Science of the Total Environment 497–498 (2014) 29–37

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short-term bioavailability of carbon in soil organic matter fractions of different particle sizes and densities in grassland ecosystems

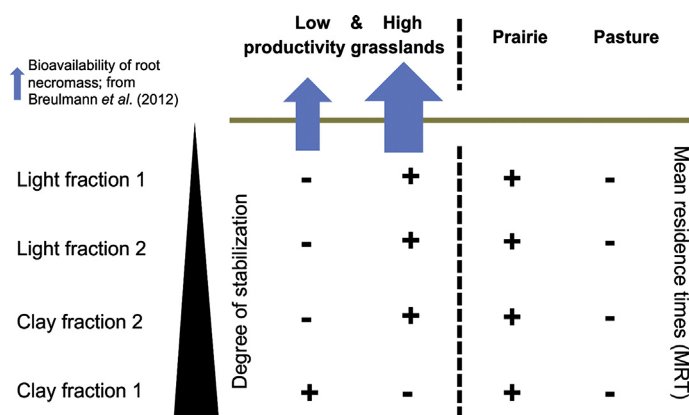
Marc Breulmann [a,e,*], Nina Petrovna Masyutenko [b], Boris Maratovich Kogut [c], Reiner Schroll [d], Ulrike Dörfler [d], François Buscot [e,f,g], Elke Schulz [e]

[a] Helmholtz-Centre for Environmental Research—UFZ, Environmental and Biotechnology Centre (UBZ), Permoserstraße 15, 04318 Leipzig, Germany
[b] All-Russia Research Institute of Arable Farming and Soil Erosion Control, Kursk, Russia
[c] V.V. Dokuchaev Soil Science Institute, Moscow, Russia
[d] Helmholtz-Centre Munich, German Research Centre for Environmental Health, Institute of Soil Ecology, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[e] Helmholtz-Centre for Environmental Research—UFZ, Department of Soil Ecology, Theodor-Lieser-Str. 4, 06120 Halle, Germany
[f] University of Leipzig, Institute of Biology, Johannisallee 21-23, 04103 Leipzig, Germany
[g] German Centre for Integrative Biodiversity Research (iDiv), Deutscher Platz 5e, 04103 Leipzig, Germany

HIGHLIGHTS

- Respiration experiment on the bioavailability of SOM fractions.
- Management intensity & the related plant community were key controlling factors.
- Further, specific traits of SOM fractions controlled decomposition processes.
- PLFA patterns highlighted differences in the active microbial community structure.
- These mechanisms could enhance the bioavailability of stabilized OC.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 April 2014
Received in revised form 21 July 2014
Accepted 21 July 2014
Available online xxxx

Editor: Charlotte Poschenrieder

Keywords:
Soil organic matter
Size–density fractionation

ABSTRACT

The quality, stability and availability of organic carbon (OC) in soil organic matter (SOM) can vary widely between differently managed ecosystems. Several approaches have been developed for isolating SOM fractions to examine their ecological roles, but links between the bioavailability of the OC of size–density fractions and soil microbial communities have not been previously explored. Thus, in the presented laboratory study we investigated the potential bioavailability of OC and the structure of associated microbial communities in different particle-size and density fractions of SOM. For this we used samples from four grassland ecosystems with contrasting management intensity regimes and two soil types: a Haplic Cambisol and a typical Chernozem. A combined size–density fractionation protocol was applied to separate clay-associated SOM fractions (CF1, <1 μm; CF2, 1–2 μm) from light SOM fractions (LF1, <1.8 g cm^{-3}; LF2, 1.8–2.0 g cm^{-3}). These fractions were used as carbon sources in a respiration experiment to determine their potential bioavailability. Measured

* Corresponding author at: Helmholtz-Centre for Environmental Research – UFZ, Environmental and Biotechnology Centre (UBZ), Permoserstraße 15, 04318 Leipzig, Germany. Tel.: +49 341 235 1015; fax: +49 341 235 1830.
E-mail addresses: marc.breulmann@ufz.de (M. Breulmann), vninp@kursknet.ru (N.P. Masyutenko), kogutbm@gmail.com (B.M. Kogut), schroll@helmholtz-muenchen.de (R. Schroll), doerfler@helmholtz-muenchen.de (U. Dörfler), francois.buscot@ufz.de (F. Buscot), elke.schulz@ufz.de (E. Schulz).

http://dx.doi.org/10.1016/j.scitotenv.2014.07.080
0048-9697/© 2014 Elsevier B.V. All rights reserved.

OC mineralization
Incubation experiment
Bioavailability
PLFA microbial community composition

CO_2-release was used as an index of substrate accessibility and linked to the soil microbial community structure, as determined by phospholipid fatty acids (PLFA) analysis.
Several key factors controlling decomposition processes, and thus the potential bioavailability of OC, were identified: management intensity and the plant community composition of the grasslands (both of which affect the chemical composition and turnover of OC) and specific properties of individual SOM fractions. The PLFA patterns highlighted differences in the composition of microbial communities associated with the examined grasslands, and SOM fractions, providing the first broad insights into their active microbial communities. From observed interactions between abiotic and biotic factors affecting the decomposition of SOM fractions we demonstrate that increasing management intensity could enhance the potential bioavailability of OC, not only in the active and intermediate SOM pools, but also in the passive pool.
© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Soil organic matter (SOM) dynamics are often described using conceptual models, since the quality, stability and availability of organic carbon (OC) in SOM can vary widely in differently managed ecosystems (Cambardella and Elliott, 1993; Schulz, 2004). These models separate the SOM into different pools based on intrinsic properties, such as microbial decomposability, stability and turnover times (Amundson, 2001; Denef et al., 2009). Clearly, it is essential to identify and characterise distinct pools of SOM to elucidate OC dynamics thoroughly (von Lützow et al., 2007). Thus, several fractionation approaches have been developed for isolating SOM fractions without substantially changing their ecologically relevant properties (Christensen, 2001; von Lützow et al., 2008). Combining fractionation methods based on particle-size and specific density is a particularly promising approach. The decomposition of SOM depends on interactions between SOM fractions and their location in the soil matrix, which provides spatially heterogeneous habitats for soil microorganisms (Sessitsch et al., 2001). Unravelling these processes is complicated by the wide range of kinetic properties that govern decomposition rates of organic compounds in soil (Davidson and Grieve, 2006). Various approaches have been used to characterise SOM, but often the relationships between SOM characteristics and decomposability have only been inferred (Denef et al., 2009). Few studies have directly examined links between SOM fractions' characteristics and microbial utilisation to evaluate their potential biological decomposability (Davinic et al., 2012). However, the bioavailability of SOM associated with different particle size fractions has been studied in respiration experiments (Oorts et al., 2006; Schutter and Dick, 2002; Semenov et al., 2010), and several studies have analysed enzyme activities in soil and DNA extracts from soil aggregates (Kandeler et al., 2000; Sessitsch et al., 2001). In addition, long-term incubations of whole soil samples followed by density fractionation have indicated that the properties and abundance of light fractions are key determinants of microbial activity and associated soil respiration (Alvarez et al., 1998; Alvarez and Alvarez, 2000; Swanston et al., 2002).

Generally, the quantity and quality of SOM are largely determined by plant community composition and land management, whereas mineralisation is mainly microbially mediated. Microbial community composition and microbial mineralisation activity have been shown to change in response to changes in organic matter (OM) (Bardgett, 2005), and when OM is added to soil mineralisation can be temporarily either accelerated or retarded, depending on the characteristics. It has also been shown that addition of fresh OM to soils, and the associated increase in nutrient availability, may stimulate the formation of a copiotrophic microbial community dominated by bacteria with high growth rates (e.g. Gram-negative bacteria) under resource-rich conditions (Fierer et al., 2003; Kramer and Gleixner, 2008). In contrast, decreasing substrate quality and the availability of easily accessible C and other nutrients may increase the relative abundance of oligotrophic community members (e.g. Gram-positive bacteria) (Bastian et al., 2009; Fierer et al., 2007). Changes in resource quality may therefore alter the relative abundance of different microbial groups in a predictable manner, with potentially important consequences for the metabolic capabilities of the soil microbial community.

