming radiation in June and July 2006 was reflected in high mean monthly temperatures (16.2 and 21.8 °C). Even though 2006 had the highest mean temperature, the 2006 growing season started late (March 25th) following a period of long lasting shallow snow cover. The 2004 growing season started on March 13th and in 2005 on March 15th. Soil surface temperatures on the grassland site were only influenced by grazing events during the growing season. Surface temperatures increased by between 1.7 and 2.5 °C after grazing events compared to the afforestation site. However, this temperature increase only lasted a few days to weeks.

3.2 Productivity determined by management and climate

Site preparation at the afforestation site in 2003 destroyed about 30% of the vegetation cover along the planting rows. Thus, in the first year of the measurements (2004) gross primary productivity (GPP), deduced from eddy fluxes partitioning, was reduced by 41% on the afforestation site compared to the grassland (Tab. 3). Even in the third year (2006) GPP on the afforestation site did not reach the level of GPP of the grassland site but was 14% smaller.

Table 3: Annual sums of GPP, TER and NEE [g C m^{-2} a^{-1}] for the afforestation and the grassland site. Fraction C remaining (NBP) in soil includes the C export via sheep grazing.

g C m^{-2} a^{-1}		**Afforestation**	**Grassland**
GPP	2004	851	
GPP	2004 (III-XII)	842	1435
GPP	2005	1024	1287
GPP	2006	1102	1279
TER	2004	816	
TER	2004 (III-XII)	766	1243
TER	2005	1044	1321
TER	2006	1041	1206
NEE	2004	-34	
NEE	2004 (III-XII)	-76	-192
NEE	2005	19	33
NEE	2006	-61	-73
NBP	2004	-34	
NBP	2004 (III-XII)	-76	-115
NBP	2005	19	67
NBP	2006	-61	-7

GPP at the grassland as compared to the afforestation was especially enhanced in April and May 2004 and 2005 and in June 2006 but only until a sheep grazing event. However, the reduction in measured standing biomass on the disturbed planting rows does not explain the lower GPP at the afforestation site: Total reduction of above ground biomass at the afforestation site due to lower biomass density on planting rows was 15, 7 and 3% in 2004, 2005 and 2006, respectively (Tab. 4). GPP on the afforestation site at the time of biomass sampling was reduced by 33, 25 and 10% compared to the grassland site. Sheep grazing six weeks before biomass sampling in 2004 may have exerted some direct effect on GPP and standing biomass. In 2005 and 2006 there was no sheep grazing before biomass sampling between the end of May and beginning of June. However, the measured standing biomass had no significant difference between either site, grassland or afforestation, on all sampling dates from 2004-2006, except May 2005 (Tab. 4).

Table 4: Mean and standard errors (SE) of above ground biomass [g DW m^{-2}] of non-disturbed grass stripes, disturbed planting rows and average biomass of the afforestation and the grassland site.

g DW m^{-2}	**Afforestation**						**Grassland**	
	planting row		grass stripe		average		average	
	mean	SE	mean	SE	mean	SE	mean	SE
20.07.2004	314	(33)	565	(31)	465	(45)	417	(23)
25.10.2004	0	-	9*	(<1)	6*	(<1)	25*	(3)
31.05.2005	218	(11)	281	(13)	262	(17)	319	(13)
09.09.2005	286	(14)	332	(31)	318	(34)	337	(17)
13.06.2006	218	(16)	243	(14)	233	(21)	211	(15)

*) only green, living biomass.

GPP was limited by dry summer conditions. The recovery of the vegetation after dry periods played an important role for the C fluxes of the sites. After the summer drought in the second half of 2004, vegetation regrowth on the grassland site increased the maximum GPP and the maximum rate of photosynthesis (A_{max}) derived from Michaelis Menthen equations (Falge et al., 2001) (Fig. 3), whereas the drying and senescence of the herbaceous vegetation at the afforestation site was hardly reversible. End of October mean standing active (green) biomass was 25 ±3 g DW m^{-2} at the grassland site and only 6 ± <1 g DW m^{-2} at the afforestation site with no active biomass left on the planting rows (Tab. 4).

Mowing on the afforestation and grazing on the grassland were the main management factors that directly influenced GPP on both sites. Mowing between planting rows at the afforestation reduced the standing biomass by 35 ±1% in 2004 and 17 ±3% in 2005. Above ground biomass reduction was more effective with sheep grazing on the grassland with 47% in June 2004, 34% in July 2005 and 51% in July 20 06 being removed.

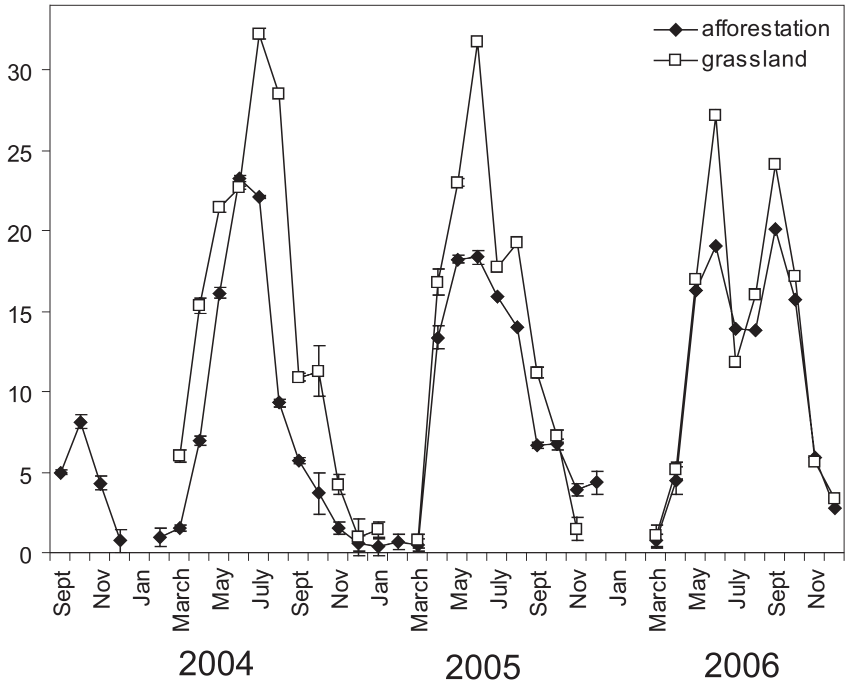

Figure 3: Time course of maximal rate of photosynthesis (A_{max}) on monthly time steps for the afforestation site (closed symbols) and the grassland site (open symbols) for the 3 investigated years.

Higher GPP at the grassland compared to the afforestation was reflected in higher maximum photosynthetic rates (A_{max}) (Fig. 3). A_{max} followed a strong seasonal cycle and was reduced by soil water limitations (e.g. in July, August 2006), sheep grazing and mowing. However, A_{max} increased in September 2006 due to improved water availability in August and September. Thus, the annual cycle of growth and senescence of the herbaceous vegetation could be accelerated by water stress but was partly reversible.

3.3 Ecosystem and soil respiration

Soil respiration followed a strong seasonal cycle with occasionally high fluxes of 8-12 μmol $m^{-2}s^{-1}$ between May and August (Fig. 4). Correlation between soil temperature and soil efflux was week but maximum soil efflux increased with increasing soil temperature and can be described with a positive linear function (not shown). However, there were also low efflux values at high temperatures which indicate low explanatory value of temperature for mean soil respiration at both sites. In fact, we found soil respiration better constrained by the soil moisture content with an optimum function and low efflux at very high and very low soil moisture contents (Fig. 5). Highest soil efflux was measured at soil moisture contents between 19 and 24%. There were no significant differences in the relations between soil respiration and soil temperature or moisture between the two sites.

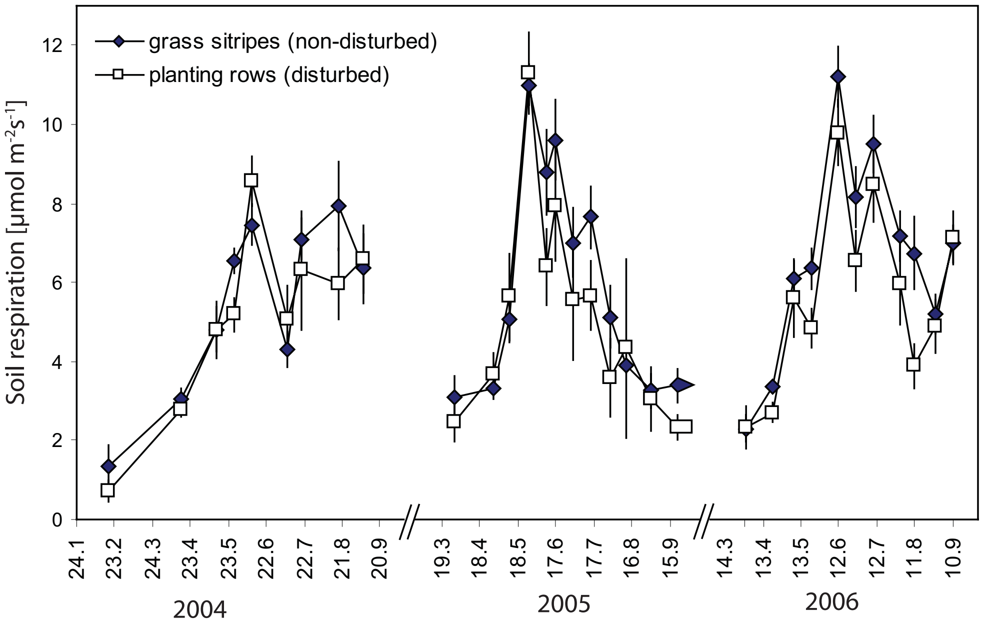

Figure 4: Time course of soil respiration at the afforestation site on the non-disturbed grass stripes (closed symbols) and on the disturbed planting rows (open symbols) during the growing season of the three investigated years.

At the afforestation site soil respiration was measured separately for the disturbed planting rows and the grass stripes in between. Mean soil respiration on the planting rows was reduced significantly by 9, 12 and 14% in 2004, 2005 and 2006 respectively, as compared to the non-disturbed grass stripes ($p<0.05$). At 44% of all measurement days the average CO_2 efflux was higher at the planting rows as compared to the grass stripes in 2004, despite lower standing biomass.

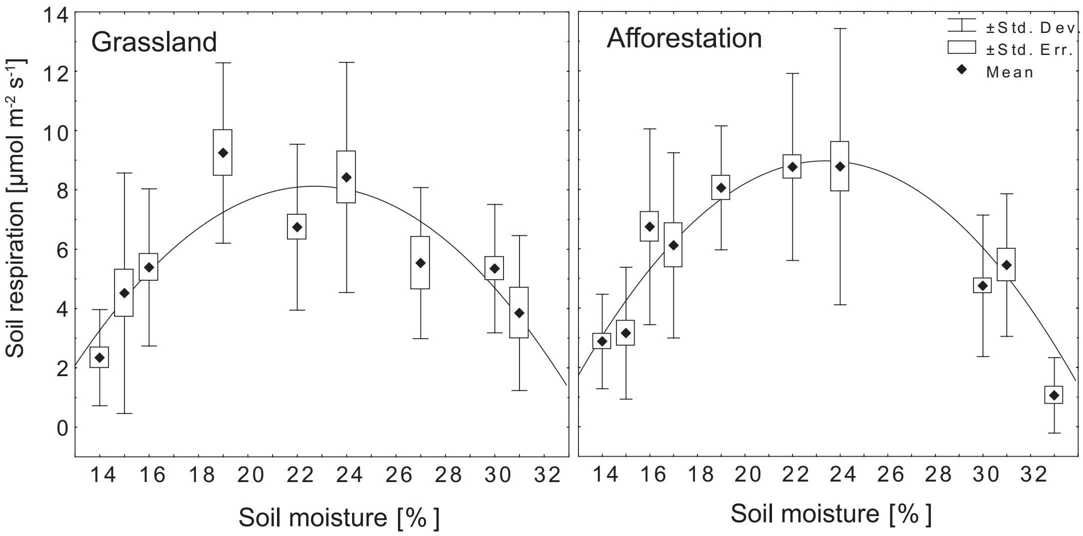

Fig. 5: Mean soil respiration vs. soil moisture content in 8 cm depth for the grassland and the afforestation site. Sample dates with >5mm accumulative rain 5 days before measurements were excluded.

Mean total ecosystem respiration (TER) derived from eddy flux partitioning was 62, 27 and 16% higher at the grassland site than at the afforestation for 2004, 2005 and 2006 (Tab. 3). Higher respiration correlated to higher GPP at the grassland compared to the afforestation. Thus, only the ratio TER/GPP allows assessing heterotrophic respiration pattern and the effect of disturbance on mobilisation of soil organic carbon. Grazing and mowing events shifted the TER/GPP to higher values for a few weeks as illustrated in a steeper slope when TER vs. GPP is plotted (Fig. 6).

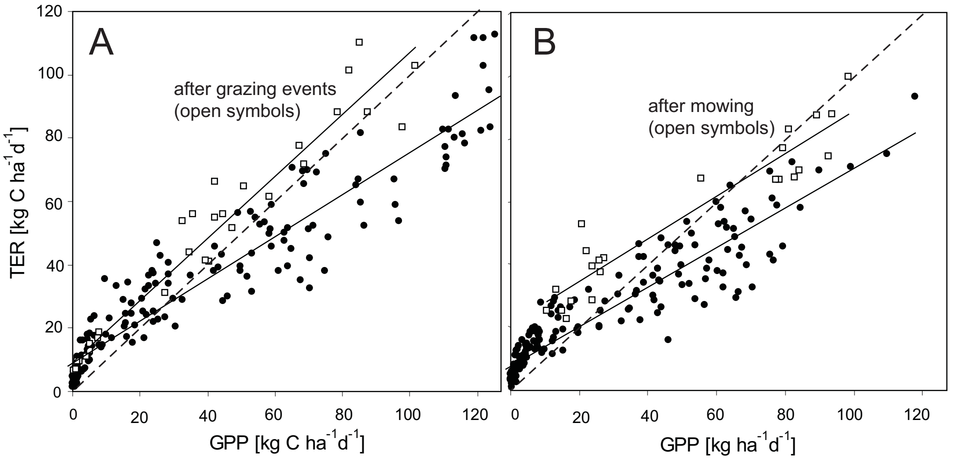

Figure 6: Correlation between gross primary productivity GPP and total ecosystem respiration TER for the afforestation site (A) and the grassland site (B). Points with open symbols indicate periods after disturbance by site management (mowing and grazing). The dashed line indicate TER=GPP (1:1).

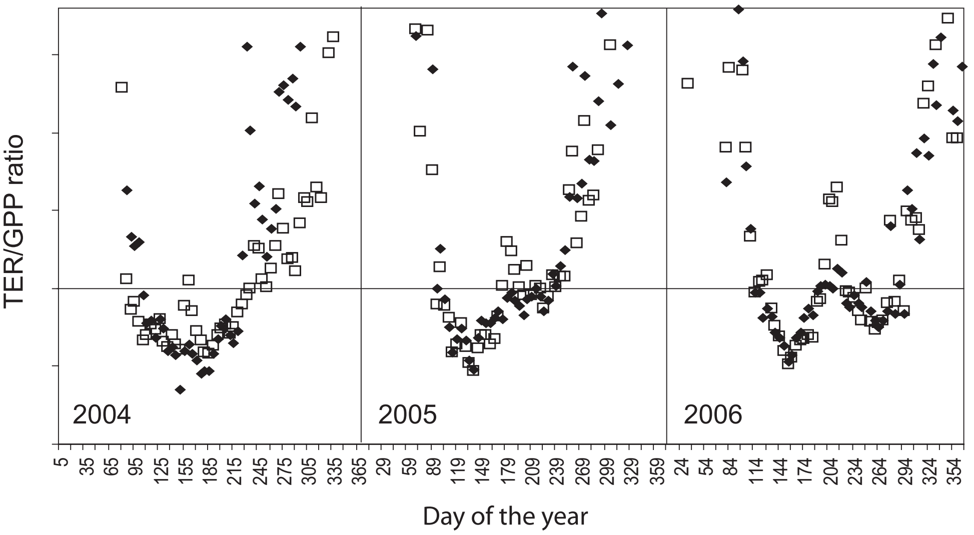

Figure 7: Time course of the TER/GPP ratio for the afforestation site (closed symbols) and the grassland site (open symbols) for 2004-2006.

The variability of the TER/GPP ratio is part of the annual cycle of growth and senescence of the herbaceous vegetation. TER/GPP ratios >1 indicate a net C loss whereas ratios <1 were measured when more C was assimilated than respired (growing season). A seasonal dynamic was superimposed on this annual cycle, mainly due to water limitations during summer; e.g. TER/GPP increased from 0.6 to 1.1 at the afforestation site and from 0.6 to 1.6 at the grassland site within 7 weeks during a dry summer period in 2006 (Fig. 7). Climatic and seasonal variability are eliminated when only ratio differences between the two towers are plotted (Fig. 8). Periods of rapid C losses at the afforestation site are indicated by differences in TER/GPP >>0 in early spring and late summer 2004 (Fig. 8). 30 g C m^{-2} was lost at the afforestation site compared to the grassland between mid March and the end of April 2004. In addition, 92 g C m^{-2} was lost between mid August and the middle of November 2004. 10-20% of the C loss in autumn may have been derived. Soil respiration measurements in August 2004 showed that additional C loss on the afforestation site was derived from the disturbed planting rows (Fig. 4).

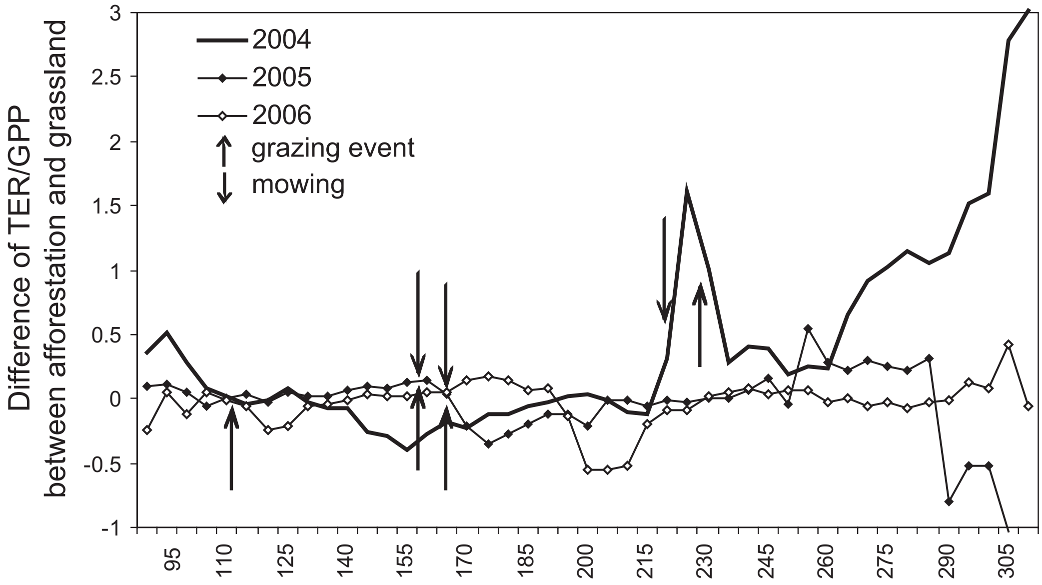

Figure 8: Difference of the TER/GPP ratio between the grassland and the afforestation site (afforestation – grassland) for the three growing seasons. Values >0 indicate C loss at the afforestation site compared to the grassland

Such C loss was no longer detected on the afforestation site in 2005 and 2006. However, after sheep grazing events in November 2005 and 2006 higher TER/GPP ratios were measured on the grassland compared to the afforestation site during winter season. Cumulative C loss at the grassland was considerable with 33 g m^{-2} in winter 2005/2006 and 6 g m^{-2} in November and December 2006. Thus, climatic and management effects on

C fluxes could be disentangled by using differences in fluxes and flux ratios between the two towers.

