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CENTRE FOR
ENVIRONMENTAL
RESEARCH - UFZ

PhD Dissertation 15/2011

Functional soil organic matter pools and soil organic carbon stocks in grasslands – An ecosystem perspective

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ISSN 1860-0387

Functional soil organic matter pools and soil organic carbon stocks in grasslands – An ecosystem perspective

Von der Naturwissenschaftlichen Fakultät
der Gottfried Wilhelm Leibniz Universität Hannover
zur Erlangung des Grades
Doktor der Naturwissenschaften
Dr. rer. nat.
genehmigte Dissertation
von

MSc Marc Breulmann
geboren am 23. März 1982 in Osnabrück

2011

Referent: Prof. G. Guggenberger
Korreferent: Prof. F. Buscot
Tag der Promotion: 25.11.2011

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List of Abbreviations

$\delta^{13}C$	Ratio of stable carbon isotopes ^{13}C:^{12}C, related to VPDB
a.s.l.	Above sea level
Al	Aluminium
Alcvul	*Alchemilla vulgaris*
ANOVA	Analysis of Variance
Antsyl	*Anthriscus sylvestris*
ASE	Accelerated Solvent Extraction
β-Gluc	β-Glucosidase activity
BP	Before Present
GHG	Greenhouse Gases
C	Carbon
^{12}C	Stable carbon isotope with atom mass 12
^{13}C	Stable carbon isotope with atom mass 13
^{14}C	Radioactive carbon isotope with atom mass 14
Camrot	*Campanula rotundifolia*
CCA	Canonical Correspondence Analysis
CF	Clay Fraction
CF1	Clay Fraction 1 with particles < 1 µm
CF2	Clay Fraction 2 with particles 1 – 2 µm
CH_4	Methane
C_{HWE}	Hot Water Extractable Carbon
$C_{HWE\ bulk\ soil}$	Hot Water Extractable Carbon of the bulk soil
$C_{HWE\text{-}fraction}$	Hot Water Extractable Carbon of a functional SOM pool
C:N ratio	Ratio of Carbon to Nitrogen
$(C:N)_{HWE}$	Ratio of Carbon to Nitrogen in Hot Water Extracts
clay cont.	Clay content
CO_2	Carbon Dioxide
DM	Dry Matter
E_{SOC}	Carbon enrichment factor
f+mU silt	Fine and medium silt
F:B	Ratio of Fungal to tot. Bacterial PLFA biomass marker

FA	Fatty acid
FAO	Food and Agricultural Organization of the United Nations
Fe	Iron
G	Gravitational constant
GC/MS	Gas Chromatograph/Mass Spectrometry
gU silt	Coarse silt
Hollan	*Holcus lanatus*
HPG	High Productivity Grasslands
HWE	Hot Water Extraction
IRMS	Isotope Ratio Mass Spectrometer
KCl	Potassiumchloride
KOH	Potassium hydroxide
Leuvul	*Leucanthemum vulgare*
LF1	Light Fraction 1 with a specific density of < 1.8 g cm^{-3}
LF2	Light Fraction 2 with a specific density of 1.8 – 2.0 g cm^{-3}
LPG	Low Productivity Grasslands
Lycflo	*Lychnis flos-cuculi*
$MgSO_4$	Magnesium sulfate
MSD	Mass Selective Detector
N	Nitrogen
N_2O	Dinitrogen monoxide (nitrous oxide)
NH_4^+- N	Ammonium - N
N_{HWE}	Hot Water Extractable Nitrogen
$N_{HWE\ bulk\ soil}$	Hot Water Extractable Nitrogen of the bulk soil
$N_{HWE-fraction}$	Hot Water Extractable Nitrogen of a functional SOM pool
NPP	Net Primary Production
NO_3^-- N	Nitrate - N
OC	Organic Carbon
OM	Organic Matter
PCA	Principal Component Analysis
PCC	Plant community composition
Pg	Petagram
Phos	Phosphatase activity

Physpi	*Phyteuma spicatum*
PLFA	Phospholipid Fatty Acid
$PLFA_{Actinomycetes}$	Actinomycetes biomass marker
$PLFA_{Bac}$	Total bacterial biomass marker
$PLFA_{Fung}$	Fungal biomass marker
$PLFA_{Gram(-)}$	Gram-negative bacteria marker
$PLFA_{Gram(+)}$	Gram-positive bacteria marker
$PLFA_{Protozoa}$	Protozoan biomass marker
$PLFA_{Tot}$	Total microbial biomass marker
POM	Particulate Organic Matter
ppm	Parts per million
Prot	Protease activity
R	Programme for statistical computing and graphics
rpm	Revolutions per minute
sand	Sand content
SAT:UNSAT	Ratio of Saturated to Unsaturated PLFA marker
SE	Standard Error
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TN	Total Nitrogen
Tripra	*Trifolium pratense*
Trirep	*Trifolium repens*
USDA	United States Department of Agriculture
VPDB	Vienna Pee Dee Belemnite, standard for $^{13}C/^{12}C$ isotope scale
w/v	Weight over volume
Xyl	Xylanase activity

Kurzzusammenfassung

Im Rahmen eines interdisziplinären Forschungsprogramms des BIOLOG–Europe Programms „Biodiversität und Globaler Wandel", wurden extensiv genutzte Graslandmodellsysteme untersucht. Die Veränderung der pflanzlichen Artzusammensetzung aufgrund einer prognostizierten globalen Erwärmung und einem damit verbundenen Anstieg an Treibhausgasen zeigt einen signifikanten Einfluss auf globale Ökosystemfunktionen wie Primärproduktion, biogeochemische Kreisläufe oder die Nährstoffspeicherung. Pflanzen sind in der Lage, Kohlenstoffdioxid (CO_2) aufzunehmen und in Form von Biomasse oder Exudaten dem Boden zuzuführen. Der Boden spielt hierbei eine entscheidende Rolle im Kohlenstoff (C) Kreislauf und damit auch für das Klimasystem. Aufgrund von klimatischen Veränderungen können höhere Abbauraten von Pflanzenmaterial bzw. organischer Bodensubstanz (OBS) durch die am Um- und Abbau beteiligten Bodenmikroorganismen erwartet werden. Dies kann somit zu einer höheren CO_2-Freisetzung führen.

Relevante Prozesse einer Steuerung der C-Speicherung in Graslandökosystemen im Rahmen des bodenbedingten Potentials wurden in der Dissertation aufgegriffen. Das Ziel der Arbeit war es, die vier Punkte: Eintrag, Transformation, Akkumulation und Verlust von C in und aus Böden im Zusammenhang mit der pflanzlichen Artzusammensetzung zu untersuchen. Dazu wurden die zwei stark voneinander abhängigen und miteinander reagierenden Fraktionen der toten OBS und der mikrobiellen Bodenbiomasse betrachtet. Besonderes Augenmerk lag auf der Verteilung des Bodenkohlenstoffs in unterschiedlich stabilen funktionellen Pools der OBS.

Das Verständnis der Schlüsselzusammenhänge im System Pflanze-Boden setzt eine Verknüpfung von abiotischen und biotischen Faktoren und Prozessen voraus. Kausale Zusammenhänge zwischen dem Eintrag von pflanzlicher Streu (Wurzelstreu) und der Qualität und Quantität der OBS wurden in dieser Arbeit nachgewiesen. Bodenchemische und biologische Prozesse wurden durch die pflanzliche Gesellschaft und ihrer funktionellen Zusammensetzung maßgeblich beeinflusst. Besonders der Abbau von Wurzelstreu und strukturelle und funktionelle Charakteristika der Bodenmikroorganismen waren signifikant betroffen. Es konnte gezeigt werden, dass weniger produktive Grasländer, deren Vegetation aus langsamer wachsenden Pflanzen besteht, ein höheres Potential haben, Bodenkohlenstoff zu speichern (*Chapter 1*). Die Identifizierung von C-Vorräten in funktionellen Pools der OBS entlang des Bodenprofils lieferte einen umfassenden Überblick über die Verteilung des Bodenkohlenstoffs. Der größte Anteil der Bodenkohlenstoffvorräte war in den oberen Bodenschichten (0-30 cm) gespeichert. In tieferen Schichten war der C primär mit Tonpartikeln assoziiert, der räumlich vor mikrobiellem Abbau geschützt ist. Bodentextur wie auch die Bodentiefe waren Haupteinflussfaktoren für diesen stabilen funktionellen Pool der OBS. Die labilen funktionellen Pools der OBS wurden stark von der Vegetation beeinflusst. Zudem erwies sich das natürliche $^{13}C/^{12}C$ Isotopenverhältnis und die Heißwasser Extrahierbarkeit von Bodenkohlenstoff und C der funktionellen Pools der OBS als solider Indikator ihrer Stabilität und Verfügbarkeit (*Chapter 2*). Im Respirationsversuch zur mikrobiellen Verwertung des Bodenkohlenstoffs in funktionellen Pools der OBS konnte gezeigt werden, dass die potentielle C-Verwertbarkeit von den spezifischen Eigenschaften der Pools, der Bewirtschaftung sowie vom Bodentyp abhängt und für die Ausprägung einer spezifischen, angepassten mikrobiellen Gesellschaft bestimmend ist. Die Verwertbarkeit der stabilen funktionellen Pools der OBS wird aufgrund der Disaggregierung erhöht, da die Zugänglichkeit zu verwertbaren C-Quellen gesteigert wird. Die Verwertbarkeit der labilen funktionellen Pools der OBS ist stark abhängig von ihrer chemischen Stabilität (*Chapter 3*).

Ein künftiger Forschungsfokus des terrestrischen C-Kreislaufes sollte auf der Untersuchung unterschiedlicher Ökosysteme, Bodentypen und klimatischen Zonen liegen und Faktoren wie die Bewirtschaftungs- und Klimaveränderungen berücksichtigen. Die Anwendung von molekular-biologischen Techniken würde es ermöglichen, Schlüsselorganismen der mikrobiellen Bodenfauna, die am Abbau der C-Quellen beteiligt sind, zu identifizieren.

Schlagwörter: C Kreislauf, Funktionelle Pools der OBS, Bodenkohlenstoff

Abstract

Within the scope of the BIOLOG–Europe Programme "Biodiversity and Global Change", extensively managed grasslands were used as model ecosystems. Changes in the composition of the plant community, due to environmental changes and rising global atmospheric concentrations of carbon dioxide (CO_2), are known to significantly influence ecosystem functions, such as net primary production, biogeochemical cycles and nutrient storage. Plants can assimilate CO_2 and can additionally influence the soil food web by determining the quantity and quality of carbon (C) compounds (e.g. organic matter) entering the soil. Terrestrial ecosystems are able to change and regulate the amount of CO_2 in the atmosphere by sequestering C in soils and releasing C from soils through respiration. Therefore, environmental changes can affect the rate of decomposition of soil organic matter (SOM).

This work describes relevant processes involved in C sequestration in soils of grassland ecosystems. The main aim of the studies was to obtain quantitative information on four crucial processes, namely the input, transformation, accumulation and loss of C in and from soils in relation to the plant community composition and its functional groups. Furthermore, the biological mechanisms behind these processes as well as the various functional pools of SOM were considered.

Concomitant analysis of the effects of the abiotic and biotic components of terrestrial ecosystems contributes to obtain a better understanding of how the key elements of the plant-soil system interact. Causal links between plant litter input (mainly roots) and the quality and quantity of SOM were found. The results indicated that the plant community composition affected the dynamics of SOM and related below-ground microbial processes (decomposition of root biomass, structural and functional parameters of the soil microbial community composition). In particular, the plant functional group of legumes, play an important role in ecosystem functioning; soils in ecosystems with a high abundance of slow-growing plants exhibit slower nutrient cycling and thus have a higher potential of soil organic carbon (SOC) accumulation, which is an important ecosystems service (*Chapter 1*). By identifying and characterising different functional SOM pools through the soil profile, a first comprehensive and detailed overview of the distribution of organic carbon (OC) in the functional SOM pools was obtained. The bulk of the SOC was stored in the top soil, at depths of 0-30 cm. In deeper soil layers, OC was primarily associated to clay particles, where it is protected from microbial decomposition. The main factors influencing this stable functional SOM pool were found to be soil texture and depth, whereas the vegetation had the strongest influence on the labile functional SOM pools. The natural $^{13}C/^{12}C$ isotopic ratios combined with the hot water extractable C fraction of SOC proved to be robust indicators of SOC stability and availability (*Chapter 2*). Under incubation conditions functional SOM pools were assessed on their potential biological decomposability. Microbial utilisation of OC in SOM pools depends on specific traits of the respective functional SOM pools, the management of the grassland and the soil type. The utilisation of stable functional SOM pools (complexed SOM: two clay fractions) depends on specific pools characteristics, such as accessibility to soil microorganisms, which was increased due to disaggregation that promoted the accessibility of previously protected C to soil microorganisms. However, the OC utilisation of the labile functional SOM pools (uncomplexed SOM: two light fractions (LF)) depends on its chemical stability. The OC of LF1 is readily degradable promoting C losses, whereas the OC within LF2 can be considered as recalcitrant, due to low levels of respired CO_2 (*Chapter 3*).

Future studies on terrestrial C cycle research should focus on assessing the C accumulation potential of different ecosystems, soil types and climatic zones, with particular emphasis on influencing factors, such as land use patterns and environmental changes. The use of molecular techniques will further help to identify potential key microbial groups involved in degrading C sources.

Keywords: C cycle, Functional SOM pools, Soil organic carbon

Extended Summary

Functional soil organic matter pools and soil organic carbon stocks in grasslands – An ecosystem perspective

(Funktionelle Pools der organischen Bodensubstanz und Bodenkohlenstoffvorräte in Graslandsystemen – Eine ökosytemare Betrachtungsweise)

1. General Introduction

1.1 Carbon sequestration in terrestrial ecosystems

The predicted increase of the mean temperature until the end of the 21st century is associated with rising global atmospheric concentrations of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (IPCC, 2007). From these, CO_2 is the most important one of the anthropogenic greenhouse gases (GHG), affecting and controlling the earth system globally in a significant way (Heimann & Reichstein, 2008). Changing environmental conditions will strongly influence plant and animal species and may affect the structure of terrestrial ecosystems on earth (Foley *et al.*, 2003). The feedback between the carbon (C) cycle and climate change has recently received much attention (e.g. Kyoto Protocol*), whereas knowledge on a better understanding of how soils will respond to changing environmental conditions is needed (Powlson *et al.*, 2010). Hence, it is important to consider that terrestrial ecosystems are able to change and regulate the amount of CO_2 in the atmosphere by sequestration of C in soils and releasing C from soils through respiration.

The oceanic C pool is the largest one, where 38.000 petagram (Pg) C is stored (Lal 2003). However, soils provide the largest terrestrial stock of C, which is particularly abundant in the cold boreal and humid tropical regions (for more detailed information see: "*global soil organic carbon map*"; http://soils.usda.gov/use/worldsoils/mapindex/soc.html). The "*global soil organic carbon map*" shows that significant amounts of soil organic carbon (SOC) are also found in temperate zones. It is assumed that the terrestrial biosphere is a CO_2 sink that counteracts the increasing anthropogenic emissions to some extent. However, there is a calculated imbalance, which suggests that the soils store the supposedly "missing" CO_2 (Schimel, 1995). The uppermost metre of the global SOC pool contains approximately 1.500 Pg C; the uppermost two metres contain roughly 2.500 Pg C, which is twice the amount stored in the atmosphere and three times the amount stored in vegetation (Jobbágy & Jackson, 2000, Batjes, 2002). While the sequestration of C in forest and agricultural ecosystems is reasonably well understood (Valentini *et al.*, 2000), comparatively little is known about the distribution of C in grasslands and how it is affected by the composition of the vegetation within these environments (Falge *et al.*, 2002, Rumpel & Kögel-Knabner, 2011). Grasslands are one of the most common ecosystem types in the world (Scurlock & Hall, 1998), covering

* Not before 2004, major ecological issues, such as the fixation of atmospheric CO_2 in soils was mentioned or described in the Kyoto Protocol (Article 3.4).

20-40% of the ice-free terrestrial surface (Cernusca *et al.*, 2008). It has been estimated that 10-30% of the global C pool is stored in grasslands (Anderson, 1991, Eswaran *et al.*, 1993), much of it below-ground. This underground pool contains around 200-300 Pg C, with turnover times ranging from 22 to 5000 years (Batjes & Sombroek, 1997, Schulze *et al.*, 2010).

Soils can act as either sinks or sources of CO_2, depending on land use and management. The accumulation potential of C in soils depends on the size, composition and turnover of its functional soil organic matter (SOM) pools. Consequently, when studying the dynamics of SOM, it is necessary to consider functional SOM pool, their distribution, bioavailability and the mechanisms by which they are stabilised.

1.2 Dynamics of soil organic matter: Functional SOM pools and SOM stabilisation

Soil organic matter is the total organic fraction of the soil. It contains living microbial biomass and a complex and heterogeneous mixture of organic compounds and minerals, including plant and soil microbial derived residues at various stages of decomposition and stabilisation degrees (Rodeghiero *et al.*, 2009). It is heterogeneously distributed between the soil compartments and its quality, availability and stability vary widely. The properties of its constituent compounds are highly variable, ranging from readily-degraded SOM compounds to compounds that are physically resistant to decomposition or whose molecular structure makes them highly stable. Current conceptual models differentiate functional SOM pools in *active*, *intermediate* and *passive* pools (Smith *et al.*, 1997, Amundson, 2001) on the basis of their different turnover rates, or equivalently, their residence times. In order to understand the dynamics of SOM and to gain insight in stabilisation and destabilisation mechanisms, it is essential to identify, isolate and characterise functional SOM pools, without altering any of their properties that might be relevant to their function in the ecosystem. This can be accomplished using methods, such as physical fractionation (Christensen, 2001, von Lützow *et al.*, 2007). Physical fractionation techniques are often applied and include size- and density-based separation of organo-mineral complexes (Christensen, 2001). By using complementary methods for fractionation on the basis of particle size and specific density, it is possible to separate *uncomplexed*, *primary* and *secondary complexed* SOM (Figure E.S. 1). Uncomplexed SOM is not associated with minerals and is separated by partitioning in heavy liquids (e.g. polytungstates, iodates or bromoform) with densities of 1.6 – 2.0 g cm^{-3} (Christensen 2001, Gregorich *et al.*, 2006, von Lützow *et al.*, 2007).

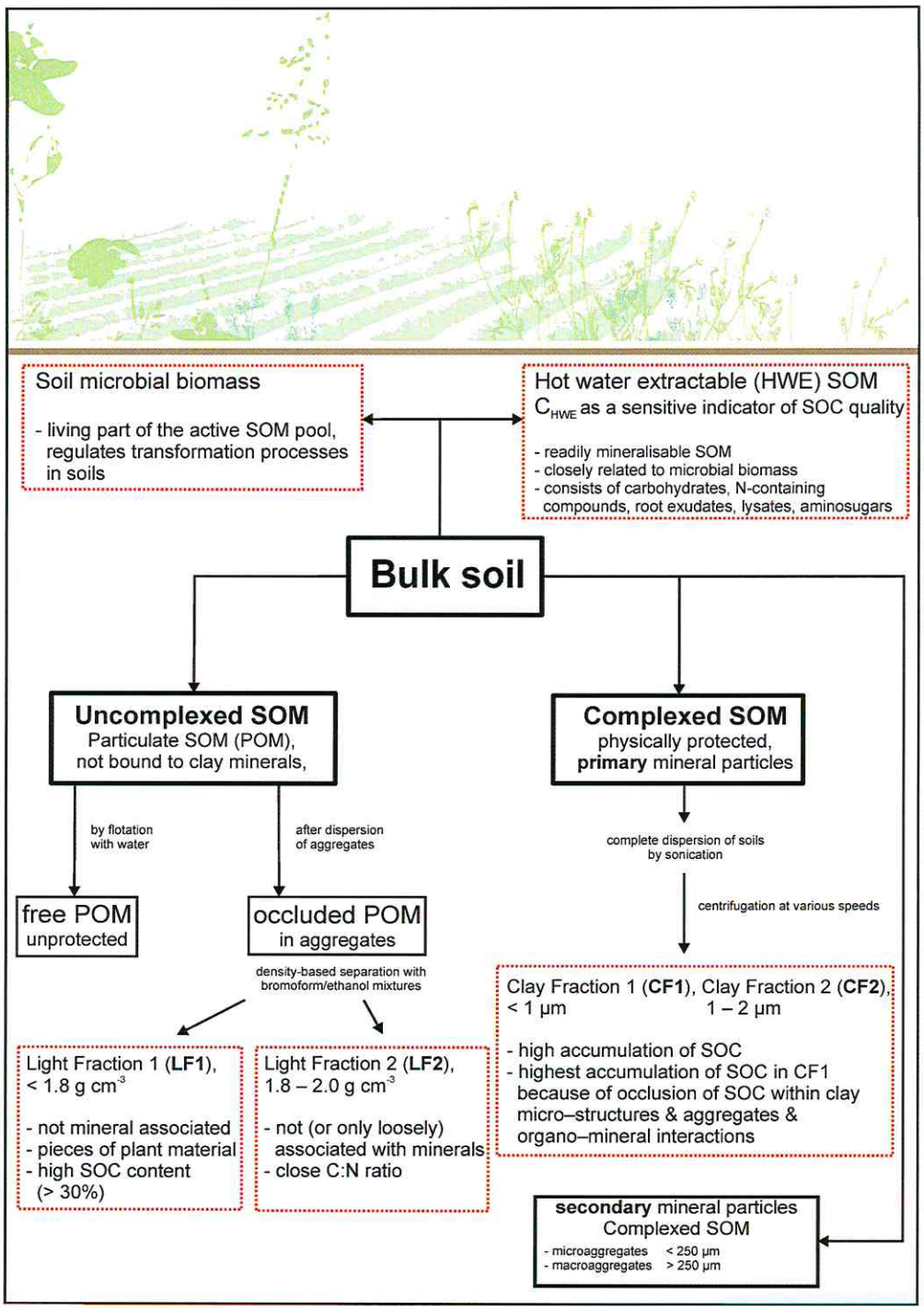

Figure E.S. 1 Conceptual model of functional soil organic matter (SOM) pools, adapted from the work of Christensen (2001), von Lützow *et al.* (2007), Böhm *et al.* (2010) and Schulz *et al.* (2011) by M. Breulmann. The work discussed in this thesis focused primarily on the organic carbon (OC) in the functional SOM pools surrounded by red frames (CF1, CF2, LF1, LF2, C_{HWE} and soil microbial biomass); C_{HWE}, hot water extractable soil C fraction; POM, particulate organic matter.

The uncomplexed SOM in soils consists of both *free* and *occluded* SOM. Free SOM includes loose organic particles, while occluded SOM is physically protected by aggregates (Christensen, 2001). Complexed SOM (primary mineral particles) is obtained by complete dispersal of the soil (e.g. by sonication in water). Since the highest concentrations of SOM (50-75% C) are associated with clay-sized particles, these are of particular importance when studying the accumulation of C in soils. Secondary complexed SOM consists of micro- (< 250 µm) and macro-aggregates (> 250 µm) (Denef *et al.*, 2009).

Functional SOM pools (uncomplexed and complexed SOM) are stabilised in soils by specific mechanisms with certain turnover rates (von Lützow *et al.*, 2007). The stabilisation of SOM can be defined as the "*protection of organic matter from mineralisation*" (Sollins *et al.*, 1996). Three general mechanisms of SOM stabilisation have been described:

(1) *Recalcitrance* (2) *Spatial inaccessibility* (3) *Interaction*

(1) *Recalcitrance* is the selective preservation of SOM due to its structural composition/molecular-level characteristics. Recalcitrance is primarily associated with plant litter and rhizodeposits, microbial products, humic polymers and charred SOM, all of which are comparatively slowly degraded by microbes and enzymes (Sollins *et al.*, 1996, von Lützow *et al.*, 2006, von Lützow *et al.*, 2008). However, "recalcitrance" implies only a relative stability - the microbial community will eventually degrade any natural SOM in soil (Guggenberger, 2005, Rillig & Mummey, 2006).

(2) *Spatial inaccessibility* refers to various processes that cause the physical occlusion of SOM, rendering it inaccessible to microbes and degradative enzymes. These include interactions with aggregates, intercalation between phyllosilicate sheets, effects related to hydrophobicity and encapsulation within organic macromolecules. Humic substances and biologically-processed organic material have a tendency to form organo-mineral complexes. Metabolic binding agents produced by the soil biota can play a major role in the formation of larger aggregates (Tisdall & Oades, 1982, Kögel-Knabner *et al.*, 2008).

(3) *Interactions* between the SOM and minerals and metal ions reduce the availability of SOM to microorganisms and enzymes. Clay minerals and/or amorphous hydroxides of iron (Fe) and aluminium (Al) play a major role in these processes because of their reactive

hydroxyl groups (Sollins *et al.*, 1996, Six *et al.*, 2002, Eusterhues *et al.*, 2003, von Lützow *et al.*, 2006). In soils, where the interaction between SOM and minerals or metal ions is a significant and important mechanism of C sequestration, stabilised SOM pools with relatively high mean ages and low turnover rates are observed (Baldock & Skjemstad, 2000, Kaiser *et al.*, 2002).

A wide range of approaches and methods exist for isolating and characterising functional SOM pools. However, reliable data on the distribution of OC in the functional SOM pools, especially in deeper soil horizons and on the influence of the aboveground plant community are sparse. Most chemically and/or physically isolated functional SOM pools as well as complementary methods are not homogeneous in terms of turnover rates and do not correspond to specific stabilisation mechanisms. Hence, these methods do not describe functional SOM pools (von Lützow *et al*, 2007).

In conceptual models, functional SOM pools are often discriminated by their turnover times. However, less information is available, especially in the context of the "actual/potential" availability of different functional SOM pools and of their effects on the C cycle (Swanston *et al.*, 2004). Combining abiotic and biotic components will contribute to a better understanding of how the organic substrate properties, functional SOM pools and the diversity of the soil communities interact.

1.3 SOM, a substrate for soil microorganisms and plant - soil feedback loops

It has been shown that above-ground and below-ground subsystems are closely linked and that the feedbacks between these play an important role in controlling ecosystem processes (van der Heijden *et al.*, 1998, Hooper *et al.*, 2000, Wardle, 2002). Plant communities influence ecosystem functions, such as net primary production (NPP) and biogeochemical cycles (Fornara & Tilman, 2008), as well as the *quantity* and *quality* of C input to soils (Batjes & Sombroek, 1997, Wardle & Lavelle, 1997, Tilman *et al.*, 2001). On the other hand the below-ground subsystems can indirectly regulate plant growth and plant community composition via their effects on the transformation of SOM and influence on the supply of available nutrients in soils (Wardle *et al.*, 2004).

The storage of C in soils is primarily dependent on the amount of C entering soils (e.g. plant biomass production) and the loss of C through respiration. However, little is known about the

significance of interactions of the above-ground and below-ground subsystems regulating the fluxes of C to and from soils.

There is a huge trait variation among plant species that drive soil microbial processes, such as litter decomposition. Fornara and Tilman (2008) demonstrated that the plant community composition influences the rates of C and nitrogen (N) accumulation in soils. De Deyn (2009) reported that changes in plant species and functional group richness influenced the storage of C in soils. The responses of plant species was related to the presence of N fixers and forbs in stimulating soil microbes by providing them a diverse range of substrates; the microbes in turn modulate soil C cycling.

Soil microorganisms are dependent on organic C that is fixed above-ground and transported into the soil by roots or via litter decomposition. They are major direct contributors to the flux of C from soils to the atmosphere and play a key role in soil biological processes (De Deyn *et al.*, 2008). They break down organic materials and mix organic residues with inorganic constituents, deriving energy and nutrients from a diverse range of SOM molecules. In turn, they have profound impact on the cycling of nutrients in the soil. For instance, soils with bacteria-dominated microbial communities typically exhibit high decomposition rates and nutrient turnover (via bacteria-based energy channels), which benefits fast-growing plant species. Conversely, slow-growing plants favour a fungal food web with a slower cycling of nutrients (fungal-based energy channel) (Bardgett & Wardle, 2010).

Studies on linking plant diversity and plant community composition with SOC storage as an ecosystem service and the dynamics of SOM are scarce (Woodward *et al.* 2009). It is widely recognised that the interaction between the above- and below-ground subsystem plays a key role in the C cycle, but the understanding of the processes occurring, remains still limited. Therefore, it is essential to elucidate the relationships between soil organisms and SOM, because these processes promote soil fertility and maintain soil structure.

2. BIOLOG – Europe Programme: The DIVA subproject

The presented work is part of an interdisciplinary research project within the BIOLOG–Europe Programme (Biodiversity and Global Change) that focuses on the effects of environmental changes on biodiversity in Europe (http://www.biolog-europe.org). The BIOLOG project is funded by the Federal Ministry of Education and Research (BMBF) in

Germany and consists of four major subprojects: (1) BIOPLEX, (2) INVASION (3) SUBICON and (4) DIVA. The research presented in this thesis was conducted as part of the DIVA subproject (grant no. 01LC0613B), whose main objectives were to investigate "*The relationship between Biodiversity and Ecosystems Functioning in Grassland Ecosystems*" (http://www2.uni-jena.de/biologie/ecology/biolog/ english.htm). Specifically, the DIVA subproject was designed to examine the relationship between plant communities and ecosystem processes and functions, such as C and N fluxes. The subproject was conducted in collaboration with researchers from the Friedrich-Schiller-University in Jena (FSU), the Georg-August University in Göttingen, the Max-Planck-Institute for Biogeochemistry (MPI-bgc) in Jena and the UFZ – Helmholtz Centre for Environmental Research in Halle. Extensively managed semi-natural grasslands in the Thuringian Forest (Thüringer Schiefergebirge) and in the Franconian Forest in Central Germany were used as model ecosystems. Experimental and theoretical approaches were used to investigate the importance of grassland ecosystems and the composition of their plant communities for the stability and functioning of ecosystems, e.g. history of land use, diversity of arbuscular mycorrhizal fungi, plant productivity, soil nutrient status and nutrient cycling.