Recently, analytical and experimental approaches have also shown that SOM stability is not solely determined by its molecular structure; instead, the availability of SOM to soil microorganisms is controlled by complex interactions between environmental and biological factors (Schmidt et al., 2011). However, no published study to our knowledge has combined analyses of bioavailability of the OC of size–density fractions and soil microbial communities, although simultaneous analysis of the abiotic and biotic components of SOM could provide valuable insights into interactions between properties of specific organic substrates, the OC in SOM fractions, and the diverse members of soil microbial communities. Thus, in the presented study we examined the potential bioavailability of the OC in SOM fractions of different particle size (clay-sized particles) and density (light fractions LF1, <1.8 g cm^{-3}; LF2, 1.8 – 2.0 g cm^{-3}) isolated from samples of soils from four grassland ecosystems in Germany and Russia with contrasting soils, climatic conditions and management intensities. Fractionated SOM fractions were also used as carbon sources for microbial utilisation in a respiration experiment. To estimate the bioavailability of OC in the fractions we linked their chemical characteristics to biological properties by using CO_2 release as an index of substrate accessibility.

We hypothesised that: (1) the bioavailability of OC in SOM fractions depends on the degree of stabilisation, (2) the quality of decomposing SOM fractions affects the structure and activity of soil microbial communities, and (3) the availability of OC modulates the abundances of functional microbial groups, which in turn control the decomposition processes.

2. Materials & methods

2.1. Study sites

The selected ecosystems were grasslands with varying management intensity on two soil types: Haplic Cambisol in Germany and Typical Chernozem in Russia. The German sampling sites were located in extensively-managed semi-natural grasslands of the Thuringian and Franconian Forests, at similar altitudes on a plateau-like mountain range in Central Germany (26.56′N 24.29′E, 606 m a.s.l., and 24.31′N 27.33′E, 633 m a.s.l., respectively). The sites, included in the BIOLOG-Europe programme, differed in plant biomass production, plant community composition and litter quality, being classified as high productivity (HPG) and low productivity (LPG), respectively (Breulmann et al., 2012). Higher abundances of grasses and legumes determined the plant community composition in HPG compared to LPG (Breulmann et al., 2012). 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). Average annual precipitation ranges from 980 to 1200 mm, with a summer maximum, and mean annual air temperature is +6.1 °C. The soils are weakly stagnic Haplic Cambisols (siltic) (FAO, 2006), a carbonate-free soil type that developed from parent rocks of schist and greywacke, with an initial soil pH (KCl) of 4.4 (Kahmen et al., 2005).

The Russian sampling sites (pasture and prairie) are parts of a long-term field experiment, conducted by the All-Russia Research Institute of Arable Farming and Soil Erosion Control in Russia, in the Central Chernozemic State Biosphere Reserve (51°57′N 36°09′E), 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 ca. +5.3 °C. Average annual precipitation amounts to 570 mm with drought periods every 3–5 years. The soils are classified as typical Chernozems (FAO, 2006) 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^{-1}, whilst the prairie sites are ungrazed and in their natural state. The plant community composition of the prairie is dominated by grasses; for a further description, see Barré et al. (2010).

2.2. Soil sampling and elemental analysis

Pooled soil samples ($n = 100$) were taken with an auger (1 cm diameter, 30 cm length) from each of the German high and low productivity sites from the top 30 cm in 2008. Soil samples from the Russian pasture and prairie sites were collected from the top 25 cm (plough layer) in 2003. About 10 single soil samples were taken, which were later pooled. All soil samples were air-dried and sieved to <2 mm. Visible plant residues and stones were removed by hand and the soil was stored in closed plastic cups at room temperature (20 °C) until analysis. Total organic carbon (TOC) and total nitrogen (TN) contents of the bulk soil samples, and the isolated SOM fractions (see below) were determined by dry combustion of triplicate sub-samples using a Vario El III C/N analyser (Elementar, Hanau, Germany). As the carbonate concentration of the soils was negligible, the total measured C concentration was considered to represent TOC.

2.3. Size–density fractionation: aim and method description

Size–density fractionation was carried out following the protocol described by Shaymukhametov (1985), as modified by Schulz et al. (2011), designed to separate SOM associated with clay sized particles (<2 μm) from SOM with specific densities less than 2 g cm^{-3}. Before fractionation, undecomposed, free particulate organic matter (POM, <1 g cm^{-3}), such as plant and root residues, was removed from four 20 g air-dried soil samples by gentle flotation in 50 ml of deionised water using a glass rod. This free POM constituted less than 1% of TOC and was not further considered. The quality of the size–density fractions was assessed by the TOC content.

The size–density fractionation consisted of two main steps. In the first step, the soil was dispersed to obtain primary clay particles. All clay-associated SOM was released from a soil–water suspension, 1/3 (*w*/*v*) ratio, by applying 15 1-minute bursts of low ultrasonic energy (44 J ml^{-1}) using a UPS 200 ultrasonic processor with an S7 ultrasonic tip (Dr. Hielscher GmbH). After each ultrasonic burst, the suspension was centrifuged (3 min at 112 *g*) to separate the clay fraction, which was collected through decantation of the supernatant and further divided into two clay sub-fractions (CF1, <1 μm and CF2, 1–2 μm) by centrifugation for 10 min at 3584 *g* for CF1, and 3 min at 2016 *g* for CF2.

The two clay fractions released after disruption of the aggregates represent the total clay-associated SOM fraction of the bulk soil sample (Schulz et al., 2011). Within this fraction the OC is protected from decomposition by strong interaction to the mineral phase; thus, these fractions represent a passive pool. The clay fraction was subdivided into two sub-fractions because of the higher proportion of OC associated with finer clay particles and the older age of coarser clay particles (von Lützow et al., 2007). Within each clay fraction, OC is protected from decomposition by strong interactions with the mineral phase.

(2) In the second step, the remaining solid phase after centrifugation (silicates, sand, and POM of varying degrees of degradation that was previously either free or occluded in aggregates) was used to separate SOM of specific densities of less than 2 g cm^{-3} with relatively high lignin contents (Gregorich et al., 2006) and C/N ratios. Two light fractions (LF: LF1, <1.8 g cm^{-3} and LF2, 1.8–2.0 g cm^{-3}) were separated by liquid–solid partitioning using a bromoform/ethanol mixture. The light fractions represent an active, readily available pool (LF1) and an intermediary pool (LF2) that have no, or limited, interactions with clay minerals (Demyan et al., 2012; Schulz et al., 2011). Previous TEM-based analysis using an element-specific sensor showed that the two light fractions were not affected by their contact with the partitioning mixture (data not shown).

The isolated fractions were dried at 60 °C in a water bath and ball-milled in preparation for further analysis. The remaining fractionation residue was almost free of OC (<0.01%) and was not further considered.

2.4. Bioavailability of OC in the SOM fractions

The isolated SOM fractions from each of the four sites were used as OC sources in a laboratory incubation experiment. For each SOM fraction, five replicate mixtures of SOM fraction and 5 g of a soil (Albic Luvisol; FAO (2006) were prepared. The soil was characterised as having low TOC and TN contents (Table 1) and served as a carrier material in the experiment. The air-dried carrier material was rewetted with water to 60% of its maximum water-holding capacity and pre-incubated for eight days in order to establish a microbial community. Then amounts of the isolated SOM fractions (CF1, CF2, LF1 or LF2) containing 12.5 mg C were added and thoroughly mixed with the carrier material. Five control mixtures without SOM were also prepared, giving a total of 85 experimental units (two German sites × two Russian sites × four SOM fractions × five replicates + five controls). (See Tables 2 and 3.)