3.4 Net ecosystem exchange NEE

Net ecosystem exchange (NEE) is the only flux which is directly measurable with eddy covariance technique. Highest C sink per day during the growing season was 3.8 g C m^{-2} d^{-1} at the afforestation and 4.9 g m^{-2} d^{-1} at the grassland. Winter season break down of photosynthetic capacity induced a time lag between the start of the growing season and the first day with negative NEE between 14 days and one month. The beginning of the growing season is marked by an accelerated C loss with positive NEE fluxes (Fig. 9). The late start of the 2006 growing season was followed by a particularly long 4 weeks period of positive daily NEE fluxes. C uptake from both sites was reduced by 4-9 g C m^{-2} compared to 2004 and 2005, during which the growing season started 10 days earlier.

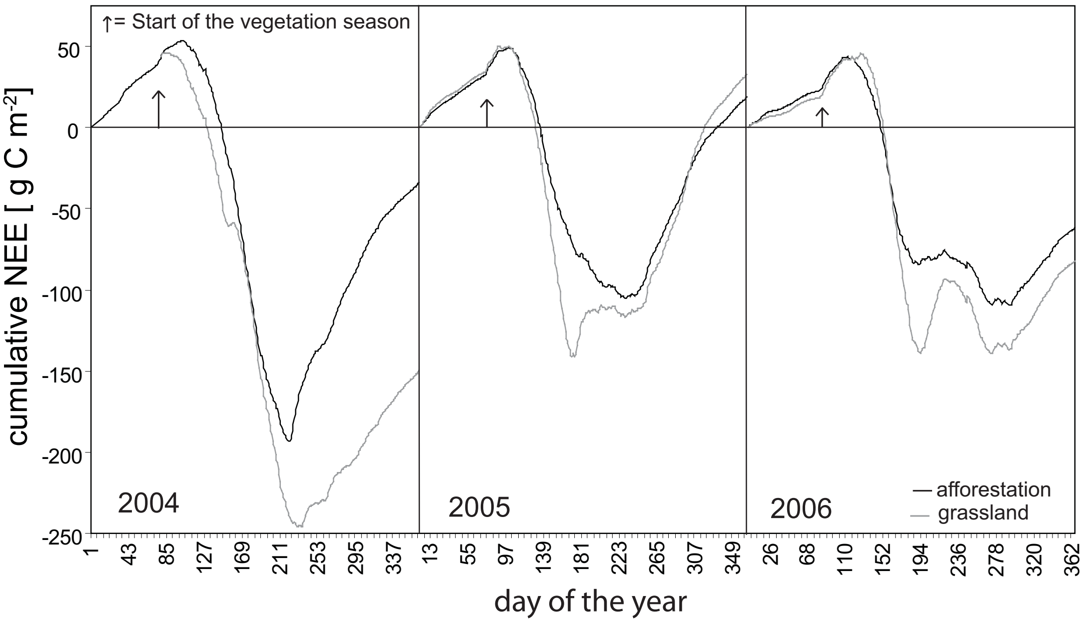

Figure 9: Time course of NEE fluxes [g C m^{-2}] at the grassland site (grey line) and the afforestation (solid line) for the years 2004-2006.

The annual NEE balance on the afforestation site is a proxy for the soil carbon stock change, as there was no C export via biomass harvest and biomass stock changes can be neglected in yearly time scales. Annual export as dissolved organic carbon was < 4 g C m^{-2} and thus, is of minor importance for the ecosystem C balances (Don et al. in prep.). At the grassland site C export by sheep has to be accounted for in the annual C balances. There were two to three grazing events per year, each lasting between one and four days (Tab. 1). Basic preconditions for the eddy covariance technique, such as a homogeneous source area and stationarity of the fluxes, were violated during this time. Thus, data from these time periods were rejected and modelled with gap filling techniques. Estimated C export derived from measurements of standing biomass inventories before and after sheep grazing was in the range of the annual NEE balance of the site (Tab. 3). In 2004 and 2006 both sites were C sinks, whereas in 2005 19 g C m^{-2} at the afforestation and 67 g C m^{-2} at the grassland site was lost to the atmosphere. In the first year (2004), the C sink was 34% smaller at the afforestation than at the grassland site. However, in the second and third year, C balance of the afforestation site was similar to the C balance of the grassland site. The strongest temporary C sink was measured at the grassland site during summer in all three investigated years. Between April and June the C sink of the grassland increased continuously compared to the afforestation (Fig. 10). Each year, a sheep grazing event stopped the C accumulation on the grassland and induced a C loss comparably high to the C accumulation before the grazing event.

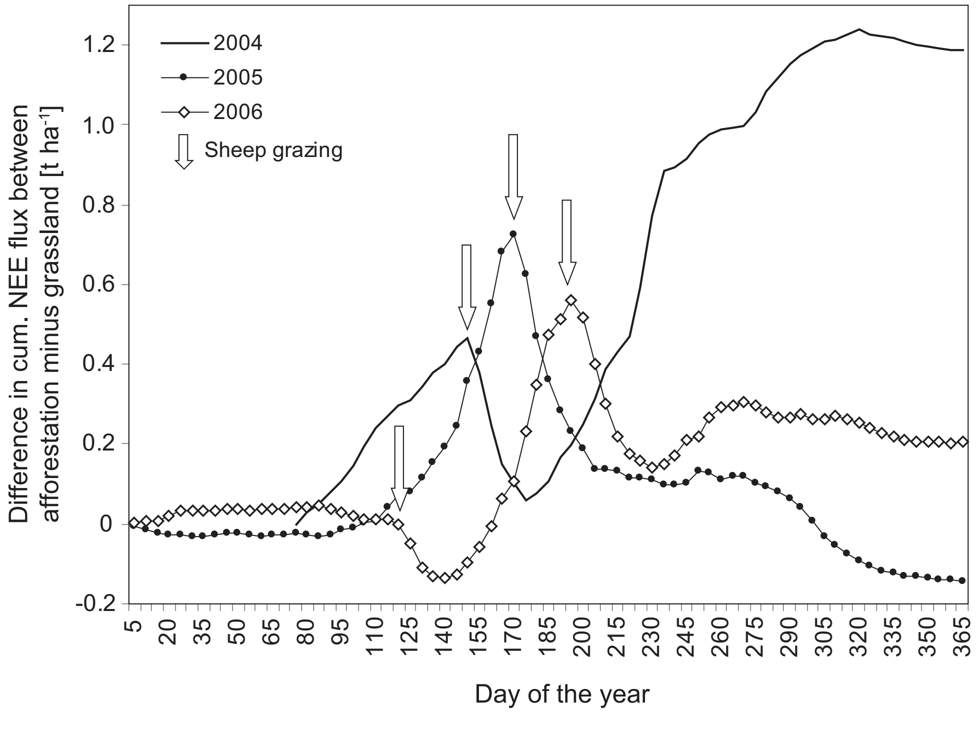

Figure 10: Differences in cum. NEE fluxes on 5 days time steps between grassland and afforestation (afforestation – grassland) for 2004-2006. Positive values indicate a stronger C sink at the grassland site compared to the afforestation.

4. Discussion

4.1 Impact of climate variability on the C fluxes

Ecosystems which have a low ability to buffer their own microclimate, such as grasslands, are highly sensitive to climate variability in their element fluxes (Novick et al., 2004). The annual C balance of the two neighbouring sites showed similar responses to climate variability during three years of flux measurements. Despite differences in the seasonal patterns between the two sites, both sites were C sinks in 2004, a small C source in 2005 and a small sink in 2006 (Tab. 3). We found increasing GPP and a built up of A_{max} with vegetation growth to follow climatic factors like temperature and radiation in spring and early summer. However, water stress period starting in June reduced A_{max} even before standing biomass was disturbed by mowing or grazing (Fig. 3). To some extent this loss is reversible, e.g. in August and September 2006. High precipitation in August 2006 led to a significant resprout in vegetation at both sites with increasing GPP, A_{max} and NEE (Fig. 3 and 9). Coupled to GPP, soil respiration was found to be not only influenced by precipitation amounts, but also by the rainfall timing, corresponding with the results from Harper et al. (2005). Irregular precipitation patterns decreased soil respiration and GPP.

Recent climate trends for central Germany indicate that large parts of the low land areas between North Thurigia, Lower Saxony and Brandenburg faced a precipitation reduction of between 30-50% during the growing season (Franke and Köstner, in press). 42% less precipitation during July and August 2005 compared to 2004 and 2006 correspond to an additional C loss of 67-137 g C m^{-2} at the grassland and 11-45 g C m^{-2} at the afforestation site, when the C balance from July to September over the three years is compared. Thus, summer droughts may cause C losses from grass-dominated systems (Ciais et al., 2005; Novick et al., 2004). On a global view across different ecosystems, NEE corresponds with the duration of the growing season (Churkina et al., 2005). Starting the vegetation 10 days earlier on both study sites increased the systems C uptake by 5 - 9 g m^{-2}. However, an earlier start to the growing season may also shift the start of the soil water limitation period to an earlier date. Sousanna et al. (2007) found no relation between the length of the growing season and the number of days with net CO_2 uptake. In our study, the end of the growing season, seen as the period with plant activity, was not related to climatic factors such as temperature, but was dependent on water availability. Thus, the C balance of the two sites depended more on precipitation amounts and pattern during the growing season than on other climatic drivers like radiation, air temperature or the duration of the growing season

(Suyker et al., 2003). However, the long-term effect of climate change on grass-dominated systems, such as summer droughts, cannot be deduced from this three year measurement period.

4.2 Impact of afforestation-associated changes in site management on the C fluxes

Flux measurements from two nearby eddy covariance towers, one on a control plot with "business as usual" (grassland) and one on a plot with land use change treatment (afforestation), allowed detecting the influence of land use change (site preparation, early stage of afforestation) without confounding influences by climate variability. More than 95% of total C stocks of Central European grasslands are stored in soils (Nöllert, 2003). Transfer of plant biomass into soil organic carbon and a possible mobilisation and depletion of soil C stocks determine the C balance of new forests established on grasslands. Most afforestation studies found decreasing soil organic carbon (SOC) stocks during the early stage of afforestations (summarized by Paul et al., 2002; Post and Kwon, 2000). Short term effects on SOC were found to be particularly high in clay rich soils like at the Mehrstedt site, since large parts of SOC might be protected in aggregates or organo mineral complexes and are released during site preparation. We found enhanced mineralisation and SOC mobilisation on the afforestation compared to the grassland only for 14 months after site preparation with ploughing of planting rows (autumn 2003). A net C loss of 122 g m^{-2} in spring and autumn 2005 was a combined effect of mobilisation of soil carbon in the disturbed planting rows and a drought induced breakdown of the vegetation. Flux differences between the two sites in the second and third year do not indicate enhanced C losses from the afforestation site compared to the grassland. Thus, C loss during the first 3 years of the afforestation was equal to about 1.4% soil organic C stocks. This is two to three times less than reported in other studies (Paul et al., 2002). Reduced soil disruption caused by only ploughing planting rows and reduced turnover of soil organic C buried into the subsoil with deep ploughing are two reasons for minimized C loss on this afforestation compared to other studied afforestation sites.

Vegetation removal on the planting rows was the major reason for reduced GPP on the afforestation site. Thuille and Schulze (2006) found that the main reason for decreasing C stocks in mineral soils was the reduced C input from non woody vegetation during transition from grassland to forest. Three years after establishing the afforestation, NEE was similar at both sites even though GPP was 14% less at the afforestation site than at the grassland site. Mulching and plugging of the senescent grasses on the non-mowed planting rows may have additionally contributed to decreased productivity on the afforestation site. The planted trees did not exert a significant impact on the C balance, as C accumulation in tree biomass during plant growth was compensated by C loss from dead seedling which comprised around 40% of the planted trees (Don et al., 2007). Standing tree biomass was only 2% of the total standing biomass during the growing season.

Seasonal patterns in the differences of the NEE fluxes between the two sites were closely linked to the mowing and grazing events. High C sequestration rates on the grassland site in spring and early summer were stopped by the grazing events (Fig. 9 and 10). GPP and NEE were reduced by mowing and grazing, however recovery was the more successful the earlier in the year the disturbance took place. Increasing sun light availability in the first half of the year made it energy efficient for plants to invest into new photosynthetic active tissue (Parsons and Chapman, 2000). Grazed plants generally produced younger leaves with higher photosynthetic capacity; thus, grazed prairie did not sequester less C than ungrazed (LeCain et al., 2002; Owensby et al., 2006). Additionally, vegetation shifts from grasses to herbs and rudural species on the afforestation can reduce the drought resistance and the capacity to recover from drought stress. The grasses locating of the apical merisitem close to ground and below ground compared to herbs is an adaptation to fast regrowth after disruptions such as grazing or drought (Owensby et al., 2006).

Our study confirms the close linkage between soil respiration or TER and GPP in grass and herb dominated systems (Craine et al., 1998; Owensby et al., 2006; Raich and Tufekcioglu, 2000; Wan and Luo, 2003). On a tallgrass prairie a reduction in above ground biomass did reduce soil respiration by 21 to 49% by the second day after clipping, even though soil temperature increased by 3°C (Bremer et al., 1998). Soil efflux was influenced by clipping for the rest of the growing season and the following winter season. In another study of a tall grass prairie, 56% of soil respiration was found to derive from soil organic carbon (Wan and Luo, 2003). Thus, recent assimilated C determines soil respiration to a large extent.

On longer, seasonal time scales TER and GPP were also correlated. The maximum cumulative C sink in early summer (Fig. 8) was 56-72 g C m^{-2} higher at the grassland site over the three years due to higher productivity on this site compared to the afforestation.

Within three weeks, most of this additional assimilated C was respired again due to enhanced C turnover after a sheep grazing event. Biomass left on the site as faeces after sheep grazing can be mineralised with higher rates than the standing biomass. Soil surface temperature was increased by up to 2.5°C for a short period after grazing, which may have stimulated soil respiration. Direct measurements of soil respiration could not detect accelerated soil organic carbon mineralisation after grazing. Soil respiration seems to be dominated by the autotrophic respiration where recently assimilated C is respired via roots and root exudates (Bremer et al., 1998). Additionally, soil and vegetation disruption caused by sheep trampling may enhance C turnover. After sheep grazing in November 2005 and 2006, higher TER/GPP ratios at the grassland site compared to the afforestation indicate that sheep grazing accelerated C turnover in the system. On the other hand, sheep grazing may stimulate productivity on the grassland site by quickly recycling nutrients and compensatory growth (Parsons and Chapman, 2000) and C input into the soil via roots (Jones and Donnelly, 2004). Higher GPP together with similar standing peak biomass at the grassland site compared to the afforestation indicate that grazing may shift the shoot-root ratio, with more below ground biomass on the grassland site.

4.3 The annual C balance

Cumulative NEE, the annual C balances, were strongly influenced by climatic variability and modified by different land use at the two investigated sites. We found NEE on both sites to be close to zero in 2005 and 2006 and a C sink of around 600 g C m^{-2} at the grassland in 2004 (Tab. 3). Average grassland C sinks of nine European grasslands was 104 ±73 g C m^{-2} a^{-1} (Soussana et al., 2007). However, these sites were managed more intensively than the sites in this study with only 45 to 75 g C m^{-2} a^{-1} C export by grazing. Several flux studies found grasslands to be C neutral, most of them conducted in native grasslands (Ammann et al., 2007; Flanagan et al., 2002; Li et al., 2005; Owensby et al., 2006). C export with harvested biomass partly offset the negative NEE (C sink) measured with eddy covariance. A negative correlation between harvest and C- intake by grazing and C sinks was found for grasslands (Soussana et al., 2007). However, negative effects of C export on the C balance of grasslands may be restricted to intensively managed grasslands.

Annual C balances derived from eddy covariance measurements are sensitive to three types of errors (Ammann et al., 2007; Goulden et al., 1996): i) uniform systematic errors, ii) selective systematic errors that occur under selective environmental conditions and

iii) sampling uncertainties due to data gaps. Some systematic errors level off, when fluxes from the two towers are compared because instrumentation and data treatment for each tower was identical. A selective systematic error was created by excluding certain wind directions at the afforestation site to restrict the fluxes to the area of interest. The same exclusion was carried out for fluxes at the grassland, even though their source area was not affected by different land use. With this exclusion, certain weather conditions are systematically under represented in the data sets. GPP and TER were only slightly effected by this exclusion with a <5% error at the grassland site. NEE was almost the same (error <1%) with and without wind direction exclusion in 2004 but had an offset of 18% in 2005 and 13% in 2006. However, when fluxes from both towers are compared this systematic error should level off.

When sheep were close to the flux tower, basic assumption on stationarity and homogeneity (surface roughness and fluxes) of the footprint were violated. Thus, respiratory fluxes from the sheep during grazing periods would have to be measured indirectly to derive the fraction of C remaining in soil (=NBP) from NEE. Only a small fraction of 3% grazed biomass C is assimilated and incorporated as meat and other sheep products. Most of the assimilated C is respired directly with a biomass intake fraction between 0.5 and 0.8 (Garcia et al., 2003). Grazed biomass was estimated as the difference between above ground biomass of grazed and caged (ungrazed) plots directly after grazing events. Estimates of the C export during grazing are difficult and suffer from systematic errors (Li et al., 2005; McNaughton et al., 1996). The error of grazed biomass was 70-80% due to Taylors errors propagation law which increases the relative errors of differences of two relatively similar numbers. The error of the grassland C balance cannot be reduced without improved estimates of the C export during grazing.