At the beginning of the first phase of the DIVA subproject in 2001, 78 sites were selected along a plant species diversity gradient, ranging from 13-45 species (Kahmen *et al.*, 2005a). The work described in this thesis was begun at the start of the third phase of the project in 2007; at that point, only 11 grassland sites remained, all of which exhibited similar plant diversity; there was no diversity gradient in the sites studied in this work.

3. Main aims of this thesis

Within the scope of the BIOLOG – Europe Programme the main objective of this work was to obtain quantitative data regarding four crucial processes, namely the *input*, *transformation*, *accumulation* and *loss* of C in and from soils and the biological mechanisms behind these processes were also studied (Figure E.S. 2).

Recently, the management of ecosystems with the aim to realise C accumulation in soils as an ecosystem function has become an important issue. Detailed studies on linking different plant communities to SOM dynamics are scarce. Hence, the BIOLOG – DIVA subproject provided an optimal platform for SOM research.

This work describes the relevant processes involved in controlling C sequestration in grassland ecosystems of Central Germany. Additionally, grasslands of the forest steppe of Russia were studied to determine whether the validity of the hypotheses constructed and the conclusions drawn remain valid in grasslands with different soil types and climates.

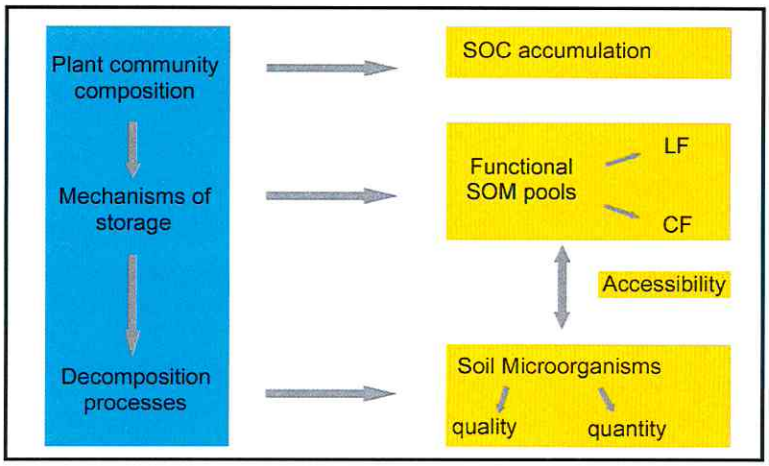

Figure E.S. 2 The primary objectives of the work reported in this thesis were to investigate: (i) the effects of the plant community composition on SOC accumulation (ii) the mechanisms of OC storage in the different functional SOM pools (LF, light fraction; CF, clay fraction) and (iii) the decomposition processes of C sources (iv) the structural and functional composition of the soil food web and (v) the potential accessibility of functional SOM pools with respect to microbial utilisation.

Although approaches of linking the effects of the plant community composition with ecosystem services, such as SOM dynamics are scarce there is evidence that grassland ecosystems may influence SOM dynamics through the quantity and quality of C input to soils (Fornara & Tilman, 2008, De Deyn, 2009, Woodward *et al.* 2009).

The purpose was to analyse the effects of the plant community composition on structural and functional soil microbial characteristics; to identify the key drivers of litter decay and to determine its significance for C accumulation in soils of grasslands.

In this context, the first study (***Chapter 1***) comprises analyses of plant communities of extensively managed grasslands in Central Germany, which differed in terms of their plant biomass production, the composition of their plant communities and their litter quality. The decomposition of shoot and root litter under incubation conditions was studied. Readily

useable labile C and N fractions in the studied soils were isolated by hot water extraction; functional microbial groups were identified by phospholipid fatty acid (PLFA) analysis and microbial activity was measured using a set of soil exo-enzymes as sensitive indicators of soil ecological stability.

Data on the depth distribution of OC in the functional SOM pools and the potential effects of the plant community composition are limited. In addition, with regard to stabilisation mechanisms of functional SOM pools, a lack of knowledge still exists (von Lützow *et al.*, 2007).

The main objective was to investigate the importance of grassland ecosystems and its vegetation composition for the distribution and stability of SOC stocks of the bulk soil and among functional SOM pools at different depths of the soil profile.

Therefore, in the second study (***Chapter 2***) two clay fractions (complexed SOM: CF1, < 1 µm; CF2, 1 – 2 µm) and two light fractions (uncomplexed SOM: LF1, < 1.8 g cm^{-3}; LF2, 1.8 – 2.0 g cm^{-3}) were considered. The stability of functional SOM pools was characterised by analysing their stable C isotope signature ($\delta^{13}C$) and C_{HWE}.

Kandeler *et al.* (2005) revealed a dominance of bacteria in clay fractions, whereas high fungal activity was detected in the sand fraction (Kandeler *et al.*, 1999). By studying abiotic and biotic components, it should be possible to obtain new insights into the interactions between the properties of individual organic substrates, functional SOM pools and the diverse members of the soil communities.

The main aim was to determine the potential decomposability and stability of functional SOM pools; to analyse how levels of functional groups within microbial communities change during decomposition of the OC of functional SOM pools and to detect how specific functional groups of soil microorganisms control decomposition processes of these pools.

Detailed information, presented in the third study (***Chapter 3***), on the decomposability and stability of functional SOM pools during microbial utilisation were obtained from incubation experiments, where these pools were used as substrates. Functional SOM pools (CF1, CF2, LF1, LF2), were isolated from grassland ecosystems in Central Germany. In addition, to confirm the controlling influence of functional SOM pool characteristics for a potential utilisation by microorganisms, soils of grassland ecosystems of Central Germany and Russia were studied. PLFA extractions were used to identify changes in the functional groups of the soil microbial communities during the decomposition of the functional SOM pools and how

specific functional groups of soil microorganisms control the decomposition processes of these pools.

4. Study area and sampling design

4.1 Site description

Thuringian Forest and Franconian Forest, Germany (BIOLOG – DIVA subproject)

The 11 experimental sites are located in semi-natural grasslands of the Thuringian Forest and Franconian Forest (11°00'-11°37'E and 50°21'-50°34'N), which is situated on a plateau-like mountain range on the border between Bavaria and Thuringia in Central Germany. All sites were at similar altitudes (606-703 m a.s.l.) and had similar exposures. The average annual temperatures vary between 6.0 and 7.0°C and the average annual precipitation in the region is 850-1200 mm. The soils were classified as carbonate free Haplic Cambisols (siltic) (FAO classification) under weak stagnic conditions and were formed mainly from schist and greywacke bedrock. The experimental sites were located within an area of 20 km by 40 km and can be treated as independent grassland sites. The grasslands could be classified as either low productivity grasslands (LPG) or high productivity grasslands (HPG), which differ in terms of their plant biomass production, plant community composition and litter quality. The grassland sites had been extensively managed in the preceding 20 years (they had been neither grazed nor fertilised and cut twice a year in early summer and autumn).

Long-term field experiment, Russia (Reference site)

The soil samples from the Pasture and Prairie sites (*Chapter 3*) were obtained from the Central Chernozemic State Biosphere Reserve and the All-Russia Research Institute of Arable Farming and Soil Erosion Control in Kursk, Russia (36°19'E and 51°73'N). The experimental sites are located in the forest steppe climatic zone of the Central Russian Heights which has an average annual precipitation of 574 mm and an average temperature of 5.3°C. The soils are classified as Typical Chernozems (http://oopt.info/index.php?oopt=1104).

4.2 Experimental and sampling design

Thuringian Forest and Franconian Forest, Germany (BIOLOG – DIVA subproject)

The size of each experimental site in the Thuringian Forest and Franconian Forest was 20 × 15 m. The sites were divided further into 5 x 5 m sub sites, which were used for various experiments; for instance, drought was induced by constructing roofs over individual sub sites to exclude precipitation (Kahmen *et al.*, 2005b), or pesticides were laid down to control above-ground and below-ground herbivory (Stein *et al.*, 2010). Soil samples were taken from the control sub site (5 x 5 m) in 2008 (*Chapter 1* and *Chapter 3*); archived soil samples of entire soil profiles down to the bedrock were taken in 2004 (*Chapter 2*)

Long-term field experiment, Russia (Reference site)

The experiment consisted of the following plots: Unmown Prairie (unused prairie in its "natural state"), Oak-Forest, Mowed Prairie (annually), Pasture (land used for cattle grazing at a stocking rate of 0.9 livestock units/ha) and Continuous Fallow. The soil samples of the Pasture and Prairie sites (*Chapter 3*) were taken from the top 25 cm.

5. General Methods

5.1 General soil and vegetation analysis

Prior to chemical analysis, the soil samples were air dried, sieved to < 2 mm and visible plant residues and stones were picked out by hand. Portions of soil samples to be used for microbial parameter analyses were frozen at -20°C after sampling (*Chapter 1 & 3*). The organic OC and total nitrogen (TN) contents of bulk soil samples and of functional SOM pools were determined by combustion in a C/H/N analyser (Vario El III, Elementar, Hanau, Germany). Soil organic carbon stocks were calculated (*Chapter 2*), as described by Don *et al.* (2007).

The vegetation in each grassland plot in Central Germany was surveyed in 2008 (*Chapter 1*) and June 2004 (*Chapter 2*) and all vascular plant species present, were identified. Plant species were classified into one of four functional groups: grasses, small herbs, tall herbs and legumes (*Chapter 2*). Above-ground biomass was harvested in each plot at peak standing biomass from four 25 cm x 30 cm quadrates, by cutting 3 cm above the ground, dried and finely ground to powder. The remaining biomass was removed from the plots in accordance

with the local management practices. Annual root biomass production was harvested and estimated in 2007 using in-growth cores (Lauenroth, 2000).

5.2 Fractionation approaches to identify functional SOM pools

5.2.1 Hot water extractable C and N

Hydrolysis of SOM under weak conditions, such as cold or hot water extractions, is often used as a sensitive tool for estimating the decomposable SOM in soils (Schulz, 2002). In this work, a labile, hot water extractable soil C fraction (C_{HWE}) was examined; changes in the composition of this fraction are likely to reflect anthropogenic changes in the soil-plant system (Figure E.S. 1).

Hot water extraction (HWE) was performed on samples of LPG and HPG sites, using the method of Schulz *et al.* (2003). The soil samples were extracted by boiling in deionised water in a reflux condenser (*Chapter 1*). Bulk soil samples and various functional SOM pools were extracted with hot water using an ASE 200 - Accelerated Solvent Extraction System (DIONEX, Idstein, Germany), because of the small fraction amounts (*Chapter 2*). HWE was also used to measure readily decomposable C and N in plant material (Klimanek, 1997). The total C and N concentrations within extracts of soil samples and plant material were determined using an elemental analyser for aqueous samples (Micro N/C and Multi N/C, Analytik Jena, Jena, Germany).

5.2.2 Particle size and density fractionation

Current models of the distribution of SOM across different functional SOM pools typically focus exclusively on the turnover time when defining the different pool types (active, intermediate and passive) and thus do not account for biological, physical and chemical mechanisms of SOM stabilisation. Numerous techniques have been developed for fractionating functional SOM pools and identifying pools with different properties. Several of these techniques are discussed in recent reviews by von Lützow *et al.* (2007) and Denef *et al.* (2009).

In the work described in this thesis, functional SOM pools were isolated by physical fractionation of bulk soil samples, with different fractions being separated on the basis of (1) particle size and (2) specific density in order to achieve a finer resolution in the separation than has been achieved to date and to identify more homogeneous pools.

(1) The physical fractionation method (*Chapter 2 & 3*) followed the protocol of Shaymukhametov *et al.* (1984) as modified by Schulz (2004). Complexed SOM (clay fractions) were isolated by complete dispersal (disaggregation) of soil samples using low-frequency ultrasound (Christensen, 2001, Figure E.S. 1). Free particulate organic matter (POM) as part of the uncomplexed SOM was removed prior to sonication by flotation in water. To respond to two of the existing classifications of clay (the International USDA: < 2 µm and the Russian classification: < 1 µm) two clay fractions were isolated: a clay fraction one (CF1, < 1 µm) and on the other hand a clay fraction two (CF2, 1 – 2 µm); (Shein, 2009).

(2) Uncomplexed SOM (light fractions) was isolated using high-density solutions after the mechanical disruption of the soil. These fractions therefore include both uncomplexed SOM that was occluded by or within aggregates and uncomplexed SOM that was associated with the aggregates' surfaces (Figure E.S. 1). While salt solutions with high specific densities (polytungstates or iodates) are often used for this purpose, a bromoform/ethanol mixture was used in these studies (Shaymukhametov *et al.* 1984); beneficially, the isolated fractions can be easily purified and contain no traces of the dense solution. Two light fractions were separated: a fraction with a density of < 1.8 g cm^{-3} (LF1) consisting mainly of particulate, partly decomposed plant residues and a second fraction with a density of 1.8 – 2.0 g cm^{-3} (LF2) consisting of SOM that was only loosely (or not at all) associated with minerals.

Soil samples of LPG and HPG (sampling date 2004 and 2008), as well as from sites of the long-term field experiment in Russia were used for isolating functional SOM pools. Due to the fractionation approach of a complete disaggregation of the soil, in this work the two stabilisation mechanisms *recalcitrance* and *interaction* with clay minerals were studied.

5.2.3 Soil microbial biomass

The soil microbial biomass is an additional and vital component of the active and labile SOM pool that regulates and effects transformation processes in soils (Prechtel *et al.*, 2009). Microbial communities in the sampled soil were characterised using PLFA analysis and microbial activity was measured using a set of soil exo-enzymes as sensitive indicators of soil ecological stability.

Phospholipid fatty acids were extracted as structural markers of the microbial community composition, based on the method of Bligh and Dyer (1959) (*Chapter 1 & 3*). Individual phospholipids were identified by GC/MS (Hewlett Packard 5971A mass selective detector,

combined with a 5890 series II gas chromatograph) and quantified using MSD ChemStation D.01.02.16 chromatography software (Agilent Technologies, United States). Phospholipid fatty acid 19:0 was used as an internal standard. According to Frostegård *et al.* (1993), Pennanen *et al.* (1996) and Zelles (1999) the following PLFAs indentify specific microbial groups: gram-positive bacteria (i15:0, a15:0, i17:0, a17:0), gram-negative bacteria (cy 17:0, cy 19:0), fungi (18:2ω6), Protozoa (20:2ω6,9c, 20:3ω6,9,12c, 20:4ω6,9,12,15c) and actinomycetes (10Me16:0, 10Me17:0, 10Me18:0). The total bacterial biomass was quantified on the basis of the sum of the following PLFAs: i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, cy 17:0, 17:0, cy 19:0.

PLFAs were extracted from three soil depths of grasslands of Central Germany (*Chapter 1*) as well as of samples of the incubation experiment (*Chapter 3*), where functional SOM pools were obtained from LPG and HPG grasslands and Prairie and Pasture sites.

5.3 Soil enzyme activities

Soil enzyme activities (*Chapter 1*) were measured in samples from grasslands of Central Germany and three soil depths. All enzyme activities were measured colorimetrically using a spectrophotometer (U-200, Hitachi Ltd., Tokyo, Japan). Blank samples were not incubated with a substrate to correct for variation in the samples' background absorbance. The following enzymes were analysed, according to the methods described by Schinner *et al.* (1993): alkaline phosphatase (EC 3.1.3.1), protease (EC 3.4.21-24), β-glucosidase (EC 3.2.1.21) and xylanase (EC 3.2.1.8).

5.4 N mineralisation

The N mineralisation within selected samples of the incubation experiment (*Chapter 3*) (functional SOM pools from sites of Central Germany and from the long-term field experiment in Russia) was determined. These samples were extracted with 1 M KCl by shaking for 1.5 h and then filtered. The ammonium - N (NH_4^+-N) and nitrate - N (NO_3^--N) concentrations within the extracts were determined using a flow injection analyser (FIAstar 5000, Foss GmbH, Rellingen, Germany).

5.5 Decomposition experiments

An Albic Luvisol with a low content of C and N was used as a standard carrier material for laboratory incubation experiments (*Chapter 1 & 3*). This soil was rewetted with water to 60%

of its maximum water holding capacity prior to incubation. The experimental units were incubated in an automatic respirometer (Respicond V, Nordgren Innovations AB, Sweden) at a constant temperature of 22°C (Nordgren, 1988). The CO_2 evolution from the experimental units was measured hourly of maximal 96 polyethylene vessels.

Plant material and bulk soil samples for decomposition experiments were used from sites of Central Germany (*Chapter 1*) and functional SOM pools *3* were additionally obtained from the long-term field experiment in Russia (*Chapter*).

5.6 Stable isotope analyses

Aliquots of bulk soil and functional SOM pools, as well as plant residues, taken in 2004 from grassland plots in Central Germany through the soil profile, were subjected to total combustion for CO_2 determination in a Vario El III elemental analyser (Elementar, Hanau, Germany), coupled via a ConFlow III (ThermoFinnigan, Bremen, Germany) to a Delta V plus isotope ratio mass spectrometer (IRMS) (Thermo Electron Corporation, Erlangen, Germany) for isotope analyses (*Chapter 2*). The C isotopic composition was expressed using the conventional δ notation, defined as $\delta^{13}C_{sample} = ({}^{13}C/{}^{12}C_{sample} - {}^{13}C/{}^{12}C_{VPDB}) / {}^{13}C/{}^{12}C_{VPDB} \times 1000‰$, where VPDB (Vienna Pee Dee Belemnite) is the standard defining the ${}^{13}C/{}^{12}C$ isotope scale (Coplen, 1995).

5.7 Radiocarbon measurements

Illustrative data obtained from samples taken at one grassland site of Central Germany (the clay fraction (CF1) of deeper soil layers) were analysed for their radiocarbon ages (*Chapter 2*) at the Leibniz-Laboratory for Radiometric Dating and Isotope Research in Kiel, Germany (Nadeau *et al.*, 1998).

6. General Results and Discussion

Soils have a pronounced influence on the global C cycle on many different levels and play a key role in controlling global atmospheric CO_2 levels (Amundson, 2001). The CO_2 concentration in the atmosphere has increased significantly in recent years; since Schlesinger (1977) published one of the first analyses of the global C budget, it has risen by ~25 parts per million (ppm). Together with declining biodiversity, changes in climate, consequential in

composition of the plant community and human activity have various effects on C storage in soils. Today soils are a focal point of scientific interest because of their ability to store large quantities of OC. The need to improve our understanding of the processes that regulate C dynamics in soils makes it important to determine how trends in the atmosphere affect SOC storage.

6.1 Relationships between abiotic factors, the composition of plant communities and the soil food web (*Chapter 1*)

The composition of plant communities has increasingly been recognised as an important driver of ecosystem functioning. Because the composition of the plant community determines the quantity and quality of C compounds transferred to the soil, it influences key aspects of ecosystem functionality, such as NPP, biochemical cycles and SOM dynamics (De Deyn & van der Putten, 2005, Fornara & Tilman, 2008, De Deyn 2009).

The first study describes an investigation aimed at elucidating the relationships between abiotic factors, the composition of plant communities and the soil food web in extensively managed semi-natural grassland ecosystems.

These grassland ecosystems differ significantly in terms of their above-ground biomass production. On the basis of their NPP, they were characterised as either LPG or HPG. In accordance with the local management practices, the above-ground biomass was removed from the sites and so the principle input of plant litter was via the root system, which was significantly greater in LPG than in HPG (Figure E.S. 3).

The results showed that the higher production of vital roots in the LPG gave these grasslands comparatively large fractions of labile C (C_{HWE}). It was found that this fraction was a key factor of the below-ground food web, determining the rate and nature of the decomposition processes that occurred. The surplus of labile C in combination with the higher root litter production resulted in high activities of cellulose degrading soil enzymes, such as xylanase and β-glucosidase. Microbial degradation experiments using root necromass showed that roots from LPG were less degradable. This was reflected in about 20% lower decomposition of root litter in LPG grassland soils, which had important consequences for soil microorganisms (Figure E.S. 3).

It can be assumed that soil microbial communities depend on the quality and quantity of root litter, which is a function of the plant species composition in these grassland ecosystems. The

comparatively high proportion of legume species in HPG had pronounced effects on the protease activity and created a positive feedback loop that promoted above-ground biomass production, increasing the growth of grasses with high N use efficiency. This complementary interaction between legumes and grasses in HPG was reflected in the structure of the soil microbial community composition. The higher N availability in HPG soil, which benefits fast-growing plants, facilitated the development of a bacteria dominated community that promotes comparatively high decomposition rates and nutrient turnover. In contrast, the higher abundance of slow-growing plants, which produce less-degradable root litter, in LPG favours the development of a fungal-dominated community in which nutrient cycling is comparatively slow. Thus suggests that LPG sites are important for SOC accumulation.

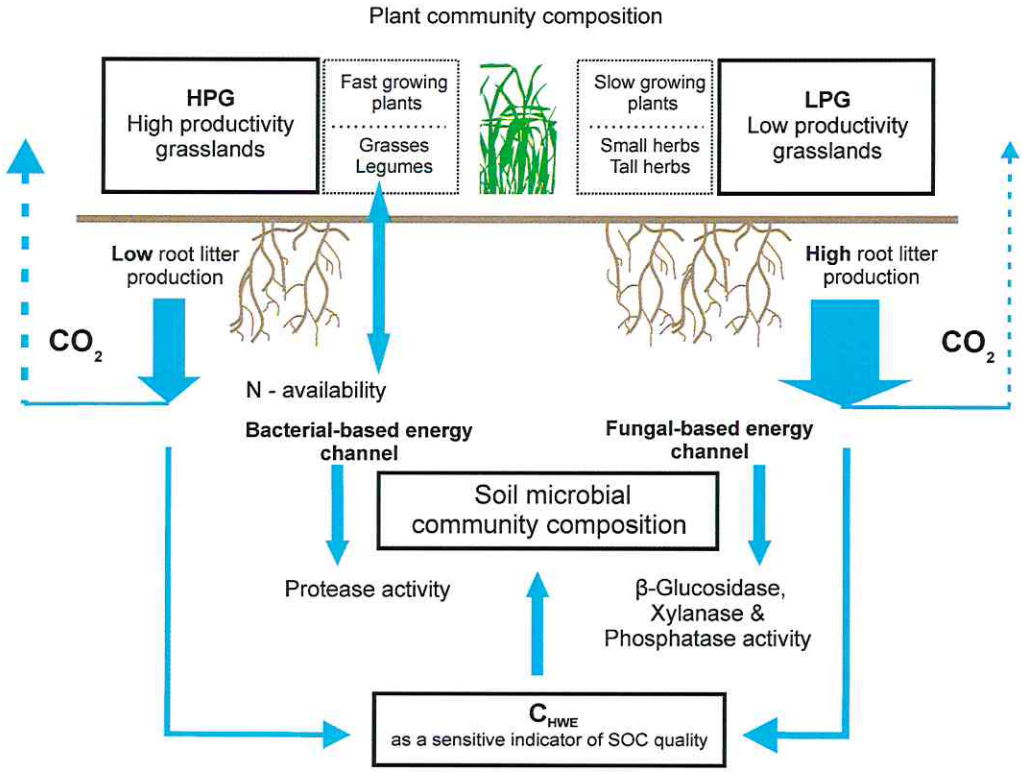

Figure E.S. 3 The main results of the first study (*Chapter 1*), illustrating the effects of the plant community composition on a labile carbon fraction (C_{HWE}) and on the soil food web in semi-natural grassland ecosystems with differing productivities. The depicted effects refer to positive and negative interactions within the two grassland types.

The results demonstrated that above-ground and below-ground subsystems in grassland ecosystems are closely linked and that the feedback loops connecting these two subsystems play an important role in controlling SOM dynamics. The composition of the plant community and its quality and quantity significantly affected SOM dynamics and related below-ground microbial processes. Environmentally-induced changes in the composition of plant communities can thus significantly influence C accumulation in soils.

6.2 Functional SOM pools: SOC stocks and stability indicators (*Chapter 2*)

Reliable data on the distribution of OC in the functional SOM pools (e.g. in deeper soil horizons), their stability and the potential influence of the plant community on these pools are sparse. Soils' C accumulation potential is determined by the soil type, size of their functional SOM pools and their turnover. It is therefore necessary to study the relevant functional SOM pools and the mechanisms by which they are stabilised in order to understand the dynamics of SOM.

The second study describes a series of analyses of isolated functional SOM pools (complexed SOM: CF1, CF2 and uncomplexed SOM: LF1, LF2) of whole soil profiles in grassland ecosystems of Central Germany, that were conducted to obtain a better understanding of the distribution and stability of OC in the functional SOM pools and therefore a comprehensive overview of the distribution of C stocks in soils.

The results showed that largest SOC stocks were found to be located in the topmost 30 cm of the soil. However, around 17% was stored at lower depth. High SOC stocks were found in the two clay fractions (CF1, CF2); most of the SOC within the subsoil was bound to clay minerals. The importance of clay particles in C accumulation in soils was confirmed by the high carbon enrichment factors (E_{SOC}) in CF1. The increase of E_{SOC} in this fraction with depth is indicative of the absorption of the mobile SOC by clay (Tiessen & Stewart, 1983, Saroa & Lal, 2003). High SOC stocks (about 19%) were also detected in LF1 of the topsoil; these originate primarily from high inputs of plant material; LF2 accounted for less (approx. 12%) of the total SOC.

The main factors affecting both CFs were soil depth and texture, suggesting relatively slow cycling of OC in these fractions because these are strongly associated with clay minerals and protected from mineralisation in undisturbed aggregated soils (Figure E.S. 4). A direct influence of the plant community was not found. Radiocarbon dating of the CF1 in deeper soil layers confirmed that the OC of the clay fractions has long residence times and is presumably

controlled by pedogenic processes. Stocks in both LFs were strongly influenced by the plant species in the topsoil and by soil chemical properties.

The isolated individual functional SOM pools were found to be more or less stabilised or decomposable, depending primarily on their interactions with mineral soil components and recalcitrance. To qualitatively discriminate between functional SOM pools of differing stability, the C hot water extractability (C_{HWE}) and the natural abundance of ^{13}C were used as stability indicators in bulk soil samples and of functional SOM pools. Organic carbon in the functional SOM pool, which is readily extracted, is likely to be highly degradable, whereas a low extractability would be expected in stable pools with a low degradation potential. The comparatively low C extractability of clay fractions relative to that of bulk soil suggests that the OC in these fractions is physically protected within aggregates that form physical barriers to microbes and enzymes. After disaggregation by sonication during the fractionation process, the OC in the clay fractions became accessible, to some extent (Cheshire *et al.*, 2000). The hot water extractability of the OC in LF1 was significantly greater than that in LF2, indicating that the OC of LF1 was rather unstable and degradable while that of LF2 was more robust and had a lower degradation potential (Figure E.S. 4).

The $\delta^{13}C$ values were used as a measure of the quality of OC in the functional SOM pools. They were found to increase slightly with soil depth in both clay fractions (CF1, -26.4‰ to -24.7‰ and CF2, -26.8‰ to -25.2‰) due to preferential utilisation of the lighter ^{12}C isotope during microbial and chemical transformation processes. In contrast, the $\delta^{13}C$ values of LF1 and LF2 remained at a relative constant negative level, indicating that fresh OC from plant material was incorporated in these fractions (LF1, -27.7‰ to -27.8‰ and LF2, -27.4‰ to -26.9‰).

The $\delta^{13}C$ values were found to correlate strongly with the ratio of the C_{HWE} extractable from the SOC of the bulk soil to the OC for individual functional SOM pools. More stable OC was closely associated with the bulk soil and clay fractions (Figure E.S. 4). The two LFs were found to differ in both stability and the availability of OC, due to the preference of microorganisms for the lighter ^{12}C isotope.

The results demonstrated that terrestrial grassland ecosystems have the ability to buffer or regulate the amount of CO_2 in the atmosphere by sequestering C in soils and releasing C to the atmosphere. The identification and analysis of different functional SOM pools provided a first comprehensive and detailed overview of the distribution of OC in these pools in grassland ecosystems. Furthermore, it has been shown that the intrinsic properties of

functional SOM pools are highly variable and depend on the extent to which the OC in these pools is stabilised, which was proved by the correlation between $\delta^{13}C$ values and the ratio of the C_{HWE} extractable C content in soils.