The prepared mixtures were pressed into small (3.85 cm^3) stainless steel tubes to obtain a standardised soil density of 1.3 g cm^{-3} across all treatments (Schroll et al., 2006). The steel tubes were then incubated in polyethylene vessels in a Respicond V automatic respirometer (Nordgren Innovations AB, Sweden; Nordgren (1988) at a constant temperature of 22 °C for 10 days. A short decomposition time of 10 days was chosen because of the small amounts of SOM used in the experiment. Measurements of CO_2-evolution from the SOM mixtures were taken hourly by determining changes in electrical conductivity induced by absorption of CO_2 in a KOH solution. To quantify the CO_2 produced during

Table 1
General description of the total organic carbon (TOC) and nitrogen (TN) concentrations in the Albic Luvisol (FAO, 2006) used as a carrier material in the incubation experiment, the bulk soils and the SOM fractions (CF1, CF2, LF1, LF2) from low and high productivity grasslands (LPG, HPG) in Germany and from pasture and prairie grasslands in Russia. Values are arithmetic means ± SE.

		TOC (%)	TN (%)	C:N ratio	C (% of SOC)	Fractionation C recovery (%)
Carrier material		0.4	0.0	13.3	–	–
LPG	Bulk soil	4.2	0.4	12.1	–	–
	CF1	9.0	1.0	9.4	31.7	97.6
	CF2	8.3	0.8	10.9	25.0	
	LF1	31.6	1.8	17.4	33.8	
	LF2	8.5	0.6	13.7	7.1	
HPG	Bulk soil	3.6	0.3	11.8	–	–
	CF1	8.1	0.9	9.3	26.4	97.8
	CF2	7.7	0.7	11.3	20.3	
	LF1	37.3	2.0	19.1	46.7	
	LF2	6.3	0.3	19.3	4.4	
Prairie	Bulk soil	5.4	0.5	11.7	–	–
	CF1	9.3	1.0	9.7	38.5	93.7
	CF2	11.7	1.0	11.2	25.0	
	LF1	33.2	2.2	15.2	27.6	
	LF2	7.8	0.7	11.7	2.6	
Pasture	Bulk soil	5.0	0.5	11.2	–	–
	CF1	8.5	0.9	9.4	34.8	90.3
	CF2	11.1	1.0	10.8	18.2	
	LF1	31.6	2.3	13.0	29.3	
	LF2	8.2	0.1	10.6	8.0	

LPG: low productivity grassland; HPG: high productivity grassland.

Table 2
Mean values of a first order exponential model of the total mineralised carbon (C_0) in SOM fractions, and their degradation rate constants (k), mean residence times (MRT) and half-lives ($t_{1/2}$), of soil from low productivity (LPG) and high productivity (HPG) grassland, and prairie and pasture grassland.

		C_0 (% of initial C)	k (year^{-1})	MRT (years)	$t_{1/2}$ (years)
LPG	CF1	1.46	0.51	1.96	1.36
	CF2	1.91	0.73	1.37	0.95
	LF1	5.40	2.19	0.46	0.32
	LF2	1.06	2.19	0.46	0.32
HPG	CF1	2.28	0.73	1.37	0.95
	CF2	0.57	0.22	4.55	3.15
	LF1	2.88	1.46	0.68	0.47
	LF2	0.46	0.22	4.55	3.15
Prairie	CF1	0.84	0.29	3.45	2.39
	CF2	0.39	0.15	6.67	4.62
	LF1	0.52	0.15	6.67	4.62
	LF2	0.36	0.15	6.67	4.62
Pasture	CF1	1.35	0.44	2.27	1.58
	CF2	0.90	0.29	3.44	2.39
	LF1	16.62	7.30	0.14	0.09
	LF2	0.68	0.22	4.55	3.15

decomposition of the SOM fractions, the CO_2 originating from the carrier material was subtracted from the total CO_2 released and the remainder was used to express respired carbon as the C loss (% of initial C). The results were not corrected for microbial resynthesis of CO_2 and thus only account for net mineralisation.

The data were fitted to an exponential decay model according to the equation $C_{pot.\ mineralised} = C_0\,(1 - e^{-kt})$, where C_0 is the amount of mineralisable C, t is the incubation time, and k is the first order mineralisation rate constant. Mean residential times were calculated from the turnover rates. The mean residence times (MRT) and half-life are equal to 1/k, and 0.693/k, respectively.

2.5. Phospholipid fatty acid analysis

Fatty acids (FA) derived from phospholipids (PLFA) were extracted according to Bligh and Dyer (1959). Triplicate samples (2 g) were taken for PLFA analysis and stored at −20 °C until analysis. The PLFAs were extracted from the samples in a chloroform, methanol and citrate buffer, loaded onto a silica-bonded solid phase extraction column (SPE-SI; Bond Elute, Varian, Palo Alto, USA) and separated into neutral lipids, glycolipids and phospholipids by sequential elution with chloroform, acetone and methanol. The extracted PLFAs were hydrolysed and methylated using a methanolic KOH solution then identified using a 5890 series II gas chromatograph coupled to a 5971A mass selective detector (Hewlett Packard) and quantified using MSD ChemStation D.01.02.16 chromatography software (Agilent Technologies, United States). Methyl nonadecanoate (19:0) was used as an internal standard. The relative abundance of specific microbial groups was quantified using PLFA markers as follows: fungi (18:2ω6), *Actinobacteria* (10Me16:0, 10Me17:0 and 10Me18:0) and bacteria (i15:0, a15:0, 15:0, i17:0, a17:0, cy 17:0, 17:0 and cy 19:0) (Frostegård et al., 1993; Zelles, 1999). The fatty acids i15:0, a15:0, i16:0, i17:0 and a17:0 were considered specific to Gram-positive bacteria (GP), whilst fatty acids cy17:0 and cy19:0 were considered specific to Gram-negative bacteria (GN). The ratio of fungal to total bacterial biomass (F:B ratio) was used as an index of the relative abundance of fungi and bacteria in the sampled soils (Bardgett and McAlister, 1999). The ratio of total saturated to total unsaturated PLFAs (SAT:UNSAT ratio) was used as an indicator of nutritional stress in bacterial communities and nutrient availability. Finally, the GP:GN ratio was used as an indicator of the relative C availability (Fanin et al., 2014).

Table 3
Relative abundances of specific microbial groups and indices of microbial community structure (as indicated by phospholipid fatty acid measurements) associated with decomposing SOM fractions (CF1, CF2, LF1, LF2) from: a) low productivity (LPG) and high productivity grasslands (HGP), and b) prairie and pasture grasslands. Values are arithmetic means ± SE. Below: summary of results of a two-way ANOVA, testing the effects of *Site* and *SOM fraction* on microbial parameters. Bold values indicate significant effects ($^{***}p \le 0.001$, $^{**}p \le 0.01$, $^{*}p \le 0.05$). Letters indicate statistically homogeneous subgroups of SOM fractions (Tukey's HSD, $p \le 0.05$).