5. Conclusions

- Paired measurements with two flux towers in close vicinity allow investigating the impact of land use change without confounding factors of annual climate variability.
- During the first year, 1.2 t C ha^{-1} was lost due to the afforestation of the former grassland site; most of the lost C derived from enhanced mineralisation of soil organic carbon. Even though GPP at the afforestation did not reach the same level as the

grassland, the C balance consolidated quickly with no further C loss compared to the grassland in the second and third years.

- Sensitivity of grass dominated ecosystems on limestone increased during the year with decreasing soil water content. Management of the two investigated sites determined the short term NEE. On an annual basis, differences between both management types seem to level off due to a strong linkage between ecosystem respiration and ecosystem assimilation.
- Climate factors like precipitation during the growing season where most dominant in determining the NEE balance of the two sites. Increasing frequencies of drought periods during summer are most likely to shift grass dominated systems from C sinks to C sources.
- The fate of biomass C grazed by sheep or cattle needs to be further constrained with complete farm yard C budgets to understand the role of grassland ecosystems for the global C cycle.

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Spatial and vertical variation of soil carbon at two grassland sites – implications for measuring soil carbon stocks

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Axel Don[1], Jens Schumacher[1, a], Michael Scherer-Lorenzen[1, 3], Thomas Scholten[2], Ernst-Detlef Schulze[1]

1) Max Planck Institute for Biogeochemistry, Hans-Knöll Strasse 10, 07745 Jena, Germany
2) University of Tübingen, Institute of Geography, Rümelinstraße 19-23, 72070 Tübingen, Germany
3) Institute of Plant Sciences, ETH Zürich, Universitätsstr. 2, 8092 Zürich, Switzerland
a) Present address: Institute for Probability, Friedrich-Schiller-University Jena, Ernst-Abbe-Platz 2, 07743 Jena, Germany

Abstract

Soil organic carbon (SOC) stocks are a function of the SOC concentration and the bulk density of the fine soil. Both variables are prone to changes and are influenced by abiotic and biotic processes. To determine the effect of variations of both variables on SOC stocks at two grassland sites, one with Stagnic Vertisols and one with Orthoeutric Arenosols, 440 soil cores were sampled down to 60 cm depth. Bulk density and C and N concentrations were measured in 5 cm intervals (0-10 cm depth) and 10 cm intervals (10-60 cm depth). SOC stocks at the clay rich site with Vertisols were almost twice as high (86 t C ha^{-1} in 0-60 cm depth) as at the sandy site with Arenosols (48 t C ha^{-1}). Variations in the SOC stocks were determined by the thickness of the loess layer at the clay rich site. Underlying clay horizons hampered the C translocation into the subsoil which resulted in lower SOC stocks of the whole profile. Semivariograms showed spatial autocorrelations of SOC concentrations within a range of 47 and 131 m, respectively. The range of autocorrelation between samples of bulk density was much shorter (39 and 51 m). Relative variances in bulk density were 1-2 magnitudes lower than the variability of SOC concentration and decreased rapidly as soil depth increased. The difference in the variation of SOC concentration and bulk density was used to revise the sampling design for SOC stocks. An unequal number of samples, i.e. more SOC concentration samples than bulk density samples, would not necessarily decrease the power of the sampling design to detect SOC stock changes. In contrast, the optimum sampling design for these sites would consist of 33-44% bulk density samples and 56-67% SOC concentration samples.

Keywords: soil organic carbon stocks, spatial variability, bulk density, geostatistics, grassland

1. Introduction

The soil is a key component of the global carbon cycle with 1500-2000 Pg of organic carbon stored in its upper 100 cm (Schulze, 2006). Soil organic carbon (SOC) stocks may be very sensitive to climate change, having a negative feedback which could enhance global warming (Bellamy et al., 2005). The biological terrestrial carbon sink may be offset by a slight shift in the fragile balance between C input and C mineralization/export via seepage water. Such small shifts are difficult to detect due to the high spatial variation of SOC stocks. SOC stocks in grasslands are similar to that in forests with ca. 70 t C ha^{-1} in 0-30 cm depth estimated for France (Arrouays et al., 2001). Model calculations assume most European grasslands to be C sinks with an average sequestration rate of 0.5 t ha^{-1} which is <1% SOC stock change per year (Vleeshouwers and Verhagen, 2002). However, there is a lack of data on SOC stocks changes in grasslands to validate these findings. Additionally, sampling soil profiles is technically difficult and sample preparation for analysis is time consuming. Thus, most studies on soil carbon were restricted to the upper 15 to 30 cm of the soil and only few include deeper sections of the soil cover (Conant and Paustian, 2002). However, in temperate climates large amounts of SOC may be stored in subsoil horizons below 30 cm depth (Lorenz and Lal, 2005). Jobbagy and Jackson (2000) reported that in temperate grasslands 59% of SOC is located below 20 cm depth in the first meter of the soil. C stock changes due to shifts in species composition of grasslands, which lead to changes in root architecture may not be detectable without deep (>30 cm) sampling (Conant and Paustian, 2002). The sensitivity of the subsoil C pool to climate change is unknown. However, minor shifts in these stocks will have considerable impact on the entire C balance.

SOC stocks are determined from two variables, namely i) SOC concentration and ii) bulk density, which are both prone to changes over time. To measure C sequestration in soils based on repeated measure designs, it is necessary to estimate both variables each time. Many studies in the past did not include bulk density measurements (Arrouays et al., 2006; Bellamy et al., 2005; Collins et al., 2000; Hassink, 1994; Jones et al., 2005). Obviously, these studies can only give a first approximation to quantify the effect of C sequestration in soils.

SOC concentrations are closely linked to biotic processes like biomass production, decomposition and the placement of above-ground litter and root litter in and onto the soil. Bulk density is mainly a function of the parent material, soil genesis as well as soil aggregate formation and may change due to land use changes, erosion, seasonal wet/dry cycles and anthropogenic perturbation by tillage and compaction (Lal and Kimble, 2001). For instance, bulk density in a grassland soil changed up to 11% due to land use changes and management changes (Conant et al., 2001). Thus, the scale and amplitude of spatial and vertical variation of the two variables may be different, even though SOC concentration and bulk density are negatively correlated with each other in many soils (Huntington et al., 1989). This correlation has been used widely to estimate bulk densities from SOC concentrations (Howard et al. 1995, Crowe et al. 2006).

SOC stock changes during short time periods, like the Kyoto commitment period 2008-2012, are especially difficult to estimate on the relevant national and global scales (Smith, 2004). Knowledge about the variability of SOC concentration and bulk density of major soil types on a plot scale can assist in generating effective sampling designs and can help to scale-up carbon inventories. The spatial variability of both variables determines how many samples are needed to detect a certain SOC stock change. Therefore, the aim of this study was to

i) investigate the spatial and vertical variation of SOC concentrations, bulk density and SOC stocks at two grassland sites with contrasting geology and loess cover thickness but similar land use history,

ii) deduce implications from the data sets for an effective sampling design for soil organic carbon stocks.

2. The two study sites

This study was conducted at two extensively managed grassland sites, Mehrstedt (10°39´ E, 51°17´ N, 270-290 m a.s.l.) and Kaltenborn (10°14´E, 50°47´N, 320-350 m a.s.l.). Both sites belong to the BIOTREE project and are situated in Thuringia/Germany within 70 km distance of each other. Both sites were used as arable land until 1980 (Mehrstedt) and 1975 (Kaltenborn) and were later used for sheep grazing. They were occasionally mown once per year. Mean annual temperatures at both sites were around 8.0°C with a slightly higher annual precipitation of 650 mm at Kaltenborn compared to 550 mm at Mehrstedt.

Table 1: Soil parameters of a representative profile at the Mehrstedt site (Stagnic Vertisol). Texture, pH, CEC (Cation exchange capacity), BS (base saturation) and amount of pedogenic iron oxides (Fe_d, dithionite extractable and Fe_o, oxalate extractable).

depth	sand	silt	clay	pH	CEC	exchangable cations								BS	Fe_d	Fe_o
[cm]	[%]	[%]	[%]	(KCl)	[$cmol_c$ kg^{-1}]	H^+	Al^{3+}	Fe^{2+}	Mn^{2+}	Ca^{2+}	Mg^{2+}	K^+	Na^+	[%]	[g/kg]	[g/kg]
0-9	2.9	61.8	35.2	6.95	27.6	<0.1	0.4	0.1	0.6	196	66	13.0	<0.1	99.6	14.9	2.6
9-17	2.4	63.4	34.1	6.78	27.0	<0.1	0.5	0.2	0.8	194	67	7.3	0.1	99.5	16.3	2.8
17-36	3.4	60.5	36.1	6.85	30.8	<0.1	0.7	0.2	0.4	220	81	5.5	<0.1	99.6	9.9	2.0
36-46	5.2	42.7	52.1	7.03	45.8	<0.1	0.9	0.3	0.1	342	109	5.8	0.3	99.7	8.3	1.6
46-61	1.6	28.0	70.4	7.16	49.9	<0.1	1.0	0.3	0.2	401	91	5.2	0.3	99.7	9.3	1.6
61-80	2.4	31.3	66.3	7.35	48.0	<0.1	0.5	0.2	0.2	396	79	3.6	0.5	99.8	11.1	4.4
80-100	1.5	30.4	68.1	7.37	42.3	<0.1	1.0	0.3	0.3	330	88	3.2	0.3	99.6	9.9	0.7

The bedrock in Mehrstedt consisted of limestone with a variable loess cover on which Stagnic Vertisols (eutric sillic) developed. Soil texture was dominated by clay which led to a characteristic shrinking and swelling dynamic resulting in prismatic aggregates in the deeper horizons (Tab. 1). In at least the deeper horizons the soil was partly influenced by carbonate, resulting in a high pH. Cation exchange capacity (CEC) was high with 27-50 $cmol_c$ kg^{-1} accompanied by a base saturation of almost 100% (Tab. 1). The vegetation was composed of herbs and grasses with above-ground peak biomass of 542 g dry mass DW m^{-2} ± 38 g m^{-2} (mean ± standard error s.e.) in 2004. The peak biomass is a proxy for the above-ground net primary production.

Table 2: Soil parameters of a representative profile at the Kaltenborn site (Orthoeutric Arenosol). Texture, pH, CEC (Cation exchange capacity), BS (base saturation) and amount of pedogenic iron oxides (Fe_d, dithionite extractable and Fe_o, oxalate extractable).

depth	sand	silt	clay	pH	CEC	exchangable cations								BS	Fe_d	Fe_o
[cm]	[%]	[%]	[%]	(KCl)	[$cmol_c$ kg^{-1}]	H^+	Al^{3+}	Fe^{2+}	Mn^{2+}	Ca^{2+}	Mg^{2+}	K^+	Na^+	[%]	[g/kg]	[g/kg]
0-8	80.5	10.8	8.7	5.0	3.6	0.4	3.0	0.1	0.2	25.3	4.4	2.3	0.8	90	2.5	0.5
8-27	80.7	10.3	9.0	4.5	5.8	0.2	2.9	0.3	0.1	44.2	6.4	3.3	0.7	94	1.6	0.8
27-38	78.3	13.8	8.1	4.7	3.6	<0.1	3.0	0.1	0.2	25.3	4.4	2.3	0.8	91	2.2	1.2
38-50	76.1	17.5	7.8	4.3	2.1	0.1	4.1	<0.1	0.2	12.7	1.6	1.7	0.6	79	1.9	1.1
50-61	77.1	16.2	6.7	4.2	2.2	0.2	4.2	<0.1	0.1	13.2	1.9	1.5	0.6	79	1.6	1.5
61-75	78.7	13.9	7.4	4.4	2.0	<0.1	2.9	<0.1	0.1	14.7	2.3	2.3	0.6	97	1.9	1.2
75-105	79.6	13.7	6.8	4.2	3.5	<0.1	7.3	0.6	0.2	19.9	2.1	3.4	1.1	75	1.9	0.9
105-117	78.5	13.7	8.5	4.4	2.8	<0.1	1.5	<0.1	0.4	21.0	1.8	3.4	0.4	94	3.2	1.1
117-135	79.3	13.3	7.4	4.5	4.3	<0.1	0.3	<0.1	0.7	31.1	7.0	3.7	0.5	98	2.5	1.0

At the Kaltenborn site, Orthoeutric Arenosols developed on sandstone from the lower German Triasian. In all horizons sand content was >75% with a loamy sand texture. There was no detectable loess cover (Tab. 2). The soil was characterised by a CEC 10 times lower than that of the Mehrstedt site and $pH_{(KCl)}$ values between 4.2 and 5.0. Above-ground peak biomass of the sampling area was similar to the Mehrstedt site with 539 g DW m^{-2} ± 50 g m^{-2}. Stone content at both sites was low (Tab. 3).

Table 3: Mean SOC stocks, carbonate concentrations and stone contents at the Mehrstedt and Kaltenborn sites derived from the soil core samples. Standard error SE is given in the second column.

depth	SOC stocks [t ha^{-1} 10cm $depth^{-1}$]				Carbonate [%]		Stone content [mass %]			
[cm]	Kaltenborn		Mehrstedt		Mehrstedt		Kaltenborn		Mehrstedt	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
0-5	16.39	0.32	28.67	0.35	1.02	0.06	2.34	0.18	3.31	0.11
5-10	15.09	0.27	23.83	0.23	1.07	0.07	3.59	0.26	4.31	0.12
10-20	10.97	0.14	19.54	0.23	1.10	0.07	3.91	0.24	3.92	0.15
20-30	9.56	0.17	16.04	0.21	1.26	0.08	4.96	0.43	4.30	0.18
30-40	4.80	0.17	10.10	0.25	2.14	0.13	9.55	0.92	3.94	0.16
40-50	3.55	0.10	7.40	0.21	3.04	0.15	11.60	1.01	4.29	0.21
50-60	3.29	0.10	6.55	0.25	3.77	0.16	12.03	1.01	5.21	0.26

3. Material and Methods

3.1 Sampling design

The two sites were sampled between October 2003 and November 2004. The Mehrstedt site comprised 17 ha which were sampled in a regular grid (24 m x 24 m) with 18 cores per ha, resulting in a total of 284 soil cores. The Kaltenborn site comprised 6 ha which were sampled in a regular grid (16 m x 24 m) with 25 samples per ha, in a total of 156 soil cores.

Soil cores were taken using an 87 mm diameter soil column cylinder auger, which was hammered up to 60 cm deep into the soil using a fuel powered mechanical hammer (Eijkelkamp, Giesbeek). In some cores, stones and very dense clay horizons inhibited sampling to 60 cm depth. Compaction of the soil column during the sampling procedure was corrected based on the measured length of the soil core and the burrow depth. Compaction was on average 1.5% ±2.1 standard deviation (SD) at the Mehrstedt site and 6.0% ±2.6 SD at the Kaltenborn site. The soil cores were segregated into 0-5, 5-10, 10-20, 20-30, 30-40, 40-50 and 50-60 cm increments and placed in sealed plastic bags. The 440 cores resulted in a total of 2890 samples. There was no permanent organic layer at both sites, thus, sampling was restricted to the mineral soil.

3.2 Soil analysis

Coarse roots, plant and animal residuals (> 1mm) were extracted by hand from the fresh samples. The samples were air dried, weighed and sieved to 2 mm using a sieving machine which also forces very hard clayish clumps through a sieve (Jehmlich GmbH, Nossen). The sieved samples were weighed again to determine the stone mass. Bulk density BD of the fine soil [mass volume^{-1}] was calculated as follows:

$$BD_{finesoil} = \frac{mass_{sample} - mass_{stones}}{volume_{sample} - \frac{mass_{stones}}{\rho_{stones}}} \qquad \text{equ. (1)}$$

ϱ=density of stones [mass volume^{-1}] was approximated by 2.6 g cm^{-3}.

A subsample was dried at 40°C until a constant weight and bulk density was corrected for residual water in the air dried samples. Soil organic carbon (SOC) concentration of the ground samples was determined by measuring total carbon concentration (TC measured with VarioMax, Elementar) subtracting the soil inorganic carbon (SIC) which represents the carbonate-C. SIC was determined after ignition of the ground soil sample at 450°C for 16 h in a muffle kiln. Total nitrogen concentration (N_{tot}) was measured simultaneously with TC and both concentrations were corrected for free water in the air dried sample. SOC stocks [mass C area^{-1}] for a specific soil depth interval were calculated as follows:

$$SOC_{stocks} = \sum_{i=1}^{n} BD_{finesoil,i} \cdot SOC_{conc,i} \cdot depth\_volume_i \qquad \text{equ. (2)}$$

where n is the number of sampled soil depth intervals i, $BD_{finesoil,i}$ and $SOC_{conc\, i}$ represent bulk density and soil organic carbon concentration of the fine soil [mass C mass fine soil^{-1}], respectively, in the soil depth increment i with fine soil volume $depth\_volume_i$.

3.3 Statistical analysis

All measured variates were first characterised by classical descriptive statistics (means and standard error of the mean) without considering the spatial nature of the data. Throughout the paper, error bars and error of mean values (± X) indicate the standard error of the mean (Webster, 2001).

Geostatistical methods have been used to study the spatial variation of soil variables. Semivariograms for fixed distance intervals of 5 m were estimated (Webster, 2001b). Maximum distance was set to 200 m for all semivariograms. A visual examination of the 3D-semivariogram plots exhibited no obvious differences in the spatial structure in any direction, on both sampling sites. We also tested for anisotropy of semivariograms (Webster, 2001b) but did not detect any anisotropic pattern. Thus, all semivariograms are calculated on an omnidirectional basis. The following models were fitted to the estimated semivariograms by nonlinear least squares regression:

Exponential without nugget:

$$\gamma(h) = c\left[1 - \exp\left(-3h/a\right)\right] \qquad \text{equ. (3)}$$

Exponential with nugget:

$$\gamma(h) = c_o + (c - c_o)\left[1 - \exp\left(-3h/a\right)\right] \qquad \text{equ. (4)}$$

h = lag distance [length],

c = variance (sill) [dimension of the variable^2],

a = range [length],

c_0 = nugget [dimension of the variable^2].

Spatial dependencies were described with parameters deduced from these models. Usually, semivariance increases with sampling distance, approaching a constant value called `sill´. The distance at which 95% of the sill c is achieved is the range a. The range indicates the maximum distance where samples are spatially autocorrelated. Ordinary kriging was used as an interpolation method to predict values for soil variables incorporating the spatial dependence structure (Webster, 2001b).

General relative semivariograms $\gamma_{GR}(h)$ were calculated as

$$\gamma_{GR}(h) = \frac{\gamma(h)}{\bar{x}(h)^2} \qquad \text{equ. (5)}$$

where $\bar{x}$ (h) is the mean of all data values that are used to calculate the semivariogram γ(h). Sill values deduced from these relative semivariograms correspond to the squared coefficient of variation (CV= SD/ $\bar{x}$) in classical statistics and are called “relative sill”. These parameters are relative measures of the variability of a variable, independent of the absolute variable values. To compare the variability of vertical profiles between the two investigated sites we used the relative sill. The relative sill is independent of the size of the sampled area.

