Response of soil carbon pools to plant diversity in semi-natural grasslands of different land-use history

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Abstract
Within the scope of the BIOLOG-DIVA project, funded by the German Federal Ministry of Education and Research (BMBF), we analysed the effects of plant diversity on soil carbon pools as well as on biotic processes within the soil food web. It is a large interdisciplinary research project focusing on the relationship between biodiversity and ecosystem functioning in grassland ecosystems. The results of our study indicate that carbon pools and microbial community composition are influenced by the quantity of plant litter input. Processes of C accumulation are closely linked to vegetation quality and composition which have to be considered in future research.

Key Words
Land-use history, carbon pools, plant diversity, microbial community.

Introduction
Environmental changes and global climate changes have reduced biodiversity due to nutrient enrichment and increasing atmospheric CO_2 concentration (Smith *et al.* 2008; Walker *et al.* 2004). Biodiversity changes can alter the functioning of ecosystems such as biochemical cycles or the stability of ecosystems. This topic has raised numerous concerns including the fact that ecosystem functions such as carbon (C) accumulation may respond either positively or negatively (Naeem *et al.* 1996; Tilman *et al,*. 1996). Plant diversity can promote the accumulation of C in grasslands by enhancing productivity (Tilman *et al.* 2006); therefore less diverse ecosystems could weaken the ability of soils to accumulate C. The C reservoir of soils is about three times higher than the atmospheric C pool (Jobbagy and Jackson 2000) and steadily increasing atmospheric CO_2 concentrations have encouraged much interest in the long-term C capture potential of soils. It is important to understand how changes in plant species number and community composition in combination with changing management practices may influence rates of C accumulation (Fornara and Tilman, 2008). So far less information is available of biodiversity effects on soil C pools. Nevertheless, Fornara and Tilman (2008) found strong effects of plant functional composition on rates of soil C and N accumulation in a grassland biodiversity experiment. Steinbeiss *et al.* (2008) focused on soil organic C in the short term as a possible indicator for future soil C development. Higher diversity and plant functional traits increased the retention of C in the soil. This indicates that more diverse ecosystems could increase the C capture potential from the atmosphere to the soil. A changing management regime could therefore strengthen the ability of increasing the diversification of grassland ecosystems as this offers a possibility to reduce and store atmospheric CO_2 in soil.

The presented work is part of an interdisciplinary research project within the BIOLOG-Europe Programme (Biodiversity and Global Change), called DIVA. The project is funded by the Federal Ministry of Education and Research (BMBF) evaluating: "The relationship between Biodiversity and Ecosystem Functioning in Grassland Ecosystems". It is a collaborative research effort of the Helmholtz Centre for Environmental Research (UFZ), Friedrich-Schiller-University and the Max-Planck-Institute for Biogeochemistry, both in Jena, and the Office for Ecological Studies in Bayreuth. Extensively managed meadows of different history in management intensity in the Thuringian and the Franconian Forest in Central Germany are used as model ecosystems to investigate the relationship between genetic/ phenotypic diversity and ecosystem processes such as C and nitrogen fluxes. The main objective of the presented work is the quantification of the four nodal points: input, transformation, accumulation, and loss of C in and from soils. We try to understand the biological and biochemical mechanisms behind these processes in relation to plant diversity and land use intensity in semi-natural grasslands.

Methods

Study site description

The study is conducted in semi-natural grasslands in Central Germany. All sites are located within an area of 20 by 40 km and are similar in elevation above sea level and exposition (Figure 1). The soil is a Stagnic Cambisol (siltic) and the bedrock material consists mainly of schist and greywacke. Average annual precipitation is above 950 mm with a slight summer maximum and the average temperature is 5.0 °C. All experimental plots are extensively managed, (no fertilization and grazing for the past 25 years) and mown twice a year in early July and September. The experimental plots represent a gradient in plant species diversity. They can be classified into two meadow types differing in species richness and biomass production: *1^st^* mountain hay meadows (high plant diversity but unproductive) and *2^nd^* rich meadows (low plant diversity but highly productive) (Table 1).

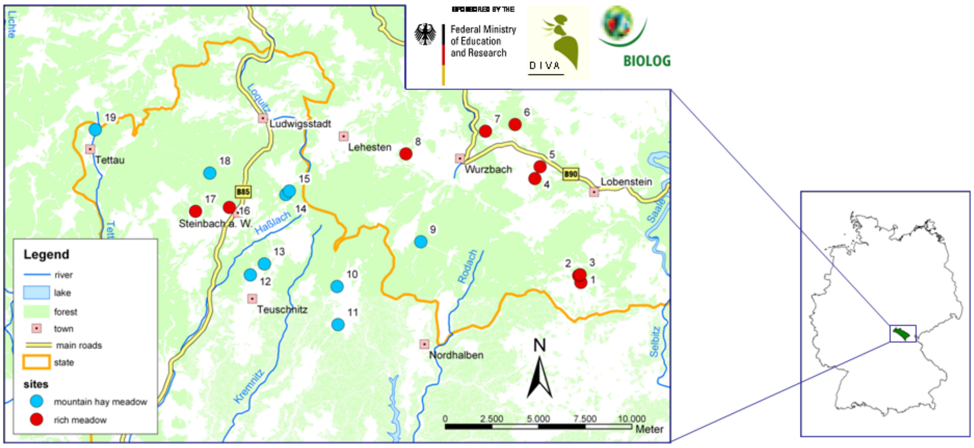

Figure 1. Overview of all experimental plots in the Thuringian and Franconian Forest in Central Germany.