		PLFA indicator groups (%)					Microbial community indices			
		Gram-positive	Gram-negative	Actinobacteria	Total bacteria	Fungi	Total PLFA nmol g^{-1} soil	F:B ratio	GP:GN ratio	SAT:UNSAT ratio
a)										
LPG	CF1	12.4 ± 0.6^{bc}	14.0 ± 0.4^{bc}	15.7 ± 0.4^{ac}	27.9 ± 1.3^{abc}	3.4 ± 0.2^{a}	4.0 ± 0.5^{ab}	0.13 ± 0.03^{bc}	0.63 ± 0.03^{bc}	1.03 ± 0.07^{bc}
	CF2	11.0 ± 0.8^{c}	17.3 ± 1.3^{a}	17.9 ± 1.7^{ab}	28.6 ± 1.4^{ab}	3.8 ± 0.2^{d}	1.9 ± 0.3^{c}	0.14 ± 0.02^{c}	0.50 ± 0.03^{c}	0.92 ± 0.04^{c}
	LF1	11.4 ± 0.5^{c}	14.8 ± 0.1^{ab}	18.0 ± 0.3^{ab}	26.9 ± 0.7^{bc}	3.3 ± 0.0^{a}	1.7 ± 0.1^{c}	0.12 ± 0.00^{abc}	0.57 ± 0.02^{c}	1.20 ± 0.05^{ab}
	LF2	14.1 ± 0.6^{bc}	11.4 ± 0.2^{c}	15.7 ± 0.5^{ac}	26.4 ± 0.4^{bc}	3.0 ± 0.2^{ac}	2.8 ± 0.5^{bc}	0.11 ± 0.01^{abc}	0.81 ± 0.03^{ab}	1.30 ± 0.02^{a}
HPG	CF1	17.7 ± 0.6^{a}	12.6 ± 0.2^{bc}	13.0 ± 0.6^{c}	31.5 ± 0.2^{a}	2.5 ± 0.1^{bc}	4.9 ± 0.5^{a}	0.08 ± 0.01^{a}	0.88 ± 0.03^{a}	1.12 ± 0.03^{abc}
	CF2	15.0 ± 0.1^{ab}	11.2 ± 0.2^{c}	11.6 ± 0.8^{b}	27.6 ± 0.9^{abc}	4.3 ± 0.3^{a}	4.4 ± 0.4^{ab}	0.15 ± 0.05^{abc}	0.85 ± 0.04^{a}	1.13 ± 0.06^{abc}
	LF1	11.5 ± 0.5^{bc}	13.6 ± 2.0^{bc}	17.1 ± 0.3^{ab}	25.4 ± 0.6^{bc}	2.8 ± 0.1^{ac}	1.6 ± 0.2^{c}	0.11 ± 0.01^{abc}	0.63 ± 0.12^{ab}	1.18 ± 0.08^{abc}
	LF2	9.2 ± 0.7^{d}	25.6 ± 1.2^{bc}	68.0 ± 5.1^{ab}	64.2 ± 4.1^{c}	2.2 ± 0.1^{b}	1.3 ± 0.1^{c}	0.14 ± 0.04^{ab}	0.70 ± 0.17^{c}	1.16 ± 0.07^{abc}
Two-way ANOVA *F* $^{p\text{-}value}$	Sites	$\mathbf{10.59}^{**}$	$\mathbf{27.78}^{***}$	$\mathbf{37.34}^{***}$	0.04 ns	$\mathbf{54.47}^{***}$	2.63 ns	$\mathbf{19.33}^{**}$	$\mathbf{30.07}^{***}$	0.47 ns
	SOM fractions	$\mathbf{9.25}^{**}$	$\mathbf{5.38}^{*}$	$\mathbf{11.15}^{**}$	$\mathbf{10.02}^{***}$	$\mathbf{14.78}^{***}$	$\mathbf{22.49}^{***}$	$\mathbf{3.77}^{*}$	2.79 ns	$\mathbf{5.91}^{**}$
	Sites × SOM fractions	$\mathbf{21.37}^{***}$	$\mathbf{15.07}^{***}$	$\mathbf{16.59}^{**}$	$\mathbf{5.19}^{*}$	$\mathbf{5.41}^{*}$	$\mathbf{9.48}^{***}$	$\mathbf{4.52}^{*}$	$\mathbf{22.81}^{***}$	$\mathbf{4.53}^{*}$
b)										
Prairie	CF1	12.4 ± 1.5^{e}	12.7 ± 0.5^{abc}	17.5 ± 0.9^{ab}	25.5 ± 1.0^{c}	2.7 ± 0.7^{a}	2.9 ± 0.5^{abc}	0.11 ± 0.03^{a}	0.68 ± 0.07^{d}	1.07 ± 0.05^{c}
	CF2	13.8 ± 0.2^{cd}	12.8 ± 0.1^{ab}	18.4 ± 0.3^{a}	26.8 ± 0.2^{bc}	2.2 ± 0.0^{a}	2.5 ± 0.1^{bc}	0.08 ± 0.00^{a}	0.73 ± 0.01^{cd}	1.19 ± 0.01^{bc}
	LF1	12.0 ± 0.5^{de}	13.1 ± 0.1^{a}	18.4 ± 0.3^{a}	25.1 ± 0.3^{c}	2.1 ± 0.1^{a}	1.8 ± 0.2^{c}	0.08 ± 0.00^{a}	0.65 ± 0.03^{d}	1.18 ± 0.03^{bc}
	LF2	16.5 ± 0.6^{ab}	10.9 ± 0.1^{d}	16.0 ± 0.1^{ab}	28.1 ± 0.6^{bc}	2.8 ± 0.2^{a}	3.5 ± 0.1^{ab}	0.10 ± 0.10^{a}	0.93 ± 0.02^{ab}	1.26 ± 0.02^{b}
Pasture	CF1	20.4 ± 1.5^{f}	10.9 ± 0.1^{d}	16.5 ± 0.2^{ab}	32.2 ± 1.3^{a}	2.3 ± 0.7^{a}	4.2 ± 0.4^{a}	0.07 ± 0.02^{a}	1.05 ± 0.04^{e}	1.28 ± 0.01^{ab}
	CF2	17.8 ± 0.1^{a}	11.4 ± 0.6^{bcd}	16.5 ± 0.8^{ab}	30.2 ± 0.8^{ab}	2.9 ± 0.6^{a}	3.4 ± 0.1^{abc}	0.10 ± 0.02^{a}	0.94 ± 0.03^{a}	1.29 ± 0.05^{ab}
	LF1	14.2 ± 1.1^{abc}	10.9 ± 0.4^{d}	15.5 ± 0.8^{ab}	26.1 ± 1.2^{bc}	3.5 ± 0.3^{a}	3.9 ± 0.6^{ab}	0.13 ± 0.02^{a}	0.84 ± 0.03^{ab}	1.41 ± 0.01^{a}
	LF2	14.5 ± 0.5^{bc}	11.2 ± 0.3^{cd}	15.3 ± 0.8^{b}	26.7 ± 0.9^{bc}	5.4 ± 0.6^{b}	4.3 ± 0.3^{a}	0.20 ± 0.02^{b}	0.83 ± 0.02^{bc}	1.29 ± 0.02^{ab}
Two-way ANOVA *F* $^{p\text{-}value}$	Sites	$\mathbf{103.24}^{***}$	$\mathbf{30.23}^{***}$	$\mathbf{14.80}^{**}$	$\mathbf{16.57}^{***}$	$\mathbf{30.83}^{***}$	$\mathbf{30.02}^{***}$	$\mathbf{13.51}^{***}$	$\mathbf{121.61}^{***}$	$\mathbf{45.02}^{***}$
	SOM fractions	$\mathbf{9.91}^{**}$	$\mathbf{4.36}^{*}$	$\mathbf{5.46}^{*}$	$\mathbf{6.02}^{**}$	$\mathbf{8.25}^{**}$	$\mathbf{4.57}^{*}$	$\mathbf{4.22}^{*}$	$\mathbf{11.00}^{***}$	$\mathbf{5.94}^{**}$
	Sites × SOM fractions	$\mathbf{50.86}^{***}$	$\mathbf{5.86}^{**}$	**0.62 ns**	$\mathbf{8.28}^{**}$	$\mathbf{7.83}^{**}$	**1.77 ns**	$\mathbf{6.27}^{**}$	$\mathbf{51.64}^{***}$	$\mathbf{5.42}^{**}$

LPG: low productivity grassland; HPG: high productivity grassland; F:B ratio: fungal:bacterial ratio; GP:GN ratio; Gram-positive bacteria:Gram-negative bacteria ratio; SAT:UNSAT: total saturated to total unsaturated PLFAs.

2.6. Statistical analyses

Data were analysed in R (version 3.0.2; R Core Team (2013). Distributions of data were tested for normality and homogeneity, and are presented as arithmetic means ± standard errors (SE). Effects were regarded as significant if $p \leq 0.05$. The effects of sample site and SOM fraction on microbial parameters were evaluated by two-way analysis of variance (ANOVA) and Tukey's HSD. Where the assumptions of the model were not fulfilled, Box–Cox transformation was applied. The PLFA profiles were compared and analysed by principal component analysis (PCA), following log-transformation of the PLFA data to satisfy the assumptions of normality and variance homogeneity.

Within the analysis only sites within one location were compared.