Geostatistical analyses were performed with the package gstat using R software (R Development Core Team, 2006).

3.4 Power analysis to evaluate different sampling designs

Power analysis was performed to calculate the minimum detectable difference (MDD) of SOC stocks for 0-60 cm depth (Schöning, 2006). In order to assess the SOC stock error, the error propagation of the SOC concentration variance and bulk density variance has to be taken into account. To quantify the effect of different sampling intensities for both variables, we performed a simulation based on the observed data. A constant total sample size $n_{total} = 100$ was apportioned to n_{SOC} (varying from $n_{SOC} = 1$ to $n_{SOC} = 99$) soil cores sampled for SOC concentration and n_{BD} cores sampled for bulk density, i.e., $n_{total} = n_{SOC} + n_{BD}$. We randomly selected n cores from the pool of measured soil cores, where $n = \min(n_{SOC}, n_{BD})$. These selected soil cores constituted the soil samples which were analysed for both variables, SOC concentration and bulk density. The remaining $n_{total} - 2n$ cores were randomly selected representing those soil cores analysed for only one of the two variables (for bulk density if $n_{SOC} < 50$ or otherwise for SOC concentration). In the first run, the value of the missing variable was set equal to the mean value of that variable derived from the first set of soil cores. In a second run, we used the correlation between bulk density and SOC concentrations to derive a linear pedotransfer function which was used to estimate the missing variables for the soil cores were only one variable was measured. SOC stocks for each soil core were then calculated using equation (2) by summing up separate values for each depth increment. The values for the individual soil cores were averaged to yield an estimate of SOC stock for the sampled area. Note that the number of analysed soil cores is smaller than n_{total} and differs for different values of n_{SOC}. The standard error *se* of the estimated average SOC stocks was estimated as the standard deviation of the respective estimates calculated from 10,000 simulation runs. The resulting estimate of the standard error also incorporates the statistical dependency between SOC stock estimates of the individual soil cores and can therefore be used to determine the minimum detectable difference MDD based on the usual approximation formula

$$MDD \geq \sqrt{2}\, se\left(z_{\alpha}^{(2)} + z_{\beta}^{(1)}\right) \qquad \text{equ. (6)}$$

where *se* is the estimated standard error of SOC stock estimates, $z_{\alpha}^{(2)}$ is the two-sided critical value of the normal distribution at a given significance level α ($z_{\alpha}^{(2)} = 1.96$ for $\alpha = 0.05$) and $z_{\beta}^{(1)}$ is the one-sided quartile of the normal distribution corresponding to a probability of type II error β ($z_{\beta}^{(1)} = 0.84$ for $\beta = 0.2$) (Krebs, 1999). We performed these simulations separately for the Mehrstedt and the Kaltenborn site.

4. Results

4.1 Soil carbon concentrations and stocks

SOC stocks at the Mehrstedt site varied from between 57 and 136 t C ha^{-1} for 0-60 cm depth with a mean SOC stock of 86 ±1.7 t C ha^{-1} (Tab. 3). Much lower SOC stocks were found at the sandy Kaltenborn site (48 ±1.3 t C ha^{-1} for 0-60 cm depth) ranging from between 34 and 67 t C ha^{-1} (Tab. 3). SOC concentrations decreased exponentially with increasing depth at both sites with SOC concentrations <1% in all horizons below 10 cm depth at Kaltenborn and below 30 cm at Mehrstedt (Fig. 1A). SOC concentration followed a normal distribution for both sites.

The former arable land use was visible in the SOC profiles of both sites. Mean SOC concentrations at 20-30 cm depth were slightly increased compared to an exponential function as remnant from the former ploughing horizon (Fig. 1A). SOC concentrations at both sites showed spatial dependencies among the neighbouring cores of the sampling grid. The semivariograms indicate a lower variance between neighbouring soil cores (Fig. 2A and 3A).

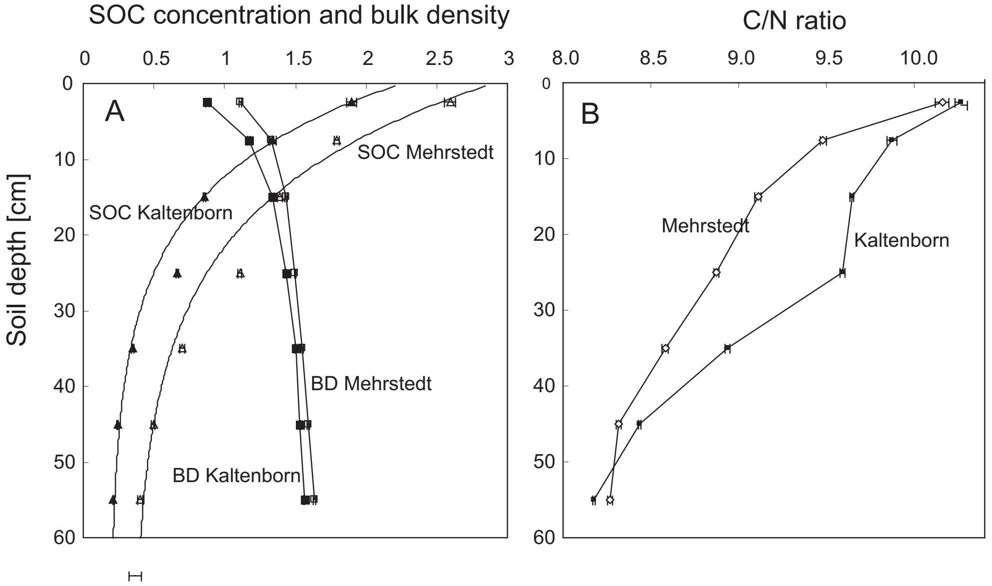

Figure 1: Profiles of (A) the mean SOC concentration [%] with fit of exponential function and mean bulk density of the fine soil [kg dm^{-3}] and (B) mean C/N ratio at the sites Mehrstedt (open symbols) and Kaltenborn (filled symbols). Bars indicate standard errors.

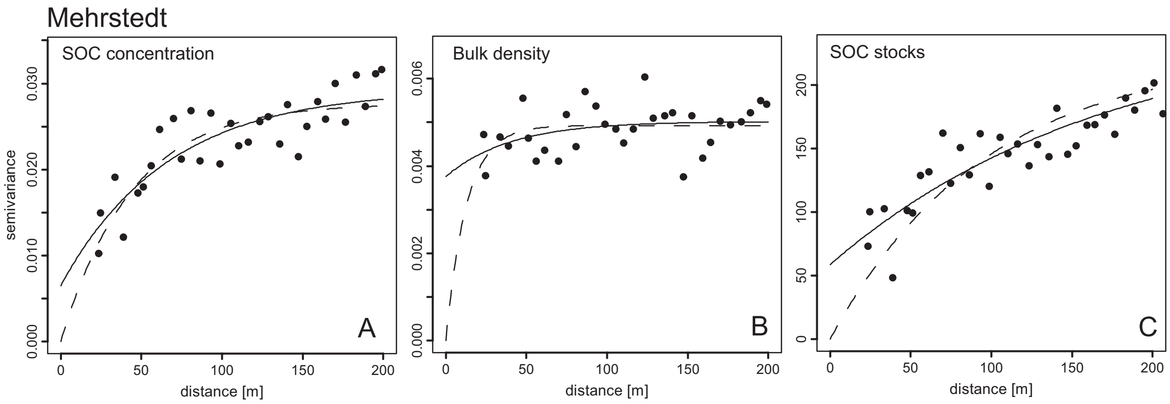

Figure 2: Semivariograms of mean SOC concentration (A), mean bulk density (B) and SOC stock (C) for 0-60 cm depth at the Mehrstedt site. Fit with exponential model with (continuous line) and without nugget (dashed line).

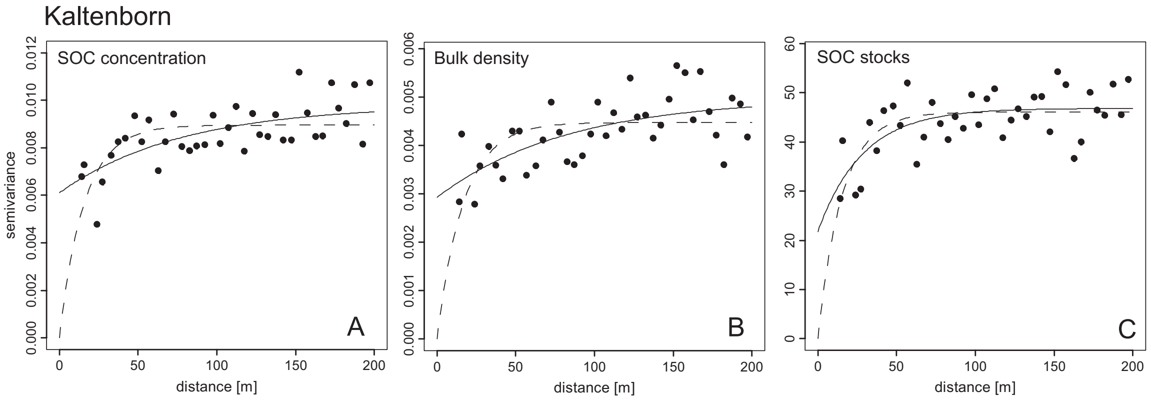

Figure 3: Semivariograms of mean SOC concentration (A), mean bulk density (B) and SOC stock (C) for 0-60 cm depth at the Kaltenborn site. Fit with exponential model with (continuous line) and without nugget (dashed line).

The range at the two grassland sites spanned from 47 to 233 m with a longer range for the models with nugget than without nugget effect (Tab. 4). The semivariograms show that at the Mehrstedt site spatial autocorrelation of SOC concentration was stronger than at the Kaltenborn site. The nugget/sill ratio as a measure of spatial correlation was 0.22 at Mehrstedt and 0.62 at Kaltenborn. A high nugget/sill ratio indicates a high degree of small scale variation and low spatial autocorrelation.

Table 4: Parameter of the semivariograms for the variables mean SOC concentration [%], mean bulk density [g cm^{-3}] and SOC stock [t ha^{-1}] estimated with an exponential model (Exp) and an exponential model with nugget (Exp+Nugg) for 0-60 cm soil depth. Nugget/sill ratio is the relative proportion of the nugget from the sill.

		Mehrstedt		**Kaltenborn**	
		Exp	Exp+Nugg	Exp	Exp+Nugg
SOC conc.	Sill c	0.0277	0.0293	0.0090	0.0098
	Range	131.2	198.8	47.3	233.1
	Nugget c_o		0.0065		0.0061
	Nugget/Sill		0.22		0.62
Bulk density	Sill c	0.0049	0.0050	0.0045	0.0050
	Range	38.8	121.5	51.0	247.1
	Nugget c_o		0.0038		0.0029
	Nugget/Sill		0.74		0.58
SOC stock	Sill c	223.7	248.3	46.1	46.8
	Range	284.6	514.4	46.5	85.9
	Nugget c_o		58.6		21.8
	Nugget/Sill		0.24		0.47

For the depth 0-20 cm there was no difference in the relative variance (sill of the general relative semivariogram) between the two sites. However, for the whole profile up to 60 cm depth, the sill was 10 times higher at the Mehrstedt site than at the Kaltenborn site. The profiles of the relative sill differ remarkably between the two sites (Fig. 4A): At the Kaltenborn site the relative variability of the SOC concentrations seems to be constant within the profile with a higher sill in the depth interval 30-40 cm and slightly higher sills for the two deepest depth intervals. The relative variability of SOC concentrations at the Mehrstedt site is rather constant within the upper 30 cm of the profile but increases continuously in the deeper horizons (Fig. 4A). The same pattern was observed for the variance of N_{tot} concentrations (not shown).

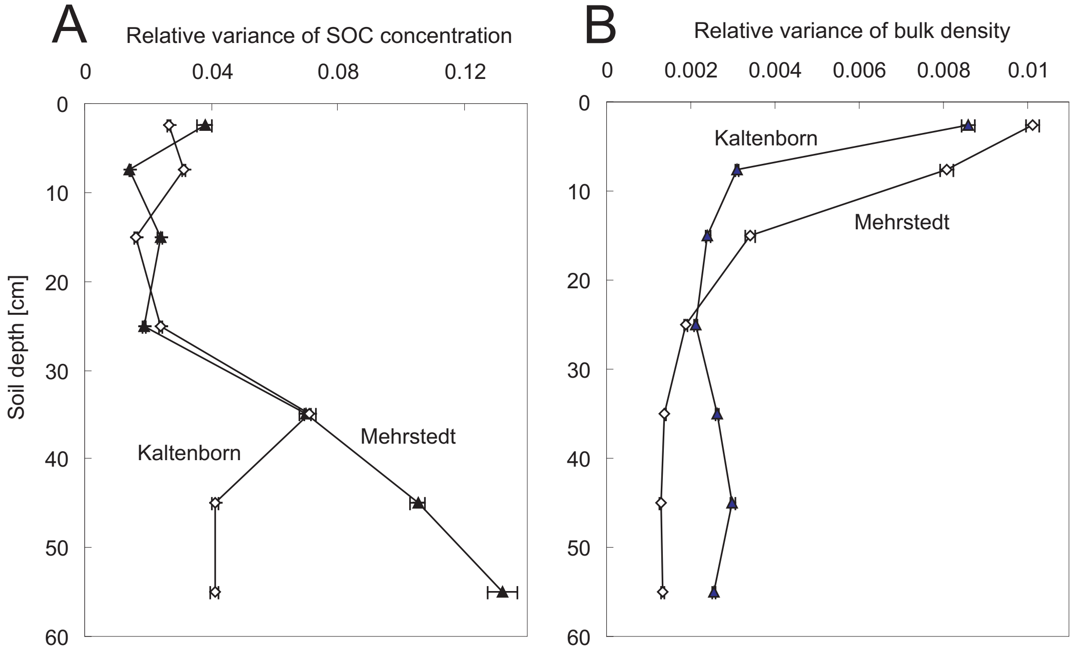

Figure 4: Profiles of the relative variance (sill) deduced from general relative semivariograms with an exponential model for SOM concentrations (A) and bulk density (B) at the sites Mehrstedt and Kaltenborn. Error bars are the standard error of the means deduced from model fit of the general relative semivariograms.

Inorganic carbon as carbonate was only present at the Mehrstedt site with mean carbonate stocks being more than twice as high as SOC stocks (195±11 t carbonate-C ha^{-1} for 0-60 cm depth compared to 86 t organic-C). Carbonate concentrations within the relict ploughing horizon Ap were low showing almost no depth gradient. Carbonate content increased steadily below the former Ap horizon. However, carbonate was distributed irregularly in patches (Fig. 5). Some patches were even carbonate free up to 60 cm sampling depth. These patches did not correspond to the topography of the site but were unpredictable from surface investigations.

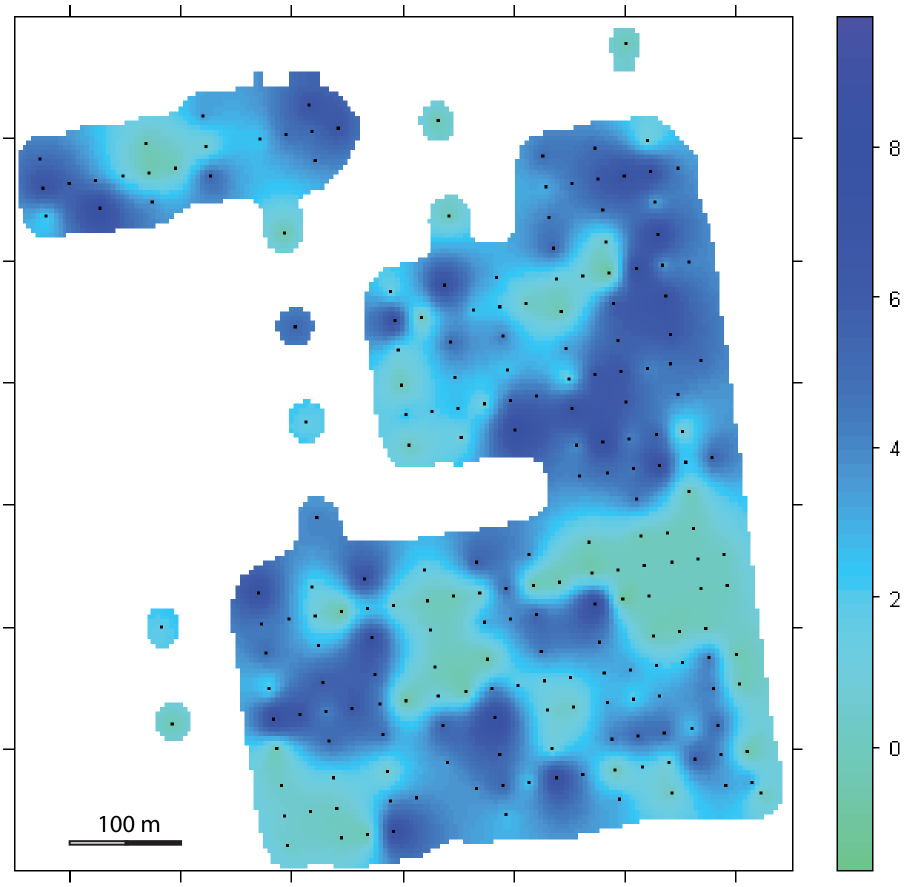

Figure 5: Ordinary kriging of the carbonate concentration [%] in 40-50 cm soil depth at the Mehrstedt site. Sampling points are marked with black dots.

Patches with high carbonate content were characterised by high clay content and higher bulk density (Fig. 6). Carbonate free patches originated from the loess cover which had been decalcified over time. They contained more SOC than clay and carbonate rich profiles, especially in the subsoil (Fig. 6).

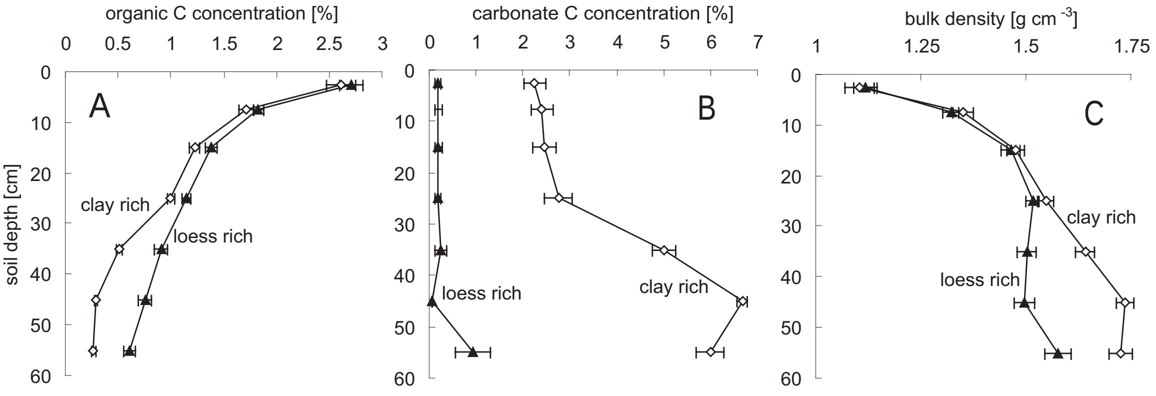

Figure 6: Mean SOC (organic-C) (A), and carbonate-C concentration (B) and bulk density (C) of selected profiles at the Mehrstedt site of luvisols with low (filled symbols) and vertisols with high carbonate content (open symbols).