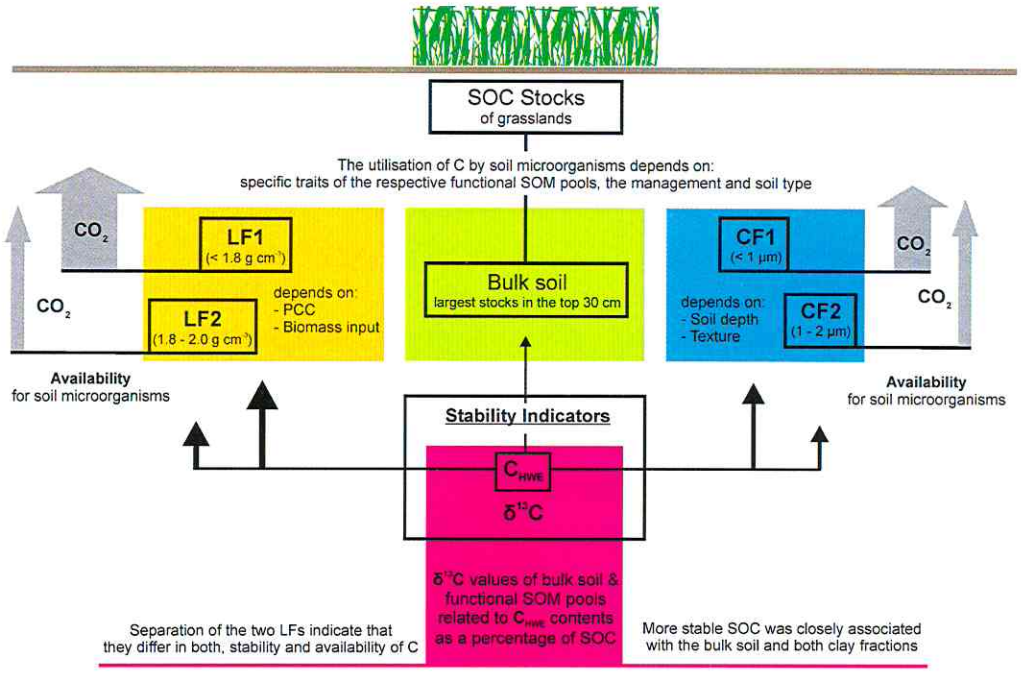

Figure E.S. 4 Main results of the second and third study (*Chapter 2* and *Chapter 3*), showing the stability and stocks of SOC in functional SOM pools (CF1, CF2, LF1, LF2) and their potential availability to soil microorganisms in grassland ecosystems. PCC; plant community composition, C_{HWE}; hot water extractable carbon.

6.3 Bioavailability of OC in functional SOM pools (*Chapter 3*)

The dynamics of SOM are often described using conceptual models, because the quality, stability and availability of SOM can vary widely (Körschens, 1980, Schulz, 2004). Consequently, the bioavailability of OC in the functional SOM pools and the soil microorganisms that are involved in transformation of the OC in these pools are still poorly understood.

The third study aimed to determine the potential decomposability and stability of functional SOM pools (complexed SOM: CF1, CF2 and uncomplexed SOM: LF1, LF2); how levels of

functional groups within microbial communities change during decomposition and how specific functional groups of soil microorganisms control decomposition processes of these pools. Soils from grassland ecosystems of different soil types, climatic conditions and management intensities were studied (Haplic Cambisol: HPG and LPG; Typical Chernozem: Pasture and Prairie).

In general, OC in CF is either protected by interaction with clay minerals or by spatial inaccessibility through occlusion by aggregates. If these aggregates are destroyed, the OC may become available to soil microorganisms as substrates, depending on the specific characteristics of the pool and the land use. Comparatively high respiration of OC was observed in (CF1) of both soil types in the fertile and more productive ecosystems, such as HPG and Pasture grasslands (Figure E.S. 4). This higher respiration of OC was associated with a significantly higher microbial biomass, due to a higher specific surface area of clay. After soil disaggregation and under optimal temperature and moisture conditions, the microbial community was able to easily degrade the OC within CF1; it can be assumed that when the aggregates are intact, the stability of this OC is primarily due to its spatial inaccessibility. However, the low levels of respired OC observed in CF2 from HPG, LPG and Prairie ecosystems indicated that CF2 is more stable; this stability presumably stems from both spatial inaccessibility and from interactions between minerals. This could be linked to higher nutrient turnover and decomposition rates, which was reflected in higher respiration of OC. It could be assumed that the differences in the clay mineralogy of the two soil types (smectites are abundant in the Typical Chernozem) and in the use of the land from which they were taken are also important determinants of the soil microbial structure and the resulting utilisation of the functional SOM pools.

Uncomplexed SOM (light fractions) typically has greater turnover rates than CF (Figure E.S. 4). However, these effects were dependent on the soil type, the site-specific management and the plant community composition, respectively. Low productivity grasslands (Haplic Cambisol) were characterised by slow-growing plants and were dominated by a fungal-based soil food web, leading to an uniformly continuous increase in the decomposition of LF1. The microbial community in the HPG grassland was dominated by gram-negative bacteria and rapidly decomposed the available OC within the first 5 days. It is reasonable to assume that the soil microbial communities of grazed grasslands (Pasture; Typical Chernozem) are dominated by bacteria and would therefore favour decomposition and the loss of C in soils. However, the level of respired OC from LF2 was very low, suggesting that the OC of this

fraction is not available for decomposition, resulting in a decrease of the abundance of functional groups of soil microorganisms; the OC in LF2 is thus likely to be stabilised by recalcitrance. Only certain specialised microorganisms found in the LPG ecosystems were capable of exploiting the OC within LF2; these microbes were responsible for much of the observed total OC respiration within these soils.

The results reported first insights into the microbial utilisation of functional SOM pools and clearly demonstrated that the utilisation of these pools is dependent on three factors: (1) specific traits of functional SOM pools, like the association to the mineral soil matrix (complexed SOM) or the chemical stability of OC compounds of uncomplexed SOM; (2) the management intensity of the grasslands. This had significant influences on the quality of the functional SOM pools, affecting the accessibility to soil microorganisms and (3) soil type, which yielded in different intensities of released CO_2. Depending on the availability of the OC in individual functional SOM pools, the three factors were responsible for the formation of a specific microbial community.

7. Final Conclusions

It is still astonishing that we know so little about soils, their ecology and the roles they play in nutrient cycling and in regulating ecosystem functions. Richard Bardgett noted in the closing comments of 'The Biology of Soil: A Community and Ecosystem Approach' (2005): "[...] *soils remains the least understood, and perhaps most abused, habitat on Earth*". However, in recent years, more and more ecologists, environmental scientists and even policy makers have begun to pay attention to soils. In addition to the interest in the diversity of soil organisms and the feedback loops connecting above-ground and below-ground communities as important drivers in ecosystem dynamics, soils have recently come into focus because of its impact on the global C cycle. The exchange of CO_2 between land and atmosphere is particularly important in this context. Since only a small percentage of the C input to soils becomes stabilised, it is important to understand the physicochemical processes that stabilise C within the soil and protect it against microbial decomposition. Likewise it is crucial to understand how management processes and vegetation changes regulate these processes, which have the potential to sequester C in grassland soils.

The results reported herein clearly demonstrated that the quality and quantity of SOC, which is a function of the plant species composition in the extensively managed grassland ecosystems, have a profound influence on below-ground transformation processes (decomposition of root biomass, soil microbial community composition and its activity). In particular, the plant functional group of legumes, plays an important role in ecosystem functioning; soils in ecosystems with a high abundance of slow-growing plants exhibit slower nutrient cycling and thus have a higher potential for SOC accumulation, as an important ecosystem service.

By identifying and characterising different functional SOM pools through the soil profile, a first comprehensive and detailed overview of the distribution of OC in the functional SOM pools of grassland ecosystems and therefore a comprehensive overview of the distribution SOC stocks was obtained. High SOC stocks were found in the two clay fractions; most of the SOC within the subsoil was associated to clay minerals. High SOC stocks were also detected in LF1 of the topsoil; these originate primarily from high inputs of plant material, whereas LF2 accounted for less of the total SOC. Both CFs were dominantly influenced by pedogenic processes, suggesting relatively slow cycling of SOC in these fractions. Both LFs were strongly influenced by recalcitrant properties and by the plant species in the topsoil and represent by these SOM pools of fast response to climate induced environmental changes. In addition, a reliable indicator of the stability of functional SOM pools was identified, which was used to ascertain the processes by which the different functional SOM pools were stabilised. This was proved by the correlation between $\delta^{13}C$ values and the percentage of C_{HWE} of the functional SOM pools from the respective total C.

The utilisation of functional SOM pools under optimum incubation conditions clearly revealed first insights into the interactions between functional SOM pools, its properties and the diverse members of the soil communities. The utilisation of OC source from the SOM pools was dependent on three factors: (1) specific traits of functional SOM pools, (2) the management intensity of the grasslands and (3) the soil type. The OC utilisation of the two clay fractions depends on their accessibility to soil microorganisms as specific pool characteristics, which was increased due to disaggregation. However, the OC utilisation of the two light fractions depends on the chemical stability of OC. The OC of LF1 is readily degradable, promoting C losses, whereas the OC within LF2 can be considered as recalcitrant, due to low levels of respired CO_2. Depending on the availability of the OC in individual

functional SOM pools, the three factors were responsible for the formation of a specific microbial community.

The work discussed in the three studies successfully accomplished the main objectives, and enabled a better and detailed understanding of the processes occurring, when studying the C cycle and thereby the dynamics of SOM. The results from grassland ecosystems clearly demonstrate the importance and necessity of linking abiotic and biotic components. Applied to the aims of the BIOLOG – DIVA subproject, grasslands of lower productivity, harbouring a specific plant community composition, play an important role in ecosystem functioning for C accumulation in soils. Furthermore, the consideration of functional SOM pools (uncomplexed and complexed SOM) resulted in a comprehensive view of the distribution of SOC and showed a first insight into the potential availability of OC in these pools.
In the future, more resources must be allocated to terrestrial C cycle research. The C accumulation potentials of soils of different ecosystems, soil types and climatic zones need to be assessed, in particular with regard to the influencing factors land use and environmental changes. The application of molecular techniques will be needed to identify potential key microbial groups involved in the degradation of specific C sources. Moreover, the availability and cycling of nutrients, such as nitrogen and phosphorus with strong effects on the dynamics of C in soils should be addressed in future approaches to improve the knowledge on the dynamics of SOM.

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1 Impact of the composition of the plant community on labile soil organic carbon and soil food webs in semi-natural grassland ecosystems of different productivity

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Submitted for publication in Plant and Soil

Date of submission: 07.04.2011

Abstract

This paper describes the effects of plant litter on soil organic carbon (SOC) and on the soil food web in extensively managed mountainous grasslands in Central Germany that vary both in biomass production and plant community composition. The decomposition of shoot and root litter was studied in a lab-incubation experiment. Labile soil C and N were isolated by hot water extraction (C_{HWE}), while functional groups of microbes were identified by phospholipid fatty acid analysis and microbial activity was measured using a set of soil exo-enzymes. The results indicated that the plant community composition affected SOC dynamics and below-ground microbial processes, especially via roots. This was reflected in about 20% lower decomposition of root litter in low productivity grassland soil. The C_{HWE} soil fraction was found to be a key driver of the below-ground food web, controlling soil microbial processes. Below-ground responses appear to be related to the presence of legume species, which affected the microbial communities, as well as the ratio between fungal and bacterial biomass and patterns of soil enzyme activity. Low productivity fungal-dominated grasslands with slow C turnover rates may play an important role in SOC accumulation. The approach used here is of particular importance, since associated biological processes are fundamental to ecosystem functioning.

Keywords

Above- below-ground feedback, Enzyme activities, Labile soil carbon fraction, Legumes, Litter decomposition, PLFA

1. Introduction

Changes in the composition of the plant community are known to influence ecosystem functions, such as net primary production and biogeochemical cycles (Fornara & Tilman, 2008), as well as the quantity and quality of carbon (C) compounds (e.g. organic matter) entering the soil food web (Wardle & Lavelle, 1997, Tilman *et al.*, 2001). In this context, the composition of the plant community, in terms of plant functional groups and species diversity can also influence C sequestration in soils, although the mechanisms that govern turnover and stabilisation of soil C are poorly understood (Steinbeiss *et al.*, 2008). The different plant functional groups (such as grasses, forbs and legumes) play a particularly important role in ecosystem functioning (De Deyn *et al.*, 2009).

Currently, little is known about the relationships and the interplay between changes in plant communities and climate, the properties of the soil and the general site conditions; likewise, the extent to which such changes affect the soil food web and soil organic carbon (SOC) dynamics remains unclear. This may be because the effects of changes in the composition of the plant community on ecosystem processes, such as changes in the amount and type of fresh organic matter entering the soil, the rate of decomposition, or loss of C are not well understood (Fontaine *et al.*, 2007). A number of studies in this area have generated a broad range of results; changes in net primary production have been reported to have positive, neutral and negative effects on components of the soil food web (Hooper & Vitousek, 1997, De Deyn *et al.*, 2004). Since the above-ground and below-ground components of terrestrial ecosystems are implicitly dependent on each other, (Porazinska *et al.*, 2003), it is necessary to study both subsystems in order to fully assess the impact of changes in the composition of the plant community on soil communities and nutrient cycling (De Deyn & van der Putten, 2005). Moreover, soil organisms release nutrients into the soil, regulate their levels and transform soil organic matter (SOM). These processes are components of feedback loops by which they can affect various aspects of the above-ground system, such as the diversity and productivity of plant communities (van der Heijden *et al.*, 2008). However, the dynamics of SOC are primarily affected by the amount of C entering the soil, for example via plant production and the loss of C through heterotrophic and autotrophic respiration.

The decomposition of plant litter is a key process in the flow of energy and nutrients in terrestrial ecosystems; it is sensitive to changes in the composition of the plant community, management practices and climatic conditions. Plant litter is one of the most readily available

C fractions (Bardgett, 2005) and is thus of pivotal importance for the structure and activity of the soil microbial community. Plant species with different litter properties can significantly influence the structure of the soil community in terrestrial ecosystems. Soil microorganisms are key drivers of litter and SOM decomposition and thus play an important role in nutrient cycling (Bardgett, 2005). However, it is difficult to quantify the amount of SOM that is available to microorganisms. Since dynamics of the soil C pool are known to be linked to changes in the composition of soil microbial communities (Wolters *et al.*, 2000), it is essential to elucidate the relationships between abiotic factors and the composition and activity of the soil microbial community (Bardgett *et al.*, 2005).

Generally, SOC and total nitrogen (TN) content are regarded as the dominant factors in soil fertility (Körschens, 2006). Since changes in SOC caused by climate or different management practices are reported to be very slow, measureable changes in SOC and TN can only be detected by monitoring changes in the levels of responsive C and N fractions and taking these to be indicative of the overall changes in the readily-available soil C and N pools (Böhm *et al.*, 2010). The hot water extractable soil C fraction (C_{HWE}) is one such fraction that can be monitored to estimate the supply of decomposable SOC (Schulz, 2002); data on changes in this labile fraction can provide insights into changes in soil-plant systems. Notably, this fraction is a sensitive indicator of SOC quality; it constitutes the readily-decomposable SOM (Körschens *et al.*, 1998, Ghani *et al.*, 2003). Although some attention has been paid to the effects of management practices on C_{HWE} in agricultural ecosystems (Schulz, 2002, 2004), little is known about the relationship between the composition of the plant community and the makeup of C_{HWE} in grassland ecosystems. In addition, useful functional and structural markers of the composition and activity of the below-ground microbial community have yet to be identified.

Extensively managed semi-natural grasslands in Central Germany with plant communities of various compositions were used as model systems in this study. The main objective was to identify the key drivers of litter decay and to determine its significance for C accumulation in soils of grasslands. We therefore performed respiration experiments using above- and below-ground plant litter as the substrate to obtain detailed information on litter quality. We then investigated the effects of plant litter input on C_{HWE} and microbial activity and determined the effects of SOC quality on structural and functional markers (the PLFA pattern and enzyme activities) of the below-ground microbial community. Finally, we tested the effect of the composition of the plant community and soil chemical parameters on the soil food web in order to identify the main determining factors.

2. Materials and Methods

2.1 Study area and plot description

The experimental sites were located in the semi-natural grasslands of the Thuringian and Franconian Forest, which is situated on a plateau-like mountain range (606-703 m a.s.l.) at the border between Bavaria and Thuringia (Central Germany) (Table 1. 1). The area's natural vegetation consists of montane mixed spruce, fir and beech forests. The forests were converted during the 15th century into an agricultural landscape with a high proportion of mountain hay meadows and pasture grasslands (Kahmen *et al.*, 2005). The study focused on 10 experimental plots (each 20 m x 15 m, which were further divided into 5 m x 5 m subsides), which were classified on the basis of their plant biomass production into six low productivity grasslands (LPG) and four high productivity grasslands (HPG). The 10 plots were located within an area of 40 km^2 and were treated for the purpose of this study as independent replicates. They differ significantly in terms of their plant biomass production, the composition of their plant communities and their litter quality (Table 1. 1, Table S1. 1, Table S1. 2) The soil was classified as a carbonate free Haplic Cambisol (siltic) under weak stagnic conditions (FAO classification) and was formed mainly from schist and greywacke bedrock. The area's average annual precipitation is about 1000 mm and the average temperature is 6.0-7.0°C. The sites are not fertilized or used for grazing and have been extensively managed for at least 20 years. Their management consists of two cuts per year, one in early summer and one in the autumn; the cuttings are removed from the sites.

2.2 Soil and vegetation analysis

About 100 single soil samples were taken with an auger (1 cm diameter, 30 cm length) from each of the ten grassland plots in June 2008 of the control sub sites. Samples were acquired at three different depths (depth 1, 0-10 cm; depth 2, 10-20 cm; and depth 3, 20-30 cm). The samples for each plot and soil depth were pooled, resulting in 30 samples for analysis. Portions of the pooled samples to be used for microbial parameter analyses (see below) were frozen at -20°C after sampling. Before chemical analyses, the soil samples were sieved to < 2 mm, visible plant residues and stones were removed by hand and the samples were air-dried and finely ground to powder. The SOC and TN contents of the bulk soil samples were determined by combustion in a C/H/N analyser (Vario El III, Elementar - Hanau, Hanau, Germany).

In June 2008, vegetation surveys were carried out on two 1 m^2 areas within each plot the cover of all vascular plant species present was estimated visually using a modification of the Londo scale (Londo, 1984) (for species per plot, see Table S1. 1). The plant species cover was used to calculate the Shannon diversity index and Evenness for each plot (Magurran, 1988). Vascular plant species were classified into one of four functional groups: grasses, small herbs, tall herbs and legumes (Table S1. 2). In addition, above-ground biomass was harvested in each plot at peak standing biomass from four 25 cm x 30 cm quadrates, by cutting 3 cm above the ground. The rest of the biomass was removed in accordance with local management practices. Annual root biomass production was harvested and estimated in 2007, using in-core growth from five cores taken from each plot (5 cm diameter, 20 cm length; Lauenroth, 2000). Plant biomass was dried at 105°C and finely ground to a powder.

2.3 Litter decomposition experiment

20 g portions of an Albic Luvisol soil (FAO classification), which was used as a standard in the laboratory incubation experiments, were placed in 250 ml polyethylene vessels. The experimental units (vessels) consisted of soil alone (control) and soil amended with the harvested root and shoot biomass at a ratio of 500 mg C kg^{-1} soil, in triplicates per grassland site. The soil was rewetted with distilled water to 60% of its maximum water holding capacity and the ground biomass was added. The vessels were incubated in an automatic respirometer (Respicond V, Nordgren Innovations AB, Sweden; Nordgren, 1988) for 35 days and measurements of CO_2 evolution were taken hourly. To quantify the CO_2 produced from each biomass sample, the CO_2 emitted by the control was subtracted from the total CO_2 evolved.

2.4 Hot water extractable C and N

Hot water extractions (HWE) of the soils were performed according to the method of Schulz *et al.* (2003). Samples (10 g) of air-dried soil (n = 3 replicates per site and soil depth) were boiled in 50 ml deionised water (extraction ratio: 1/5, w/v) for 1 h in a reflux condenser. After cooling to room temperature, 0.1 ml 1 M $MgSO_4$ was added to each resulting suspension, which was then centrifuged for 10 min at 2000 rpm to obtain clear extracts. The extracts were filtered to eliminate microorganisms, using RC 25 Minisart single-use syringe membrane filter units with a 0.45 µm pore size (Sartorius AG, Göttingen, Germany).

To detect readily decomposable C and N compounds in plant material, we used hot water extractions (Klimanek, 1997). Plant material was extracted as follows: 250 mg plant material

(n = 3) was boiled for 2 h in 25 ml deionised water under reflux conditions. The extracts were filtered twice (1st filter: Whatman folded filters, Ø 150 mm, 595 ½ Sartorius AG, Göttingen, Germany; 2nd filter: 0.45 µm Minisart filters, Sartorius AG, Göttingen, Germany). The extracts of the soil samples and plant material were analysed for their total C and N concentrations (C_{HWE}, N_{HWE}) using an elemental analyser for aqueous samples (Micro N/C and Multi N/C, Analytik Jena, Jena, Germany).

2.5 Soil enzyme activities

Alkaline phosphatase (EC 3.1.3.1) and protease activities (EC 3.4.21-24) were analysed in 1 g soil portions. The activities of β-glucosidase (EC 3.2.1.21) and xylanase (EC 3.2.1.8) were analysed in 5 g soil portions, testing three replicates in all cases. All enzyme activities were measured colorimetrically using a spectrophotometer (U-200, Hitachi Ltd., Tokyo, Japan); activities were calculated on the basis of soil dry matter (DM). Blank samples were not incubated with a substrate to correct for variation in the samples' background absorbance. The methods used for the enzyme activity measurements were described in detail by Schinner *et al.* (1993).

2.6 PLFA analysis

Phospholipid fatty acids (PLFA) were extracted from triplicate subsamples of the 30 pooled soil samples using the method of Bligh and Dyer (1959). Soil lipids were extracted using a mixture of chloroform, methanol and citrate buffer (pH 4.0) from fresh soil samples (equivalent to 2 g, DM). The lipid material was separated into neutral lipids, glycolipids and phospholipids, by sequential elution from a silica-bonded solid phase extraction column (SPE-SI; Bond Elute, Varian, Palo Alto, USA) using chloroform, acetone and methanol. Phospholipids were hydrolysed and methylated by adding a methanolic KOH solution. Individual fatty acid (FA) methyl esters were then identified by GC/MS (Hewlett Packard 5971A mass selective detector, combined with a 5890 series II gas chromatograph) and quantified using MSD ChemStation D.01.02.16 chromatography software (Agilent Technologies, United States). PLFA 19:0 was used as an internal standard.

Individual PLFA markers were used to quantify the relative abundance of specific microbial groups, as described by Frostegård *et al.* (1993), Pennanen *et al.* (1996), White *et al.* (1996) and Zelles (1999). Bacterial biomass ($PLFA_{Bac}$) was quantified as the sum of the i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, cy 17:0, 17:0, 18:1ω7, cy 19:0 fatty acids. The fatty acids i15:0,

a15:0, i17:0 and a17:0 were considered to be specific for Gram-positive ($PLFA_{Gram(+)}$) and cy 17:0 and cy 19:0 for Gram-negative bacteria ($PLFA_{Gram(-)}$). The quantity of the PLFA 18:2ω6 was used as an indicator of fungal biomass ($PLFA_{Fung}$). The biomass of Protozoa ($PLFA_{Protozoa}$) was estimated from 20:2ω6,9c, 20:3ω6,9,12c and 20:4ω6,9,12,15c fatty acids; that of the Actinomycetes ($PLFA_{Actinomycetes}$) was estimated from the FAs 10Me16:0, 10Me17:0 and 10Me18:0. The total sum of all PLFAs found ($PLFA_{Tot}$) was used to estimate the total microbial biomass. The ratio of fungal to total bacterial biomass (F:B) was used as an index of the relative abundance of fungi and bacteria in the soils (Bardgett *et al.*, 1996). The ratio of total saturated to total unsaturated FAs (SAT:UNSAT ratio) was also determined, since it can be used as an indicator of nutritional stress in bacterial communities and nutrient availability (Bossio *et al.*, 1998).

2.7 Data analysis

All data were tested for normality and homogeneity of variance and are presented as arithmetic means ± standard errors (SE). The effects were regarded as significant if $P \leq 0.05$. The effects of soil depth (0-10 cm, 10-20 cm and 20-30 cm) and grassland type (LPG and HPG) on hot water extractable C and N and microbial parameters, were analysed by two-way analysis of variance (ANOVA). Grassland plots were included in the model as a non-interacting factor and were eliminated in cases of non-significance using model simplification. Differences between CO_2 accumulations amongst grassland types were tested by a one-way ANOVA, followed by a *Tukey's post hoc test*. These analyses were performed in R, version 2.9.2 (R Development Core Team, 2009). If the assumptions of the model were not met, a Box-Cox transformation was carried out.

PLFA profiles obtained from the sampled soils were compared and analysed by Principal Component Analysis (PCA), using the SPSS software package (SPSS Statistics 17.0.1, Chicago, USA), following log-transformation of the PLFA data to satisfy assumptions of normality and variance homogeneity.

To investigate the responses of soil microbial parameters to environmental variables, Canonical Correspondence Analysis (CCA) was applied. All data for the CCA were also log-transformed to satisfy assumptions of normality and variance homogeneity (except data on the presence or absence of plant species). The CCA was performed using CANOCO software Version 4.5 for Windows (Ter Braak & Smilauer, 2002). Automatic selection by means of Monte Carlo permutations (4999 unrestricted permutations) was used to test the significance of the variables. A forward selection of environmental variables was performed in order to

determine which variables best explained the microbial data. At each step, a Monte Carlo statistical test was carried out to assess the significance of the selected variable.

Table 1. 1 General plot descriptions, including vegetation data, soil texture and soil chemical properties, for the two grassland types studied. Annual temperature and total annual precipitation data were provided by "Bayerische Landesanstalt für Wald und Forstwirtschaft in Freising" and data on clay, silt and sand contents were obtained from Gründling (2010). Vascular plant species were identified and classified into the following functional groups on a percentage cover basis: grasses, small herbs, tall herb and legumes. All values are given as means ± SE.

	Grassland type	
	Low productivity grasslands	High productivity grasslands
Average annual temperature (°C)	6.43	
Elevation a.s.l. (m)	645.43 (± 14.04)	638.25 (± 2.06)
Total annual precipitation (mm)	1346.40	
Species richness (N)	19 (± 2)	16 (± 2)
Shannon Diversity index	1.84 (± 0.20)	1.99 (± 0.17)
Evenness	0.69 (± 0.06)	0.72 (± 0.08)
Shoot biomass ($g_{dm}\ m^{-2}$)	134.59 (± 11.05)	217.85 (± 28.36)
Root production (g)	0.72 (± 0.18)	0.44 (± 0.05)
C_{HWE} (mg kg^{-1}) of shoots	108010 (± 2690)	98213 (± 2770)
N_{HWE} (mg kg^{-1}) of shoots	36580 (± 146)	30127 (± 1570)
C_{HWE} (mg kg^{-1}) of roots	59210 (± 7698)	78585 (± 1052)
N_{HWE} (mg kg^{-1}) of roots	2460 (± 175)	3584 (± 519)
Grasses (%)	73.3 (± 9.1)	84.9 (± 8.5)
Small herbs (%)	35.6 (± 9.1)	42.1 (± 6.2)
Tall herbs (%)	18.3 (± 2.7)	13.5 (± 4.6)
Legumes (%)	1.8 (± 0.5)	12.0 (± 1.93)
Soil type	Haplic Cambisol (siltic)	
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)

3. Results

3.1 Decomposition of plant litter

In the decomposition experiment, input of plant material (particulate organic matter of root and shoot necromass) yielded a significant increase in released CO_2 ($P \leq 0.001$; Table 1. 2). The evolution of CO_2 was saturated on day 30 in incubations with the above-ground biomass, but continued to increase until the end of the experiment in incubations with the root biomass. The percentage of respired shoot biomass-derived C was significantly higher than that from root biomass ($P \leq 0.001$, Figure 1. 1a, b). No significant differences were detected in the CO_2 evolution from incubations of shoot biomass from the two grassland types. During the 35 days of incubation, root biomass from HPG plots decomposed significantly ($P \leq 0.001$; Table 1. 2) faster than root biomass from LPG plots (Figure 1. 1). About 43% of the HPG root biomass decomposed, compared to just 37% of the LPG root biomass.

3.2 Labile soil C and N fraction

The C_{HWE} and N_{HWE} fractions ($P \leq 0.001$; Table 1. 2) of the bulk soil samples declined significantly with depth (Figure 1. 2a, d). The largest pools were detected in the first soil layer (0-10 cm). However, the $(C:N)_{HWE}$ ratio was not affected by depth. The grassland type had a highly significant effect on the C_{HWE} fraction ($P \leq 0.001$) and the $(C:N)_{HWE}$ ratio ($P \leq 0.001$) (Table 1. 2). The $(C:N)_{HWE}$ ratio was about 11% and the C_{HWE} fraction about 21% higher in LPG. In addition, the labile soil C and N fraction displayed a significant two-way interaction between soil depth and grassland type (C_{HWE}, $P \leq 0.001$; N_{HWE}, $P \leq 0.05$; Table 1. 2).

The percentage share of C_{HWE} and N_{HWE} from SOC and TN respectively was not significantly influenced by grassland type (Table 1. 2). However, considering just the top 10 cm, a significant higher percentage of C_{HWE} and N_{HWE} from SOC and TN were detected in LPG (*Tukey-test*).

Table 1. 2 Summary of results of a two-way ANOVA testing the effects of two factors (soil depth and grassland type) and their interaction on C and N contents in hot water extractable SOM (C_{HWE}, N_{HWE}), on the percentage of C_{HWE} and N_{HWE} pools in the respective total pools of C and N and soil enzyme activities and PLFA parameters. Individual grassland sites were included in the model as a non-interacting factor. Bold values indicate significant responses.