3. Results

3.1. Mass and carbon distributions amongst SOM fractions

Carbon and mass balances of all isolated SOM fractions indicated mean recovery rates of approximately 95% (Table 1). The highest levels of carbon were found in the clay-associated fractions (CF1 and CF2). Across all four grassland sites, 28–47% of OC was stored in the LF1 fraction, and only 3–8% in the LF2 fraction (Table 1).

3.2. Mineralisation of SOM fractions

The cumulative C loss during the decomposition experiment was plotted as a function of incubation time for each of the four isolated SOM fractions from low productivity (LPG) and high productivity (HPG) grasslands in Germany (Fig. 1) and from prairie and pasture grasslands in Russia (Fig. 2). For all analysed samples, R^2 was greater than 0.79. The total mineralised carbon in each SOM fraction, their degradation rate constants, mean residence times and half-lives are listed in Table 2.

Differences in C loss were observed both between SOM fractions and between sites after 10 day incubation. Amongst German grasslands CF1 samples, total cumulative C loss due to respiration was higher from the HPG than from the LPG samples (2.28% and 1.46% of initial C, respectively; Fig. 1a). Amongst CF1 samples of Russian sites, the respiration was significantly higher in pasture samples than in prairie samples (1.35 and 0.84% of initial C, respectively) (Fig. 2a, Table 2). Respiration of CF2 resulted in a lower cumulative C loss from HPG (0.57% of initial C) and a significant higher C loss from LPG samples (1.91% of initial C; Fig. 1b) than from corresponding CF1 samples. However, C losses from CF2 isolated from both prairie and grasslands were lower than those from corresponding CF1 samples (0.39% and 0.90% of initial C, respectively; Fig. 2b).

The SOM in LF1 incubations started to decompose within 24 h. Amongst German grassland samples, C loss was generally higher from LPG (5.40% of initial C), than from HPG samples (2.88% of initial C; Fig. 1c). Amongst Russian samples, C losses were much smaller in LFI prairie samples than in pasture LFI samples (0.52% and 16.62% of initial C, Fig. 2c).

Carbon losses from LF2 fractions were higher in LPG than in HPG samples (1.06 and 0.46% of initial C), and higher in pasture than in prairie samples (0.68 and 0.46% of initial C, respectively) (Figs. 1d and 2d).

As shown in Table 2, the *k* and MRT values obtained from the incubations of CF1 samples from the LPG site were 0.51 $year^{-1}$ and ca. two years, respectively. Corresponding values for incubations of CF1 from the HPG site were higher (0.73 $year^{-1}$) and longer (ca. five years), respectively. The *k* values for CF2, LF1 and LF2 incubations were clearly

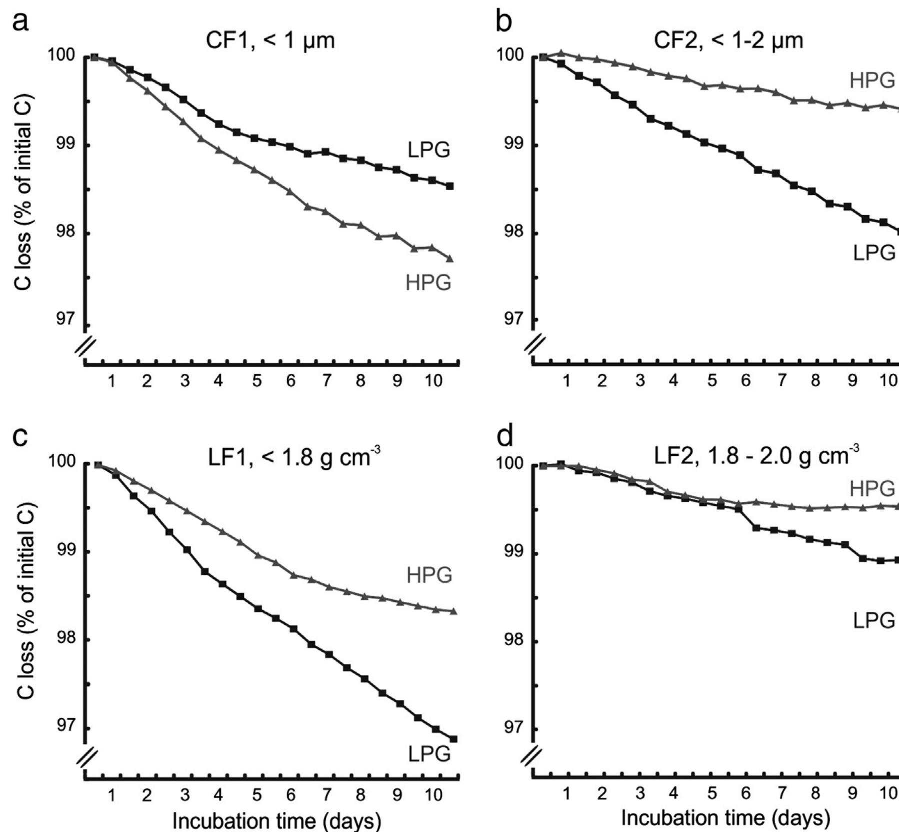


Fig. 1. Cumulative C loss in the laboratory respiration experiment as a function of incubation time for four isolated SOM fractions of low productivity (LPG) and high productivity (HPG) grassland soils in Germany. For all analysed samples R^2 was >0.88. For clarity, only every twenty-fourth measured data point (average of five replicates) is shown. a) CF1: clay fraction 1 with a particle size of <1 μm; b) CF2: clay fraction 2 with a particle size of. 1–2 μm; c) LF1. Light fraction 1 with a specific density of <1.8 g cm^{-3} and d) LF2: light fraction 2 with a specific density of 1.8–2.0 g cm^{-3}.

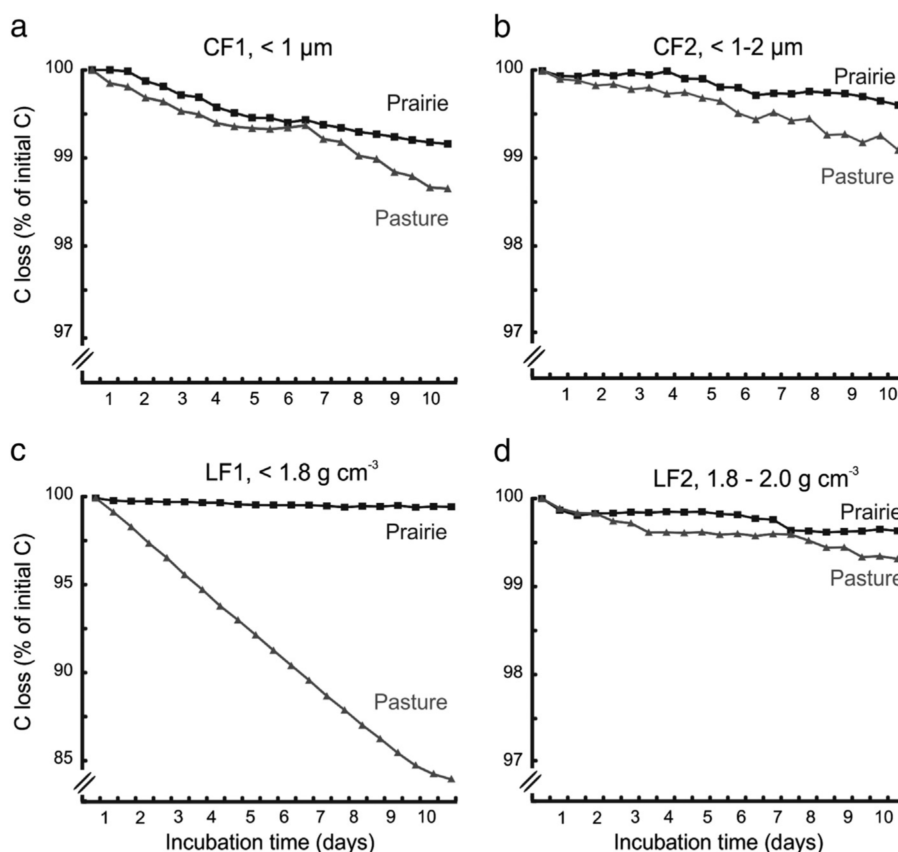


Fig. 2. Cumulative C loss in the laboratory respiration experiment as a function of incubation time for four isolated SOM fractions of prairie and pasture grassland soils in Russia. For all analysed samples R^2 was >0.79. For clarity, only every twenty-fourth measured data point (average of five replicates) is shown. a) CF1: clay fraction 1 with a particle size of <1 μm; b) CF2: clay fraction 2 with a particle size of 1–2 μm; c) LF1. Light fraction 1 with a specific density of <1.8 g cm^{-3} and d) LF2: light fraction 2 with a specific density of 1.8–2.0 g cm^{-3}.

higher for LPG samples than for HPG samples, and the MRT ranged between 0.5 and 1.5 years. The calculated k values for incubations of all prairie fractions tended to be lower than values for corresponding pasture fractions (0.2–0.3 versus 0.2–7.3 $year^{-1}$), and thus the MRT values were higher (3.5–6.7 versus 0.1–4.6 years).