Table 1. Study site and general plot description including soil texture and soil chemical properties. "Bayerische Landesanstalt für Wald und Forstwirtschaft in Freising" provided the climatic data. All values are given as means ± SE.

	Mountain hay meadows	Rich meadows
Average annual temperature [°C]	6.43	
Elevation (m)	645 (± 14.04)	638 (± 2.06)
Total annual precipitation [mm]	1346.4	
Species richness [N]	19 (± 2)	16 (± 2)
Aboveground biomass [g_{dm}/m^2]	134 (± 11.05)	218 (± 28.36)
Soil type	Stagnic cambisol	
pH (KCl)	4.13 (± 0.05)	4.88 (± 0.11)
SOC [%]	4.14 (± 0.35)	3.52 (± 0.26)
TN [%]	0.34 (± 0.03)	0.31 (± 0.02)
Clay [%]	27.03 (± 1.14)	23.91 (± 0.66)
Silt [%]	48.89 (± 1.49)	50.28 (± 1.19)
Sand [%]	24.08 (± 1.85)	26.93 (± 1.20)

Soil and vegetation analysis

Soil samples were taken in June 2008 from mountain hay meadows and rich meadows and separated into three depths (0-10 cm, 10-20 cm, 20-30 cm). The soil was sieved to < 2 mm, handpicked free from visible plant residues and stones. Soil samples for microbial parameters were frozen after sampling at -20 °C. Aboveground biomass was harvested at peak standing biomass and in accordance to the local management practices the aboveground biomass was removed from the plots. Soil organic carbon (SOC) and total nitrogen content of bulk soil samples were determined by combustion in a C/H/N analyser (Vario El III, Elementar-Hanau).

Particle size, density fractionation and C stocks

Physical fractionation methods include the separation according to particle size and density of primary organo-mineral complexes after disrupting the soil (von Lützow *et al.* 2007). The procedure follows the protocol of Shaymukhametov (1985) and was modified by Schulz (2004). For the fractionation procedure two replicates of 20 g (DM) bulk soil are used. Plant residues are separated by flotation with deionised water.

The fractionation procedure consists of two steps: (i) the separation of SOM associated to an easily dispersible clay fraction (particles < 2 µm) by applying ultrasonic energy to a soil/water suspension; by different centrifugation speed and time the clay fraction (CF) was subdivided into a CF1: < 1 µm and CF2: 1 - 2 µm. (ii) After centrifugation the sediment was shaken with bromoform/ethanol mixtures of a density of 2 g/cm^3 and 1.8 g/cm^3, respectively, to stepwise separate two light fractions (LF); LF1: < 1.8 g/cm^3 and LF2: 1.8 - 2.0 g/cm^3. Under consideration of the bulk soil density and the stone mass content, SOC stocks (mass C/area) were calculated as described in Don *et al.* (2007).

Hot water extractable C and N
According to the method of Schulz and Hoffmann (2003), hot water extractions (HWE) were realized. Air dried soil samples were boiled in 50 ml deionised water or 1 h under a reflux and then membrane filtered. The plant material was boiled for 2 h under a reflux and the extracts were membrane filtered twice. Extracts of soil samples and plant material was analysed for the total C and N concentrations using an elemental analyser for liquid samples (Micro N/C and Multi N/C, Analytik Jena, Germany).

Substrate incubation experiment
The experimental units consisted of: soil by itself (Albic Luvisol, control) and soil amended with the respectively biomass (shoot- and root biomass). The soil was rewetted with distilled water and the finely powdered biomass was added to the soil on the basis of 500 mg C/kg. The soil was incubated in an automatic respirometer (RESPICOND, Nordgren 1988) for 30 days and CO_2 evolution was measured hourly.

PLFA and soil enzyme analysis
PLFAs will be extracted based on the method of Bligh and Dyer (1959) and Bausenwein *et al.* (2008). All enzyme activities (alkaline phosphatase, protease, β-glucosidase and xylanase) will be measured colorimetrically according to Schinner *et al.* (1993).

Results
It is accepted that the SOC dynamics represent an equilibrium between C input (e.g. primary productivity) and C output (e.g. decomposition) processes. These processes are driven by the interaction of abiotic and biotic factors. To better understand the SOC dynamics, the biotic linkages and the factors that regulate the transformation of C in managed grasslands we applied the following approaches:

Plant diversity effects on C pools and SOC stocks
Ecological relevant pools of organic matter were isolated through the combination of fractionation methods according to differences in particle size and specific density. The effects of plant diversity and land-use history on these pools will be presented.

Response of labile HWE SOM pool to plant diversity and its relation to the soil microbial community
We will extract a labile C pool through HWE and show how this pool is affected by plant species and litter quality. Additionally, the effects of this labile pool on phospholipid fatty acids and enzyme activities will be presented.

Litter quality as a key component of SOC pool formation
Decomposition of litter can be seen as a key process for the flow of energy and nutrients in terrestrial ecosystems and therefore we tested in a respiration experiment the decomposability of plant litter of plots differing in species richness.

Conclusions
From our results it can be concluded that decomposable but also stable or stabilized SOM pools should be considered for predicting the long-term C capture potential of soils for atmospheric CO_2. Future work of biodiversity effects on C accumulation in soils needs to consider the whole soil profile, since a huge amount of C is expected to be stored in deeper soil layers.
Details on actual results and on those of ongoing experiments will be presented at the conference.

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