4.2 Bulk density of the fine soil

Bulk density of the fine soil increased inversely to the decrease of the SOC concentration with increasing soil depth (Fig. 1A). Bulk density was higher throughout the entire profile at the Mehrstedt site compared to Kaltenborn, which is opposite the expected trend. No influence in either sites bulk density was detected from the former cropland management. Below 40 cm depth, bulk density was approaching a site specific constant high value.

Semivariograms showed a weak spatial dependency at both sites (Fig. 2B and 3B). Spatial models described the semivariogram more adequately than the pure nugget model, which assumes no spatial dependency. Nevertheless, the nugget/sill ratio was higher at Mehrstedt (0.74) than at Kaltenborn (0.58) indicating a weaker spatial dependency. For some depth intervals, especially at Mehrstedt, it was impossible to fit exponential or spherical models with a nugget due to low spatial dependencies. There may have been lower variance between neighbouring samples on a small scale of <20 m distances which was not detected by our sampling grid.

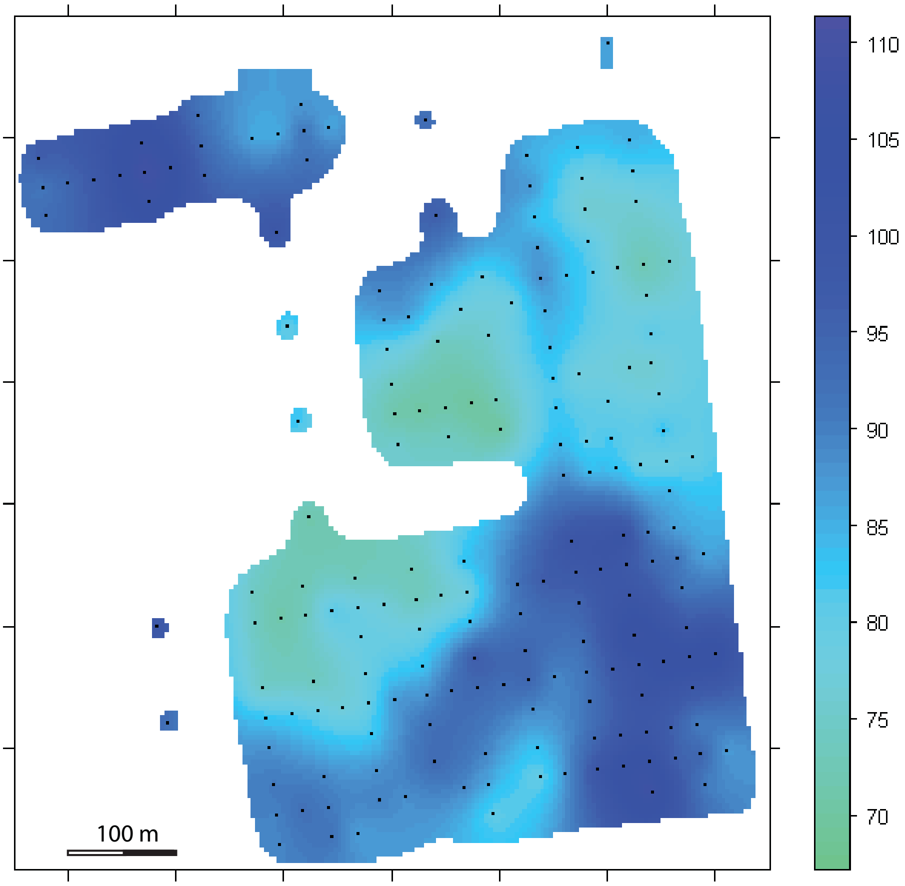

Figure 7: Ordinary kriging of SOC stocks 0-60 cm depth for the Mehrstedt site [t C ha^{-1}]. Sampling points are marked with black dots.

General relative semivariograms for both sites showed that the relative variability of the bulk density strongly depends on the soil depth (Fig. 4). The relative sill of the bulk density was low for the depth intervals below 20 cm depth and increased sharply by a factor 3-5 in the upper 20 cm of the soil. The relative bulk density variance was magnitudes lower (0.001) than the SOC concentration variance for the whole soil profile (0.01 Kaltenborn and 0.11 Mehrstedt), which is important for the optimum SOC sampling design.

Bulk density was negatively correlated with SOC concentrations with a correlation coefficient R between 0.36 and 0.50 for different soil depth increments at the Mehrstedt site and between 0.35 and 0.58 for depth increments of the surface soil at Kaltenborn. In the subsoil (>30 cm depth) at the Kaltenborn site correlations between bulk density and SOC concentrations were not significant ($0.01 \leq R \leq 0.08$). Correlations were used to derive pedotransfer functions to decrease the uncertainty of the SOC stocks estimates in the simulation model described below.

4.3 C/N ratio of the organic matter

Soil C/N ratios depend on the plant species of the above and below-ground litter, the nutrient status of the site and the degradation level of the organic matter (Prescott, 2005; Vestgarden, 2001). In general, C/N ratios strongly decreased from values ≈10 in the upper layers to ≈8.3 in 50-60 cm depth. The SOC to N_{tot} concentration plot showed a linear relationship between both variables for each separate depth interval (not shown). Intercepts for linear regression between both variables were never significantly different from 0. Thus, N_{tot} is almost entirely organically bound. Profiles of the C/N ratio of the two sites were distinct from each other with a clear ploughing effect visible only at the Kaltenborn site (Fig. 1B). Fresh organic matter with a higher C/N ratio left its fingerprint in the upper 10 cm of the soil profiles. Lower C/N ratios throughout the whole profile at the Mehrstedt site compared to the Kaltenborn site indicate a higher proportion of stabilised highly degradated organic matter.

4.4 Minimum detectable difference of SOC stocks using different sampling designs

The minimum detectable difference (MDD) of SOC stock changes is a measure for the effectiveness of a sampling design to detect C sequestration rates based on repeated SOC stock inventories. MDD is proportional to the standard deviation of SOC stocks and the standard deviation of SOC stocks is determined by the variance of the SOC concentration and the bulk density (equ. 2). The higher the variance of a variate, the more samples are needed to estimate this variable with equal precision. For our study, SOC concentration and bulk density were measured at each measurement point, thus, the sample amounts of each variable were the same. But similar sample amounts for SOC concentration and bulk density did not result in the highest statistical power: With a simulation of different sample intensities of SOC concentration and bulk density we found minimum MDD when SOC concentrations were sampled more often than bulk density (65 sample cores SOC concentration and 35 bulk density sample cores at the Mehrstedt site without using the pedotransfer function and 64/36 with pedotransfer function; 56/44 without and 68/32 with pedotransfer function at the Kaltenborn site) (Fig. 8).

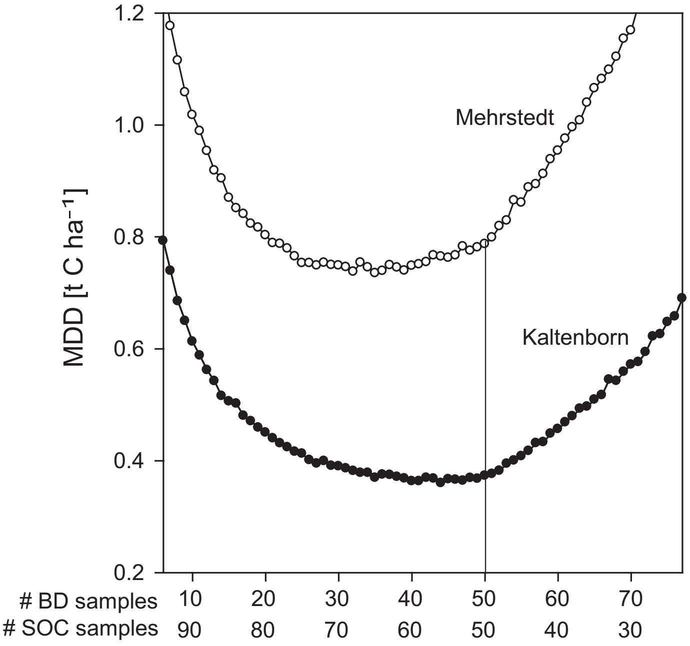

Figure 8: Minimum detectable difference (MDD) of SOC stocks for 0-60 cm depth [t C ha^{-1}] depending on the number of SOC concentration and bulk density samples for Mehrstedt (rhombus symbols) and Kaltenborn (triangle symbols) without (open symbols) and with pedotransfer function from regression between SOC concentration and bulk density (filled symbols). Constant total sample number $n_{total} = 100$.

MDD of similar sample amounts for bulk density and SOC concentration (50/50) was 7% and 11% higher at the Mehrstedt site and 4% and 7% higher at the Kaltenborn site compared to the optimal design without and with pedotransfer function respectively. The inclusion of the correlation between SOC concentration and bulk density (pedotransfer function) decreased the MDD on average by 4% in Mehrstedt and 7% in Kaltenborn.

The reduced standard deviation of SOC stocks with this design can be used to either increase the precision of the SOC stock estimate or to reduce the sample number. In this example, sample core amounts could be reduced from 100 to 81 samples at the Mehrstedt site and to 88 samples at the Kaltenborn site. Thus, bulk density sample amounts can be reduced by about 30% at both sites compared to equal sample amounts for both variables without higher MDD when they are substituted by SOC concentration samples. At both sites there is a range from 30/70 to 50/50 sample ratio bulk density/SOC concentration with MDDs that are only slightly above the minimum MDD. However, extreme unevenness of the sampling ratio bulk density/SOC concentration (< 10/90 and > 90/10) resulted in exponentially increasing MDD in both directions (Fig. 8).

Thus, if bulk density sample amounts drop below a certain threshold, the uncertainty of the SOC stock estimation increases rapidly: If only 5 bulk density and 95 SOC concentration samples had been collected, MDD would have increased by 87% at the Mehrstedt site and by 138% at the Kaltenborn site. With only one bulk density sample for each site, the uncertainty of the SOC stock would be more than 4 times higher in Mehrstedt and more than 5 times higher in Kaltenborn compared to the optimal sampling design.

5. Discussion

5.1 The stocks

SOC stocks at the clay rich Mehrstedt site were almost twice as high as at the sandy Kaltenborn site. This is most likely due to effective stabilisation mechanisms of clay (Burke et al., 1989; Leifeld et al., 2005). Inaccessibility of C in aggregates and micopores and adsorption on clay surfaces are acknowledged as major stabilisation mechanisms (Six et al., 2002; von Lützow et al., 2006). Land use history and productivity at Kaltenborn were comparable to Mehrstedt and the profiles of SOC concentrations and stocks had a very similar form, but at different concentration levels. In Flemish seminatural and intensively managed grassland soils, SOC stocks increased from sandy to clayey soil (Mestdagh, 2006). In this study of extensively grazed grassland, the soils with clay texture contained on average 123 t C ha^{-1} ± 3.9 for 0-60 cm depth (mean ± s.d.). A compilation of 121 soil profiles of temperate grasslands, mainly from North America from several databases, resulted in a mean C stock of 91 t ha^{-1} ±5.1 for 0-60 cm depth (mean ± s.d.)(Jobbagy and Jackson, 2000). However, the range of C stocks in temperate grasslands may be between 30 and 80 t ha^{-1} (Conant and Paustian, 2002b). SOC stocks of the two investigated grassland sites thus represent the lower and upper end of this range.

Besides the effect of clay surfaces on C stabilisation, there is an additional mechanism of C stabilisation in soils. Metal ions are well known for their interaction with SOC which leads to more recalcitrant complexes (Tipping, 2002). High concentrations of Ca^{2+} and pedogenic Fe at the Mehrstedt site (Tab. 1) have the potential to stabilise SOC (Baldock and Skjemstad, 2000; Oades, 1988). Pedogenic Al and Fe content were both higher at the Mehrstedt site compared to Kaltenborn, due to a more advanced weathering status of the site and approximately higher primary iron content of the parent material. Results of the C/N ratio measurements confirm this hypothesis. The C/N ratio of the litter input seems to be comparable between the Mehrstedt and the Kaltenborn site, with similar C/N ratios in the uppermost depth interval. Lower C/N ratios in 10-40 cm depth in Mehrstedt indicate a higher proportion of highly degraded organic matter in these depth intervals.

5.2 The vertical spatial dimension

Since the conversion from arable land into grassland 23 years ago in Mehrstedt and 27 years ago in Kaltenborn, SOC input has mainly been deposited in the uppermost horizons. Above-ground biomass and root litter are the main C sources and more than 55% of the root biomass was found in the upper 5 cm of the soil at both sites (Don et al., subm.). Sheep grazing lead to detritus input of faeces on the soil surface. A new Ah horizon developed in the upper part of the former ploughing horizon (Ap). Variability of SOC concentrations was slightly increased in the top 5-10 cm of the soil indicating a non homogenous C input at the soil surface (Fig. 4).

The profiles started to reshape when the land was converted into grassland. However, the impact of ploughing during cropland use on the SOC distribution is still visible in the profiles. Degradation of the organic matter which was mixed within the ploughing horizon was slower than the new C input which formed the new C profiles. In contrast, there seems to be no legacy of ploughing on bulk density. C accumulation after conversion of cropland into grassland persists over several decades without decreasing annual C sequestration rates (Conant et al., 2001). Thus, the two investigated sites may still be in the re-accumulation phase of SOC which was lost during cropland use.

C transport in the soil profile emerges as an important process for C accumulation. We found the highest SOC stocks at the Mehrstedt site in profiles where loess altered the C transport into the subsoil. Silty textured loess has a higher permeability than clay (Bhogal et al., 2000). Besides seepage water transportation, earthworm bioturbation plays an additionally important role for C translocation within the profile (Alban and Berry, 1994). Differences in SOC stocks at the Mehrstedt site were mainly determined by different amounts of C stored in the subsoil (30 to 60 cm depth). A positive correlation between clay content and SOC stocks hardly exists when clay restricts the vertical C transport or rooting.

Decreasing relative variability of bulk density with increasing depth was also confirmed in other grassland studies (Bowman, 1991; Potter et al., 1999). High relative variability of bulk density in the upper most depth interval may partly be explained by perturbations during the soil core sampling. Sample core compression corrections could only be applied to the entire core. However, compaction was of minor importance with 1.5% at the Mehrstedt site and 6.0% at the Kaltenborn site. Sampling was conducted during autumn and spring, when soil was wet enough to prevent considerable perturbation of the soil cores during the sampling procedure. Most perturbations to bulk density derive from soil surface or the upper soil horizons such as bioturbation by animals (small mammals, earthworms etc.), rooting, erosion, drying and rewetting. Additionally, the static pressure of the overlying soil mass increases the energy needed to move particles with increasing soil depth. Thus, a decreasing variability with increasing soil depth is expected.

Relative variability of SOC concentrations were more than 100 times higher at the Mehrstedt site and 7 times higher at Kaltenborn for the whole profile, compared to the relative variability of bulk density. The general trend to higher variability (coefficients of variation) of the SOC concentration compared to the bulk density in mineral soils was found as well in forest ecosystems (Davis et al. 2004).Variability increased with soil depth at the Mehrstedt site (Fig. 4). An abrupt change in the substrate from loess to residual limestone clay was observed, which was associated with changes in SOC concentrations and bulk density (Fig. 6 and 8). High variability of SOC concentrations in the deeper soil depth intervals cannot be explained by the varying thickness of the overlying loess layer, as the variability of the bulk density did not increase with increasing soil depth. Preferential transport of C on cracks during dry periods could cause increasing heterogeneity of SOC in the deeper soil horizons. Such transport in cracks did not exist in the Arenosols of the Kaltenborn site. In accordance with this, increasing SOC variability in the subsoil was not observed at this site (Fig. 4).

5.3 The horizontal spatial dimension

Semivariograms of bulk density and SOC concentration were different in shape and scale indicating independent driving factors for the variability of both variables. A high nugget/sill ratio for bulk density indicates that spatial dependencies were low or on a smaller scale (< 20 m) than the sampling grid. Both investigated sites are characterised by a simple topography with a gentle slope (1-3°). However, spatial variability of SOC stocks were high with a patchy structure at the Mehrstedt site (Fig. 7) and a small scale irregular variability at the Kaltenborn site, which cannot be explained by the surface topography (Tab. 4, Fig. 3C, spatial distribution of SOC stocks not shown for site Kaltenborn). Almost no spatial autocorrelation was detectable for the bulk density with high nugget/sill ratios of about 0.75 (Tab. 4). The patchy distribution of SOC at the Mehrstedt site can be attributed to the different depth of the deposited loess layer. Loess derived horizons were decalcified and carbonate was only present in the underlying limestone derived clay. There was a strong negative correlation between SOC and carbonate concentration below the former Ap horizon (r=-0.65). We found highest SOC stocks where loess layers were thickest and low C stocks in clay rich soils where water and C translocation is hampered by high bulk density (Fig. 5, 6 and 7).

The role of transport processes for SOC accumulation is often underestimated. The missing correspondence between surface topography of the site and the loess layer thickness may be explained by a small scale irregular surface topography of the weathered limestone on which the loess was deposited during the Pleistocene. SOC stocks within a distance of up to 284 m at Mehrstedt and up to 46 m at Kaltenborn were autocorrelated. The higher range at the Mehrstedt site may be explained by the discrete changes of the loess layer thickness within 50-150 m distance, the scale of the semivariograms. Such a mesoscale pattern could not be observed at the Kaltenborn site, where the variability of SOC was on a smaller scale and a lower magnitude. Thus, the range is a sensible parameter which is influenced by the nugget/sill ratio and the mesoscale pattern like the loess layer thickness at the Mehrstedt site. Due to a minimum 20 m sampling distance, the small scale variability is not captured. The two model version, with and without nugget, represent the two extremes in predicting the small scale variability and indicate the possible range of the parameter range.

The spatial distribution of SOC stocks was mainly determined by the distribution of the SOC concentration. Relative sill of bulk density was one to two magnitudes lower than for SOC concentration. Chevallier et al. (2000) proposed two explanations for short-range variation of SOC concentrations on Vertisols managed as pastures: i) plant cover distribution, which occurs in tufts and ii) cracking patters and swelling-shrinking properties of the Vertisols with higher nutrient and water availability in cracks. The effect of preferential SOC transport in Vertisol cracks was shown above. The high nugget/sill ratios at the Kaltenborn site can be only attributed to the spatial variability of the C input by the plants. Due to weak stabilisation mechanisms at the Kaltenborn site the patchy structure of the plant cover directly shapes the SOC distribution in the soil. At the Mehrstedt site, a higher proportion of stabilised SOC obscures the heterogeneity of the C input.