3.3. Microbial community structure

At the end of the incubation period, PLFA markers were identified in the mixtures of SOM fractions and carrier soil. The relative abundance of most PLFA markers varied significantly between sites and SOM fractions (Table 3a & b). Accordingly, PCA of the PLFA markers clearly showed that microbial community structure varied between SOM fractions and grassland ecosystems (Fig. 3a & b). For the LPG site, significant associations were found between CF1 and fungi, between LF1 and *Actinobacteria*, and between LF2 and the SAT:UNSAT ratio. For the HPG site, strong associations were found between GP and the two clay fractions (CF1 and CF2) (Fig. 3a). The CF2 and LF1 of the prairie site were clearly associated with *Actinobacteria*. For the pasture site, CF1 and CF2 were associated with the total abundance of bacteria, GP and the GP:GN ratio (Fig. 3b).

Analyses of the relative abundance of specific microbial groups and calculated indices of the microbial community structure in each of the SOM fractions isolated from the sampled grasslands revealed the following variations in assemblages (Table 3a & b).

3.3.1. Clay fraction 1

In the LPG soil GN, fungi and *Actinobacteria* were highly abundant and (presumably) largely responsible for utilisation of the OC sources. In contrast, a higher abundance of bacteria, and GP in particular, were found in this fraction of HPG soil, resulting in significantly higher F:B (LPG, 0.6; HPG, 0.9) and GP:GN ratios (LPG, 0.63; HPG, 0.88).

Similar trends were found for CF1 of the Russian sites, but the differences between the prairie and pasture sites were more pronounced, e.g. GP:GN ratios of 0.7 and 1.1, respectively.

3.3.2. Clay fraction 2

All detected PLFA markers were less abundant in the CF2 fraction of LPG soil than in the same fraction of HPG soil. However, at both sites GN were major constituents of the microbial communities (LPG, 17.3%; HPG, 11.2%). The differences between the two sites were particularly large for total abundance of PLFA (LPG, 1.9 nmol g^{-1} soil; HPG, 4.4 nmol g^{-1} soil), fungi (LPG, 3.8%; HPG, 4.3%) and GP:GN ratio (LPG, 0.5; HPG, 0.9).

Actinobacteria were major constituents (18.4%) of the microbial community in this fraction of prairie soil, and the pasture samples had significantly higher total abundance of PLFAs, significantly higher quantities of PLFAs indicative of GP and a significantly higher GP:GN ratio.

3.3.3. Light fraction 1

Gram-negative bacteria and *Actinobacteria* were more proportionally abundant in this fraction of LPG samples (14.8% and 18.0%, respectively) than in HPG samples (13.6% and 15.5%, respectively). Amounts of all other markers were similar at both sites. The microbial community of the prairie was significantly smaller than that of the pasture (1.8 nmol g^{-1} soil and 3.9 nmol g^{-1} soil, respectively).

The prairie samples had a higher proportion of *Actinobacteria* than the pasture soil (prairie, 18.4%, pasture, 15.5%), but significant lower

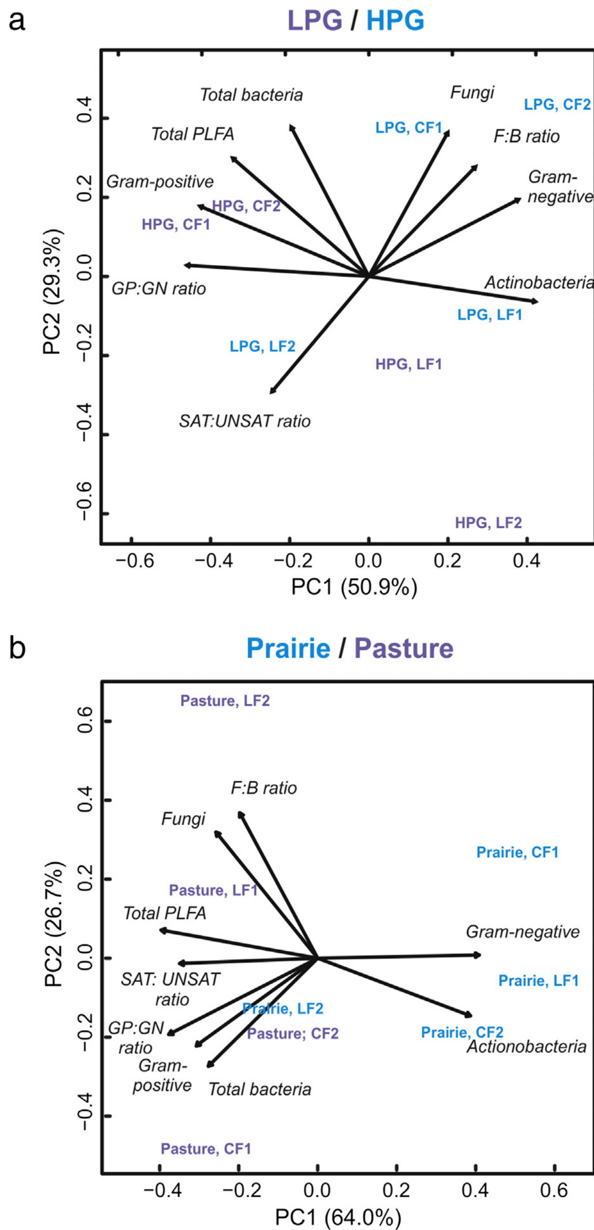


Fig. 3. Principal components analysis biplots visualising differences in the relative abundance of specific microbial groups and indices of the microbial community structure (indicated by phospholipid fatty acids, PLFA; black arrows) between different grassland sites, a) low productivity (LPG) and high productivity grasslands (HGP) and b) prairie and pasture grasslands.

GP:GN (prairie, 0.65, pasture, 0.84) and SAT:UNSAT ratios (prairie, 1.18; pasture, 1.41).

3.3.4. Light fraction 2

In this fraction of LPG soil, the GP:GN ratio (0.81) and SAT:UNSAT ratio (1.3) were significantly higher than in HPG soil (0.70 and 1.16, respectively). The microbial community in HPG samples was dominated by *Actinobacteria* (68.0%) and GN (25.6%), but these groups were not as abundant in the LPG soil (15.7% and 11.4%, respectively).

At the Russian sites, high proportions of bacterial PLFA markers were detected in this fraction of prairie soil. Furthermore, after incubation, the fungal abundance and the F:B ratio were significantly higher in soil from the pasture (5.4% and 0.2, respectively) than in soil from the prairie (2.8% and 0.1, respectively).

4. Discussion

4.1. Microbial utilisation and community structure of SOM fractions isolated by particle size (clay fractions)

Clay minerals play an important role in soil organic carbon sequestration, due to the high association (50–75%) of OC with clay-sized particles (<2 μm) and their high specific densities (>2 g cm^{-3}; heavy fractions) (Kaiser and Kalbitz, 2012; von Lützow et al., 2007). The formation of very stable, clay-associated OM can also be promoted by abiotic aggregation, and the bioavailability of clay-associated OM depends on its accessibility to soil microorganisms. Thus, CO_2-release reportedly increases following destruction of such aggregates (Franzluebbers, 1999), and in the presented study the soil was disaggregated and the accessibility of previously in the clay fractions protected OM was increased.