	Soil depth		Grassland type		Soil depth x Grassland type	
	F-value	*P*	*F*-value	*P*	*F*-value	*P*
C_{HWE}	$F_{2,24}$ = **80.85**	≤ 0.001	$F_{1,24}$ = **8.50**	≤ 0.001	$F_{2,24}$ = **7.74**	≤ 0.001
N_{HWE}	$F_{2,24}$ = **69.44**	≤ 0.001	$F_{1,24}$ = 1.98	0.172	$F_{2,24}$ = **4.95**	≤ 0.05
$(C:N)_{HWE}$ ratio	$F_{2,23}$ = 2.29	0.124	$F_{1,23}$ = **25.18**	≤ 0.001	$F_{2,23}$ = 0.44	0.648
C_{HWE} (% of SOC)	$F_{2,24}$ = **21.27**	≤ 0.001	$F_{1,24}$ = 0.30	0.595	$F_{2,24}$ = 2.37	0.092
N_{HWE} (% of TN)	$F_{2,23}$ = **28.85**	≤ 0.001	$F_{1,23}$ = 0.48	0.690	$F_{2,23}$ = 1.92	0.152
Alkaline phosphatase	$F_{2,24}$ = **22.23**	≤ 0.001	$F_{1,24}$ = **4.29**	≤ 0.05	$F_{2,24}$ = 2.06	0.149
Protease	$F_{2,23}$ = **31.11**	≤ 0.001	$F_{1,23}$ = **50.80**	≤ 0.001	$F_{2,23}$ = 1.41	0.265
Xylanase	$F_{2,24}$ = **85.53**	≤ 0.001	$F_{1,24}$ = **4.32**	≤ 0.05	$F_{2,24}$ = 1.41	0.975
β-glucosidase	$F_{2,24}$ = **31.76**	≤ 0.001	$F_{1,24}$ = **14.60**	≤ 0.001	$F_{2,24}$ = 1.21	0.316
$PLFA_{Tot}$	$F_{2,24}$ = **79.69**	≤ 0.001	$F_{1,24}$ = 2.85	0.104	$F_{2,24}$ = 1.06	0.363
$PLFA_{Fung}$	$F_{2,24}$ = **36.92**	≤ 0.001	$F_{1,24}$ = 2.33	0.140	$F_{2,24}$ = 1.06	0.930
$PLFA_{Bac}$	$F_{2,24}$ = **37.30**	≤ 0.001	$F_{1,24}$ = 0.35	0.560	$F_{2,24}$ = 0.53	0.594
$PLFA_{Gram(+)}$	$F_{2,24}$ = **21.33**	≤ 0.001	$F_{1,24}$ = **21.37**	≤ 0.001	$F_{2,24}$ = 0.93	0.408
$PLFA_{Gram(-)}$	$F_{2,23}$ = **36.97**	≤ 0.001	$F_{1,23}$ = 3.07	0.071	$F_{2,23}$ = 1.93	0.168
$PLFA_{Protozoa}$	$F_{2,23}$ = **21.17**	≤ 0.001	$F_{1,23}$ = **8.83**	≤ 0.05	$F_{2,23}$ = 0.63	0.540
$PLFA_{Actinomycetes}$	$F_{2,24}$ = **34.79**	≤ 0.001	$F_{1,24}$ = 0.76	0.391	$F_{2,24}$ = 2.01	0.155
F:B ratio	$F_{2,24}$ = 0.98	0.383	$F_{1,24}$ = **5.77**	≤ 0.05	$F_{2,24}$ = 0.11	0.896
SAT:UNSAT ratio	$F_{2,24}$ = **4.04**	≤ 0.05	$F_{1,24}$ = 3.23	0.085	$F_{2,24}$ = 0.67	0.521

3.3 Soil microbial properties

A significant decline in the activity of all measured soil enzymes were found with increasing soil depth, in both grassland types ($P \leq 0.001$; Figure 1. 2b, c, e, f). The ANOVA also showed that protease, xylanase and β-glucosidase activities were all significantly influenced ($P \leq 0.001$, 0.05 and 0.001, respectively) by grassland type and there were close-to-significant effects on alkaline phosphatase activity ($P = 0.050$; Table 1. 2). The enzyme activities were generally higher in LPG than in HPG and the highest activities were detected in the upper soil layer (0-10 cm). Overall, the alkaline phosphatase and β-glucosidase activities were approximately 31% and the xylanase activity approximately 15% higher in LPG. In contrast, the protease activity was about 48% higher in HPG ($P \leq 0.001$; Figure 1. 2c).

The Principal Component Analysis (PCA) of the PLFA profiles revealed that the structure of the soil microbial community varied with both soil depth and grassland type (Figure 1. 3). Principal Component 1 explained 52.9% of the variation in PLFA composition and Principal Component 2 a further 44.6%. While $PLFA_{Gram(+)}$ ($P \leq 0.001$), $PLFA_{Protozoa}$ ($P \leq 0.05$) and the F:B ratio ($P \leq 0.05$) were significantly higher in LPG (Table 1. 2, Table 1. 3), the SAT:UNSAT ratio ($P = 0.082$) increased from 0.86 (± 0.14 SE) in LPG to 0.94 (± 0.15 SE) in HPG (Table 1. 3). $PLFA_{Tot}$, $PLFA_{Fung}$, $PLFA_{Bac}$ and $PLFA_{Actinomycetes}$, were not significantly influenced by grassland type (Table 1. 2).

Table 1. 3 Relative abundance of phospholipid fatty acids (PLFA) signatures, expressed in nmol g^{-1} DM, in low productivity and high productivity grasslands. All values are given as means ± SE.

	Grassland type	
	Low productivity grasslands	High productivity grasslands
$PLFA_{Tot}$	33.02 (± 3.8)	29.26 (± 4.1)
$PLFA_{Fung}$	1.65 (± 0.2)	1.41 (± 0.3)
$PLFA_{Bac}$	7.71 (± 1.0)	9.18 (± 1.9)
$PLFA_{Gram(+)}$	3.28 (± 0.5)	1.99 (± 0.3)
$PLFA_{Gram(-)}$	4.08 (± 0.6)	3.83 (± 0.6)
$PLFA_{Protozoa}$	3.27 (± 0.5)	3.21 (± 0.7)
$PLFA_{Actinomycetes}$	0.39 (± 0.1)	0.47 (± 0.1)
F:B ratio	0.22 (± 0.0)	0.15 (± 0.0)
SAT:UNSAT ratio	0.86 (± 0.1)	0.94 (± 0.1)

3.4 Relation between environmental factors and microbial parameters

The environmental variables that best explained the microbial data were examined using Canonical Correspondence Analysis (CCA; Figure 1. 4). The CCA showed that the C_{HWE} fraction explained most of the variance ($P \leq 0.001$) between the estimated microbial parameters (Figure 1. 4). In addition, $PLFA_{Gram(-)}$, $PLFA_{Protozoa}$ and $PLFA_{Actinomycetes}$ were influenced by the C_{HWE} fraction. In addition, the C_{HWE} fraction was also found to have weak effects on $PLFA_{Gram(+)}$, the F:B ratio, $PLFA_{Fungi}$, soil enzyme activities, $PLFA_{Bac}$, $PLFA_{Tot}$ and the SAT:UNSAT ratio. CCA axis 1 suggested a correlation between the F:B ratios and $PLFA_{Fungi}$ on the one hand and the presence of certain plant species on the other - specifically, *Lychnis flos-cuculi* ($P \leq 0.05$), *Campanula rotundifolia* ($P \leq 0.01$) and the two legume species present, *Trifolium repens* and *T. Pratense*. In addition, plant species, such as *L. flos-cuculi* and *C. rotundifolia* affected $PLFA_{Gram(+)}$, while *Holcus lanatus* affected $PLFA_{Actinomycetes}$ ($P \leq 0.05$). The eigenvalues of the first and second axes in the ordination diagram were 0.028 and 0.004, indicating that the majority of the variance within the data was associated with axis 1.

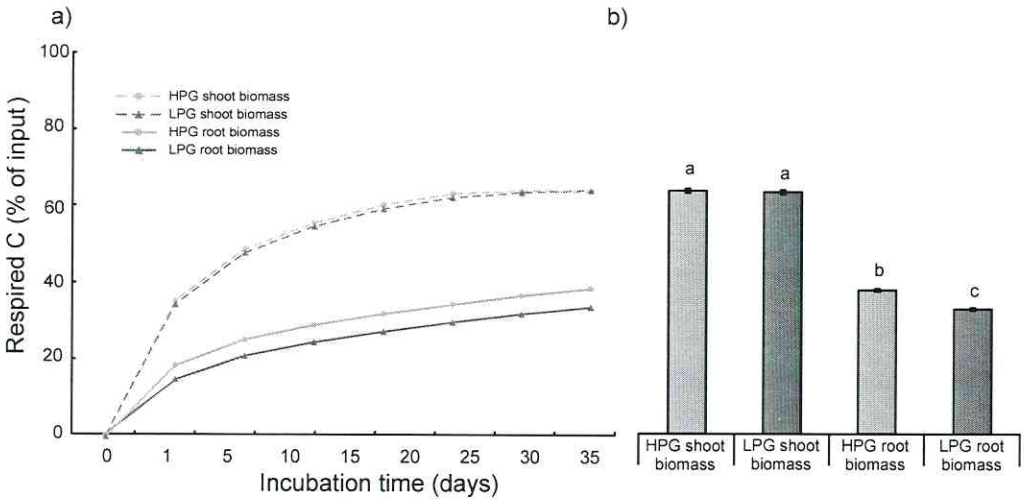


Figure 1. 1 a) Respired C (% of input) over time and b) total respired C (% of input) after 35 days of incubations of soil + root biomass and soil + shoot biomass samples from two grassland types. Values are arithmetic means ± SE and values with the same letter are not significantly different at $P \leq 0.05$.

4. Discussion

4.1 The decomposability of plant litter and its effect on labile soil C and N

Recently, soil respiration experiments have been used to determine the potential effects of cellulose or litter addition on macro-aggregate dynamics (Helfrich *et al.*, 2008), the stability of organic C in soil-size fractions (Ohm *et al.*, 2007) and microbial competition (Fontaine *et al.*, 2003). The respiration experiment presented here provided information on the decomposability of the particulate necromass in relation to litter type, even though no significant differences in their C:N ratios were found. Total soil respiration strongly increased after the addition of shoot and root litter. During the decomposition of shoot litter, CO_2 respiration was significantly higher than that during root litter decomposition. This was probably due to the higher levels of readily available C and N compounds and lower levels of recalcitrant compounds in shoot litter. However, no significant differences in shoot litter decomposition were found between the two grassland types. But there was a significant difference in root litter decomposition between the grassland types. Roots of LPG were less degradable (Figure 1. 1a, b), which is consistent with their lower C and N hot water extraction contents (Table 1. 1). The hot water extraction contents of plant material proved to be useful indicators of readily decomposable plant C and N compounds, reflecting a close correlation between extraction contents and cellulose, hemicellulose and lignin contents (Klimanek, 1997). The quality of root litter, reflecting plant species composition, is a key factor controlling mineralisation processes. A higher proportion of legume species with a higher litter quality (low C:N ratio) in HPG and a greater species richness of small and tall herbs in LPG could be expected to affect decomposition rates. We suggest that these differences are responsible for the observed decomposition rates and that these differences have important consequences for soil biota and the processes they influence. Fresh organic C input from litter activates the growth of microorganisms and alters the structure of soil microbial communities, depending on the quantity and quality of available substrates (Griffiths *et al.*, 1999, Fontaine *et al.*, 2003).

Besides the differences in the composition of the plant community, the below-ground biomass production was higher in the LPG. Despite a lower hot water extraction content from root necromass in the LPG, the higher biomass production of vital roots contributed to increases in the sizes of the labile C_{HWE} and N_{HWE} fractions, especially in the uppermost soil layer. As a consequence, the ratios of C_{HWE} to the total SOC and of N_{HWE} to the total N pool were higher

in the topsoil layer of LPG. The C_{HWE} fraction, which is a component of the labile soil C fraction and consists mainly of simple organic compounds that are hydrolysed under the extraction conditions (100°C), originates from soil microbial biomass, root exudates and lysates present in the soil solution (Leinweber *et al.*, 1995). Water extractions at lower temperatures, such as those described by Sparling (1998), are mainly used to assess microbial biomass. Thus, the extracted C is of predominantly microbial origin. In contrast, according to Schulz *et al.* (2003) and Hoffmann *et al.* (2006), C_{HWE} can be seen as a sensitive indicator of decomposable SOC. Körschens *et al.* (1998) reported that SOC is closely related to microbial activities, such as soil respiration and NO_3-release. Thus, C_{HWE} can be regarded as an indicator of these processes.

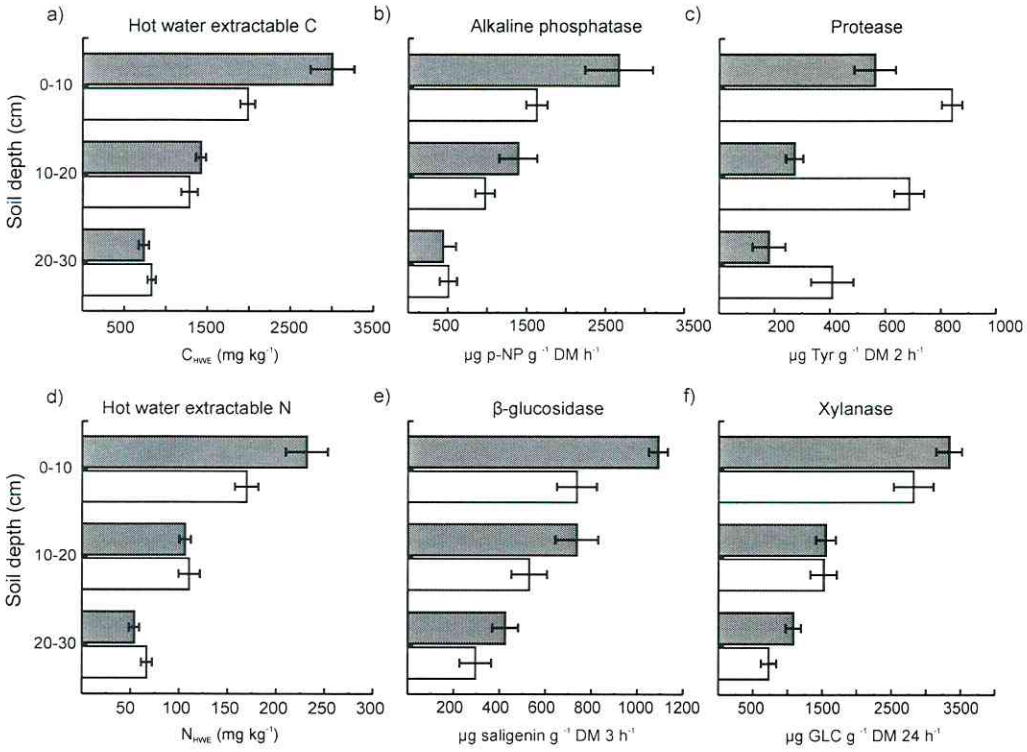


Figure 1. 2 Labile soil C and N in hot water extracts (HWE) and enzyme activities in samples from three soil layers in high and low productivity grasslands: a) Hot water extractable C, b) Alkaline phosphatase, c) Protease, d) Hot water extractable N, e) β-glucosidase and f) Xylanase. Grey bars represent low productivity and white bars high productivity grasslands. Bars represent arithmetic means ± SE.

4.2 Soil microbial components

The soil microbial community regulates transformation processes in soils (Paul, 2007) and requires extracellular enzymes that break down SOM components during the decomposition of plant litter. The production and activity of these enzymes are closely linked to soil community dynamics and the functioning of the ecosystem (Sinsabaugh *et al.*, 2002). For example, high concentrations of labile soil C in the upper soil layer (0-10 cm) were responsible for the high activity of cellulose degrading soil enzymes in LPG sites.

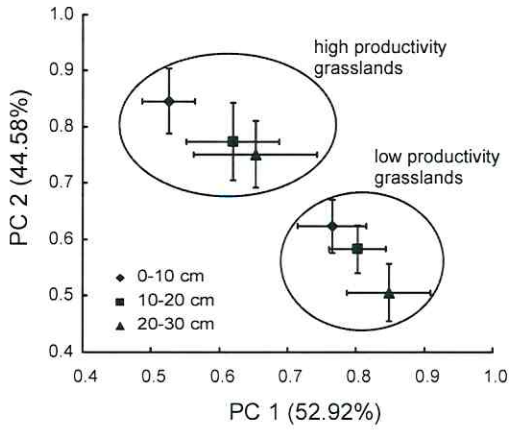


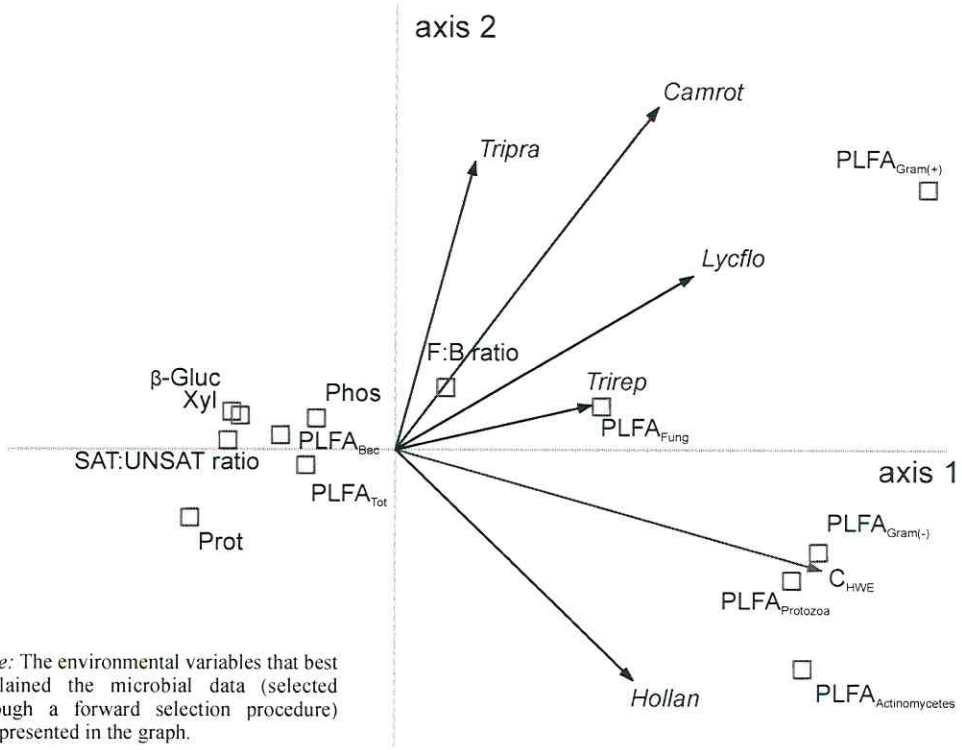
Figure 1. 3 Principal Component Analysis (PCA) of PLFA patterns obtained from soil samples from low productivity and high productivity grasslands at three depths. Data are presented as arithmetic means ± SE.

In addition, to the microbial community, the influences of particular plant species in driving soil microbial processes are highly variable. De Deyn *et al.* (2009) reported that changes in plant species and functional group richness influenced the storage of C in soils and these responses were related to the presence of N-fixing species and forbs. A higher proportion of legume species, such as *Trifolium repens* and *T. pratense*, in HPG had pronounced effects on the above-ground biomass production, increasing the growth of grasses that have high N use efficiency (Table 1. 1). The activity of protease, one of the most important soil enzymes involved in organic matter mineralisation (Sardans *et al.*, 2008), was significantly higher in HPG than in LPG sites. This activity coincided with higher cover of *Trifolium* spp., a keystone genus and one of the most effective nitrogen-fixing species in European grasslands (Spehn *et al.*, 2002). In general, legumes have low nutrient use efficiency and strong effects on N availability and N supply rates, in both natural and agricultural systems

(Fornara & Tilman, 2008). The significant enhancement of protease activity in HPG might be related to the first phase of N release when peptide bonds between amino acids are hydrolysed. However, higher N availability in HPG soils, resulting from the presence of legume species, did not increase the labile N_{HWE} fraction. In HPG, there was higher above-ground biomass production and overall N uptake by plants. The clear effects of the differences in plant species composition on the soil microbial community were supported by the results of the PLFA analysis (Figure 1. 3). Highest fungal:bacterial (F:B) ratios were detected in LPG, indicating that a more fungal-dominated microbial community was present. This is associated with large amounts of less degradable root litter. Comparing differently managed grasslands, Bardgett and McAlister (1999) and Grayston *et al.* (2004) found a reduction in soil F:B ratios with more intense management, while increases in the F:B ratio were attributable to more efficient nutrient cycling and the abundance of mycorrhizal fungi. Similarly, Smith *et al.* (2008) found an association between high F:B ratios and plant species typical of traditionally managed grasslands in Northern England. Since fungal-dominated soils have slow C turnover rates, extensively managed grasslands with low productivity may play an important role in enhancing SOC accumulation.

4.3 Relation between environmental factors and microbial parameters

The quantity and quality of the labile soil C fraction is a key driver of the activity and structure of the microbial community. As an indicator, C_{HWE} values for soil were even more significant than SOC in affecting structural and functional components of the soil food web (Figure 1. 4). In addition, legume species, such as *Trifolium repens* and *T. pratense*, were responsible for significantly higher above-ground biomass production in HPG. It is likely that legumes have strong effects on N availability in soils. It is also likely that this N is used for biomass production by grasses, such as *Holcus lanatus*, that dominate the plant communities in HPG. Similar complementary effects between grasses and legumes have been reported by Fornara and Tilman (2008). Pronounced effects of legumes on soil fungi and the F:B ratio were identified in the present study. The higher N availability in HPG soils facilitates the development of a bacteria-dominated community, which is linked with higher decomposition rates and nutrient turnover. This benefits fast-growing plant species and is known as the bacterial-based energy channel. The comparatively high F:B ratio and $PLFA_{Fung}$ content of LPG soils, together with the higher abundance of slow growing plants with slower nutrient cycling in LPG areas (which have fungal-based energy channel) indicate that LPG sites are important for SOC accumulation.



Note: The environmental variables that best explained the microbial data (selected through a forward selection procedure) are presented in the graph.

Figure 1. 4 Canonical Correspondence Analysis (CCA) of soil structural and functional parameters. PLFA data and soil enzyme parameters were set as species variables (represented by squares) and individual plant species composition of all grassland sites and all measured soil chemical parameters as environmental variables (represented by arrows). The following abbreviations are used for environmental variables: Camrot, *Campanula rotundifolia*; Hollan, *Holcus lanatus*; Lycflo, *Lychnis flos-cuculi*; Tripra, *Trifolium pratense*; Trirep, *Trifolium repens* and C_{HWE}, hot water extractable soil carbon. The abbreviations used for the microbial data are: Phos, phosphatase; Prot, protease; Xyl, xylanase; β-Gluc, β-Glucosidase; $PLFA_{Bac}$, total bacterial biomass; $PLFA_{Gram(+)}$, gram-positive bacteria; $PLFA_{Gram(-)}$, gram-negative bacteria; $PLFA_{Fung}$, fungal biomass; $PLFA_{Protozoa}$, protozoan biomass; $PLFA_{Actinomycetes}$, actinomycete biomass; F:B ratio, fungal to bacterial biomass ratio; SAT:UNSAT ratio, ratio of total saturated to total unsaturated fatty acids; $PLFA_{Tot}$, total microbial biomass.

4.4 Conclusions

The results indicate that the composition of the plant community affects SOC dynamics and related below-ground microbial processes, especially via the roots. The C_{HWE} of soil provided an indicator of the labile soil C fraction, which in turn proved to be a suitable tool for

detecting early changes of the readily available SOC and its effects on the soil food web. The composition of the plant community and particularly the presence of legumes, had major effects on the soil food web. The presence of legume species in high-productivity grassland systems was associated with specific below-ground responses; specifically, the high N availability in the soils of these systems had pronounced effects on protease activity. In keeping with the high input of root litter in LPG systems, soil enzymes involved in the degradation of cellulose were found to be most active in LPG soils. Traits represented in the above-ground plant community had impacts on the soil food web structure (bacterial- vs. fungal-based energy channel), affecting root litter decay. Leaving aside boundary conditions, such as climate (temperature, precipitation) and soil conditions (soil texture, clay content, water holding capacity), we have demonstrated that the quality of SOC is a key controlling factor for below-ground transformation processes that also depend on the composition of plant communities. Clearly, it is essential to understand the interactions between abiotic and biotic factors.

To understand better the relationships between plant community changes and soil processes in terrestrial ecosystems, further work on vegetation effects on C accumulation in soils is needed. In addition, analyses of the whole soil profile are required, since significant amounts of C can be expected to be stored in deeper soil layers. Such studies will also add to our understanding of biologically and biochemically mediated processes that affect ecosystem function. In order to predict the potential long-term efficacy of soils to incorporate and accumulate atmospheric CO_2, stable or stabilised SOC pools need to be considered. Our results indicate that low productivity grassland soils have the potential to provide more C storage than had previously been thought.

Acknowledgments

This study is part of an interdisciplinary research project of the BIOLOG–Europe programme called DIVA (DIVA–Verbundprojekt Jena III) that was funded by the German Federal Ministry of Education and Research (BMBF), grant no. 01LC0613B. We thank Beate Michalzik for her pre-review, as well as Sabina Christ and Gabor Kovaçs for valuable comments on the manuscript. We also thank Jacqueline Rose and Gabriele Henning for technical assistance in the field and the laboratory, Juliane Specht for providing the root

biomass data, Uwe Langer for his help with the PLFA analysis, Ingolf Kühn for statistical advice and Ilka Egerer for technical coordination of the BIOLOG DIVA–Verbundprojekt.

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Supplementary Materials

Table S1. 1 Individual plant species determined on the ten grassland sites.

Site	S1	S2	S3	S17	S9	S11	S12	S13	S15	S19
Coordinates (E 011° ; N 50°)	37.43°E 24.25°N	37.35°E 24.33°N	37.42°E 24.33°N	27.33°E 24.31°N	23.15°E 24.43°N	24.29°E 26.56°N	20.16°E 26.21°N	20.15°E 27.28°N	27.29°E 224.31°N	27.24°E 23.45°N
Grasland type	HPG	HPG	HPG	HPG	LPG	LPG	LPG	LPG	LPG	LPG
Achillea millefolium	x	x		x	x		x		x	
Agrostis capillaris										x
Ajuga reptans									x	
Alchemilla vulgaris agg.	x		x	x	x	x	x	x	x	x
Alopecurus pratensis									x	
Anthoxanthum odoratum			x	x	x	x	x	x	x	x
Anthriscus sylvestris	x	x	x							
Arrhenatherum elatius		x	x	x	x				x	
Bellis perennis				x					x	
Bistorta officinalis							x		x	
Bromus hordeaceus	x	x	x							
Campanula patula						x				
Campanula rotundifolia				x	x	x	x	x	x	x
Cardamine pratensis					x	x			x	
Centaurea pseudophrygia					x	x			x	
Cerastium holosteoides	x	x	x	x				x	x	
Cirsium palustre				x						
Cynosurus cristatus						x			x	
Dactylis glomerata	x	x	x	x		x		x	x	
Elytrigia repens		x								
Epilobium angustifolium				x						
Festuca pratensis	x	x							x	
Festuca rubra				x	x	x	x	x	x	x
Geranium sylvaticum									x	
Heracleum spondylium	x									
Hieracium pilosella							x			x
Holcus lanatus	x	x		x		x	x	x	x	
Hypericum maculatum			x		x	x				x
Hypochaeris radicata										x
Knautia arvensis					x	x	x		x	
Lathyrus linifolius					x		x			
Leontodon hispidus				x						
Leucanthemum vulgare				x			x	x		
Lolium multiflorum	x									
Lolium perenne		x								
Luzula campestris				x	x	x	x	x	x	x
Lychnis flos-cuculi								x		
Meum athamanticum					x	x	x	x	x	x
Phleum pratense			x							
Phyteuma spicatum					x	x	x	x	x	x
Plantago lanceolata	x		x	x	x	x	x	x	x	x
Poa pratensis	x	x	x	x	x		x	x	x	
Poa trivialis	x	x	x	x				x	x	
Ranunculus acris	x		x		x	x			x	x
Ranunculus repens		x								
Rhinanthus minor						x				
Rumex acetosa	x	x	x	x	x	x	x	x	x	x
Rumex acetosella										x
Rumex obtusifolius			x							
Stellaria graminea					x		x	x	x	x
Taraxacum officinale agg.	x	x	x	x	x	x		x	x	
Trifolium pratense	x	x		x	x	x		x	x	x
Trifolium repens		x	x	x				x	x	
Trisetum flavescens			x	x		x				
Veronica arvensis	x	x								
Veronica chamaedrys		x	x	x	x	x	x	x	x	x
Veronica officinalis				x			x	x	x	
Vicia cracca									x	

Table S1. 2 Assignment of all plant species growing in 10 grassland sites to plant functional groups.