5.4 Implications for measuring soil carbon stocks

The estimation of SOC stocks for a certain area requires estimations of mean SOC concentrations and mean bulk density. In many studies bulk density was derived from relations between SOC concentration and bulk density using pedotransfer function (Howard et al. 1995, Huntington et al. 1989, Crowe et al. 2006). We found correlations between SOC concentration and bulk density on the investigated plot scale to be too weak ($0.01 \leq R \leq 0.50$) to achieve reliable SOC stocks estimates. The uncertainty of the SOC stock estimate could be reduced by only 4% in Mehrstedt and 7% in Kaltenborn by including the regression between SOC concentration and bulk density as a pedotranfer function.

The lack of bulk density data is due to labour and time intensive bulk density sampling work. The conventional method for bulk density measurements with 100 cm^3 volume small sampler rings requires an accessible soil pit which is relative free of stones and coarse roots. In stone rich soils, cracking soils, organic coils and single grained soils other, even more difficult methods have to be used (Lal and Kimble, 2001). The method used in this study had the advantage that bulk density and SOC concentration could be determined on the same sample. However, SOC concentration measurement alone would at least be less time consuming as smaller hand driven corers could be used. We propose an uneven distribution of samples with higher amounts of SOC concentration measurements (56-67%) and only 33-44% bulk density measurements. The low bulk density variability compared to SOC concentration variability allows for a reduction in the amount of bulk density samples. Using this optimal sampling design would allow detecting significant short term SOC stocks changes at these grassland sites of < 2% with less than 20 sample cores for 0-60 cm soil depth. However, if bulk density is sampled with less than 10% of the total sample core number, standard error of SOC stocks would increase exponentially. Our simulation of SOC stock uncertainties illustrates that the error of SOC inventories with only one or very few bulk density samples would be too high to serve as a basis for scaling up SOC stock to larger areas.

6. Conclusion

The variability of SOC concentration and bulk density is caused by different factors which lead to distinct vertical and horizontal pattern of variability of both variables. SOC stock inventories cannot be made using only one of these two variables, even though SOC concentration and bulk density were weakly correlated with each other. Geostatistics provided a tool to explore variability pattern beyond simple descriptions.

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Manuscript 6

Organic carbon sequestration in earthworm burrows

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Axel Don(1) , Bert Steinberg(1), Ingo Schöning(1)a) , Karin Pritsch(2), Monika Joschko(3), Gerd Gleixner(1), Ernst-Detlef Schulze(1)

1) Max Planck Institute for Biogeochemistry, Hans-Knöll Strasse 10, 07745 Jena, Germany,
2) Institut für Bodenökologie, GSF Forschungszentrum für Umwelt und Gesundheit GmbH, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany
3) Leibniz-Centre for Agricultural Landscape Research, Eberswalder Strasse 84, 15374 Muencheberg, Germany
a) current affiliation: Friedrich Schiller University, Institute for Ecology, Dornburger Str. 159, 07743 Jena, Germany

Abstract

Earthworms strongly affect soil organic carbon cycling. The aim of this study was to determine whether deep burrowing anecic earthworms enhance carbon storage in soils and decrease C turnover. Earthworm burrow linings were separated into thin cylindrical sections with different distances from the burrow wall to determine gradients from the burrow wall to the surrounding soil. Organic C, total N, radiocarbon (^{14}C) concentration, stable isotope values (δ^{13}C, δ^{15}N) and extracellular enzyme activities were measured in these samples. Anecic earthworms increased C stocks by 270 and 310 g m^{-2} accumulated in the vertical burrows. C enrichment of the burrow linings was spatially highly variable within a distance of millimetres around the burrow walls. It was shown that C accumulation in burrows can be fast with C sequestration rates of about 22 g C m^{-2} yr^{-1} in the burrow linings but, accumulated C in the burrows may be mineralised fast with turnover times of only 3 to 5 years. C stocks in earthworm burrows strongly depended on the earthworm activity which maintains continuous C input into the burrows. The enhanced extracellular enzyme activity of fresh casts was not persistent, but was 47 % lower in inhabited burrows and 62 % lower in abandoned burrows. Enzyme activities followed the C concentrations in the burrows and were not further suppressed due to earthworms. Radiocarbon concentrations and stable isotopes in the burrow linings showed an exponential gradient with the youngest and less degraded organic matter in the innermost part of the burrow wall. C accumulation by anecic earthworm is restricted to distinct burrows with less influence to the surrounding soil. Relaxation time experiments with nuclear magnetic resonance spectroscopy (NMR) did not reveal any enhanced adsorption of C on iron oxides with C stabilising effect in burrow walls compared to the surrounding soil. Contrary to the initial hypothesis, that organic C is stabilised due to earthworms, our results suggest that the increased surface for litter input along the burrows walls provides new storage room for carbon and the potential to increase C concentrations in C poor subsoil horizons.

***Keywords*:** Anecic earthworms, C turnover, earthworm burrows, *Lumbricus terrestris,* soil organic carbon

1. Introduction

Soil carbon dynamics are influenced by an array of biotic and abiotic factors among which earthworms play a prominent role (Wolters, 2000). They consume up to 2 t litter ha^{-1} yr^{-1} which can be 100% of the annual litter fall, and simultaneously ingest 1200 t yr^{-1} of mineral soil (Raw, 1962). While processing mineral soil and detritus, less than 9% of the ingested organic C is assimilated by the earthworms (Lee, 1985). Even in an extreme example where endogeic (horizontally burrowing) earthworms were newly introduced to North America causing rapid decline of the organic layer thickness, organic matter was only mixed into the mineral soil. C loss of the entire system were small after 14 year of invasion of earthworm (Alban and Berry, 1994).

Earthworms are present in almost all ecosystems around the globe with particularly high abundances in grasslands, where they increase productivity (Partsch et al., 2006). They have been categorized into three functional groups, determined by their feeding behaviour and habitat (Bouché, 1977): (1) Epigeic earthworms are litter feeders that live in the organic layer, (2) endogeic earthworms feed their way horizontally through the upper mineral soil and (3) anecic earthworms generally inhabit one single vertical burrow for their whole life. Anecic earthworms can build burrows down to 5 m depth at maximum, mostly reaching between 1 and 2 m depth, and transport fresh organic detritus from the soil surface into the burrows while mixing with mineral soil (Lee, 1985). C stored in the deeper mineral soil is assumed to be more protected from mineralisation than C in the organic layer or the A horizon (Paul et al., 1997). The mechanisms behind the stabilisation are still unclear. Moreover, anecic earthworms can influence a horizontal soil volume of up to 1 cm thickness all along the burrows wall (Jégou et al., 2000). This zone is called the "drilosphere" (Lavelle, 1988) and C turnover may differ here to the surrounding soil.

On the one hand, earthworms enhance aggregation and are assumed to stimulate the formation of organo-mineral complexes. The formation of microaggregates within macroaggregates which contain protected occluded C seems to be enhanced by passage through the earthworm gut (Bossuyt et al., 2005). However, rapid disintegration of aggregates may occur when polysaccharides and other organic gluing agents are mineralized and the protected carbon may again be released (Ge et al., 2001; Guggenberger et al., 1996). The passage of soil and detritus through the earthworm gut facilitates the contact between C and mineral particles by grinding and mixing both components. Adsorption of C on mineral surfaces is considered to be an important stabilisation mechanism which may be enhanced by earthworm activity (von Lützow et al., 2006). Bonds with iron and aluminium oxides are particularly strong and lead to decreased C turnover (Sollins et al., 1996). Solid state nuclear magnetic resonance (NMR) spectroscopy was successfully applied to determine the formation of Fe-C bonds (Schöning et al., 2005; Smernik and Oades, 1999).

On the other hand, several studies demonstrated a short term increase of C turnover in earthworm casts due to priming: Earthworm guts behave like bioreactors where microbial activity and biomass are increased due to favourable conditions with readily available C of mucus and water (Lavelle et al., 2004; Tiunov and Scheu, 2000). Priming with mucus and water may persist for only a short time. This effect can be demonstrated by measuring extracellular enzyme activities. Enzymes are excreted by microorganisms to hydrolyse and break down organic molecules to facilitate their ingestion. Enzymes are short lived and therefore, extracellular enzymes serve as indicators for the carbon turnover in soil. Whether enzyme activity and decomposition are suppressed in the long term, or if C stabilisation is enhanced by earthworm activity is unknown. Most existing earthworm studies were performed with mesocosms for only short periods of a few months maximum. Results from mesocosm studies can not be used directly to scale up to larger scales. To our knowledge, there is no comprehensive field study that quantified the effect of anecic earthworm on C transport within the soil profile and C sequestration in burrows over a longer time scale.

The following study was undertaken to answer the question as to whether anecic earthworms increase soil C stocks in the long term and decrease C turnover in the drilosphere. The overall effect of anecic earthworms on C stocks and turnover in a temperate grassland system was quantified by analysing drilosphere micro samples. Short term and long term effects caused by earthworms (*L. terrestris*) were investigated measuring enzyme activity, stable isotopes, NMR and ^{14}C age of the burrow linings. We hypothesised that persistent burrow structures provide space for additional C in soils and that the C turnover in the burrows should be decreased, due to physical stabilisation of organic C.

2. Material and methods

2.1 Site description

The study was carried out at two extensively managed grassland sites located within Thuringia/Germany. The site `Jena´ is situated next to the river Saale in a floodplain with 587 mm average annual precipitation and a mean annual temperature of 9.3 °C (11°37'27E, 50°57'07N; 130 m.a.s.l.) (Roscher et al., 2004). The soil is a Eutric fluvisol (WRB classification) developed from alluvial loam with groundwater depth of approximately 2 m in summer. The site was used as cropland between the early 1960s and 2000 and managed as grassland before and after this period. The sampling for this study was conducted on grassland which was four years old. At the site `Mehrstedt´, Stagnic vertisols developed on clay rich limestone sediments, covered by loess (10°39'22E, 51°16'17N; 280 m.a.s.l.). The site was very dry (mean annual temperature 8.0°C) due to the limestone bedrock and low mean annual precipitation (551 mm). It was converted from cropland to grassland 23 years prior to sampling for this study (Scherer-Lorenzen et al., in press).

2.2 Sampling

Horizontal cross sections measuring 1 x 2 m² at 10, 30, 60, 85 and 110 cm depths were excavated on both sites. Mehrstedt additionally had a 140 cm depth. Each site was prepared carefully and cleaned with a small broom and vacuum cleaner. These cross sections were used to measure burrow numbers, size (with digital sliding caliper), and the location of burrows. Only burrows with a diameter >3mm were included in this study. Burrows were sampled using 5 cm diameter core samplers to extract burrow increments, 4 cm long (Fig. 1). Between 5 and 13 burrows were sampled at each sampling depth. Distances between sampled burrows were large enough (0.8-2.0 m) to insure the independence of the samples.

At the Jena site, we distinguished between inhabited and abandoned burrows: After excavating to a certain soil sampling depth, we left the soil pit open and only loosely covered with thermoplastic foil for 20 h. Anecic earthworms closed the openings of their burrows with faeces within a few hours to protect them. Closed burrows were sampled as "inhabited" and open burrows were assumed to be "abandoned". The fresh casts on the burrow openings were used to measure enzyme activity compared to that in the burrow linings.

For enzyme analysis, earthworm burrows were sampled at 30 cm depth at the Jena site. We sampled (1) inhabited, (2) abandoned and (3) inhabited burrows, which were incubated under field conditions in place (in 30 cm soil depth in 100 m³ soil corers) without earthworm access for 11 days (treatment "11d"). Thus, with the exception of earthworm activity, the burrow conditions remained unchanged. All three treatments were sampled with 4 replicates, stored at 2°C and analysed within 14 days.

2.3 Sample preparation

The burrow linings (drilosphere) were divided into four sub-samples. Using a sequence of four drills with increasing diameters, we manually collected soil samples with increasing distances from the burrow wall (0.75, 1.5, 2.5, 3.5 mm) (Fig. 1). The two inner sample cylinders were 0.75 mm thick; the outer two cylinders were 1.0 mm thick. Additionally, the soil outside the drilosphere (ca. 20 mm distance from burrow wall) was sampled (reference soil) to measure the background concentrations. C concentrations in these reference samples were similar to C concentrations of independent bulk samples for both sites (Don et al., in press, Steinbeiss et al. in prep.). Only three different drilosphere cylinders plus the reference soil were collected for enzyme measurements. The inner sample cylinder was on average 1.1 mm thick; the other two were 1.25 mm.

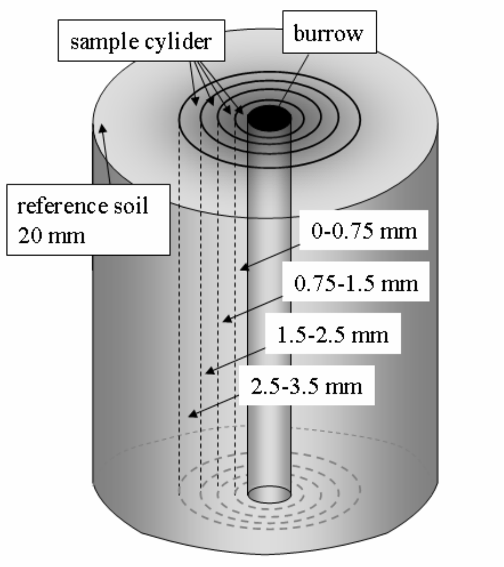

Figure 1: Sampling scheme of an earthworm burrow increment, illustrating the four cylindrical samples taken around the burrow, which were between 0.75 and 1.0 mm thick and the reference sample which was taken for each burrow outside the drilosphere 20 mm from the burrow.

2.4 Analysis

2.4.1 C, N analysis

Samples were dried at 40°C for 24 h and ground to powder. C concentrations of 250 mg ground subsamples were determined by ashing (VarioMax, Elementar) before (C_{tot}) and after (C_{in}) ignition at 450°C for 16 h. Organic carbon (C_{org}) concentrations were calculated as C_{tot}-C_{in}. Total N was measured simultaneously with C_{tot}. Limit of determination of C was 0.12% and of N 0.05%. Through out the following manuscript \`C´ is used as equivalent to \`C_{org}´.

Burrowing by anecic earthworms is mainly done by compression of the surrounding soil with radial pressure of 72-93 kPa and axial pressure of up to 65 kPa. Carbon within the compacted soil mass (Schrader et al., 2002) was accounted for in the following equation for the C enrichment of the drillosphere [mass C] of each horizon k:

$$C\_enrichment = B\,\frac{1}{n}\left(\pi\rho\frac{1}{2}\left(D_{h-1} - D_{h+1}\right)\sum_{j=1}^{n}\ \sum_{i=1}^{4}\left(c_{i,j} - c_{bulk,j}\right)\left(r^{2}{}_{i,j} - r^{2}{}_{i-1,j}\right)\right)$$

B: Number of burrows in the horizon k,

ϱ: Bulk density of horizon k [mass volume^{-1}] (data from Don et al. subm. and I. Kreuziger & J. Baade pers. com.),

D_h: Depth of the sampled horizon [length],

$c_{1...4}$: C concentration of the cylindrical burrow sample [$mass_C$ $mass_{soil}^{-1}$],

c_{bulk}: C concentration of the bulk soil outside the drilosphere [$mass_C$ $mass_{soil}^{-1}$],

$r_{1...4}$: Radius of the cylindrical burrow sample [length],

n: Number of samples j.

The C enrichment is a measure for additional C in a certain soil depth due to the earthworm activity, taking into account the various C concentrations of the reference soil in different horizons. As burrow stability was high, we assumed that the soil was not directly influenced by earthworms if no burrow was visible (Hagedorn and Bundt, 2002).

Turnover times τ for the upper 30 cm of the soil at the Jena site were calculated as (Paul and Clark, 1996):

$$\tau = \frac{(t - t_0)}{\ln(\frac{c_t}{c_{t_0}})}$$

where $(t - t_0)$ = time span between C deposition by earthworms and burrow sampling,

c_t = organic C concentration in abandoned burrows,

c_{t0} = organic C concentration in inhabited burrows.

We assumed a three year mean time of burrows abundance, as all burrows had been destroyed by ploughing four years before sampling and, the earthworms mean life time was approximately one year. Inhabited burrows were assumed to represent C concentration of newly built burrows at time t.

2.4.2 Stable isotope and radiocarbon analysis

$\delta^{13}C$ and $\delta^{15}N$ values were measured with continuous flow isotope ratio mass spectrometry (IRMS). The isotope ratio mass spectrometer (Delta+, Finnigan MAT) was combined with an elemental analyser (EA 1100, CE Instruments). The natural abundance of ^{15}N is reported as

$$\delta^{15}N = (R_{sample} / R_{standard} - 1) \times 1000 \ [‰]$$

where R is the ratio of $^{15}N/^{14}N$ in samples and standards. The standard deviation of repeated measurements of laboratory standards was 0.13‰.

Radiocarbon concentration (^{14}C) was determined with accelerator mass spectroscopy, 3MV AMS (High Voltage Engineering Europe) (Steinhof et al., 2004). Where necessary, samples were decalcified with HCl and freeze dried to remove carbonate-C. CO_2 evolved during dry combustion of 60-200 mg sample was reduced to graphite by heating a mixture of H_2 and CO_2 with iron powder at 650°C. The graphite was pressed into targets and measured with the AMS facility. All values were corrected for fractionation using $\delta^{13}C$ values. The average error was 0.25 pMC. The ^{14}C content was expressed in percent modern carbon (pMC). The fraction of fresh, recent C within the earthworm burrows and casts was calculated with a 2-pool model. Fresh C was assumed to contain recent atmospheric ^{14}C concentrations and old C ingested with the mineral soil was assumed to have ^{14}C concentrations of the corresponding soil horizon.

2.4.3 Enzyme analysis

The activities of several enzymes involved in C-cycling were measured following the method of (Pritsch et al., 2005): α-glucosidase (α-gls), β-glucosidase (β-gls), cellobiohydrolase (cel), xylosidase (xyl), N-acetylglucosaminidase (Nag), and as a very common enzyme, phosphatase (pho). Slight modifications were made due to the specific requirements of samples from earthworm casts and burrows. In brief, a mixture of 0.4 g of field-moist soil and 40 ml of distilled water was shaken by hand for a few seconds, shaken with an extractor for 2 x 15 minutes and exposed in an ice-cooled ultrasonic bath with low energy input for 2 x 3 minutes. The remaining macroaggregates were carefully crushed with a spatula. An aliquot of 50 µl of the suspension was transferred into a microplate. The sample suspension was diluted with 50 µl of distilled water and 50 µl of Methylumbelliferon (MU)-labelled substrates were added to start the incubation. All samples were measured in three replicates. Optimum substrate concentrations were 400 µM for all except phosphatase (800 µM). All substrate-sample solutions were incubated for 60 min except MU-phosphate, which was incubated for just 20 min. Blank samples with distilled water instead of sample suspension were added with six replicates to test the substrates auto-fluorescence.