In incubations of the smaller clay fraction (CF1), higher respiration rates, and significantly lower mean residence times, were detected in samples from the fertile and more productive ecosystems (i.e. HPG and pasture sites). The higher respiration was associated with higher microbial biomass, which is consistent with the high specific surface area of clay (Coleman et al., 2004). Small-sized clay fractions can protect bacteria from predation by protozoa by providing abundant protective microhabitats (England et al., 1993). High abundances of bacteria (e.g. GP) and low F:B ratios were found in the CF1 of both productive sites (HPG and pasture). Bardgett and McAlister (1999) have shown that fungal biomass and the F:B ratio are often significantly lower in intensively managed grasslands than in unmanaged or lightly managed grasslands (see also Breulmann et al., 2012). Here, we show that this is also the case for clay-associated SOM fractions.

However, the respiration rate was significantly higher in LPG than HPG samples of the coarser clay fraction, indicating that the association between OC and the mineral surface was more strongly disrupted by the destruction of aggregates in CF2 than in CF1. In contrast, OC tends to associate much more strongly with the mineral surfaces in natural, unmanaged ecosystems like the prairie, thereby increasing its stability. For the Russian grasslands, similar results for the composition of the microbial community were found for the coarser clay fraction compared to the German samples, but the slightly lower C respiration rate in the prairie CF2 fraction resulted in MRTs of about seven years. The high respiration rates in CF2 from LPG compared to HPG were also associated with lower microbial biomass, GP abundance and GP:GN ratios. This shift in the microbial community structure is indicative of a copiotrophic community, which can form when nutrient availability increases. Gram-negative bacteria preferentially mineralise easily-degradable OC (Kramer and Gleixner, 2008), whereas GP bacteria (as indicators for oligotrophic microorganisms) favour nutrient-poor conditions. The quality of the OC entering the soil, a key controlling factor for below-ground transformation processes, primarily depends on plant community composition and land management. Breulmann et al. (2012) have shown that C turnover is slow in low-productivity, fungal-dominated grasslands and that high inputs of root litter in LPG increase the activity of soil enzymes involved in cellulose degradation. Furthermore the authors have demonstrated that complementary effects between grasses and legumes have been reported to result in significantly higher above-ground biomass production in HPG. These results suggest that the degraded OM likely consists of low molecular weight compounds.

Clay fractions have well-known stabilising effects on SOM (Schulz et al., 2011; von Lützow et al., 2008), but the high respiration rates observed in this study suggest that accessibility of the OC to soil microbes is substantially increased by disaggregation of the soil under optimal conditions. Thus, the results provide better understanding of the quality of the OC stabilised within specific fractions. Clay minerals can clearly promote microbial growth by providing appropriate surfaces and maintaining pH within an optimal range (Filip, 1973; Six et al., 2006). However, our data suggest that differences in clay mineralogy, site specific abiotic factors like temperature and precipitation, as well as land use

and plant community composition, may also be important determinants of the soil microbial community structure and associated utilisation of OC sources, in accordance with previous findings (Breulmann et al., 2012; Sollins et al., 2006). It has been shown, that a higher proportion of legume species with a higher litter quality in HPG and a greater species richness of small and tall herbs in LPG were responsible for different decomposition rates. This in turn has important consequences for soil biota and the processes (e.g. aggregation) they influence. Furthermore, soil management strongly affected the importance of the stabilisation processes in both clay fractions. This is consistent with expectations as land management intensity (e.g. prairie versus pasture) and plant community composition (e.g. LPG versus HPG) are likely to affect diverse soil properties, including effective cation exchange capacity, specific or reactive surface area, and mineral charge. All of these properties have significant effects on the chemical composition and turnover of OC in clay fractions (Schöning et al., 2005; Wattel-Koekkoek et al., 2003).

Our results emphasise that increasing management intensity enhances the bioavailability of OC, not only in the active and intermediate SOM pools, but also in the passive pool, represented by the clay-associated fractions. This relationship was evident in the results from the Russian SOM fractions, but was not as clear in the data from the German soils. In our experiment, because of the lower respiration, only the OC of the smaller-sized clay fraction of LPG formed a stable, clay-associated complex. This indicates that the OC after physical destruction of soil aggregates is not as bioavailable as previously thought in HPG. Breulmann et al. (2012) concluded that the relatively high F:B ratio and fungal content of LPG soils, together with the higher abundance of slow-growing plants with slower nutrient cycling in LPG, indicate the importance of this type of grassland ecosystem for SOC accumulation.

4.2. Microbial utilisation and community structure of SOM fractions isolated by specific density (light fractions)

SOM fractions of specific densities < 1.8 g cm^{-3} (LF1) generally have significantly faster turnover rates than those associated with clay minerals (Schulz et al., 2011; Swanston et al., 2002). In an experiment involving long-term incubation of bulk soil samples followed by isolation of LF and heavy (clay) fractions, Alvarez et al. (1998) found that the light fraction lost approximately five times more C than the heavy fraction. Our results are consistent with these findings and indicate that OC in LF1 is readily available for microorganisms (although its availability is also dependent on its chemical composition); thus, OC losses by respiration are significantly higher in this fraction than in heavier fractions. The conclusion that the degraded OM of LPG largely consists of low molecular weight compounds is corroborated by the high observed respiration rates, low MRT (six months) and low GP:GN ratios (characteristic of high nutrient availability). For LF1 of Russian soils, the effects of the two management types on the respiration of OC differed much more, with significantly more OC being respired in soil from the pasture. These results are supported by findings of Klumpp et al. (2009), that intensified grazing alters plant rooting and the soil microbial community and promotes decomposition and soil C losses, presumably by affecting the chemical composition of LF1. The microbial community in LF1 of prairie soil was dominated by *Actinobacteria* (which have well-known ability to slowly mineralise relatively stable OC substrates; (Potthast et al., 2010) and characterised by an extremely low respiration rate and high MRT of about seven years. These results indicate that the plant litter (above- and below-ground) and root exudates entering prairie soils have a specific composition that has the ability to significantly reduce its microbial mineralization. Furthermore, less OC was respired in LF2 (1.8–2.0 g cm^{-3}), than in LF1 samples, indicating that the LF2 fraction was more stable, since the microbial community was less able to utilise it as an OC source. In addition, the higher GP:GN ratio in LF2 than in LF1 of LPG soil indicates that OC is more stable in LF2, in accordance with previous results of isotopic and spectroscopic analyses (Demyan et al., 2012; Schulz et al., 2011).

5. Conclusions

The potential bioavailability of SOM fractions is highly important for predicting changes in ecosystem services of soils in response to climate change, and for assessing trace gas emissions and the potential of soils for carbon sequestration. By studying abiotic and biotic parameters during the decomposition of SOM fractions, we found interactions between the properties of individual SOM fractions and the diverse members of the soil microbial communities. Management intensity and plant community composition of the grasslands, which differed significantly, were identified as the key factors controlling the decomposition processes, and thus the potential bioavailability of SOM fractions, by affecting the chemical composition and OC turnover. Specific properties of SOM fractions, such as the degree of stabilisation, further influenced the OC transformation process.

Increasing management intensity enhances the bioavailability of OC in the active, intermediate and passive SOM pools. The SOC accumulation potential of a grassland ecosystem depends on the relative abundance of fungi (fungal to bacterial biomass, F:B ratio), the growth rates of the plants and the associated rates of nutrient cycling. In our respiration experiment, strong association was found only between OC and the smaller-sized clay fraction. Although it is difficult to extrapolate to natural ecosystems, we propose that the bioavailability of OC increases when soil aggregates are destroyed. The PLFA patterns demonstrated the dependence of the active microbial community's structure on the specific SOM fraction available.