Grasses	Small herbs	Tall herbs	Legumes
Agrostis capillaris	*Ajuga reptans*	*Achillea millefolium*	*Lathyrus linifolius*
Alopecurus pratensis	*Alchemilla vulgaris agg.*	*Anthriscus sylvestris*	*Trifolium pratense*
Anthoxanthum odoratum	*Bellis perennis*	*Bistorta officinalis*	*Trifolium repens*
Arrhenatherum elatius	*Campanula patula*	*Centaurea pseudophrygia*	*Vicia cracca*
Bromus hordeaceus	*Campanula rotundifolia*	*Cirsium palustre*	
Cynosurus cristatus	*Cardamine pratensis*	*Epilobium angustifolium*	
Dactylis glomerata	*Cerastium holosteoides*	*Geranium sylvaticum*	
Elytrigia repens	*Hypericum maculatum*	*Heracleum spondylium*	
Festuca pratensis	*Hypochaeris radicata*	*Hieracium pilosella*	
Festuca rubra	*Knautia arvensis*	*Meum athamanticum*	
Holcus lanatus	*Leontodon hispidus*	*Ranunculus acris*	
Lolium multiflorum	*Leucanthemum vulgare*	*Rumex acetosa*	
Lolium perenne	*Lychnis flos-cuculi*	*Rumex obtusifolius*	
Luzula campestris	*Phyteuma spicatum*		
Phleum pratense	*Plantago lanceolata*		
Poa pratensis	*Ranunculus repens*		
Poa trivialis	*Rhinanthus minor*		
Trisetum flavescens	*Rumex acetosella*		
	Stellaria graminea		
	Taraxacum officinale agg.		
	Veronica arvensis		
	Veronica chamaedrys		
	Veronica officinalis		

2 Stability and stocks of organic carbon in functional SOM pools through the soil profile of semi-natural grassland ecosystems

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Submitted for publication in the European Journal of Soil Science

Date of submission: 02.02.2011

Abstract

Distribution, stability of soil organic matter (SOM) and the vegetation composition are crucial determinants of C dynamics in terrestrial ecosystems. However, precise data that separate between different functional pools of SOM within soil organic carbon (SOC) stocks are sparse. This is in particular the case for grassland ecosystems. This study describes the vertical distribution and stability of SOC stocks in functional SOM pools obtained from samples of semi-natural grasslands of the Thuringian and Franconian Forest in Central Germany. Two clay fractions (CF1, < 1 µm; and CF2, 1 – 2 µm) and two light fractions (LF1, < 1.8 g cm^{-3}; and LF2, 1.8 – 2.0 g cm^{-3}) were separated. The organic carbon (OC) within these fractions and their stability were characterised by analysing their stable carbon isotope composition and carbon hot water extractability (C_{HWE}). The results reveal the major proportion of SOC stored in the topsoil. In the subsoil, SOC is primarily bound to smaller clay particles, where it is protected from decomposition. Soil texture and depth were found to be the main factors influencing the two CFs, whereas the vegetation had the strongest influence on both LFs. The carbon hot water extractability (C_{HWE}) of the bulk soil and functional SOM pools, combined with the natural abundance of ^{13}C, proved to be robust indicators of SOC stability. Measurements of these parameters show that the stability of SOC is higher (and more similar) in bulk soil and CFs than in LFs.

Keywords

Soil organic carbon stocks, Functional SOM pools, Hot water extractable carbon, Depth distribution, Semi-natural grasslands, Natural ^{13}C abundance, ^{14}C radiocarbon age

1. Introduction

Ongoing global warming is considered as a result of the marked increases in global atmospheric concentrations of CO_2 (IPCC, 2007). Associated changes in environmental conditions are expected to strongly influence plants and animals and may affect the structure and distribution of global terrestrial ecosystems (Foley *et al.*, 2003). Terrestrial ecosystems can affect the amount of CO_2 in the atmosphere by sequestering carbon (C) in their soil compartments or releasing it to the atmosphere. Depending on their use, soils can therefore either moderate or accelerate climate change. However, the mechanisms that govern the stability and dynamics of soil organic carbon (SOC) are still poorly understood.

Globally, the C reservoir of soils is estimated to be about three times higher than the atmospheric C pool (Jobbágy & Jackson, 2000). Each soil has a specific C-accumulation potential, depending on the vegetation it supports and climatic factors (Guo & Gifford, 2002). Substantial information on the C sequestration of forest ecosystems and agricultural ecosystems are available (e.g. Valentini *et al.*, 2000). In contrast, information on the SOC dynamics of non-forest ecosystems (e.g. grasslands) and the influence of the vegetation composition is underrepresented. Although grasslands represent one of the most widespread vegetation types worldwide, covering about 20-40 % of the global terrestrial ice-free surface (Cernusca *et al.*, 2008), their contributions to SOC fluxes into the atmosphere and SOC sequestration remains highly uncertain. Indeed, estimates of SOC stocks in grassland ecosystems range between 10% (Eswaran *et al.*, 1993) and 30% (Anderson, 1991) of total global SOC stocks. In addition, Conant *et al.* (2001) have reported that improved management of converted grasslands could lead to the sequestration of considerable amounts of atmospheric C. Thus, there is a profound need for more information on SOC stocks their distribution and their stability (Rumpel & Kögel-Knabner 2011).

Uncertainties regarding SOC stocks are exacerbated by the fact that most relevant studies have focused on stocks in the top 30 cm of the soil (Arrouays *et al.*, 2001, Bellamy *et al.*, 2005) and neglected stocks in the subsoil. Analysing the subsoil is difficult but essential for both calculating SOC stocks in relation to land use management and environmental changes and for predicting SOC accumulation along soils profiles (Schulze *et al.*, 2010). For instance, Don *et al.* (2009) reported that SOC changes are not necessarily restricted to the top 30 cm or Meersmans *et al.* (2009) found small losses of SOC from 1960 to 2006 in the top soil of grasslands in northern Belgium but gains when 0-100 cm of the soil profile was considered. It has only been recognised recently that SOC located in

deeper soil horizons contributes considerably to total SOC stocks (Jobbágy & Jackson, 2000, Schöning & Kögel-Knabner, 2006) and factors governing the stability and dynamics of SOC in the subsoil are not well understood.

Since soil organic matter (SOM) is heterogeneously distributed among soil compartments and its quality, stability and availability vary widely, it is essential to characterise different fractions of SOM. These fractions represent distinct functional SOM pools and can be isolated by various methods, e.g. size-density fractionation, without substantially changing properties relevant to their functions in ecosystems. For instance, Christensen (2001) has shown that organic carbon OC associated with clay particles (< 2 μm) of functional SOM pools play an important role in C sequestration in soils. In contrast, density fractionation enables to isolate SOM that is not or only loosely associated with soil minerals (Kölbl & Kögel-Knabner, 2004). This light fraction reflects a functional SOM pool that is rapidly turned over and strongly contributes to the short-term structural stability of soil. Schulz (2004) and Gulde *et al.* (2008) showed that in agricultural ecosystems biomass residue inputs predominantly accumulate in light fractions with a fast turnover. Thus, combining particle-size and density fractionation has the potential to provide detailed information on SOM dynamics in terrestrial ecosystems.

Little is known about the stability of functional SOM pools in soils and the influencing processes. However, three kinds of stabilisation mechanisms are known: (1) chemical stabilisation, (2) physical protection through its interaction/association with minerals and (3) biochemical stabilisation or low accessibility for biological degradation (Six *et al.*, 2002, von Lützow *et al.*, 2006, Chabbi *et al.*, 2009).

Generally the hot water extractability of soil C (C_{HWE}) is a sensitive indicator of labile SOM (Hoffmann *et al.*, 2006). Schulz *et al.* (2011) applied the C_{HWE} as a stability indicator to functional SOM pools. High hot water extractability indicates the presence of OC that is potentially readily degradable, whereas low extractability indicates the presence of stable C with low degradability. In addition, analyses of the natural abundance of ^{13}C allow the residence time and size of functional SOM pools to be estimated (Gleixner *et al.*, 2002); provide information on the vertical distribution of young SOC and the dynamics of functional SOM pools (Balesdent & Mariotti, 1996); and facilitate investigations of the stability and availability of C in different functional SOM pools (Schulz *et al.*, 2010).

The present study used to investigate the importance of grassland ecosystems and its vegetation composition for the distribution of SOC stocks among functional SOM pools and describes the vertical distribution and stability of SOC stocks. Two clay fractions (CF1,

< 1 µm; and CF2, 1 – 2 µm) and two light fractions (LF1, < 1.8 g cm^{-3}; and LF2, 1.8 – 2.0 g cm^{-3}), representing functional SOM pools (complexed SOM: CFs and uncomplexed SOM: LFs), were isolated from the soil profile of grassland ecosystems of the Thuringian and Franconian Forest in Central Germany. The quality of these pools was characterised by analysing their carbon hot water extractability (C_{HWE}) and stable isotope ($\delta^{13}C$) composition. The main objectives were: (1) to analyse the SOC stored along soil profiles in the bulk soil and in functional SOM pools (including the subsoil for a more exhaustive SOC stock estimation), (2) to determine the importance of clay fractions for estimating C sequestration in soils of semi-natural grasslands, (3) to identify the main factors affecting the quality and abundance of functional SOM pools and (4) to examine the utility of C hot water extractability and the natural abundance of ^{13}C as indicators to discriminate between functional SOM pools of differing stability.

2. Materials and Methods

2.1 Site description

Our study focused on four experimental plots (each 20 m x 15 m) in semi-natural grasslands of the Thuringian and Franconian Forest in Central Germany, a region with a high proportion of mountain hay meadows and grasslands. All investigated plots had been neither grazed nor fertilised in the preceding 20 years and are cut and harvested twice a year in early summer and autumn. The above-ground biomass was removed in accordance with local management practices. The four grassland plots were located within an area of 20 by 40 km^2 (11°00'-11°37'E and 50°21'-50°34'N) with similar altitudes (606-706 m a.s.l.) and exposure and can be regarded as independent grassland plots. The average annual precipitation in the region ranges from 980 to 1200 mm, with a slight summer maximum. The mean annual air temperature is 6.1°C and the soils are weakly stagnic Haplic Cambisols (siltic) that have developed from a carbonate-free bedrock material, mainly consisting of schist and greywacke.

2.2 Sampling

Samples (in triplicate subsamples per horizon) of entire soil profiles down to bedrock (i.e. 80 + cm) were taken from each of four independent sites in early June 2004 (Table 2. 1) from the control plot. The samples for each plot and soil depth were pooled, resulting in

21 analytical bulk soil samples, which were subjected to size-density fractionation, as described below. Before chemical analysis, the soil samples were air dried, sieved to < 2 mm, visible plant residues and stones were picked out by hand and the remaining soil was stored in closed plastic cups at room temperature until analysis.

The vegetation in each plot was surveyed in June 2004. All vascular plant species were identified in two 1 m^2 quadrates at each grassland sites. Above-ground biomass was harvested at peak standing biomass in four 25 cm x 30 cm rectangles in each plot, dried at 105°C and finely ground to powder. Plant species found in each of the grassland plots are listed as supplementary material in Table S2. 1.

2.3 Elemental analysis

Carbon and total nitrogen (TN) contents of the bulk soil samples and functional SOM pools (n = 3) were determined by dry combustion using a C/H/N analyser (Vario El III, Elementar, Hanau, Germany). As the carbonate concentration of the soil was negligible, the total measured C concentration was considered to correspond to SOC.

Table 2. 1 General soil chemical and texture parameters through the soil profile of four grassland sites. With the exception of soil organic carbon (SOC) and total nitrogen content (TN), the data were obtained from Gründling (2010); n.a., not available.

	Site1						Site2					
Soil horizon	Ah	(Sew-)Bv	(Sew-)Bv	(Sew-)Bv	II (Sd-)(i)ICv	II (i)ICv	Ah	Bv	Bv	Sew	Sew	II Sd
Thickness	0-12	12-47	12-47	12-47	47-72	72-100+	0-10	10-40	10-40	40-75	40-75	75-120
Sampling depth (cm)	0-10	10-20	20-30	30-50	50-80	80+	0-10	10-20	20-30	30-50	50-80	80+
pH ($CaCl_2$)	4.5	4.1	4.4	4.5	4.0	3.8	4.6	4.6	4.5	4.4	4.0	n.a.
SOC (%)	6.8	1.9	1.6	1.4	0.3	0.3	5.7	2.5	1.1	0.6	0.5	n.a.
TN (%)	0.5	0.2	0.1	0.1	0.1	0.1	0.5	0.2	0.1	0.1	0.1	n.a.
Clay (%)	27.2	23.5	22.3	23.4	14.3	19.2	27.2	24.1	22.2	21.5	23.5	n.a.
Stone (%)	25.7	25.7	n.a.	n.a.	n.a.	n.a.	22.9	22.9	22.9	38.5	35.8	n.a.
Sand (%)	22.3	25.9	25.7	25.2	35.5	30.6	17.6	23.6	22.3	20.3	22.0	n.a.
f+mU silt (%)	34.0	32.3	34.3	34.1	28.2	32.0	37.8	33.1	33.4	38.5	36.4	n.a.
gU silt (%)	16.5	18.3	17.7	17.3	22.0	18.2	17.5	19.2	22.1	19.7	18.1	n.a.
	Site3						Site4					
Soil horizon	rAp	rAp	Bv	Sw-Bv	II Sd-ICv	II Sd-ICv	Ah	Bv	Bv	Bv-ICv	II ICv	II ICv
Thickness	0-18	0-18	18-28	28-40	40-84+	40-84+	0-12	12-24	12-24	24-50	50-110	50-110
Sampling depth (cm)	0-10	10-20	20-30	30-50	50-80	80+	0-10	10-20	20-30	30-50	50-80	80+
pH ($CaCl_2$)	4.6	4.7	4.9	4.8	n.a.	n.a.	4.5	4.6	4.7	4.7	4.3	4.0
SOC (%)	5.1	3.4	1.4	0.8	n.a.	n.a.	5.1	3.4	2.4	1.4	0.4	0.2
TN (%)	0.5	0.3	0.2	0.1	n.a.	n.a.	0.5	0.3	0.2	0.1	0.1	0.1
Clay (%)	24.7	22.8	22.4	19.7	n.a.	n.a.	24.4	22.0	22.2	17.2	9.3	7.8
Stone (%)	57.8	57.8	56.1	56.1	n.a.	n.a.	22.4	22.4	21.5	21.5	21.5	n.a.
Sand (%)	21.4	27.7	25.2	22.4	n.a.	n.a.	20.7	26.6	24.2	21.2	50.0	48.3
f+mU silt (%)	36.0	33.9	34.3	39.6	n.a.	n.a.	36.3	34.7	33.8	39.7	24.9	26.3
gU silt (%)	18.0	15.5	18.1	18.5	n.a.	n.a.	18.7	16.7	19.8	21.9	15.8	17.6

2.4 Calculation of C stocks and carbon enrichment factors (E_{SOC})

Soil organic carbon stocks (mass C per unit area and depth) were calculated, as described in Don (2007), for both bulk soil samples and functional SOM pools isolated from each soil depth.

Carbon enrichment factors (E_{SOC}) of functional SOM pools were calculated for each site and soil depth, following Christensen (2001); Figure 2. 2, as the ratio of the SOC content of each functional SOM pool to that of the bulk soil ($E_{SOC} = SOC_{fraction}/SOC_{bulk\ soil}$).

2.5 Size-density fractionation

The physical fractionation method involves the separation of primary organo-mineral complexes according to particle size and density, after disaggregating the soil (von Lützow *et al.*, 2007), following the protocol of Shaymukhametov *et al.* (1984) as modified by Schulz (2004). Before fractionation, particular organic matter (POM) in form of plant and root residues were removed by flotation with deionised water from two replicate portions of 20 g air-dried soil.

The size-density fractionation to obtain uncomplexed (light fraction) and complexed (clay fraction) SOM involved two main steps. In the first step, SOM associated with an easily dispersible clay fraction (< 2 µm particles) was separated by applying 44 J ml^{-1} of ultrasonic energy for 15 x 1 min to a soil:water (1:3; w/v) suspension, using a UPS 200 instrument with an S7 ultrasonic tip (Hielscher GmbH, Teltow, Germany). After each ultrasonic treatment, the suspension was centrifuged for 3 min at 112 G to separate the clay fraction. This clay fraction was subsequently subdivided into two sub-fractions (CF1, < 1 µm; and CF2, 1 – 2 µm) by centrifugation for 10 min at 1792 G and 3 min at 1008 G, respectively. In a second step, after isolating clay-related SOM, two light fractions (LF1, < 1.8 g cm^{-3}; and LF2, 1.8 – 2.0 g cm^{-3}) were separated from the remaining solid phase via liquid-liquid partitioning, by suspending it in a bromoform (*tribrommethane*)/ethanol mixture, then adding excess water to break and separate phases containing LF1 and LF2. The CFs and LFs were dried at 60°C in evaporation dishes and then ground using a mortar and pestle for further analyses. The residue remaining after fractionation was almost free of organic carbon (OC) and was not further considered in this study. Carbon and mass balances of all isolated fractionations resulted in recovery rates of > 95% and > 98%, respectively.

2.6 Hot water extractable C and N

The samples were extracted by hot water extraction (HWE) using an ASE 200 - Accelerated Solvent Extraction System (DIONEX, Idstein, Germany), essentially following the method of Schulz *et al.* (2003). Briefly, four replicates of 200 mg of each bulk soil and functional SOM pools samples were added to 1 ml preconditioned extraction cells with a calcined silica sand carrier, then extracted with deionised water for 11 min under 50 bar pressure at 50°C (with one cycle of preheating for 1 min, heating for 5 min, 5 min static, 30% flushing and purging for 90 s). The resulting extracts were filtered using RC 25 Minisart single-use syringe membrane filter units, with 0.45 µm pore size membranes (Sartorius AG, Göttingen, Germany).

The total C and N concentrations of the extracts of bulk soil samples and functional SOM pools ($C_{HWE\ bulk\ soil}$, $N_{HWE\ bulk\ soil}$, $C_{HWE-fraction}$, $N_{HWE-fraction}$) were analysed using an elemental analyser for aqueous samples (Micro N/C and Multi N/C, Analytik Jena, Jena, Germany) and results presented here are expressed as mg kg^{-1}.

2.7 Isotope analyses

Bulk soil and functional SOM pools as well as plant residues were homogenised, dried, weighed into tin capsules and then combusted to CO_2 at 950°C in a Vario El III (Elementar, Hanau, Germany) elemental analyser, coupled via a ConFlow III (ThermoFinnigan, Bremen, Germany) to a Delta V plus isotope ratio mass spectrometer (IRMS) (Thermo Electron Corporation, Erlangen, Germany) for isotope analyses. The carbon isotopic composition was expressed using the conventional δ notation, defined as $\delta^{13}C_{sample} = (^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{VPDB}) / {}^{13}C/^{12}C_{VPDB} \times 1000$ [‰], where VPDB (Vienna Pee Dee Belemnite) is the standard defining the $^{13}C/^{12}C$ isotope scale. Three replicates of 70 mg of each bulk soil and functional SOM pools samples were analysed. The reproducibility of the carbon isotope analysis was better than ± 0.2‰ (Böttger *et al.*, 2007).

2.8 Data analysis

The effects of soil depth and functional SOM pools on SOC stocks, E_{SOC}, C_{HWE} and stable isotope ratios were analysed by two-way ANOVA. A common *Tukey-test* was used to examine the significance of differences in SOC stocks, E_{SOC} and C_{HWE} between the sites, for samples of the same depth. Before these analyses, a *Fisher's F test* was carried out to check whether the variances were significantly different from one another (Crawley, 2007). C_{HWE} and $\delta^{13}C$

values of samples of varying depths and types were compared by Principal Components Analysis (PCA), after log-transforming the data to meet assumptions of normality and variance homogeneity. These analyses were performed in R (Version 2.11.0).

To investigate the responses of SOC stocks to environmental variables, Canonical Correspondence Analysis (CCA) was applied, after log-transforming the data to meet assumptions of normality and variance homogeneity (except data on the presence/absence of plant species), using CANOCO Version 4.5 software for Windows (Ter Braak & Smilauer, 2002). Automatic selection with Monte Carlo permutations (4999 unrestricted permutations) was used to test the significance of the variables. To determine which variables best explained the SOC stocks, forward selection of environmental variables was performed, applying a Monte Carlo permutation test at each step to evaluate the significance of the selected variable. All data were tested for normality and homogeneity of variance and are presented as arithmetic means ± standard errors (SE). Effects were regarded as significant at $P \leq 0.05$.

3. Results

Generally, high variations in SOC stocks among the four grassland plots were detected and the two-way ANOVA showed there were significant differences ($P \leq 0.0001$) in SOC stocks, E_{SOC}, C_{HWE} and $\delta^{13}C$ values between the functional SOM pools (Table 2. 2). Soil depth had a significant effect ($P \leq 0.05$) on SOC stocks, E_{SOC} and C_{HWE}, but only a marginally effect ($P \leq 0.10$) on $\delta^{13}C$ values. In addition, significant two-way interaction effects of functional SOM pools and soil depth on all response variables were detected (Table 2. 2).

3.1 SOC stocks

Overall, total SOC stocks amounted to about 99.3 t ha^{-1}. About 83% (82.6 t ha^{-1}) of the total SOC stocks were found in the top 30 cm of the bulk soil and the remaining 17% (17 t ha^{-1}) in the subsoil (Figure 2. 1 & Table S2. 2). Similar trends with depth were found for functional SOM pools (CF1, CF2, LF1, LF2). Apart from about 5% C stored in plant and root litter (POM), most C (about 59%) was stored in the clay fractions, with a significantly higher proportion ($P \leq 0.05$) in the smaller particle-size clay fraction, CF1 (34.6%), than in the large fraction, CF2 (24.7%). Two light fractions were separated down to a 50 cm soil depth: LF1, < 1.8 g cm^{-3} and LF2, 1.8 – 2.0 g cm^{-3}. In total, about 32% of the total SOC stocks were found in these LFs and significantly higher SOC stocks ($P \leq 0.05$) were detected in LF1 (18.6%)

than in LF2 (11.5%). Of the 17% of the total SOC found in the subsoil, most (54%) was associated with the smaller particle-size clay fraction (CF1).

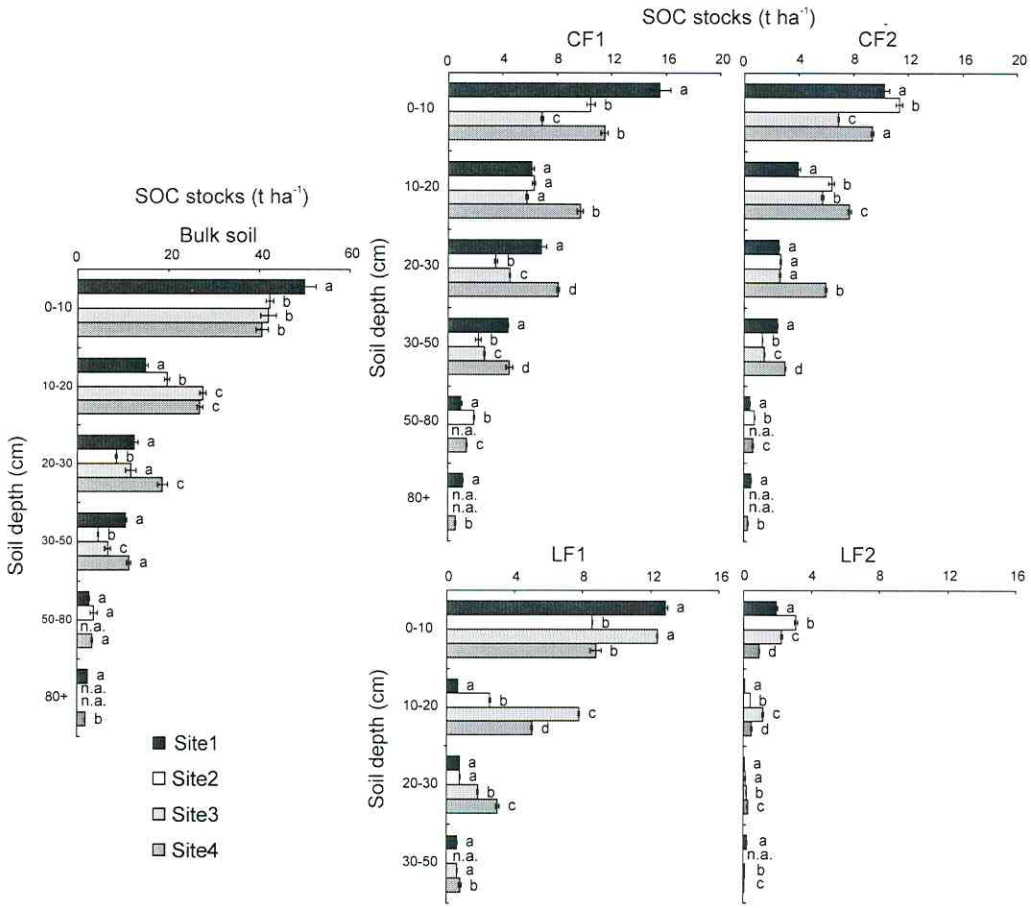

Figure 2. 1 Soil organic carbon (SOC) stock distribution with depth, in bulk soil, clay fractions (CF1; CF2) and light fractions separated by density fractionation (LF1; LF2) in samples from four grassland sites. Values are means ± SE and values within each soil depth marked by the same letter are not significantly different; n.a., not available.

3.2 Carbon enrichment factors (E_{SOC})

Values of E_{SOC} > 1 indicate enrichment of OC in the corresponding functional SOM pools, while values < 1 indicate depletion of OC. Carbon enrichment factors were generally > 1, indicating an enrichment of OC in all functional SOM pools. The enrichment factor of CF1 increased significantly ($P \leq 0.001$) with soil depth (Figure 2. 2), whereas that of CF2 was only marginally affected by soil depth. E_{SOC} values were considerably lower for LF2 than for LF1, but they increased with soil depth in both cases.

Table 2. 2 Summary of results of a two-way ANOVA testing the effects of two factors (functional soil organic matter (SOM) pools and soil depth) and their interaction on SOC stocks, Carbon enrichment factors (E_{SOC}), Hot water extractable carbon (C_{HWE}) and stable isotopes ($\delta^{13}C$). Bold values indicate significant responses ($P \leq 0.05$).

	Functional SOM pools		Soil depth		Functional SOM pools x Soil depth		
	F-value	*P*	*F*-value	*P*	*F*-value	*P*	
d.f.	5		4		18		*Residuals*
SOC stocks	142.443	**≤ 0.0001**	96.119	**≤ 0.0001**	22.113	**≤ 0.0001**	69
E_{SOC}	135.492	**≤ 0.0001**	13.187	**≤ 0.0001**	10.977	**≤ 0.0001**	69
C_{HWE}	134.776	**≤ 0.0001**	2.688	≤ 0.01	3.115	**≤ 0.001**	69
$\delta^{13}C$	108.944	**≤ 0.0001**	2.168	≤ 0.10	2.941	**≤ 0.001**	69

3.3 Factors influencing SOC stocks

Canonical Correspondence Analysis (CCA) showed that SOC stocks were significantly influenced by several factors (Figure 2. 3). Clay fractions (CF1 & CF2) were influenced by soil depth (30-50 cm versus 50-80 cm, $P \leq 0.05$) and texture (sand versus coarse silt (gU silt), $P \leq 0.05$). In contrast, stocks in both light fractions within the top 20 cm were significantly influenced (0-10 cm, $P \leq 0.001$; 10-20 cm, $P \leq 0.0001$) by the presence of various plant species in the plots, such as *Alchemilla vulgaris*, *Anthriscus sylvestris*, *Leucanthemum vulgare* and *Phyteuma spicatum* ($P \leq 0.0001$). Additional factors, such as the total nitrogen content of the bulk soil ($P \leq 0.05$), clay content ($P \leq 0.05$), hot water extractable nitrogen content ($P \leq 0.05$), C:N ratio of hot water extracts ($P \leq 0.001$) and fine & medium silt content (f+mU silt) ($P \leq 0.0001$), also had significant effects on stocks in the LFs.

3.4 Hot water extractability of OC in functional SOM pools ($C_{HWE-fraction}$)

$C_{HWE-fraction}$ was used as an indicator of the stability of OC in functional SOM pools (Figure 2. 4). For the entire soil profile, values of this parameter for all functional SOM pools significantly differed from the bulk soil value and in both clay fractions the hot water extractability decreased significantly ($P \leq 0.0001$) with soil depth. Compared with bulk soil, the extractability was 160% higher in CF1, 159% higher in CF2, 522% higher in LF1 and 258% higher in LF2.

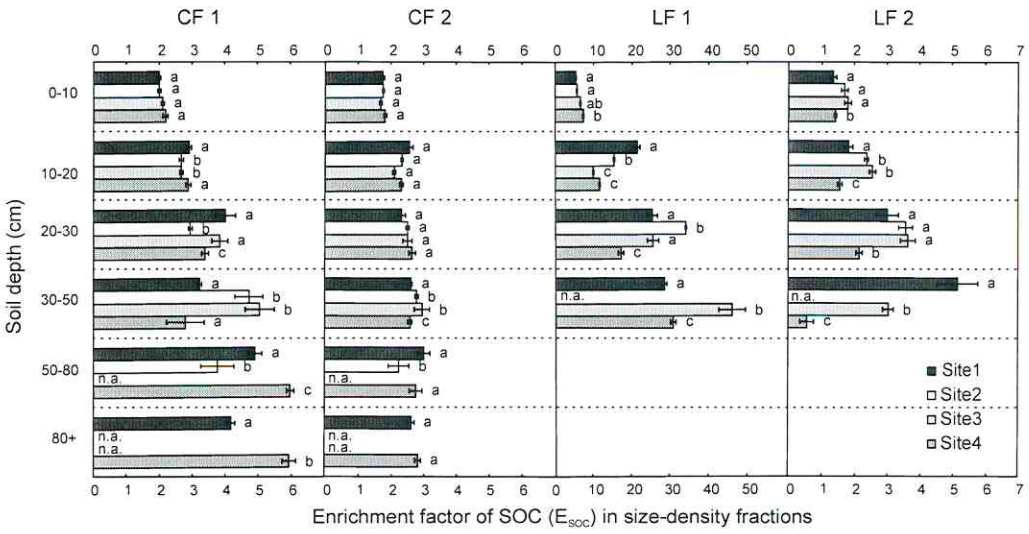

Figure 2. 2 Carbon enrichment factors (E_{SOC}) in functional SOM pools through the soil profile. Values are means ± SE and values within each soil depth with the same letter are not significantly different; n.a., not available.