A calibration with all soil suspensions was done with six different MU concentrations (0, 100, 200, 300, 400, 500 pmol MU in 50 µL) thus accounting for a possible quenching effect of the organic molecules in the suspension. As there was no difference in quenching between all three treatments (abandoned, inhabited, 11d), we applied the same calibration curves for all treatments. Incubation was stopped with 2.5 M Tris (pH>10). Microplates were centrifuged for 10 minutes and fluorescence emission of MU was measured using a fluorescence spectrophotometer (Cary Eclipse, Varian, Australia; excitation wave length: 360 nm, emission 450 nm). The average emission rates of the samples without added substrate were subtracted from the emission values of all samples. Calibration curves were used to calculate the enzyme activity expressed as pmol MU mg^{-1} dry weight (DW) soil sample h^{-1}.

2.4.4 NMR relaxation time experiment

A variable contact time ^{13}C solid state NMR experiment was conducted to determine the spin-lattice relaxation time of protons in the rotating frame ($T_{1\rho}H$) in the Mehrstedt samples (30 cm depth) from the innermost part of an earthworm burrow as well as in the surrounding soil outside the drilosphere (reference soil). Close association between Fe^{3+} and organic compounds may compromise the cross polarisation between ^{1}H and ^{13}C by shortening the relaxation time $T_{1\varrho}H$. ^{13}C solid state NMR spectroscopy was done with a Bruker DSX 200 spectrometer. Samples were spun in the magic angle at a frequency of 6.8 KHz. To study the effect of iron on relaxation times we used untreated samples as well as samples which were previously treated with 10% hydrofluoric acid (HF). Treatment with HF was applied to remove the paramagnetic iron from the soil and to concentrate the soil organic carbon (Schmidt et al., 1999). The total number of scans with a constant recycle delay of 200 ms was 65.000 or 42.500 for untreated samples and 14.000 for HF treated samples. NMR spectra were acquired using an array of 15 different contact times varying from 0.05 to 4.5 ms in untreated samples and using 24 different contact times from 0.01 ms to 6 ms in HF treated samples. All NMR spectra were subjected to Fourier transformation, phasing and baseline correction. The values of signal intensities of alkyl C (0-45 ppm), N-alkyl C (45-60 ppm) and O-alkyl C (60-110 ppm) at different contact times were fitted with a two component approach to determine the spin relaxation time of protons (Abelmann et al., 2004; Mehring, 1983). The fitting was applied using Mathcad 2000 Professional software according to the procedure described by (Abelmann et al., 2004).

2.4.5 Species and biomass of earthworms

On the 26th of May 2005, one soil block measuring 1 m x 1 m x 0.5 m was excavated at both sites and hand sorted for earthworms. In addition, a mustard suspension was applied to the pit in order to expel earthworms from the soil. The worms were subsequently transported to the laboratory for further analysis. The fresh weight of earthworms was determined gravimetrically in a beaker with water and species were determined according to (Sims and Gerard, 1985).

2.4.6 Statistical analysis

Variates were characterised using classical descriptive statistics (means and standard error of the mean). Throughout the paper, error bars and the error of mean values (± X) indicate the standard error of the mean (Webster, 2001). The significance of different treatments was accessed using t-tests with α=0.05. Statistical analysis was performed using Statistica 5.5 software.

3. Results

3.1 Burrow systems and the earthworm community

The number of earthworm burrows increased with increasing soil depth up to a maximum at the 110 cm depth at the Mehrstedt site and at 30 cm at the Jena site (Fig. 2). High burrow densities of >500 burrows per m² were measured at both sites. 344 new burrows per m² were constructed during a four year period in the former ploughing horizon at the Jena site, accounting for almost 2/3 of the maximum burrow numbers at this site. Burrows are aerated and increase the available surface for gas exchange and litter input. Taking into account all burrows >3mm in diameter up to 110 cm depth the soil surface exposed to the atmosphere increased by a factor 8 at both sites, thus considerably increasing the available surface for gas exchange and litter input. The mean burrow diameter was around 6 mm at both sites and increased with increasing soil depth due to an increase of larger burrows in the deeper soil horizons (Tab. 1).

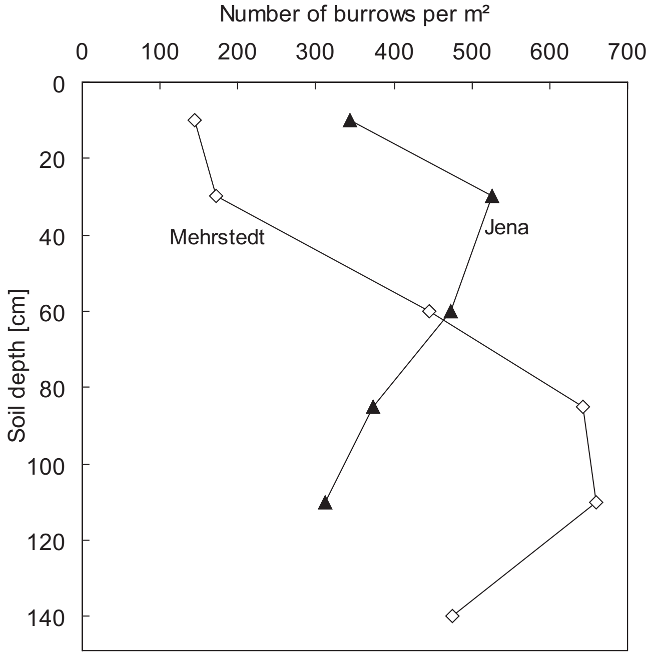

Figure 2: Number of earthworm burrows >3mm diameter at the sites Mehrstedt (open symbols) and Jena (closed symbols).

Table 1: Earthworm burrow distribution for different size classes and sampling depths at Jena and Mehrstedt sites [burrow number m^{-2}] and mean burrow diameter [mm].

Diameter [mm]	**Jena**			**Mehrstedt**				
	10 cm	**30 cm**	**60 cm**	**10 cm**	**30 cm**	**60 cm**	**85 cm**	**110 cm**
3≤x<4	130	144	63	18	30	75	124	73
4≤x<5	91	134	69	29	44	58	95	115
5≤x<6	62	75	70	19	31	76	101	78
6≤x<7	36	52	65	15	30	71	98	113
7<x<8	9	44	79	30	20	82	78	108
8≤x<9	6	39	58	16	9	38	65	100
9≤x<10	6	20	29	12	9	24	40	40
10≤x<11	4	11	22	5	1	17	27	23
≥11	0	8	17	2	0	4	15	8
Mean burrow diameter	4.5	5.3	6.5	5.9	5.4	6.0	5.9	6.3

The abundance of earthworms was low compared to the burrow numbers with 228 earthworms at the Jena site and 233 at the Mehrstedt site. Anecic earthworms accounted for only 14 % at the Jena site (32 individuals) and 21% (49 individuals) of total earthworms at the Mehrstedt site. Anecic earthworms made up 31 % of total earthworm biomass in Jena (22 g m^{-2}) and 57% in Mehrstedt (93 g m^{-2}). This was due to their body size, which is considerably larger than other earthworm groups. The only anecic earthworm species at both sites was *Lumbricus terrestris,* which inhabited only 6-7% of the burrows at the horizon with maximum burrow density.

3.2 Increased C stocks due to earthworm burrows

At each depth, C concentrations were the highest in the inner most part of the burrows and decreased exponentially with distance from the burrow wall (Fig. 3). Total N-concentrations followed the same trend (data not presented). Down to 140 cm depth, the drilosphere was C- and N-enriched compared to the surrounding soil. However, the absolute and relative differences between the drilosphere and the surrounding soil became smaller as soil depth increased.

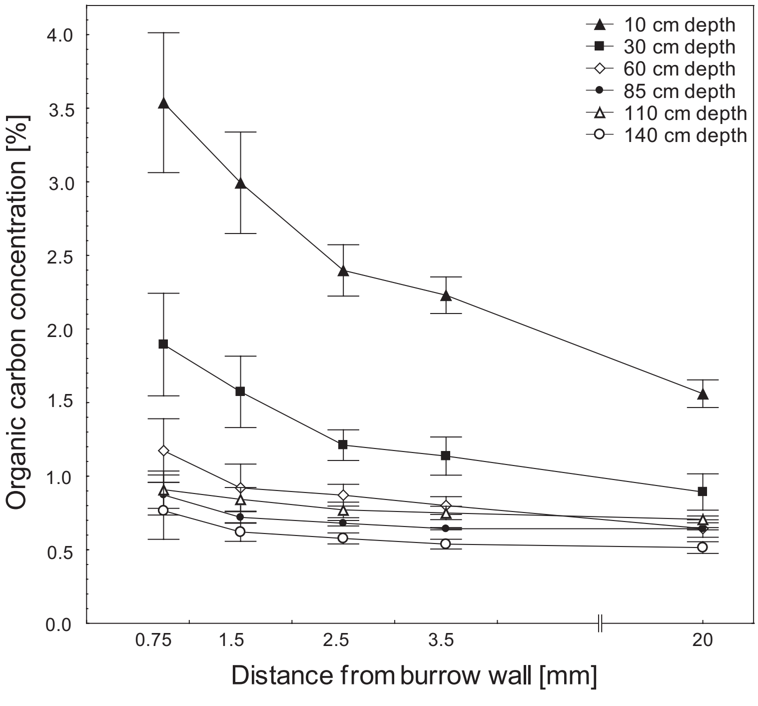

Figure 3: Organic C concentration [%] in the different layers around the earthworm burrows for different soil depths at the Mehrstedt site.

At the Jena site, inhabited and abandoned burrows were sampled separately. We found a strong enhancing effect of earthworm presence on the C concentrations in the burrows. To separate the effect of the earthworms from the C dynamic in the soil profile, we calculated the additional C in the drilosphere – called "C enrichment" - by subtracting the background C concentration. Inhabited burrows showed significantly higher C enrichment compared to abandoned burrows (Fig. 4). The C enrichment in abandoned burrows was on average for all horizons only 33% compared to the inhabited burrows. The calculated C enrichment of the whole soil profile caused by anecic earthworms based on measured burrow volume and C concentrations in the drilosphere was 310 g C m^{-2} at the Mehrstedt site and 270 g C m^{-2} at the Jena site, taking into account the different fractions of inhabited and abandoned burrows.

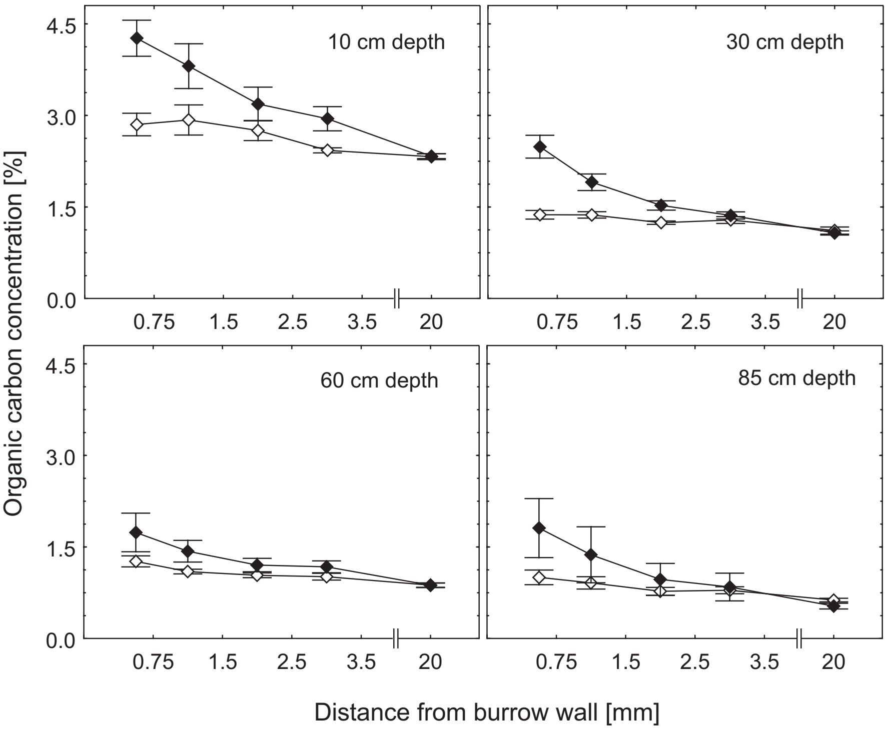

Figure 4: Organic C concentrations [%] in the different layers around the earthworm burrow from inhabited and abandoned burrows at fours different depths at the Jena site.

The highest additional C stock was detected in the subsoil between 30 and 60 cm depth (Fig. 5). Compared to the total C stocks of 11.200 g C m^{-2} for 0-100 cm depth at the Jena site and 8.600 g m^{-2} at the Mehrstedt site the additional C of the drilosphere comprised 1.5% at the Jena site and 2.8% at the Mehrstedt site (Steinbeiß, submitted; Don, submitted). At the Jena site, the drilosphere was sampled four years after the conversion of cropland to grassland. C enrichment within the drilosphere of the former ploughing horizon (0-30 cm depth) took place during this period and was calculated as 22 g C m^{-2} yr^{-1}. Even if additional C stocks within the drilosphere are small, C sequestration rate due to earthworm activity can be considerable.

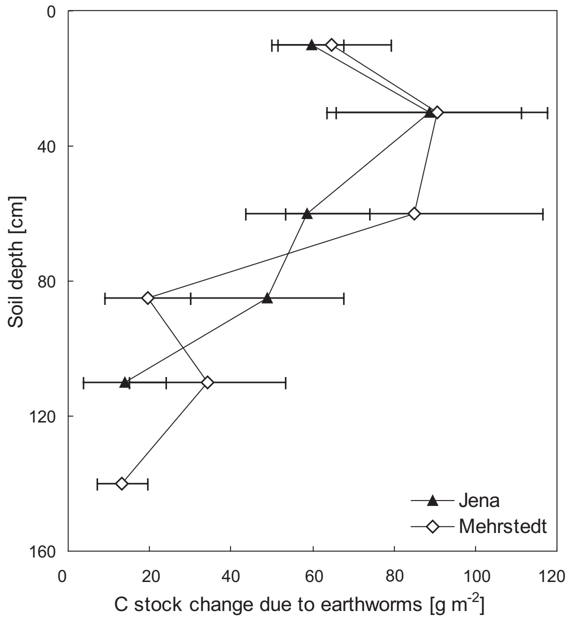

Figure 5: C stock change [g C m^{-2}] due to C enrichment in anecic earthworm burrows for different soil depth intervals at the Jena site (closed symbols) and the Mehrstedt site (open symbols).

C-turnover times (mean residence times) were calculated for newly built burrows at 10 and 30 cm soil depths. They provided information on C dynamics over a scale of time in years. In the innermost burrow wall, C turnover was about five years at 10 cm depth and three years at 30 cm. Turnover times increased with increasing distance to the burrow wall with eight and six years (0.75-1.5 mm distance from burrow wall), 14 and 10 years (1.5-2.5 mm distance from burrow wall) and 10 and 35 years (2.5-3.5 mm distance from burrow wall) at 10 cm and 30 cm soil depths, respectively.

3.3 Origin and ^{14}C age of carbon in the drilosphere

The horizontal gradients of C and N from the burrow wall to the bulk soil resembled those of vertical gradients within soil profiles. Along with the C and N gradients, we found $\delta^{15}N$ values increased from the inner burrow wall to the surrounding reference soil (Fig. 6).

Low stable isotope values indicated a low level of degradation of the organic matter within the drilosphere.

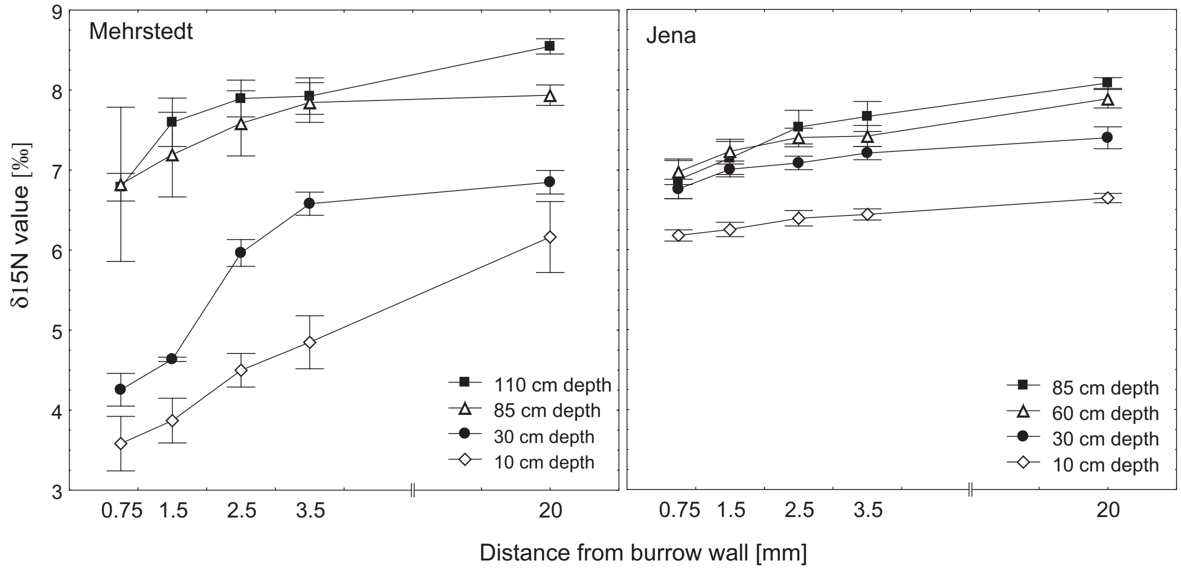

Figure 6: δ^{15}N values in the different distances from the earthworm burrow wall for different soil depth at the sites Jena and Mehrstedt.

Fresh detritus had a low stable δ^{15}N value of around 1.9‰ at the Mehrstedt site and 3.8‰ at the Jena site. The δ^{15}N value difference between burrow wall and surrounding reference soil corresponded to about one trophic level of the food web (Minagawa and Wada, 1984). δ^{13}C values showed the same tendencies but had an offset of several per mill depending on the carbonate content of the samples (not shown). In most horizons, the whole sampled drilosphere up to 3.5 mm distance from the burrow wall was influenced by fresh organic matter.