Our results highlight the importance of considering the relationship between microbial community structure and degradation of OC in various SOM fractions for understanding the functional significance of microbial communities in C cycling.

Acknowledgments

This 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 under the BIOLOG-Europe programme, coordinated by Prof. W.W. Weisser and Ilka Egerer. We thank Prof. H. Knicker and Prof. B. Michalzik for valuable comments on the manuscript. We are also grateful to Jacqueline Rose and Gabriele Henning for technical assistance.

References

Alvarez R, Alvarez CR. Soil organic matter pools and their associations with carbon mineralization kinetics. Soil Sci Soc Am J 2000;64:184–9.

Alvarez CR, Alvarez R, Grigera S, Lavado RS. Associations between organic matter fractions and the active soil microbial biomass. Soil Biol Biochem 1998;30:767–73.

Amundson R. The carbon budget in soils. Annu Rev Earth Planet Sci 2001;29:535–62.

Bardgett RD. The biology of soil: a community and ecosystem approach. Oxford: Oxford University Press; 2005.

Bardgett RD, McAlister E. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biol Fertil Soils 1999;29:282–90.

Barré P, Eglin T, Christensen BT, Ciais P, Houot S, Kätterer T, et al. Quantifying and isolating stable soil organic carbon using long-term bare fallow experiments. Biogeosciences 2010;7:3839–50.

Bastian F, Bouziri L, Nicolardot B, Ranjard L. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biol Biochem 2009;41:262–75.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.

Breulmann M, Schulz E, Weißhuhn K, Buscot F. Impact of the plant community composition on labile soil organic carbon, soil microbial activity and community structure in semi-natural grassland ecosystems of different productivity. Plant and Soil 2012;352:253–65.

Cambardella CA, Elliott ET. Methods for physical separation and characterization of soil organic-matter fractions. Geoderma 1993;56:449–57.

Christensen BT. Physical fractionation of soil and structural and functional complexity in organic matter turnover. Eur J Soil Sci 2001;52:345–53.

Coleman DC, Crossley DAJ, Hendrix PF. Fundamentals in soil ecology. San Diego: Elsevier Academic Press; 2004.
Davidson DA, Grieve IC. Relationships between biodiversity and soil structure and function: evidence from laboratory and field experiments. Appl Soil Ecol 2006;33:176–85.
Davinic M, Fultz LM, Acosta-Martinez V, Calderón FJ, Cox SB, Dowd SE, et al. Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. Soil Biol Biochem 2012;46:63–72.
Demyan MS, Rasche F, Schulz E, Breulmann M, Müller T, Cadisch G. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. Eur J Soil Sci 2012;63:189–99.
Denef K, Plante AF, Six J. Characterization of soil organic matter. In: Kutsch WL, Bahn M, Heinemeyer A, editors. Soil carbon dynamics: an integrated methodology. Cambridge: Cambridge University Press; 2009. p. 91–126.
England LS, Lee H, Trevors JT. Bacterial survival in soil: effect of clays and protozoa. Soil Biol Biochem 1993;25:525–31.
Fanin N, Hattenschwiler S, Fromin N. Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil. Plant and Soil 2014;379:79–91.
FAOWorld reference base for soil resources—a framework for international classification, correlation and communication, vol. 103. Rome: Food and Agricultural Organization of the United Nations; 2006.
Fierer N, Schimel JP, Holden PA. Variations in microbial community composition through two soil depth profiles. Soil Biol Biochem 2003;35:167–76.
Fierer N, Bradford MA, Jackson RB. Toward an ecological classification of soil bacteria. Ecology 2007;88:1354–64.
Filip Z. Clay minerals as a factor influencing the biochemical activity of soil microorganisms. Folia Microbiol 1973;18:56–74.
Franzluebbers AJ. Potential C, and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biol Biochem 1999;31:1083–90.
Frostegård A, Bååth E, Tunlid A. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty-acid analysis. Soil Biol Biochem 1993;25:723–30.
Gregorich EG, Beare MH, McKim UF, Skjemstad JO. Chemical and biological characteristics of physically uncomplexed organic matter. Soil Sci Soc Am J 2006;70:975–85.
Kahmen A, Perner J, Audorff V, Weisser W, Buchmann N. Effects of plant diversity, community composition and environmental parameters on productivity in montane European grasslands. Oecologia 2005;142:606–15.
Kaiser K, Kalbitz K. Cycling downwards—dissolved organic matter in soils. Soil Biol Biochem 2012;52:29–32.
Kandeler E, Tscherko D, Bruce KD, Stemmer M, Hobbs PJ, Bardgett RD, et al. Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. Biol Fertil Soils 2000;32:390–400.
Klumpp K, Fontaine S, Attard E, Roux XL, Gleixner G, Soussana J-F. Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. J Ecol 2009;97:876–85.
Kramer C, Gleixner G. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. Soil Biol Biochem 2008; 40:425–33.
Nordgren A. Apparatus for the continuous, long-term monitoring of soil respiration rate in large numbers of samples. Soil Biol Biochem 1988;20:955–7.
Oorts K, Nicolardot B, Merckx R, Richard G, Boizard H. C and N mineralization of undisrupted and disrupted soil from different structural zones of conventional tillage and no-tillage systems in northern France. Soil Biol Biochem 2006;38:2576–86.
Potthast K, Hamer U, Makeschin F. Impact of litter quality on mineralization processes in managed and abandoned pasture soils in Southern Ecuador. Soil Biol Biochem 2010; 42:56–64.
R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013. URL http://www.R-project.org/.
Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, et al. Persistence of soil organic matter as an ecosystem property. Nature 2011;478:49–56.
Schöning I, Knicker H, Kögel-Knabner I. Intimate association between O/N-alkyl carbon and iron oxides in clay fractions of forest soils. Org Geochem 2005;36:1378–90.
Schroll R, Becher HH, Dörfler U, Gayler S, Grundmann S, Hartmann HP, et al. Quantifying the effect of soil moisture on the aerobic microbial mineralization of selected pesticides in different soils. Environ Sci Technol 2006;40:3305–12.
Schulz E. Influence of site conditions and management on different soil organic matter. Arch Agron Soil Sci 2004;50:33–47.
Schulz E, Breulmann M, Boettger T, Wang KR, Neue HU. Effect of organic matter input on functional pools of soil organic carbon in a long-term double rice crop experiment in China. Eur J Soil Sci 2011;62:134–43.
Schutter ME, Dick RP. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil published as Paper No. 11590 of the Oregon Agric. Exp. Stn., Oregon State Univ., Corvallis, OR. Soil Sci Soc Am J 2002;66:142–53.
Semenov VM, Ivannikova LA, Semenova NA, Khodzhaeva AK, Udal'tsov SN. Organic matter mineralization in different soil aggregate fractions. Eurasian Soil Sci 2010;43:141–8.
Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Appl Environ Microbiol 2001;67:4215–24.
Shaymukhametov MS. Use of physical fractionation methods to characterize soil organic matter. Eurasian Soil Sci 1985;25:70–88.
Six J, Frey SD, Thiet RK, Batten KM. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 2006;70:555–69.
Sollins P, Swanston C, Kleber M, Filley T, Kramer M, Crow S, et al. Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. Soil Biol Biochem 2006;38:3313–24.
Swanston CW, Caldwell BA, Homann PS, Ganio L, Sollins P. Carbon dynamics during a long-term incubation of separate and recombined density fractions from seven forest soils. Soil Biol Biochem 2002;34:1121–30.
von Lützow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, et al. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol Biochem 2007;39:2183–207.
von Lützow M, Kögel-Knabner I, Ludwig B, Matzner E, Flessa H, Ekschmitt K, et al. Stabilization mechanisms of organic matter in four temperate soils: development and application of a conceptual model. J Plant Nutr Soil Sci 2008;171:111–24.
Wattel-Koekkoek EJW, Buurman P, van der Plicht J, Wattel E, van Breemen N. Mean residence time of soil organic matter associated with kaolinite and smectite. Eur J Soil Sci 2003;54:269–78.
Zelles L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol Fertil Soils 1999;29:111–29.