3.5 ^{13}C isotopic abundance of functional SOM pools and bulk soil related to the C_{HWE} percentage from SOC

In total, all functional SOM pools were depleted in the light stable isotope ^{12}C than material of plant residues, which were extracted from bulk soil ($\delta^{13}C_{plant\ residues}$: -28.0 ± 0.1‰). All $\delta^{13}C$ values of the bulk soil and functional SOM pools shifted to significant more positive values. Furthermore, the mean $\delta^{13}C$ values of the bulk soil, CF1 and CF2 differed (-26.4 ± 0.1‰, -25.8 ± 0.1‰ and -26.2 ± 0.1‰, respectively) from those of the light fractions LF1 and LF2

(-27.3 ± 0.1‰ and -27.5 ± 0.1‰, respectively), which were similar to those of plant residues (Figure 2. 5).

To obtain indications of the SOC quality of the bulk soil and functional SOM pools, their $\delta^{13}C$ characteristics was related to their hot water extractable C contents (C_{HWE}), as a percentage of SOC. The Principal Component Analysis (PCA) of the bulk soil and functional SOM pools, shown in Figure 2. 5, revealed that the two clay fractions and bulk soil were similar, but the two LFs clearly differed from each other and from the bulk soil and clay fractions, in this respect. Principal Component 1 explained 44.15% of the variation and Principal Component 2 a further 38.72%.

4. Discussion

4.1 SOC stocks and C enrichment factors (E_{SOC})

The largest SOC stocks were found in the top 30 cm of the bulk soil, but a proportion (17%) was also stored at lower depths (Figure 2. 1). The goal of this work was not only to characterise the allocation of SOC stocks but also to depict the mechanisms behind this repartition in terms of turn over and sequestration of C in the different soil layers. Therefore, fractionation procedures of SOM have been used in attempts to characterise factors promoting the stability, or enhance the degradation, of SOM (Christensen, 2001, von Lützow *et al.*, 2007). Notably, Christensen (2001) showed in temperate arable soils that clay-sized particles (< 2 µm) contain higher amounts of SOC than fractions associated with larger particles and thus are more effective for C sequestration.

The importance of clay particles for C accumulation was confirmed by the high carbon enrichment factors (E_{SOC}) in CF1 and the increase of E_{SOC} in this fraction with depth (Figure 2. 2), which is indicative of absorption of the mobile C fraction by clay (Tiessen & Stewart, 1983, Saroa & Lal, 2003). The SOC that was allocated to the subsoil was bound to clay minerals (see also Table 2. 1). Carbon enrichment factors in LF1 were significantly higher than in LF2 and CFs, due to high SOC contents (> 30%), whereas E_{SOC} values in CF2 and LF2 remained at fairly constant (but lower) levels down the soil profile (Figure 2. 1).

About 18.6% of the total C stocks were detected in LF1 of the topsoil. This fraction consists of particulate soil organic matter (von Lützow *et al.*, 2007), it is not associated with clay minerals, but is relatively labile and readily decomposable and thus constitutes a high quality resource and that can be regarded as an active pool (Schulz, 2004, Gregorich *et al.*, 2006). In

addition, wide C/N ratios observed in LF1 indicates that it is strongly influenced by plant residues. Hence, the C stocks in LF1 presumably resulted from high inputs of plant material. Functional SOM pools of higher specific densities (LF2) resulted in lower SOC stocks (about 12%).

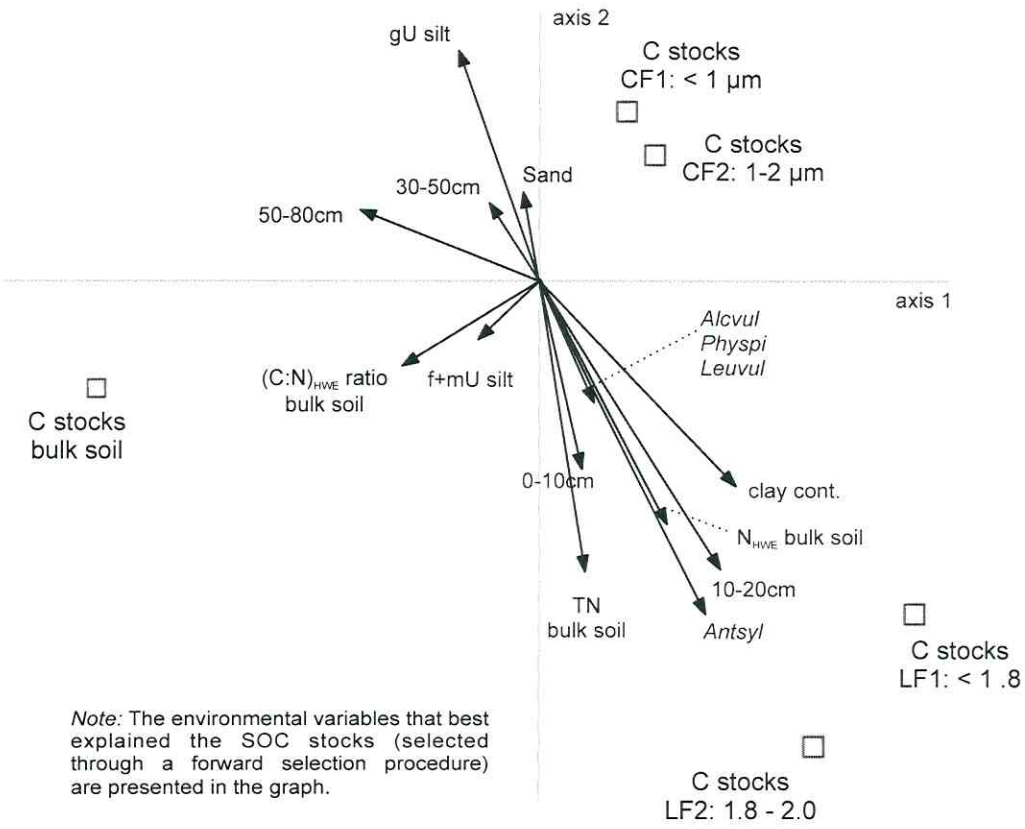

Figure 2. 3 Canonical Correspondence Analysis (CCA) of SOC stocks. Calculated SOC stocks in functional SOM pools (CF1, < 1 µm; CF2, 1–2 µm; LF1, < 1.8 g cm^{-3}; LF2, 1.8 – 2.0 g cm^{-3}) and bulk soil were set as species variables (represented by squares) and related to presence/absence of individual plant species, individual grassland sites (Site1, Site2, Site3, Site4), soil chemical and texture parameters and soil depth, set as environmental variables (represented as arrow heads). The environmental variables that best explained SOC stocks (selected through a forward selection procedure) are presented in the graph. Abbreviations for non-biological variables: TN bulk soil = total nitrogen of bulk soil; N_{HWE} bulk soil = hot water extractable nitrogen in bulk soil; $(C:N)_{HWE}$ ratio bulk soil = hot water extractable C to N ratio; f+mU = silt, fine and medium silt; gU silt = silt, coarse silt; sand = sand content; clay cont. = clay content. Abbreviations for the plant species: Alcvul = *Alchemilla vulgaris*; Antsyl = *Anthriscus sylvestris*; Leuvul = *Leucanthemum vulgare*; Physpi = *Phyteuma spicatum*.

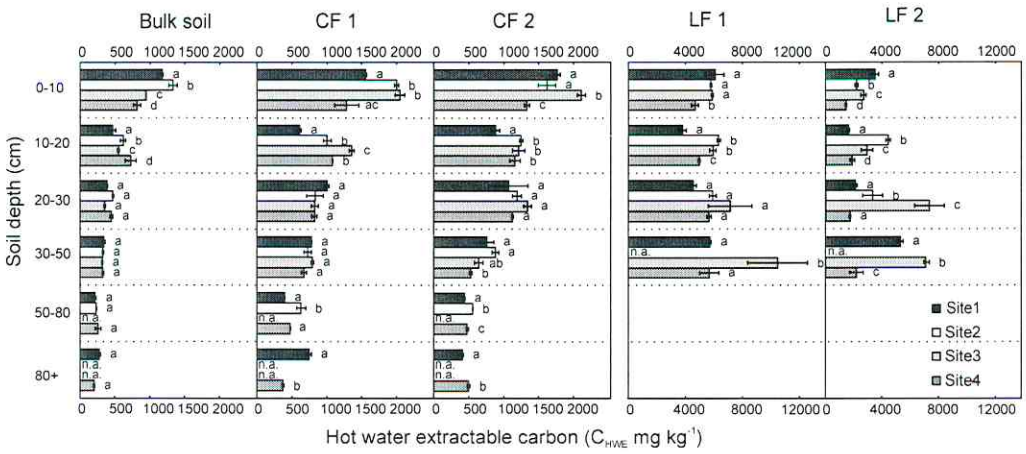

Figure 2. 4 Hot water extractable carbon (C_{HWE}) in bulk soil samples and functional SOM pools through the soil profile. Values are means ± SE and values within each soil depth marked by the same letter are not significantly different; n.a., not available.

These results are consistent with findings of Breulmann *et al.* (2011), that root litter productivity in semi-natural grasslands is positively related with the size of labile $C_{HWE\ bulk\ soil}$ and $N_{HWE\ bulk\ soil}$ pools, especially in the top soil layers (Figure 2. 4). The amount and presumed lability of LF1 may be important determinants of soil respiration, since they are strongly correlated with mineralisation processes and microbial activity in soils. This suggests that LF1 is a key driver of soil biological transformation processes in grassland ecosystems (Alvarez & Alvarez, 2000). Biederbeck *et al.* (1994) also reported that the nature and abundance of light fractions could be controlled by using specific management practices. Thus, LF1 may also be a convenient early indicator of management-induced SOC changes in grassland ecosystems.

Fontaine *et al.* (2007) postulated that increases in supplies of fresh OC could enhance the decomposition of recalcitrant SOC and thus decrease C sequestration. Results of the present study indicate that high amounts of labile and easily decomposable compounds may be key drivers of decomposition and turnover processes in semi-natural grassland soils. However, no reduction in SOC stocks of the stable functional SOM pools (CF1 & CF2) due to high inputs of fresh OC was found. During the decomposition process, fresh OC is mineralised to carbon dioxide and, simultaneously, fragments and radicals can recombine to form stabilised humic-like compounds (Kiem, 2002). Further analyses are needed to evaluate the extent to which

functional pools can be utilised by microorganisms and how they may contribute to the formation of humic substances.

4.2 Factors influencing SOC stocks

For C stocks of both clay fractions, soil depth and texture were the best predictors. The results suggest that proportions of relatively slowly cycling functional SOM pools (pools that are strongly associated with clay minerals and protected from mineralisation) tend to increase with depth (Figure 2. 3). Radiocarbon ages of CF1 in the subsoil range from 1125 Before Present (BP) at 20-30 cm to 3470 years BP at 80+ cm, according to illustrative data obtained from samples taken at one site *(analysed at the Leibniz-Laboratory for Radiometric Dating and Isotope Research in Kiel, Germany; Nadeau et al., 1998),* confirming that these clay fractions have long residence times and are presumably controlled by pedogenic processes (von Lützow *et al.*, 2008). Stocks in both light fractions were strongly influenced by plant species in the top 20 cm and soil chemical properties, such as TN and N_{HWE} (Figure 2. 3).

4.3 Stability of functional SOM pools

The hot water extractable carbon component of labile SOC consists of simple organic compounds, originating from microbial biomass, root exudates and lysates in the soil solution (Leinweber *et al.*, 1995). Therefore, it represents a source of readily decomposable SOC (Landgraf *et al.*, 2006). Here, $C_{HWE\ bulk\ soil}$ was affected by the C input from plant material and the largest labile pools were detected in the top soil (Figure 2. 4).

Hot water extraction was also applied to soil functional SOM pool and their extractability was used for qualitatively discriminating between them in terms of the stability (Schulz *et al.*, 2011). High extractability of C indicates a functional SOM pool that is potentially readily degradable, while low extractability indicates a pool that is stable with low degradation potential. Compared with bulk soil, significantly higher (ca. 160%) C hot water extractabilities of CF1 and CF2 were found (Figure 2. 4). It can be assumed that C in the clay fractions is physically protected in aggregates that formed physical barriers to microbes and enzymes (Six *et al.*, 2002). After disaggregation by sonication during the fractionation process, the C within clay fractions becomes accessible, to some extent, for degradation (Cheshire *et al.*, 2000). The $C_{HWE-fraction}$ was ca. 522% higher in LF1 (0-10 cm) than in bulk soil, indicating that LF1 contained a SOC of low stability and high degradability, while the

LF2 fraction had a lower SOC content and ca. 37% lower $C_{HWE-fraction}$ value than LF1, indicating that the LF2 pool has higher stability and lower potential for decomposition.

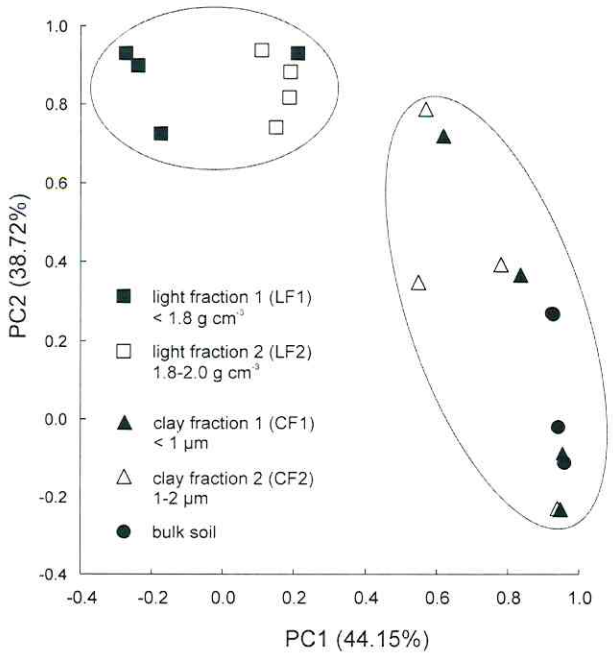

Figure 2. 5 Principle component analysis (PCA) of hot water extractable carbon and natural stable isotope signatures in functional SOM pools and bulk soil samples from four grasslands. Hot water extractable carbon and natural stable isotope signatures serve as stability indicators.

The relative natural abundance of heavy isotope ^{13}C of investigated functional SOM pools provides important information on the depth distribution of young C and the dynamics of C within functional SOM pools (Balesdent & Mariotti, 1996). One of the objectives of the present study was to use the natural abundance of $\delta^{13}C$ in functional SOM pools for indicating C quality. The $\delta^{13}C$ value of plant residues extracted from bulk soil was about -28‰, typical for C_3 vegetation and the composition of the vegetation cover was presumably stable during the 20 years preceding the study. In clay fractions (CF1, CF2) $\delta^{13}C$ values increased slightly with soil depth (CF1, -26.4‰ to -24.7‰ and CF2, -26.8‰ to -25.2‰) due to preferably utilisation of the lighter ^{12}C isotope during microbial and chemical transformation processes (Gregorich *et al.*, 1994). In contrast, in both the topsoil and subsoil, the $\delta^{13}C$ values of LF1 and LF2 remained at a relatively constant negative level, indicating that fresh OC was

incorporated in these fractions from plant material (LF1, -27.7‰ to -27.8‰ and LF2, -27.4‰ to -26.9‰).

The Principal Component Analysis (PCA) of $\delta^{13}C$ values and the amount of C_{HWE} present, as a proportion of SOC in the bulk soil and functional SOM pools (Figure 2. 5), indicated that these parameters were strongly, positively correlated, since abundant, relatively mobile ^{12}C was enriched in LF1 and LF2, while more stable SOC was closely associated with the bulk soil and clay fractions. Secondly, SOC appears to be physically protected from decomposition in clay fractions and is only accessible for degradation after disaggregation. Thirdly, further differentiation of the two LFs indicated that they differed in both stability and availability of C, due to the preference of microorganisms for the lighter ^{12}C isotope (Kuzyakov & Bol, 2004).

4.4 Conclusions

Most C was stored in the topsoil, where it is likely to be rapidly turned over; the subsoil is important for soil C preservation and need to be considered in C stock evaluations (protection, mainly by occlusion within clay microstructures and aggregates, as well as organo-mineral interactions). The identification and analysis of different functional SOM pools provided a first comprehensive and detailed overview of the distribution of OC in theses pools. Small clay particles (< 1 µm) are of particular importance for C accumulation in the investigated semi-natural grassland ecosystems wherefore texture and depth affected SOC stocks. Light fractions were influenced by plant biomass input, especially in the topsoil layer. In particular, LF1 (< 1.8 g cm^{-1}) proved to be sensitive for reflecting rapid responses to land use or management dependent changes in plant functional composition. The hot water extractability of C from functional SOM pools can be used to help elucidate C stabilisation processes. Together with $\delta^{13}C$ values, bulk soil and functional SOM pools could be differentiated in terms of the stability of OC; hence these measures could be used as stability indicators.

Terrestrial ecosystems, such as grasslands, have the ability to buffer or regulate the amount of CO_2 in the atmosphere by sequestering C in soils and releasing C to the atmosphere. Future research needs to focus on the microbial use of functional SOM pools and the key components of the microbial community that are involved in transformation of C in these pools.

Acknowledgments

This work was financially supported by the German Federal Ministry of Education and Research (BMBF), grant no. 01LC0613B. The study is part of DIVA, an interdisciplinary research project of the BIOLOG–Europe programme. We thank Sabina Christ for valuable comments on the manuscript, Jacqueline Rose, Gabriele Henning and Ute Helmstedt for technical assistance in the laboratory and Ilka Egerer for technical coordination of the BIOLOG DIVA–Verbundprojekt.

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Supplementary Materials

Table S2. 1 Individual plant species determined at the four grassland sites.

Site	Site1	Site2	Site3	Site4
Achillea millefolium	x	x		
Agrostis capillaris	x	x		
Ajuga reptans	x			
Alchemilla vulgaris agg.	x	x		x
Anthoxanthum odoratum	x	x	x	x
Anthriscus sylvestris	x		x	
Arrhenatherum elatius	x	x	x	x
Bellis perennis		x		x
Campanula patula		x		
Campanula rotundifolia	x	x		
Cardamine pratensis	x			
Centaurea pseudophrygia	x			
Cerastium arvense		x		
Cerastium holosteoides	x	x	x	
Cynosurus cristatus		x		
Dactylis glomerata	x	x	x	x
Elytrigia repens			x	
Festuca pratensis			x	
Festuca rubra	x	x		x
Galium mollugo agg.			x	
Galium saxatile				x
Helicotrichon pubescens	x			
Holcus lanatus		x		
Holcus mollis			x	
Hypericum maculatum	x	x	x	x
Knautia arvensis	x	x		
Lathyrus linifolius	x			
Leucanthemum vulgare	x	x		
Listeria spec	x			
Lolium multiflorum			x	
Lolium perenne			x	
Luzula campestris	x	x		x
Meum athamanticum	x	x		
Phleum pratense			x	
Phyteuma spicatum	x	x		
Plantago lanceolata	x		x	x
Poa pratensis	x	x	x	x
Poa trivialis			x	
Potentilla erecta	x			
Prunella vulgaris		x		
Ranunculus acris	x	x	x	x
Rhinanthus minor		x		
Rumex acetosa	x	x	x	x
Rumex obtusifolius			x	
Stellaria graminea	x	x	x	
Taraxacum officinale agg.		x	x	
Trifolium dubium		x		
Trifolium pratense	x	x		x
Trifolium repens	x	x	x	
Trisetum flavescens	x	x		x
Veronica arvensis			x	
Veronica chamaedrys	x	x	x	x
Vicia cracca	x			x

Table S2. 2 Individual soil organic carbon (SOC) stocks (t ha^{-1}) of bulk soil, clay fractions (CF1 & CF2) and light fractions (LF1 & LF2) through the soil profile of four grassland sites. Values are means ± SE; n.a., not available.

Sample	Soil depth (cm)	Site1	Site2	Site3	Site4
Bulk soil					
	0-10	47.9 ± (1.8)	42.3 ± (0.9)	40.5 ± (1.3)	39.2 ± (0.8)
	10-20	26.3 ± (0.6)	19.7 ± (0.6)	27.0 ± (0.4)	27.0 ± (0.7)
	20-30	22.6 ± (1.6)	8.6 ± (0.2)	10.7 ± (0.3)	18.7 ± (1.0)
	30-50	27.4 ± (0.1)	4.6 ± (0.1)	6.7 ± (0.7)	11.3 ± (0.5)
	50-80	5.9 ± (0.0)	3.7 ± (0.3)	n.a	3.3 ± (0.0)
	80+	2.3 ± (0.0)	n.a	n.a	1.9 ± (0.0)
CF1					
	0-10	17.3 ± (1.8)	10.9 ± (0.2)	6.9 ± (0.2)	11.5 ± (0.3)
	10-20	11.1 ± (0.3)	6.3 ± (0.1)	5.9 ± (0.1)	9.7 ± (0.2)
	20-30	12.3 ± (0.7)	3.5 ± (0.1)	4.6 ± (0.1)	8.0 ± (0.1)
	30-50	11.0 ± (0.1)	2.3 ± (0.3)	2.7 ± (0.1)	5.4 ± (0.3)
	50-80	2.8 ± (0.0)	1.9 ± (0.1)	n.a	1.4 ± (0.0)
	80+	1.2 ± (0.0)	n.a	n.a	0.6 ± (0.0)
CF2					
	0-10	11.1 ± (0.5)	11.7 ± (0.1)	6.0 ± (0.1)	9.4 ± (0.1)
	10-20	7.8 ± (0.1)	6.4 ± (0.2)	5.7 ± (0.1)	7.7 ± (0.1)
	20-30	4.6 ± (0.1)	2.7 ± (0.1)	2.6 ± (0.1)	6.0 ± (0.1)
	30-50	6.1 ± (0.1)	1.4 ± (0.0)	1.5 ± (0.1)	3.0 ± (0.0)
	50-80	1.3 ± (0.0)	0.8 ± (0.0)	n.a	0.7 ± (0.0)
	80+	0.5 ± (0.0)	n.a	n.a	0.3 ± (0.0)
LF1					
	0-10	13.1± (0.3)	9.7 ± (0.1)	14.8 ± (0.1)	8.8 ± (0.3)
	10-20	1,2 ± (0.1)	2.5 ± (0.0)	10.4 ± (0.0)	5.1 ± (0.1)
	20-30	1.4 ± (0.0)	0.8 ± (0.0)	1.8 ± (0.0)	2.9 ± (0.1)
	30-50	0.08 ± (0.0)	n.a	0.6 ± (0.0)	0.8 ± (0.0)
	50-80	n.a	n.a	n.a	n.a
	80+	n.a	n.a	n.a	n.a
LF2					
	0-10	1.9 ± (0.1)	3.1 ± (0.1)	2.2 ± (0.1)	0.9 ± (0.0)
	10-20	0.1 ± (0.1)	0.4 ± (0.1)	1.1 ± (0.0)	0.5 ± (0.3)
	20-30	0.2 ± (0.1)	0.1 ± (0.0)	0.2 ± (0.0)	0.3 ± (0.1)
	30-50	0.1 ± (0.0)	n.a	0.1 ± (0.0)	0.0 ± (0.0)
	50-80	n.a	n.a	n.a	n.a
	80+	n.a	n.a	n.a	n.a

3 Microbial utilisation of functional SOM pools in grassland ecosystems

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Submitted for publication in Soil Biology and Biochemistry

Date of submission: 17.06.2011

Abstract

Various approaches have been applied to characterise SOM, but its relationships with decomposability have often only been inferred. There have been limited direct investigations linking functional SOM pools to their decomposition by soil microbial communities. We obtained detailed information on the decomposability and stability of functional SOM pools during microbial utilisation from incubation experiments. Functional SOM pools (complexed SOM: CF1, < 1 µm; CF2, 1 – 2 µm and uncomplexed SOM: LF1, < 1.8 g cm^{-3}; LF2, 1.8 – 2.0 g cm^{-3}) were isolated by size-density fractionation from grassland ecosystems with varying management on two soil types: a Haplic Cambisol and a Typical Chernozem. The functional SOM pools were incubated as substrates to obtain information on their decomposability and stability. Phospholipid fatty acid (PLFA) extractions were used to analyse the soil microbial communities of the different functional SOM pools and how specific functional groups controlled decomposition processes of these pools. The results indicate that the utilisation of functional SOM pools depends on three factors: (1) specific traits of the functional SOM pools, (2) the management intensity of the grasslands and (3) the soil type. The OC utilisation of the two clay fractions (CF1, CF2) depends on specific pools characteristics such as their accessibility to soil microorganisms, which was increased due to disaggregation. The OC utilisation of the two light fractions depends on the chemical stability of OC. The OC of LF1 is readily degradable promoting C losses, whereas the OC within LF2 can be considered as recalcitrant. Depending on the stability/availability of the OC in individual functional SOM pools, the three factors were responsible for the formation of a specific microbial community. The utilisation of functional SOM pools under optimum incubation conditions clearly revealed first insights into the interactions between functional SOM pools, its properties and the diverse members of the soil communities.

Keywords

Functional SOM pools, OC utilisation, Decomposability, Incubation experiment, Microbial community composition, PLFA

1. Introduction

The dynamics of soil organic matter (SOM) is often described using conceptual models, as the quality, stability and availability of SOM can vary widely (Körschens, 1980, Cambardella & Elliot, 1993, Schulz, 2004). These conceptual models distinguish between various pools, e.g. uncomplexed and complexed SOM with differing intrinsic properties, such as microbial decomposability, stability and turnover times (Smith *et al.*, 1997, Amundson, 2001). It is essential to identify and characterise distinct pools of SOM to elucidate thoroughly SOM dynamics. To gain such pools, approaches have been developed for isolating functional SOM pools without substantially changing properties relevant to their functions in ecosystems (Christensen, 2001, von Lützow *et al.*, 2007). For instance, a combination of fractionation methods according to particle-size and specific density (size-density fractionation) allows the separation of uncomplexed and complexed SOM (Christensen, 2001).

The intrinsic properties of functional SOM pools are highly variable and depend on their degree of stabilisation. In general, the stabilisation of SOM can be defined as the protection of SOM from mineralisation (Sollins *et al.*, 1996). Three stabilisation mechanisms of SOM have been described: (1) recalcitrance due to molecular-level resistance, (2) spatial inaccessibility through occlusion by aggregates and (3) the interaction between SOM with minerals and metal ions (Sollins *et al.*, 1996, von Lützow *et al.*, 2006). Consequently, the decomposition processes of functional SOM pools depend on their location and interactions within the soil matrix, which provides spatially heterogeneous habitats for soil microorganisms (Sessitsch *et al.*, 2001). Unravelling these processes is difficult, because the soil organic compounds have a wide range of kinetic properties that determine their decomposition rates in soils (Davidson & Janssens, 2006).

Various approaches have been applied to characterise SOM, but its relationships with decomposability have often only been inferred (Denef *et al.*, 2009). There have been few direct investigations linking functional SOM pool characteristics to microbial utilisation, in order to evaluate their potential biological decomposability. However, the utilisation of SOM associated with different sizes of particles has been studied in respiration experiments (Schutter & Dick, 2002, Oorts *et al.*, 2006, Semenov *et al.*, 2010). In addition enzyme activities and DNA extracts of soil aggregates have been analysed (Kandeler *et al.*, 2000, Sessitsch *et al.*, 2001). Furthermore, incubations of functional SOM pools with different specific densities have indicated that these pools are a driving factor of microbial activity and

associated soil respiration (Alvarez *et al.*, 1998, Alvarez & Alvarez, 2000, Swanston *et al.*, 2002). However, to our knowledge, no combined direct analyses of functional SOM pool utilisation and the soil microbial community have been published to date.

By studying abiotic and biotic components, it could be expected to obtain new insights into the interactions between the properties of individual organic substrates, functional SOM pools and the diverse members of the soil microbial communities. To estimate the utilisation of functional SOM pools, we linked the chemical pool characteristics to biological properties by using CO_2 release as an index of substrate accessibility. To confirm the controlling influence of functional SOM pool characteristics for a potential utilisation by microorganisms (Crow *et al.*, 2007), we studied soils of grassland ecosystems differing in soil type, climatic conditions and management intensities.

The main aim of this work was to determine the potential decomposability of uncomplexd and complexed functional SOM pools, isolated by combined size-density fractionation resulting in two clay fractions (CF1, < 1 µm; CF2, 1 – 2 µm) and two light (LF1, < 1.8 g cm^{-3}; LF2, 1.8 –2.0 g cm^{-3}) fractions of four grassland ecosystems: a Low Productivity (LPG) and a High Productivity (HPG) grassland in Central Germany and a Pasture and a Prairie in the forest steppe of Russia. The functional SOM pools were used as substrates, which was added to a carrier material, in a respiration experiment, in order (1) to obtain detailed information on the decomposability and stability of these pools, (2) to analyse how levels of functional groups within microbial communities change during decomposition of the organic carbon (OC) of functional SOM pools and (3) to detect how specific functional groups of soil microorganisms control decomposition processes of these pools. The soil microbial communities within functional SOM pools were characterised by phospholipid fatty acid (PLFA) analyses.