^{14}C concentrations of the samples confirmed the C age gradient casts<burrows <surrounding soil (Tab. 2). This gradient showed that the burrow walls were under continuous construction. Casts had younger ^{14}C ages than burrows and the inner and outer part of the burrows had different ^{14}C ages too. The fractions of fresh C in the burrow walls were calculated under the assumption that, ^{14}C ages measured in the burrow walls derive from a mixture of recent detritus C and C of the corresponding soil horizon.

Table 2: ^{14}C concentrations [percentage Modern Carbon pMC %] and estimated fraction of fresh C with recent atmospheric ^{14}C concentration, burrows and surrounding soil samples from the sites Jena and Mehrstedt.

Site	Soil depth [cm]	Sample	pMC [%]	Fraction fresh C [%]
Jena	60	cast	101.5	77
Jena	60	inner burrow (0-1 mm)	100.8	71
Jena	60	outer burrow (1-2 mm)	96.5	35
Jena	60	reference soil	92.2	0
Mehrstedt	10	inner burrow (0-1 mm)	102.0	-
Mehrstedt	10	surrounding soil	102.9	-
Mehrstedt	30	inner burrow (0-1 mm)	101.5	72
Mehrstedt	30	reference soil	94.4	0
Mehrstedt	60	cast	102.7	94
Mehrstedt	60	inner burrow (0-1 mm)	95.2	64
Mehrstedt	60	outer burrow (1-2 mm)	98.9	79
Mehrstedt	60	reference soil	78.7	0

Casts contained up to 94% fresh recent C whereas the inner burrow wall contained 64-71% and the outer burrow wall 35-79% fresh C. These are rough estimations, which illustrate the aging pathway of C from casts to burrows. The ^{14}C concentrations in fresh casts at 60 cm on both sites were similar to recent atmospheric ^{14}C concentration (102.7 and 101.5 pMC in casts, 104.3 pMC in recent biomass). Thus, fresh detritus was transported directly into deep soil horizons. High ^{14}C ages at 60 cm depth in the surrounding soil, especially at the site Mehrstedt with 79 pMC (>2000 yr turnover time), showed that there is little input of fresh C into these horizons. C transport into deep horizon is a process which normally takes centuries or even longer (Römkens et al., 1998). However, earthworms speed up the transport processes to time ranges of days to years but accumulation of new C is restricted to distinct burrows. The differences between ^{14}C ages of burrows and surrounding soil was less in the surface soil horizons. At 10 cm soil depth, the input of fresh roots led to similar ^{14}C ages in the surrounding soil to that in the burrows.

3.4 Enzyme activity in burrows and casts

The activity of all measured extracellular enzymes was enhanced in the drilosphere compared to bulk soil (Tab. 3). This difference was significant ($p<0.05$) for five out of six enzymes in inhabited burrows and three out of six enzymes in abandoned burrows.

Table 3: Extracellular enzyme activities at 30 cm depth in Jena [pmol MU mg^{-1} DW h^{-1}] in fresh earthworm casts, in the drilosphere (average 0-3 mm distance from burrow wall) and in the inner burrow wall of inhabited burrows, in burrows abandoned for 11 days and in abandoned burrows. n.s.) No significant difference between inhabited and abandoned burrows.

	Enzyme activity [pmol MU mg-1 DW h-1]					**Enzyme activity/C**	
	cast	**drilosphere**	**inner burrow wall 0-1 mm**				
Enzyme			*inhabited*	*11 days*	*abandoned*	*inhabited*	*abandoned*
α-gls	1352	290	780	527	262	54	44 $^{n.s.}$
β-gls	374	71	134	121	61	313	191 *
cel	229	44	74	71	38	30	28 $^{n.s.}$
Nag	506	75	154	136	66	62	48 $^{n.s.}$
pho	263	216	256	242	226	103	164 *
xyl	797	158	230	271	135	92	99 $^{n.s.}$

*) Significant difference ($p<0.05$) between inhabited and abandoned burrows.

Similarly to the C and N gradients, enzyme activity gradients from the burrow wall to the surrounding soil were smaller in abandoned burrows compared to inhabited burrows. Our results indicate a slight decrease in enzyme activity after the earthworm burrow was abandoned. Within 11 days after earthworm removal the enzyme activity dropped on average by 8% in the innermost ring of the earthworm burrow (Tab. 3). Only for xylosidase we found increased activity 11 days after earthworm removal, however this increase was not significant ($p=0.52$). The earthworm casts derived from the same soil horizon as the drilosphere samples, had up to seven times higher activity than the average drilosphere enzyme activity, 0-3.4 mm from the burrow wall (Tab. 3).

3.5 Long term stabilisation due to adsorption on mineral surfaces – NMR relaxation time experiment

Adsorption on mineral surfaces may stabilise C in soils (Sollins et al., 1996). Iron oxides in particular, reveal large specific surfaces and can interact with organic compounds. Here, a sample from the earthworm burrow was compared with the surrounding soil (reference soil). ^{13}C solid state NMR spectra were obtained from untreated and HF treated samples. The C recovery in HF treated samples was 81% for the burrow sample and 73% for the reference soil sample. No clear differences were visible in the chemical composition of the NMR spectra acquired at a contact time of 1 ms. Both samples were dominated by O-alkyl C with the main peak being at 71 ppm and alkyl C with the main peak being at 27 ppm. NMR relaxation experiments provide an insight into Fe-Organic matter interactions (Schöning et al., 2005). Fe^{3+} impacts on ^{13}C NMR spectra if it is in close vicinity to ^{13}C-H groups by shortening the spin lattice relaxation times ($T_{1\rho}H_1$ and $T_{1\rho}H_2$) of protons. Relaxation times of protons surrounding the major carbon species did not show any distinct difference between earthworm burrows as compared to the reference soil (Tab. 4).

Protons in the surrounding of Alkyl-C and N-Alkyl-C showed slightly shorter relaxation times $T_{1\rho}H$ in the reference soil compared to the burrow. However, the same tendency was found in the HF treated samples, which indicates that the different relaxation times of burrow and reference soil are not a result of different degrees of iron influence. In general, the proton relaxation times for alkyl, N-alkyl and O-alkyl in the untreated sample from the burrows as well as in the reference soil were in a similar range and were all > 2 ms. There was no indication for a preferential interaction between iron oxides and any specific carbon species in the earthworm burrow. Our results showed that earthworms did not contribute to an increased adsorption of any compound of soil organic C on iron oxides.

Table 4: Cross polarisation behaviour for alkyl C (0-45 ppm), N-alkyl C (45-60 ppm) and O-alkyl C (60-110 ppm) of the untreated and HF treated samples from the burrow wall and the reference soil. Indices were deduced from the two component model.

			M_{01}[a]	M_{02}[a]	Slow domain		Fast domain		MEF[d]
					T_{CH1}[b]	$T_{1\rho}H_1$[c]	T_{CH2}[b]	$T_{1\rho}H_2$[c]	
Burrow	Untreated	Alkyl	0.68	0.12	0.03	3.2	0.79	3.2	0.96
Reference soil	Untreated	Alkyl	0.95	0.79	0.03	2.1	2.32	2.1	0.92
Burrow	Untreated	N-Alkyl	0.36	0.01	0.02	4.0	0.16	4.0	0.97
Reference soil	Untreated	N-Alkyl	0.39	0.06	0.03	3.6	0.64	3.6	0.92
Burrow	Untreated	O-Alkyl	0.93	0.34	0.02	3.2	0.92	3.2	0.96
Reference soil	Untreated	O-Alkyl	0.75	0.48	0.03	3.6	0.65	3.6	0.77
Burrow	HF treated	Alkyl	0.33	0.33	0.01	8.4	0.33	5.0	0.99
Reference soil	HF treated	Alkyl	0.48	0.48	0.01	3.5	0.41	3.5	0.94
Burrow	HF treated	N-Alkyl	0.20	0.20	0.01	9.1	0.35	4.3	0.99
Reference soil	HF treated	N-Alkyl	0.25	0.25	0.01	4.0	0.49	3.5	0.98
Burrow	HF treated	O-Alkyl	0.72	0.72	0.02	9.2	0.38	4.4	0.99
Reference soil	HF treated	O-Alkyl	0.75	0.75	0.02	7.0	0.46	3.5	0.98

a) M_0: Equilibrium ^{13}C magnetisation (theoretical reachable maximum magnetisation)

b) T_{CH}: Time required for a transfer of magnetisation from ^1H to ^{13}C nuclei

c) $T_{1\rho}H$: Spin lattice relaxation time in the rotating frame of protons

d) MEF: Model efficiency

4. Discussion

4.1 The burrow system of anecic earthworms – persistent biogenic structures

For temperate grasslands 100-800 burrows per m² have been reported by (Lavelle, 1988). Thus, the two sites in this study belong to the upper third of burrow rich sites with maximum burrow numbers of 527 and 660 (Tab. 1). Higher numbers of burrows in the deep horizons compared to upper horizons indicate that burrows had been destroyed at the surface (bioturbation, ploughing) but persist in the depth (Fig 2). The loamy texture of both sites supported the stability of the burrows in the subsoil. Ehlers (1975) found earthworm burrow numbers to increase with increasing soil depth from 2 to 60 cm. Particularly in tilled plots, as compared to no-tillage plots, most burrows were found below the ploughing horizon.

Increasing burrow diameter with depth (Tab. 2), as found in the present study, indicates high ages of the burrows compared to surface soil burrows. While moving up and down inside the burrows, pressure is exerted onto the burrow walls which increase the burrow diameter (Schrader et al., 2002). Hagedorn and Bundt (2002) investigated the age of burrows and cracks with radionuclides and found burrows to be persistent over decades. However, the age of burrows is difficult to determine because abandoned burrows can be reused by other earthworms (Bastardie et al., 2005). Thus, a burrow can be dated young although it already exists for decades up to centuries.

Dry conditions during the summer in Mehrstedt, may have forced the earthworms to build deeper burrows than those found in Jena. Anecic earthworms use their burrows to escape unfavourable climatic conditions. Anecic earthworms inhabit only one burrow per lifetime, if food scarcity does not force them to move. Thus, the high proportion of abandoned burrows suggests that C storage in abandoned burrows plays a major role in the total C balance of the drilosphere.

In addition to earthworm's ability to transport C into the soil directly, burrows as persistent structures may also facilitate other C transport processes. Roots use earthworm burrows to access deep soil horizons, which are difficult to penetrate through the soil matrix (Springett and Gray, 1997). C input in burrows with root biomass has never been quantified and was restricted in our studies sites to few burrows. Facilitation of plant roots excess to soil water and nutrients by earthworm burrows may increase C-sequestration along with increased productivity, thus increasing the long term sustainability of the ecosystem (Logsdon and Linden, 1992; Partsch et al., 2006; Stockdill, 1982). However, missing earthworm free control plots makes it difficult to quantify these long term effects.

C input with seepage water into the earthworm burrows may be another C transport path. The numerous burrows with missing or blocked connections to the soil surface limit the water infiltration. Only under heavy rain conditions and water saturation of the soil can the burrows high matrix potential be overcome to allow water to flow from the soil into the burrow system. Additionally, hydrophobic organic surfaces in the burrow suppress the water infiltration. During 12 summer storms, an average of only 3.9% of rain was transported through the burrows, with 70% of the burrows never conducting any seepage water (Edwards et al., 1990). Thus, C transport in burrows with seepage water may play only a minor role for C translocation compared to active C transport by earthworms.

4.2 Additional C storage within earthworm burrows

The high numbers of earthworm burrows led to a large increase in the soil surface through the burrow surfaces that can be regarded as micro-A-horizons with similar features as the surface soil A-horizon. C and N gradients in the micro-A-horizons, as well as stable isotope values, were comparable to the A-horizon of the soil profiles (Fig. 3, 4 and 6). As in A-horizons, there is a more or less continuous C input in the micro-A-horizons of the burrows. The burrow diameter must be kept within a certain range, to prevent the earthworm sliding into the burrow (Graff, 1983). Therefore, earthworms permanently cast material onto their burrow wall to reduce the burrow diameter. The range of reported C enrichments in the anecic burrow linings compared to surrounding soil is between 5 and 500 % (Hagedorn and Bundt, 2002; Jégou et al., 2000; Stehouwer et al., 1993). We showed that C enrichment in the drilosphere is temporarily (e.g. Chapter 3.2) and spatially highly variable within a distance of millimetres (Fig. 3 and 4). Therefore, sample thickness of the burrow linings is crucial and has to be considered. From our results it is also evident, that a distinction between inhabited and abandoned burrows is necessary to assess the carbon sequestration potential due to up to three times higher C concentrations in inhabited compared to that found in abandoned burrows (Fig. 4).

Once ploughing ended in Jena, many earthworm burrows were newly constructed within four years. Anecic earthworms, in particular, are suppressed in cropland systems as a result of continuous tillage disturbances (Wardle, 1995). Conversion from cropland to grassland led to a fast recovery in the earthworm population. While new burrows were being constructed in the young Jena grassland, C transport into the burrows was fast with a sequestration rate of 22 g C m^{-2} yr^{-1} a for 0-30 cm soil depth. Grasslands after conversion from cropland were estimated to sequester about 50 g C m^{-2} yr^{-1} (IPCC, 2000; Potter et al., 1999; Soussana et al., 2004). Thus, earthworm activity could account for 44% of this C accumulation. This is a conservative estimate which does not include the deeper horizons where we found the highest C enrichment of the drilosphere. C enrichment of the burrows may reach a steady state, which is determined by the size of the earthworm population, the available substrate and the mineralisation rate of the deposited C in the burrows. Compared to total C stocks, the additional C in the entire drilosphere was small with just 1.5% of the total C stock at the Jena site and 2.8% at the Mehrstedt site. However, only continuous earthworm activity can maintain this C enrichment in the subsoil.

4.3 Earthworm burrow - shortcut for C translocation within soils

Anecic earthworms diurnally move up and down in their burrows to feed on organic detritus and microflora on the soil surface (Edwards and Bohlen, 1995). Earthworms concurrently feed on mineral particles for detoxification and to grind the substrate in the gut. ^{14}C age of casts and burrows at 60 cm depth showed that fresh organic C with a ^{14}C concentration of the recent atmosphere is incorporated into the casts and deposited onto the burrow walls (Tab. 3). ^{14}C measurements of bulk soil were sensitive enough to detect the translocation of "bomb" ^{14}C into the subsoil by earthworms (Stout and Goh, 1980). The activity of anecic earthworms may violate the assumption of a steady state C input in certain soil horizons. A steady C input state must be assumed for many C models, e.g to calculate turnover times from ^{14}C concentrations. Earthworm's C translocation speeds up the vertical C transport into the subsoil and causes a heterogeneous side by side of both very young and very old C. Our data suggest that lateral mixing and diffusion is of minor importance with strong ^{14}C gradients between earthworm burrows and the surrounding soil.

4.3 Short term and long term effects of earthworm activity on C turnover

High C turnover in fresh earthworm casts is explained by high enzyme activities catalysing the mineralisation process (Tiunov and Scheu, 2000). The analyzed extra cellular enzymes are produced by the microbial community to break up large organic molecules into ingestionable fragments. We found up to seven times higher enzyme activity in fresh casts compared to the drilosphere (Tab. 3). The earthworm gut provides favourable conditions for microbial activity mainly because of the addition of mucus, a readily available C substrate (Brown et al., 2000). High microbial activity can also be measured in fresh casts but rapidly decreases in aging casts, indicating a short-term high C turnover. Moreover, lower enzyme activities in abandoned compared to inhabited burrows, and a decrease in burrows already 11 days after earthworm removal suggests a rapid change in available nutrients for microbial populations. Lower enzyme activities in abandoned burrows were in line with lower C concentrations compared to inhabited burrows, confirming that enzyme activities and organic C concentration and particularly the quality of organic C are closely linked (Stemmer et al., 1998). Thus, lower enzyme activities in abandoned burrows can be explained by decreasing substrate availability rather than effective stabilisation mechanisms. There was no significant decrease in enzyme/C-ratio in the abandoned burrows compared to the inhabited ones except for α-glucosidase (Tab. 3).

Turnover times of C are known to increase with increasing soil depth (Paul et al., 1997). However, C stabilisation is not a direct function of C location within the soil but depends on the degradation level, the concentration of C in a certain soil horizon and different stabilisation mechanisms. Burrows are structural units in soils which differ profoundly from the soil matrix: aeration along the burrows prevents any O_2-deficit in the burrows; addition of fresh, easily available C allows co-metabolic degradation of aryl-C (Hamer and Marschner, 2002). In this sense, burrow linings resemble surface soil A horizons, where fresh C is deposited and gas exchange with the atmosphere is facilitated. C dynamics in A horizons are driven by C input and characterised by lower C turnover times than those in the subsoil. Görres et al. (1997) found higher mineralisation rates and lower turnover times in incubated samples from burrow linings, than in the surrounding soil. We calculated low C turnover times for the inner burrow walls of three to eight years. Turnover times found in other grassland studies for 0 to 20 cm soil depth were higher with 10 years for the non stabilised light fractions and 46 and 57 years for mineral associated C (Römkens et al., 1999). In addition to the readily available C in burrows for mineralisation, higher moisture content in the drilosphere could enhance C turnover (Görres et al., 1997).

Only physical protection could decrease C turnover rates in the drilosphere. However, we could not confirm the hypotheses of enhanced mineral-C bonding with NMR relaxation time experiments (Tab. 4). Fresh detritus as particulate organic matter has to be further degraded and comminuted to be absorbable on mineral surfaces. Conceptually, it is necessary to distinguish between drilosphere C enrichment by recent and past earthworm activity. The main part of C enrichment in the burrow systems is stored in inhabited burrows. There is a continuous process of abandonment and re-use of the burrows which starts when the first generation of newly introduced earthworms dies. Most additional drilosphere C will be lost when earthworm activity terminates. However, there is a lack of data on the age and the time since abandonment of the burrows. It was beyond our measurement precision to determine whether abandoned burrows will on the long term contain slightly higher C stocks than the surrounding soil. Long term experiments with bare fallow showed that without C input from plants over many decades, soil C stocks will decrease to a minimum C concentration which depends on the clay and silt content (Ruhlmann, 1999). We found no evidence that C enrichment in the drilosphere would persist long after the burrows were abandoned. Thus, earthworm burrows are an input driven system where C concentrations may drop to the same base level as determined in long term bare fallow experiments.

5. Conclusions

From our results, we conclude that the C distribution in soils is changed by anecic earthworm's activity with more C stored in the subsoil where earthworms increase the C stocks. The underlying mechanisms are based on enlarged surfaces through which C enters the soil and can be stored (burrow linings). Up to now, we found no indication to suggest that C in burrow linings has a lower turnover rate and is stabilised on long term. The hypothesis that C stabilisation is enhanced as a result of absorption on mineral surfaces could not be confirmed. However, the translocation of C from organic layer to the subsoil will decrease the C vulnerability to mineralisation. C in the organic layer and the surface soil is much more prone to disturbances with rapid C loss, than subsoil-C.

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