2. Materials and Methods

2.1 Study sites

The High Productivity (HPG) and Low Productivity (LPG) sampling sites were located in semi-natural grasslands of the Thuringian and Franconian Forest with similar altitudes (606 and 633 m a.s.l., respectively). The grasslands are situated on a plateau-like mountain range in Central Germany (24.29°E-26.56°N and 27.33°E-24.31°N) and were extensively-managed experimental sites of the BIOLOG-Europe programme (http://www.biolog-

europe.org/diva). They differ in plant biomass production, plant community composition and litter quality. The average annual precipitation in the region ranges from 980 to 1200 mm, with a slight summer maximum. The mean annual air temperature is 6.1°C and the soils in the region are weakly stagnic Haplic Cambisols (siltic), that have developed a carbonate-free soil from parent rocks of schist and greywacke, with an initial soil pH (KCl) of 4.4 (Kahmen *et al.*, 2005).

The Pasture and Prairie sites are in long-term field experimental sites of the Central Chernozemic State Biosphere Reserve and the All-Russia Research Institute of Arable Farming and Soil Erosion Control in Russia (36°09'E and 51°57'N), located in the forest steppe climatic zone of the Central Russian Heights (211-223 m a.s.l.). The climate is temperate-cold, with an average temperature of about 5.3°C (http://oopt.info/index.php?oopt=1104). Annual average precipitation amounts to 570 mm with drought periods every 3-5 years. The soils are classified as Typical Chernozems with an initial soil pH (KCl) of 6.5. The Pasture sites have been used for cattle grazing with stocking rates of 0.9 livestock units/ha and the Prairie sites are in their natural state and ungrazed. For more information see Barré *et al.* (2010).

2.2 Soil and vegetation analysis

Pooled soil samples (n = 3) from HPG and LPG sites were taken in 2008 from the top 30 cm and soil samples from the Pasture and Prairie sites were taken in 2003 from the top 25 cm. Before chemical analysis, the soil samples were air-dried, sieved to < 2 mm, visible plant residues and stones were picked out by hand and the remaining soil was stored in closed plastic cups at room temperature until analysis. Carbon (C) and total nitrogen (TN) contents of the bulk soil samples and of the functional SOM pools were determined in triplicates per sample by dry combustion using a C/H/N analyser (Vario El III, Elementar, Hanau, Germany).

2.3 Size-density fractionation

The method used for the size-density fractionation followed the protocol of Shaymukhametov *et al.* (1984) as modified by Schulz (2004). Before fractionation, plant and root residues (particulate organic matter) were removed by flotation with deionised water from four replicates of 20 g air-dried soil samples. The size-density fractionation consisted of

two main steps. To combine two of the existing classifications of clay (the International USDA: < 2µm and the Russian classification: < 1 µm) two clay fractions were isolated. In the first step, complexed SOM (the SOM associated with an easily dispersible clay fraction) was separated by applying low ultrasonic energy (44 J ml^{-1}) to a soil/water suspension (1:3; w/v). After each ultrasonic treatment, the suspension was centrifuged to separate a clay fraction, which was further subdivided into two clay sub-fractions: CF1, < 1 µm and CF2, 1 – 2 µm using different centrifugal forces (revolutions per minute) and times. In the second step, the remaining solid phase (silcates) was used to separate uncomplexd SOM (LF1, < 1.8 g cm^{-3} and LF2, 1.8 – 2.0 g cm^{-3}) by liquid-liquid partitioning, using a bromoform/ethanol mixture. While salt solutions with high specific densities (polytungstates or iodates) are often used for this purpose, the bromoform/ethanol mixtures used in these studies has the advantage that the isolated fractions can easily be purified and contain no traces of the dense solution.

The isolated fractions, representing functional SOM pools, were dried at 60°C and milled for further analyses. The remainder was almost free of OC (< 0.01 %) and was not considered further. Carbon and mass balances of all isolated functional SOM pools resulted in recovery rates of > 95% and > 98%, respectively.

2.4 Utilisation of functional SOM pools

The decomposition experiment was conducted over 10 days to examine the degradability of functional SOM pools and to examine the impact of its decomposition on soil biological parameters. An Albic Luvisol (FAO classification) was used as carrier material for the decomposition experiment of OC in functional SOM pools. The carrier material was characterised as having low C and N contents (Table 3. 1) and low microbial biomass (Table S3. 1). The carrier material was rewetted with water to 60% of the maximum water holding capacity and pre-incubated for 8 days. After pre-incubation of the carrier material, functional SOM pools (CF1, CF2, LF1, LF2) fractionated from four sites were used as substrates in the incubation experiment.

The substrates were added at a concentration of 5 mg C to 5 g carrier material and well mixed. In total, 85 experimental units for the incubation experiment consisted of either the carrier material and carrier material amended with one of the respective isolated functional SOM pools (2 sites Haplic Cambisol × 2 sites Typical Chernozem × 4 functional SOM pools × 5 replicates + 5 × only the carrier material). In addition 51 samples were prepared for the identification of the microbial community structure and analysis of NH_4^+ - N and NO_3^- - N

concentrations at the beginning of the experiment (2 sites Haplic Cambisol × 2 sites Typical Chernozem × 4 functional SOM pools × 3 replicates + 3 × only the carrier material).

The carrier material/functional SOM pool mixtures for the incubation experiment were pressed into small stainless steel tubes with a volume of 3.85 cm^3 to achieve a soil density of 1.3 g cm^{-3}, in order to ensure optimal decomposition conditions (Schroll *et al.*, 2006). The steel tubes were incubated in polyethylene vessels in an automatic respirometer (Respicond V, Nordgren Innovations AB, Sweden) at a constant temperature of 22°C (Nordgren, 1988). Measurements of CO_2 evolution from the 85 experimental units were taken hourly. To quantify the CO_2 produced from functional SOM pools, the CO_2 accumulated from the carrier material was subtracted from the total CO_2 released and expressed as the percentage of C input that was respired. For clarity, only the percentages of C input respired every 24 hours are presented here (Figure 3. 1 and Figure 3. 2).

Table 3. 1 Carbon and nitrogen concentrations of functional SOM pools (CF1, CF2, LF1, LF2), the carrier material and the bulk soil of the LPG, HPG, Pasture and Prairie sites. C and N concentrations of the carrier material used for the incubation experiment are also shown. Values are arithmetic means ± SE; ND = not determined.

		Bulk soil	Clay fractions		Light fractions	
			CF1	CF2	LF1	LF2
Carrier material	C (%)	0.37 (± 0.06)	ND	ND	ND	ND
	N (%)	0.03 (± 0.00)	ND	ND	ND	ND
	C:N ratio	11.78 (± 0.06)	ND	ND	ND	ND
LPG						
	C (%)	4.22 (± 0.17)	8.98 (± 0.13)	8.33 (± 0.06)	31.58 (± 0.06)	8.47 (± 0.07)
	N (%)	0.35 (± 0.01)	0.96 (± 0.02)	0.76 (± 0.02)	1.82 (± 0.02)	0.62 (± 0.01)
	C:N ratio	12.11 (± 0.10)	9.40 (± 0.10)	10.91 (± 0.23)	17.36 (± 0.19)	13.67 (± 0.18)
HPG						
	C (%)	3.58 (± 0.12)	8.06 (± 0.06)	7.71 (± 0.01)	37.32 (± 0.49)	6.28 (± 0.06)
	N (%)	0.31 (± 0.01)	0.86 (± 0.02)	0.68 (± 0.00)	1.96 (± 0.02)	0.33 (± 0.01)
	C:N ratio	11.76 (± 0.06)	9.33 (± 0.26)	11.32 (± 0.04)	19.05 (± 0.06)	19.29 (± 0.51)
Pasture						
	C (%)	5.02 (± 0.02)	11.11 (± 0.16)	8.46 (± 0.12)	31.63 (± 0.44)	8.24 (± 0.26)
	N (%)	0.45 (± 0.00)	1.03 (± 0.03)	0.90 (± 0.02)	2.27 (± 0.05)	0.78 (± 0.04)
	C:N ratio	11.22 (± 0.01)	10.84 (± 0.16)	9.43 (± 0.05)	13.94 (± 0.12)	10.59 (± 0.23)
Prairie						
	C (%)	5.44 (± 0.17)	11.67 (± 0.18)	9.29 (± 0.07)	33.17 (± 0.18)	7.84 (± 0.08)
	N (%)	0.46 (± 0.01)	1.04 (± 0.02)	0.96 (± 0.01)	2.18 (± 0.01)	0.67 (± 0.01)
	C:N ratio	11.73 (± 0.06)	11.22 (± 0.04)	9.68 (± 0.05)	15.22 (± 0.04)	11.73 (± 0.16)

2.5 Microbial community

Fatty acids derived from phospholipids (PLFA) were extracted based on the method of Bligh and Dyer (1959). The PLFAs were detected from triplicate samples at two time points, at the beginning and the end of the incubation experiment. 51 units were analysed in order to obtain information on the microbial structure at the beginning of the experiment and 51 units were analysed at the end of the experiment. All samples were stored at -20°C until analyses. The PLFAs were extracted from samples in a chloroform, methanol and citrate buffer mixture and then separated into neutral lipids, glycolipids and phospholipids by sequential elution with chloroform, acetone and methanol from a silica-bonded solid phase extraction column (SPE-SI; Bond Elute, Varian, Palo Alto, USA). PLFAs were hydrolysed and methylated using a methanolic KOH solution and individual PLFAs were then identified by GC/MS (Hewlett Packard 5971A mass selective detector, combined with a 5890 series II gas chromatograph) and quantified using MSD ChemStation D.01.02.16 chromatography software (Agilent Technologies, United States). The PLFA methyl nonadecanoate (19:0) was used as an internal standard. According to Frostegård *et al.* (1993) and Zelles (1999), individual PLFA markers can be used to quantify the relative abundance of specific microbial groups: fungi (phospholipid 18:2ω6), actinomycetes (10Me16:0, 10Me17:0 and 10Me18:0), total bacterial biomass (i15:0, a15:0, 15:0, i17:0, a17:0, cy 17:0, 17:0, cy 19:0). The fatty acids i15:0, a15:0, i16:0, i17:0 and a17:0 were considered to be specific for gram-positive bacteria and cy 17:0 and cy 19:0 for gram-negative bacteria. The ratio of fungal to total bacterial biomass (fungal:bacterial ratio) was used as an index of the relative abundance of fungi and bacteria in soils (Bardgett *et al.*, 1996) and the ratio of total saturated to total unsaturated PLFAs (SAT:UNSAT ratio) was determined as an indicator of nutritional stress in bacterial communities and nutrient availability (Bossio *et al.*, 1998).

2.6 Microbial activity

In total, 51 units were analysed for N mineralisation at the beginning and the end of the incubation experiment. All samples were stored at -20°C until analyses. Samples were extracted with 1 M KCl (1:4; w/v) by shaking horizontally for 1.5 h and then filtered (Schleicher & Schuell 595 ½, Dassel, Germany). The extracts were analysed for ammonium - N (NH_4^+ - N) and nitrate - N (NO_3^- - N) immediately after filtration with a flow injection analyser (FIAstar 5000, Foss GmbH, Rellingen, Germany).

2.7 Statistical analyses

Data were analysed with R, version 2.12.2; R Development Core Team (2011) and all data were tested for normality and homogeneity and are presented as arithmetic means ± standard errors (SE). Effects were regarded as significant if $P \leq 0.05$. Differences in N mineralisation and soil microbial properties between sites and functional SOM pools were analysed using a two-way ANOVA, followed by a *Tukey's post hoc* test. The independent variables were the treatments (LPG, HPG, Pasture, Prairie), the carrier material and functional SOM pools (CF1, CF2, LF1, LF2). The dependent variables were N mineralisation (NH_4^+ - N and NO_3^- - N) and soil microbial properties (total PLFA, total bacterial PLFA, gram-positive bacteria, gram-negative bacteria, fungal PLFA, actinomycetes, fungal:bacterial ratio and SAT:UNSAT ratio). If the assumptions of the model were not met, a Box-Cox transformation was applied. Furthermore, a *Tukey's post hoc test* was used to compare the total respired C (% of input) after 10 days between the respective functional SOM pools.

To visualize the structural and functional differences in the soil microbial community over time, *heatmaps* were created. For this purpose the changes between the end and beginning of the incubation experiment were calculated for each of the measured variables. The data were log-transformed to satisfy assumptions of normality and variance homogeneity.

Table 3. 2 Summary of a two-way ANOVA, testing the effects of sites (LPG, HPG, Pasture, Prairie and the carrier material) and functional SOM pools (CF1, CF2, LF1, LF2) on soil nutrient and microbial parameters at the start and end of the incubation. Bold values indicate significant responses at $P \leq 0.05$.

		Sites	Functional SOM pool	Sites × Functional SOM pool			Sites	Functional SOM pool	Sites × Functional SOM pool	
	d.f.	4	3	9	Residuals	d.f.	4	3	9	Residuals
		Start of the incubation experiment					End of the incubation experiment			
NH_4^+ - N	*F*	**31.01**	1.18	**4.33**	34	*F*	**18.22**	**5.86**	**4.01**	34
	P	**≤ 0.0001**	0.3316	**0.0010**		*P*	**≤ 0.0001**	**0.0024**	**0.0014**	
NO_3^- - N	*F*	0.80	**5.20**	**3.27**	34	*F*	**98.97**	**5.06**	**9.67**	34
	P	0.5311	**0.0046**	**0.0058**		*P*	**≤ 0.0001**	**0.0053**	**≤ 0.0001**	
Total PLFA	*F*	**11.17**	**4.44**	1.75	34	*F*	**3.61**	**7.32**	**2.40**	34
	P	**≤ 0.0001**	**0.0098**	0.1152		*P*	**0.0147**	**0.0007**	**0.0311**	
Total bac. PLFA	*F*	**9.32**	2.86	0.82	34	*F*	**4.68**	**9.47**	**2.40**	34
	P	**≤ 0.0001**	0.0510	0.6017		*P*	**0.0041**	**0.0001**	**0.0316**	
Gram-positive bac.	*F*	**10.02**	**3.07**	0.52	34	*F*	**5.88**	**8.23**	**2.34**	34
	P	**≤ 0.0001**	**0.0407**	0.8507		*P*	**0.0012**	**0.0003**	**0.0360**	
Gram-negative bac.	*F*	**7.95**	**6.00**	**3.05**	34	*F*	2.53	**10.37**	**2.77**	34
	P	**≤ 0.0001**	**0.0022**	**0.0087**		*P*	0.0584	**≤ 0.0001**	**0.0150**	
Fungal PLFA	*F*	1.05	1.51	1.65	34	*F*	2.64	**3.05**	**2.23**	34
	P	0.3946	0.2286	0.1404		*P*	0.0504	**0.0417**	**0.0446**	
Actinomycetes	*F*	**10.92**	1.32	**4.71**	34	*F*	**3.85**	**5.58**	1.36	34
	P	**≤ 0.0001**	0.2834	**0.0004**		*P*	**0.0110**	**0.0032**	0.2455	
Fungal:bac. ratio	*F*	2.20	0.86	1.54	34	*F*	1.79	2.33	**2.23**	34
	P	0.0895	0.4673	0.1732		*P*	0.1531	0.0915	**0.0440**	
SAT:UNSAT ratio	*F*	**5.38**	**6.38**	2.07	34	*F*	**18.36**	**10.48**	**3.70**	34
	P	**0.0018**	**0.0015**	0.0605		*P*	**≤ 0.0001**	**≤ 0.0001**	**0.0026**	

3. Results

3.1 Decomposition of functional SOM pools

The decomposition experiment revealed a significant ($P \leq 0.0001$) difference in released CO_2 after 10 days of incubation between functional SOM pools. Total respired CO_2 was significantly higher from CF1 than from CF2 and significantly higher from LF1 compared with LF2. There were also differences in amounts of respired OC between the soil types. In the Cambisol, decomposition of CF1 started within the first two days after addition. Total respired OC after 10 days of incubation of CF1 was significantly higher from the HPG (6.8%) than from the LPG site (5.4%; Figure 3. 1a). For the Chernozem, total respired OC was highest from CF1 of the Pasture soil (14.5%), with significantly lower respiration in the Prairie soil (4.1%) (Figure 3. 2a). Compared with CF1, microbial utilisation of CF2 resulted in less total respired OC. During the first five days of incubating CF2, CO_2 evolution was similar in all sites, but thereafter the Pasture site had higher CO_2 release (7.3%); 10 day totals - HPG, 2.7%, LPG, 2.3% (Figure 3. 1b) and the Prairie site of the Chernozem 1.1% (Figure 3. 2b).

Light fraction 1 started to decompose within 24 h after its addition. In general, respired CO_2 was significantly higher and was still increasing from LPG (13.4%), compared with HPG (9.4%) soils, which reached a saturated phase after 10 days of incubation (Figure 3. 1c). Only the Prairie soil showed a lag phase of about two days (Figure 3. 2c). The released CO_2 was significantly higher and still increasing in the Pasture (17.9%) soil compared with a slower CO_2 evolution from the Prairie soil (3.6%).

Respired OC from LF2 after 10 days was significantly higher from the LPG soil (10%) (Fig. 1d) compared with HPG (1.1%). The HPG soil showed a lag-phase of about four days before the CO_2 respiration slowly started and reached a saturated phase after seven days of incubation (Figure 3. 1d). Respiration of LF2 from both Pasture and Prairie soils showed a lag of 2-4 days (Figure 3. 2d) and the total respired OC was significantly higher from the Pasture (7.1%) compared with the Prairie (3.6%) soils.

3.2 Soil microbial properties

The soil microbial properties were analysed at the beginning and the end of the 10 day incubation experiment, in order to examine the influence of functional SOM pool quality on decomposition processes and related changes in microbial community composition and

activity. The carrier material, with low C and N contents (Table 3. 1), had a low total abundance of PLFA at the beginning of the incubation experiment (Table S3. 1). Mixing the carrier material with functional SOM pools of the different soils (LPG, HPG, Pasture, Prairie) generally resulted in significantly higher PLFA abundances and the formation of a microbial community with a specific composition (Figure S3. 1a).

Significant site × functional SOM pool interactions of the two-way ANOVA are described in the supplementary materials.

3.2.1 Start of the incubation experiment

Across all functional SOM pools, the nutrient availability in the Pasture and Prairie soils was similar. Higher nutrient availability (NH_4^+ - N and NO_3^- -N) was found in LPG than in HPG for both clay fractions. However, nitrogen availability was significantly higher for both light fractions of LPG soil.

Overall, there were significant differences between sites ($P \leq 0.0001$) in the total abundance of PLFA, total bacterial PLFA, gram-positive and gram-negative bacteria, actinomycetes and SAT:UNSAT ratio ($P \leq 0.0018$) at the start of the incubation experiment (Table 3. 2). These variables were all higher in HPG soil compared with LPG and higher in the Pasture compared with the Prairie. The NH_4^+ - N content was higher in the carrier material but similar for the sites on both soil types (Table S3. 2).

Functional SOM pools significantly influenced the total abundance of PLFA ($P \leq 0.0098$), gram-positive bacteria ($P \leq 0.0407$) and gram-negative bacteria ($P \leq 0.0022$) (Table 3. 2), with a rank order CF1 > CF2 > LF2 > LF1. The highest ratios of SAT:UNSAT ($P \leq 0.0015$) were detected in LF1 and the NO_3^- - N ($P \leq 0.0046$) content was higher in CF2 (Table S3. 3).

3.2.2 End of the incubation experiment

The NH_4^+ - N and NO_3^- - N concentrations were significantly affected by sites ($P \leq 0.0001$), as well as the following microbial parameters: total abundance of PLFA ($P \leq 0.0147$), total bacterial PLFA ($P \leq 0.0041$), gram-positive bacteria ($P \leq 0.0012$), actinomycetes ($P \leq 0.0110$) and the SAT:UNSAT ($P \leq 0.0001$) (Table 3. 2). All of these parameters, except gram-negative bacteria, were higher from HPG compared with LPG (Table S3. 2). For the Chernozem soil, these parameters were higher from the Pasture compared with the Prairie (Table S3. 2).

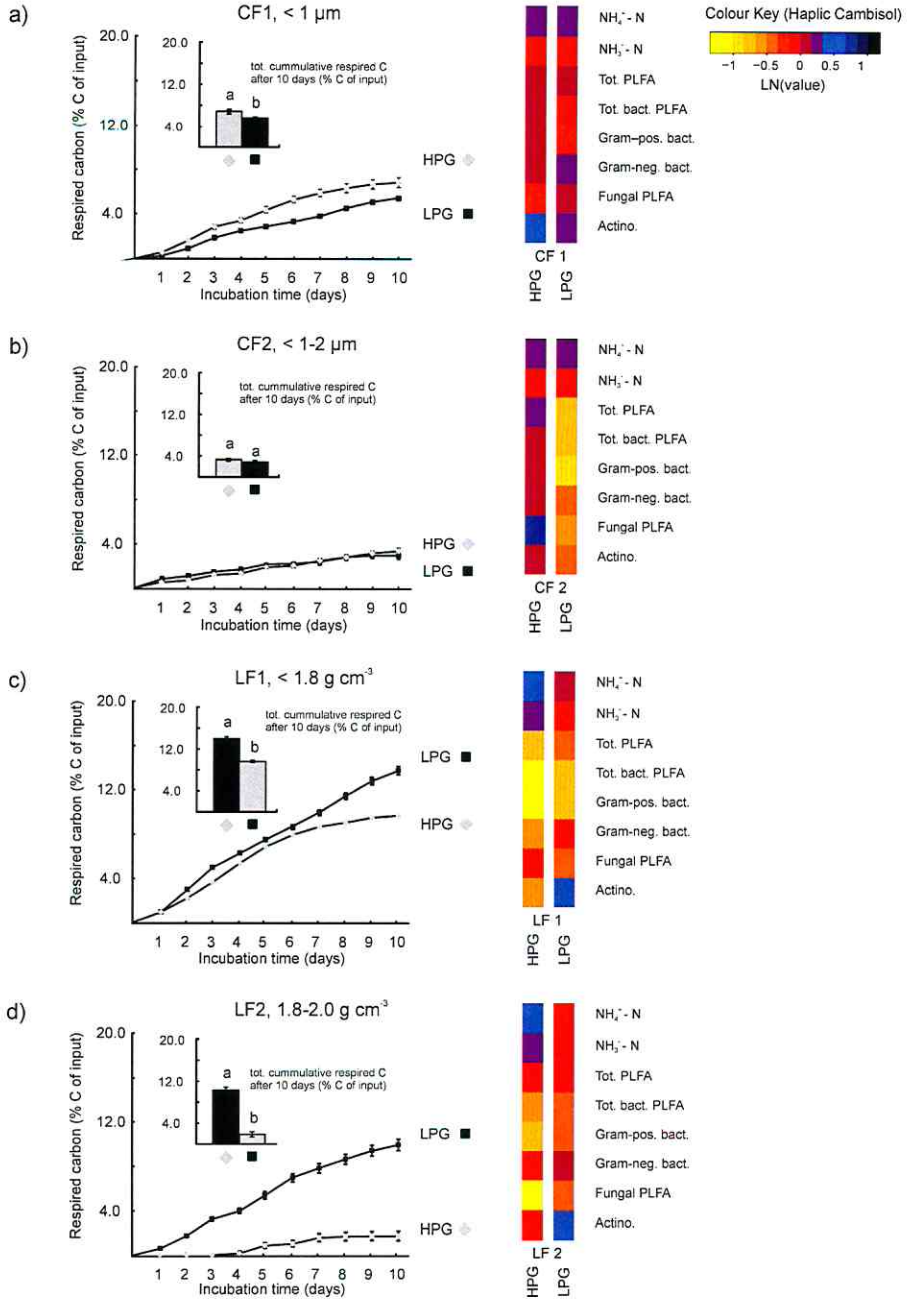

Figure 3. 1 Respiration and functional and structural parameters of the microbial community of functional SOM pools of a *Haplic Cambisol* soil from high productivity (HPG) and low productivity grasslands (LPG), respectively). a) clay fraction 1 (CF1, < 1µm), b) clay fraction 2 (CF2, 1 – 2 µm), c) light fraction 1 (LF1, < 1.8 g cm^{-3}) and d) light fraction 2 (LF2, 1.8 – 2.0 g cm^{-3}). On the left: the respired carbon (% of input) of functional SOM pools in LPG (black squares) and HPG (light grey diamonds) over an incubation time of 10 days. Insets illustrate the cumulative respired C (% of input) after 10 days; a Tukey's *post hoc* test was used to compare the total respired C between sites. Values are arithmetic means ± SE and values with the same letter are not significantly different. On the right: heatmaps representing the changes of functional and structural parameters of the microbial community between the end and the start of the incubation experiment. Darker and lighter rectangles indicate higher and lower abundances/activities of the microbial community, respectively.

Differences in soil microbial properties were also detected between functional SOM pools at the end of the incubation experiment. The NH_4^+ - N and NO_3^- - N concentrations ($P \leq 0.0024$, $P \leq 0.0053$), the total abundance of PLFA ($P \leq 0.0007$), bacterial PLFA ($P \leq 0.0001$), gram-positive bacteria ($P \leq 0.0003$), gram-negative bacteria ($P \leq 0.0001$) fungal PLFA ($P \leq 0.0417$) and actinomycetes ($P \leq 0.0032$) were significantly influenced by functional SOM pools and means were ranked CF1 < CF2 and LF2 < LF1. Only the ratio of SAT:UNSAT followed the reverse order, with higher ratios in both light fraction; LF2 < LF1 and clay fractions; CF2 < CF1 (Table 3. 2 and Table S3. 3).

3.2.3 Change of microbial properties during utilisation of functional SOM pools

To better understand the structural and functional differences in the soil microbial communities that developed over incubation time of the different functional SOM pools, *heatmaps* were created (Figure 3. 1 and Figure 3. 2). The values used to create the heatmaps were the calculated differences between the end and the beginning of the incubation experiment for each of the measured variables.

Clay Fraction 1: Within the smaller clay fraction of HPG soil, the microbial community was dominated by bacteria, particularly gram-negative, gram-positive bacteria and actinomycetes. The same effects were found for the Pasture site on the Chernozem soil (Figure 3. 1a and Figure 3. 2a). In both soil types, the NH_4^+ - N and NO_3^- - N contents were similar.

Clay Fraction 2: Positive shifts were found for the total abundance of PLFA and the fungal PLFA in HPG soil. Negative shifts were found for the abundances of all detected parameters in LPG soil, which were dominated by gram-negative bacteria and fungi. In both sites on the Haplic Cambisol soil, the NH_4^+ - N and NO_3^- - N contents were similar. The microbial community in the Pasture was dominated by fungi and in the Prairie site by gram-negative bacteria and actinomycetes, with higher NH_4^+ - N and NO_3^- - N concentrations (Figure 3. 1b and Figure 3. 2b).

Light Fraction 1: In HPG soil, the total abundances of PLFAs shifted to lower levels with an increasing level of the NH_4^+ - N and NO_3^- - N content. In contrast, positive shifts of the total abundance of PLFA, gram-negative bacteria and actinomycetes were recorded for LPG soil. For Chernozem grassland soils, changes for the Pasture (positive) and Prairie (negative) were opposite for all the measured microbial parameters (especially gram-negative bacteria, fungal PLFA and actinomycetes, NH_4^+ - N and NO_3^- - N content; Figure 3. 1c and Figure 3. 2c).

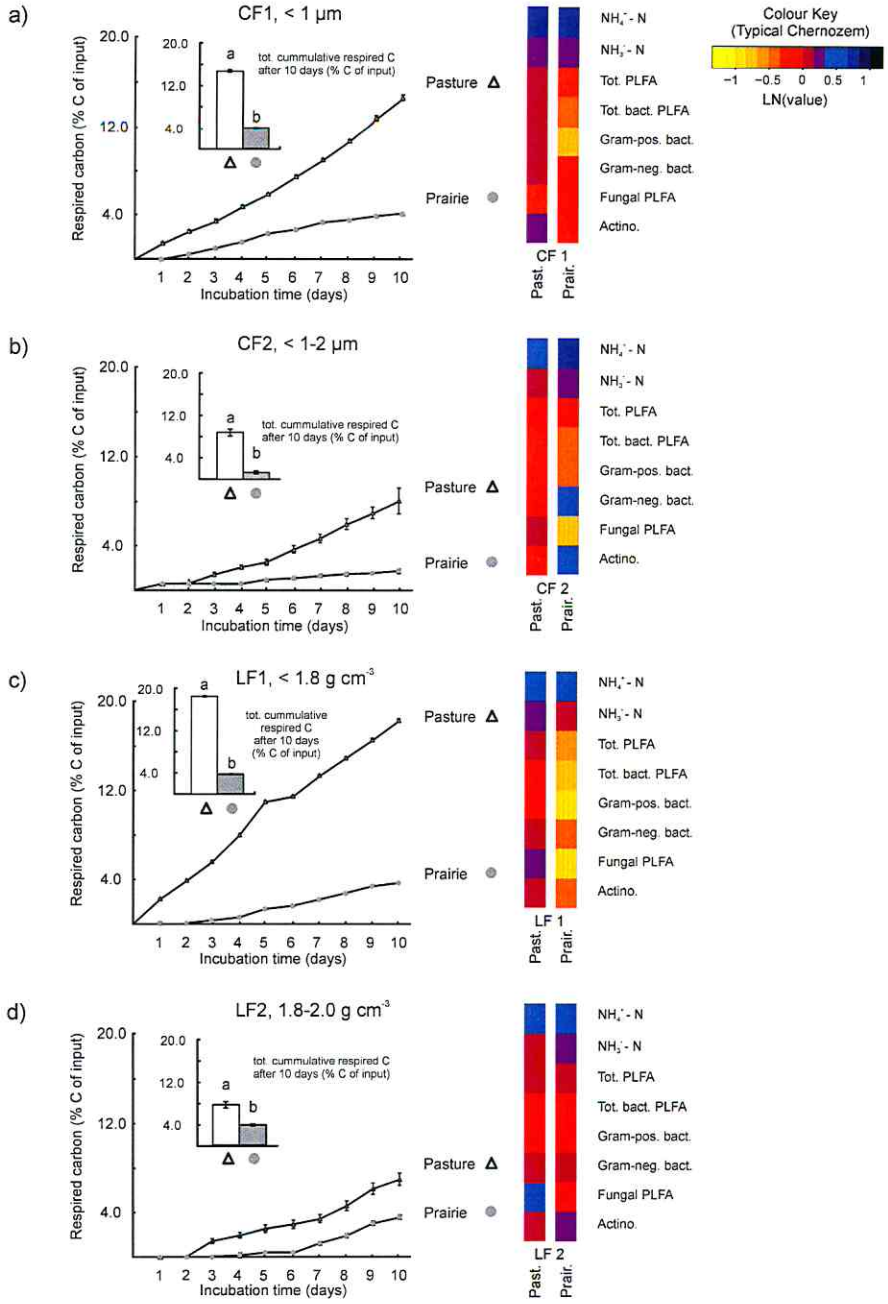

Figure 3. 2 Respiration and functional and structural parameters of the microbial community of functional SOM pools of a *Chernozem* from Pasture and Prairie soils. a) clay fraction 1 (CF1, < 1µm), b) clay fraction 2 (CF2, 1 – 2 µm), c) light fraction 1 (LF1, < 1.8 g cm^{-3}) and d) light fraction 2 (LF2, 1.8 – 2.0 g cm^{-3}). On the left: the respired carbon (% of input) of functional SOM pools in the Pasture (white triangles) and Prairie (grey circles) soils over an incubation time of 10 days. Insets illustrate the cumulative respired C (% of input) after 10 days; a Tukey's *post hoc* test was used to compare the total respired C between sites. Values are arithmetic means ± SE and values with the same letter are not significantly different. On the right: heatmaps representing the changes of functional and structural parameters of the microbial community between the start and end of the incubation experiment. Darker and lighter rectangles indicate higher and lower abundances/activities of the microbial community, respectively; Past = Pasture, Prair = Prairie.

Light Fraction 2: In HPG soil, all microbial parameters were reduced compared with LPG soil, whereas the NH_4^+ - N and NO_3^- - N contents increased. In LPG, the microbial community was dominated by gram-negative bacteria and actinomycetes. The abundances of gram-negative bacteria and actinomycetes in the Prairie increased. The fungal PLFA dominated in the Pasture (Figure 3. 1d and Figure 3. 2d).

4. Discussion

The addition of functional SOM pools to the carrier material was accompanied by an input of microbial biomass. It can be assumed that microorganisms attached to the respective functional SOM pools are primarily dormant and become active during the incubation, under conditions, which have been identified as optimal for microbial degradation processes (Ilstedt *et al.*, 2000, Schroll *et al.*, 2006).

4.1 Functional SOM pools isolated by particle size

Organic carbon in functional SOM pool, such as the clay fractions, which were separated by disaggregation of the soil structure (CF1, < 1µm; CF2, 1 – 2 µm), is either protected by interaction with clay minerals or spatially inaccessible through occlusion by aggregates. This influences access to the OC by microbes and enzymes (Kögel-Knabner *et al.*, 2008). These stabilised functional SOM pools represent an important component for SOC sequestration, due to their older mean age and higher turnover rates (Baldock & Skjemstad, 2000, Kaiser *et al.*, 2002). This is suggested by microbial community analyses indicating that particles < 2 µm provide a niche for aerobic and anaerobic microorganisms (Sessitsch *et al.*, 2001). Hence, if aggregates are destroyed, e.g. through anthropogenic activities and become available as a substrate for soil microorganisms, these functional SOM pools can be depleted, depending on land use and the quality of the C source (Figure 3. 1a and Figure 3. 2a; Schulz *et al.*, 2011). For both clay fractions (CF1, CF2) from the two soil types (Haplic Cambisol and Typical Chernozem), higher respiration of OC was detected in the fertile and more productive ecosystems, i.e. HPG and Pasture. Higher respiration of OC from the smaller sized fraction (CF1) was associated with a higher microbial biomass, which is consistent with the higher specific surface area of clay (Coleman *et al.*, 2004). Smaller sized clay fractions can protect bacteria from predation by protozoa by increasing the number of protective microhabitats available (England *et al.*, 1993). In HPG soils, higher nutrient availability and

plant nutrient uptake through legume species (Breulmann *et al.*, 2011), as well as through the deposition of animal wastes in the Pasture soil (Bardgett & Wardle, 2010) facilitate the development of a bacterial-dominated community. This can be linked to higher nutrient turnover and decomposition rates, which is reflected in higher respiration of OC in this functional SOM pool. Hence, the structure of the microbial community of CF1 was in general dominated by bacteria in both soil types (e.g. gram-negative bacteria, gram-positive bacteria and actinomycetes, Figure 3. 1a, Figure 3. 2a). Organic carbon in the Cambisol soils was respired at similar rates, but there were huge differences between the Pasture and the Prairie on the Chernozem soils. Significantly higher OC respiration in the Pasture (Figure 3. 2a) suggests that under optimal conditions and with soil aggregates destroyed, the microbial community is able to easily degrade the OC within CF1. Similar results were found for CF2 in the Pasture (Figure 3. 2b). However, amounts of respired OC from CF2 in HPG and LPG soils were both low. Low levels of respired OC were also detected in the Prairie soil, indicating a functional SOM pool of higher stability (due to interaction with minerals and spatial inaccessibility) within this system, since the microbial community was not able to use the OC source.

Clay minerals can clearly promote microbial growth by providing appropriate surfaces and maintaining the pH within an optimal range (Filip, 1973, Six *et al.*, 2006). However, in addition, our data suggest that differences in the clay mineralogy of the two soil types, as well as the land use, may be further important determinants of soil microbial structure and associated utilisation of the C source. The specific surface area of clay minerals can range from 50-100 m^2 g^{-1} for kaolinitic clays, through 300-500 m^2 g^{-1} for vermiculites to 700-800 m^2 g^{-1} for well-dispersed smectites (Coleman *et al.*, 2004). Different soil types may also harbour different types of clay particles (van Loosdrecht *et al.*, 1990). Smectite, vermiculite and chlorite are the dominant clay minerals in Chernozem soils around the world (Weaver, 1989). It can be assumed that a higher abundance of montmorillonite (smectites) in the Chernozem, in combination with a higher abundance of microbial activity in the Pasture, could stimulate bacterial respiration, reflecting a greater cation exchange capacity between the soil and the soil solution. However, this is just a hypothetical option for explanation and cannot be confirmed using the parameters investigated here.

The utilisation of the two CFs depended on their accessibility to soil microorganisms as specific functional SOM pool characteristics, which was increased due to disaggregation that promoted the accessibility of previously protected OC to soil microorganisms. Furthermore,

the management intensity and soil type were important factors affecting the utilisation of the two clay fractions.

4.2 Functional SOM pools isolated by specific density

Functional SOM pools isolated by a specific density solution (e.g. bromoform/ethanol) yield light fractions (LF) that are defined as active and intermediate fractions with significantly faster turnover rates (< 10 years) than clay fractions (Swanston *et al.* 2002, von Lützow *et al.*, 2007, Schulz *et al.*, 2011). Our results are consistent with these findings and indicate that LF1 (< 1.8 g cm^{-3}), depending on its quality, is readily available for microorganisms, resulting in general in significantly more total respired OC. However, these effects depend on the site-specific management and the soil type (Figure 3. 1c and Figure 3. 2c). For the LF1 fraction from HPG soil, a high percentage of OC was decomposed quickly within the first five days of incubation; this activated a gram-negative dominated microbial community capable of using OC the source. After consumption of the available OC, respiration reached a saturated phase (Figure 3. 1c). In LPG, a significantly denser system of living roots affects soil resources, such as nitrogen and labile OC and controls soil microbial communities (Breulmann *et al.*, 2011). LPG sites are characterised by slow-growing plants and are dominated by a fungal soil food web, leading to steadily increasing decomposition of LF1 and more complex OC sources. On the Chernozem soil, the effects on respired OC differed much more between the two grasslands, with more OC respired in the Pasture soil (Figure 3. 2c). In contrast to undisturbed Prairie grasslands, grazed grasslands are dominated by fast growing plant species with decreased litter quality, which significantly affect the soil food web (Wardle *et al.*, 2004, Bardgett & Wardle, 2010). It can be assumed that the soil communities are bacteria-dominated. In general, the LF1 fraction has the highest OC content of the isolated functional SOM pools, consisting of pieces of plant material and activating a gram-negative dominated microbial community in the Pasture soil (see Langer & Rinklebe, 2011). These results are consistent with findings by Klumpp *et al.* (2009), that intensified grazing alters plant rooting and the soil microbial community and promotes decomposition and soil C losses.

LF2 (1.8 – 2.0 g cm^{-3}) is also significantly influenced by site-specific management and soil type (Figure 3. 1d, Figure 3. 2d). Our results indicate that despite its loose association with mineral surfaces, there was less respired OC with LF2 than LF1. These results are supported by isotopic analyses reported by Schulz *et al.* (2011), indicating that OC in LF2 is stabilised by recalcitrance. In HPG soil, the level of respired OC from LF2 was very low, suggesting

that the OC was not available for decomposition and resulting in a decrease in abundance of functional groups of soil microorganisms (Figure 3. 1d). However, the total abundance of PLFA in HPG and LPG soils were similar, so it can be assumed that in LPG specialist microorganisms are capable of using the OC within LF2 and were responsible for a significantly higher total cumulative respiration of OC. In both grasslands on the Chernozem soil, there was a lag phase of two days before respiration of LF2 started, but total respired C in the Pasture was significantly higher (Figure 3. 2d), probably due to a higher abundance of fungi that were able to degrade more complex C sources (Bardgett, 2005).

The utilisation of the two LFs depended also on specific pools characteristics, as described above for the two clay fractions and on the chemical stability of OC compounds. The OC within LF2 can be considered as recalcitrant, due to low levels of respired CO_2, albeit a specialised microbial community present was capable of degrading LF2 in LPG. The OC of LF1 is readily degradable promoting OC losses. The utilisation of the light fractions depended furthermore on the management intensity and the soil type.

4.3 Conclusions

Detailed information on the microbial utilisation of functional SOM pools of two soil types of grassland ecosystems with varying management were obtained from an incubation experiment, where functional SOM pools were used as substrates. From the interactions between functional SOM pools, its properties and the diverse members of the soil community, three controlling factors for the microbial utilisation of OC were identified: (1) specific traits of functional SOM pools (accessibility to soil microorganisms), (2) the management intensity of the grasslands and (3) the soil type. Due to a complete disaggregation of the soil structure according to the fractionation approach used in this study, the accessibility for microbes was not limited and consequently the utilisation of OC of CFs was increased as compared to the aggregated bulk soil. The utilisation of the two LFs depends on the chemical stability of OC (recalcitrance). Management intensity promotes decomposition and soil C losses and the soil type induced different levels of released CO_2, reflecting the particular stability of SOM in unused Chernozems (Prairie). Furthermore, the PLFA analyses show that different microbial communities established, depending on the soil type and functional SOM pools examined. These microbial communities are responsible for OC utilisation of the different functional SOM pools. By studying abiotic and biotic components, it was possible to obtain new insights into the interactions between the properties of individual organic substrates, functional SOM

pools and the diverse members of the soil communities. In future studies, the application of molecular techniques will help to identify key microbial groups involved in degrading the different OC sources.

Acknowledgments

The work was funded by the German Federal Ministry of Education and Research (BMBF) as part of the DIVA – Jena project (grant no. 01LC0613B), an interdisciplinary research project of the BIOLOG-Europe programme, coordinated by Prof. Dr. W. W. Weisser (since April 2011 TU München) and Ilka Egerer for technical coordination. We thank Prof. Dr. B. Michalzik and Sabina Christ for valuable comments on the manuscript. We are also grateful to Jacqueline Rose, Gabriele Henning and Jana Schmidt for technical assistance.

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Supplementary Materials

Significant site × functional SOM pool interactions

Start of the incubation experiment

For the microbial community composition, significant interactions between sites and functional SOM pools (site × functional SOM pool interaction) were detected for gram-negative bacteria ($P \leq 0.0087$) and actinomycetes ($P \leq 0.0004$), with high abundances in LPG for both clay fractions but significantly lower abundances in both light fractions. In contrast, the abundance of gram-negative bacteria was higher in HPG soil for CF1, but significantly lower in the remaining functional SOM pools. In all functional SOM pools except CF1 from the Pasture, the abundance of gram-negative bacteria was higher compared with the Prairie (Table 3. 2, Figure S3. 1a). The abundance of actinomycetes was higher from the Prairie than from the Pasture site.

End of the incubation experiment

At the end of the incubation, the NH_4^+ - N content was higher in CF2 and both light fractions from HPG compared with LPG. But the NH_4^+ - N content was higher for LPG within CF1. For the Chernozem the NH_4^+ - N content was highest from the Prairie for both clay fractions and highest from the Pasture for both light fractions ($P \leq 0.0014$ for the site × functional SOM pool interaction). Similar results were detected for the NO_3^- - N content for both soil types ($P \leq 0.0001$ for the site × functional SOM pool interaction). High abundances of total PLFA ($P \leq 0.0311$), total bacterial biomass ($P \leq 0.0316$), gram-positive bacteria ($P \leq 0.0360$), gram-negative bacteria ($P \leq 0.0150$), fungi ($P \leq 0.0446$) and SAT:UNSAT ($P \leq 0.0026$) were detected in both clay fractions from HPG and in both light fractions from LPG. Within all functional SOM pools, the highest abundances of these parameters were detected in the Pasture (for the soils × functional SOM pool interaction). A site × functional SOM pool interaction was detected for the fungal:bacterial (F:B) ratio ($P \leq 0.0440$). Higher F:B ratios were detected in CF1 and LF1 from LPG. In general, the ratio was higher in the Pasture for CF2 and both light fractions (Table 3. 2).

Table S3. 1 Effects of soil microbial activity on the carrier material, in terms of nitrogen NH_4^+ - N and NO_3^- - N content (expressed as mg kg^{-1}) and the relative abundance of phopholipid fatty acids (PLFA; expressed as pmol fatty acids g^{-1} soil) at the start and end of the incubation experiment. Values are arithmetic means ± SE and values.

	Carrier material	
	Start of the incubation experiment	End of the incubation experiment
NH_4^+ - N	9.81 (± 1.1)	8.89 (± 0.1)
NO_3^- - N	26.78 (± 0.2)	23.03 (± 0.6)
Total PLFA	1678.35 (± 183.1)	3356.72 (± 141.5)
Total bac. PLFA	496.43 (± 20.9)	967.00 (± 4.9)
Gram-positive bac.	247.71 (± 25.2)	495.60 (± 12.2)
Gram-negative bac.	179.12 (± 17.5)	435.71 (± 24.5)
Fungal PLFA	221.26 (± 83.3)	87.8 (± 10.91)
Actinomycetes	68.90 (± 8.8)	82.78 (± 7.7)
Fungal:bac. ratio	0.14 (± 0.0)	0.09 (± 0.0)
SAT:UNSAT ratio	2.73 (± 0.1)	2.85 (± 0.2)

Table S3. 2 Differences between sites (LPG and HPG, Pasture and Prairie) in soil microbial activity (NH_4^+ - N and NO_3^- - N content expressed as mg kg^{-1}) and the relative abundance of phospholipid fatty acids (expressed as pmol fatty acids g^{-1} soil) at a) the start and b) end of the incubation experiment. Values are averaged over functional SOM pools and are arithmetic means ± SE.

a)

	HPG	LPG	Pasture	Prairie
	Start of the incubation experiment			
NH_4^+ - N	7.98 (± 0.4)	8.75 (± 0.2)	6.70 (± 0.2)	6.66 (± 0.1)
NO_3^- - N	25.77 (± 0.3)	25.90 (± 0.2)	25.96 (± 0.4)	25.74 (± 0.3)
Total PLFA	3656.84 (± 252.8)	3220.99 (± 208.6)	3881.31 (± 152.5)	3230.20 (± 94.1)
Total bac. PLFA	1167.25 (± 92.4)	1058.06 (± 45.3)	1210.95 (± 51.0)	1020.67 (± 25.9)
Gram-positive bac.	660.52 (± 56.3)	528.25 (± 32.7)	715.14 (± 32.2)	598.73 (± 21.4)
Gram-negative bac.	442.67 (± 38.6)	380.62 (± 28.6)	427.63 (± 15.1)	357.27 (± 28.8)
Fungal PLFA	112.02 (± 15.5)	116.35 (± 6.7)	104.71 (± 7.5)	105.26 (± 14.5)
Actinomycetes	478.26 (± 24.7)	373.88 (± 47.6)	556.69 (± 20.72)	462.51 (± 39.9)
Fungal:bac. ratio	0.09 (± 0.0)	0.11 (± 0.0)	0.09 (± 0.0)	0.10 (± 0.0)
SAT:UNSAT ratio	2.80 (± 0.2)	2.61 (± 0.1)	3.25 (± 0.1)	3.05 (± 0.1)

b)

	HPG	LPG	Pasture	Prairie
	End of the incubation experiment			
NH_4^+ - N	12.25 (± 0.5)	10.29 (± 0.5)	13.15 (± 0.5)	13.27 (± 0.5)
NO_3^- - N	28.93 (± 1.4)	23.00 (± 0.3)	31.74 (± 0.6)	31.59 (± 0.6)
Total PLFA	3047.40 (± 509.0)	2615.00 (± 318.4)	3947.49 (± 190.5)	2688.78 (± 218.5)
Total bac. PLFA	862.18 (± 169.7)	719.66 (± 93.9)	1135.02 (± 63.9)	716.47 (± 67.0)
Gram-positive bac.	462.45 (± 100.6)	326.89 (± 47.2)	660.05 (± 49.1)	380.55 (± 46.4)
Gram-negative bac.	367.01 (± 62.2)	363.94 (± 39.53)	336.45 (± 20.6)	326.38 (± 20.3)
Fungal PLFA	98.38 (± 25.2)	87.8 (± 10.91)	139.74 (± 20.1)	67.77 (± 8.1)
Actinomycetes	405.33 (± 52.6)	427.27 (± 43.5)	626.07 (± 28.7)	466.20 (± 31.6)
Fungal:bac. ratio	0.12 (± 0.0)	0.12 (± 0.0)	0.13 (± 0.0)	0.09 (± 0.0)
SAT:UNSAT ratio	2.17 (± 0.1)	2.08 (± 0.1)	2.74 (± 0.1)	2.25 (± 0.1)

Table S3. 3 Differences between functional SOM pools (CF1, CF2, LF1, LF2) in soil microbial activity (NH_4^+ - N and NO_3^- - N content expressed as mg kg^{-1}) and the relative abundance of phospholipid fatty acids (expressed as pmol fatty acids g^{-1} soil) at a) the start and b) end of the incubation experiment. Values are averaged over sites and are arithmetic means ± SE.

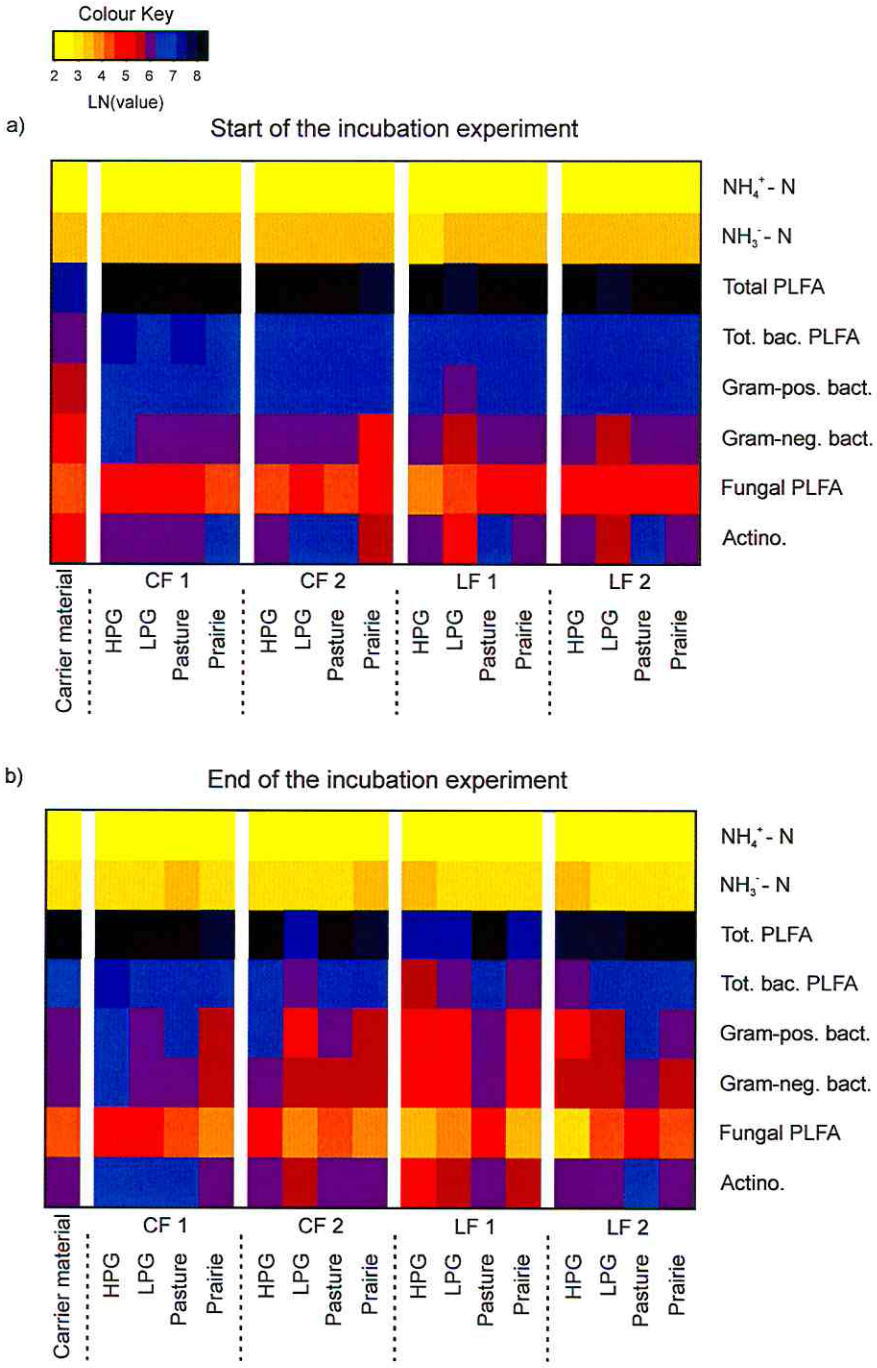
a)

	CF1	CF2	LF1	LF2
	Start of the incubation experiment			
NH_4^+ - N	7.47 (± 0.4)	7.40 (± 0.4)	7.33 (± 0.3)	7.76 (± 0.3)
NO_3^- - N	25.31 (± 0.3)	26.70 (± 0.4)	25.47 (± 0.3)	25.88 (± 0.3)
Total PLFA	3970.6 (± 221.1)	3413.31 (± 164.2)	3201.41 (± 162.4)	3404.01 (± 191.6)
Total bac. PLFA	1249.70 (± 82.4)	1087.23 (± 50.8)	1035.12 (± 45.1)	1084.87 (± 47.4)
Gram-positive bac.	716.16 (± 55.2)	601.08 (± 33.4)	567.88 (± 34.9)	617.52 (± 31.9)
Gram-negative bac.	471.05 (± 31.9)	408.92 (± 38.3)	362.83 (± 17.5)	365.40 (± 17.5)
Fungal PLFA	120.57 (± 12.0)	114.31 (± 16.4)	90.44 (± 8.9)	113.04 (± 4.5)
Actinomycetes	509.53 (± 30.5)	472.40 (± 38.1)	444.65 (± 46.4)	444.75 (± 40.5)
Fungal:bac. ratio	0.10 (± 0.0)	0.10 (± 0.0)	0.09 (± 0.0)	0.11 (± 0.0)
SAT:UNSAT ratio	2.64 (± 0.2)	2.81 (± 0.2)	3.21 (± 0.1)	3.05 (± 0.1)

b)

	End of the incubation experiment			
NH_4^+ - N	13.31 (± 0.5)	12.03 (± 0.5)	11.33 (± 0.6)	12.27 (± 0.6)
NO_3^- - N	28.30 (± 1.3)	27.83 (± 1.4)	29.35 (± 1.2)	29.77 (± 1.3)
Total PLFA	4003.3 (± 297.4)	3048.41 (± 299.8)	2274.62 (± 322.4)	2972.62 (± 356.0)
Total bac. PLFA	1195.7 (± 115.8)	863.46 (± 88.4)	588.61 (± 84.4)	785.56 (± 104.6)
Gram-positive bac.	651.56 (± 80.1)	456.68(± 60.7)	291.08 (± 51.22)	430.62 (± 63.3)
Gram-negative bac.	500.11 (± 38.2)	380.66 (± 24.3)	282.95 (± 29.82)	330.06 (± 39.0)
Fungal PLFA	105.78 (± 9.9)	106.16 (± 23.00)	70.34 (± 12.5)	111.42 (± 23.9)
Actinomycetes	612.72 (± 35.1)	443.68 (± 26.1)	381.15 (± 43.5)	467.53 (± 53.6)
Fungal:bac. ratio	0.10 (± 0.0)	0.12 (± 0.0)	0.11 (± 0.0)	0.14 (± 0.0)
SAT:UNSAT ratio	2.10 (± 0.1)	2.14 (± 0.1)	2.48 (± 0.1)	2.51 (± 0.1)

Figure S3. 1 Heatmap showing functional and structural parameters of the microbial community within the carrier material and mixed with different functional SOM pools from the four sites at a) the start and b) the end of the incubation experiment. Darker and lighter rectangles indicate higher and lower abundances and activities of the soil microbial community, respectively.



Acknowledgments

I would like to thank *Prof. Dr. F. Buscot* for the opportunity of doing my dissertation in the Department of Soil Ecology, UFZ – Helmholtz Centre for Environmental Research in Halle, the topic, for his encouragement, helpful comments on the manuscripts as well as for acting as a reviewer of my dissertation.
I am grateful to *Prof. Dr. G. Guggenberger* for his support of submitting my dissertation to the Gottfried Wilhelm Leibniz Universität Hannover and for acting as the principal reviewer of my thesis.
Furthermore, I would like to thank *Prof. Dr. R.D. Bardgett* of the Lancaster Environment Centre, for his willingness of acting as an external reviewer of my dissertation and for the pleasant and interesting arrangement during our visit.

My particular gratitude goes to my supervisor *Dr. E. Schulz* for her continuous support, numerous constructive discussions and her help, being a constant source of excellent advice.

Many thanks to the Soil Ecology Group, in particular *G. Henning* and *J. Rose* for technical assistance, *B. Schnabel* for her help on formatting the dissertation, *G. Kovaçs* for final comments and *B. Hoppe* for adding some pleasure to the office life.

Dr. C. Dormann and *Dr. I. Kühn* for statistical advises and *Dr. B. Böer* for his support during my internship and the involvement in several projects of the UNESCO.

Special thanks to *Dr. T. Eggers,* who inspired my interest in the field of ecology (soil- and experimental ecology), above- below-ground interaction and statistics during my studies at the University of Osnabrück and for proof reading.

I am grateful and honoured to have been part of an interdisciplinary research project (BIOLOG–Europe) of the Federal Ministry of Education and Research (BMBF) and thankful to the members of the DIVA subproject for the good collaboration and coordination.

Special thanks to *Sabina* for her patience, love and support and my parents for their continuous support during the last years.

Curriculum Vitae

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Declaration

according to §6(1) of the doctoral degree regulations of the Faculty of Natural Science, Gottfried Wilhelm Leibniz Universität Hannover

I declare that the work presented is my own and has not been submitted previously in any form for another degree at any university or institution. All additional help by others and sources of information used have been acknowledged within individual chapters.

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Marc Breulmann

Erklärung

gemäß §6(1) der Promotionsordnung der Naturwissenschaftlichen Fakultät der Gottfried Wilhelm Leibniz Universität Hannover für die Promotion zum Dr. rer. nat.

Hierdurch erkläre ich, dass ich meine Dissertation selbstständig verfasst und die benutzten Hilfsmittel und Quellen sowie gegebenenfalls die zu Hilfeleistungen herangezogenen Institutionen vollständig angegeben habe.

Die Dissertation wurde nicht schon als Masterarbeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

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Marc Breulmann

List of Publications

Peer reviewed publications

Schulz E., **Breulmann, M.**, Boettger, T., Wang, K.-R. & Neue, H.-U. (2011) Effect of organic matter input on functional pools of soil organic carbon in a long-term double rice crop experiment in China. *European Journal of Soil Science*, **62**, 134-143.

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Demyan, S., Rasche, F., Schulz, E., **Breulmann, M.**, Müller, T. & Cadisch, G. "Relationship between DRIFTS of bulk soil and SOM fractions in the Static Fertilization Experiment Bad Lauchstädt", **submitted**.

Eggers, T., **Breulmann, M.**, Fieberg, K., Möhlmeyer, A. & Gromes, R. "Combined effects of above-ground herbivores and below-ground decomposers (earthworms) on soil enzymes" **in preparation**.

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