Biol Fertil Soils (2015) 51:427–442
DOI 10.1007/s00374-014-0987-7

ORIGINAL PAPER

Clay mineral composition modifies decomposition and sequestration of organic carbon and nitrogen in fine soil fractions

Cordula Vogel · Katja Heister · Franz Buegger · Irina Tanuwidjaja · Stephan Haug · Michael Schloter · Ingrid Kögel-Knabner

Received: 10 September 2014 / Revised: 14 December 2014 / Accepted: 16 December 2014 / Published online: 9 January 2015

Abstract The interaction between minerals and organic matter (OM) is a key to the turnover of OM in soils. In particular, clay minerals, iron oxides and charcoal are considered as important constituents affecting the sequestration of carbon (C) and nitrogen (N). Here, we incubated pre-produced artificial soils (842 days) and a natural soil (Ap, Luvisol) with ^{13}C- and ^{15}N-labelled plant litter over 63 days to follow OM turnover and the formation of organo-mineral associations regarding different compositions (montmorillonite (MT), illite (IL), montmorillonite + charcoal (MT+CH), illite + ferrihydrite (IL+FH)). The microbial biomass, salt extractable organic C, the isotopic C and N composition in the bulk soil and the soil fractions (combined density and particle size fractionation) were determined. By comparison of the artificial soils with the natural soil, we were able to show that the produced soil-like systems have OM dynamics comparable to the natural soil. We found out that the decomposition of the added plant litter was affected by the type of clay mineral that formed the artificial soils, as the soil MT exhibited a slower mineralisation compared to IL, which was in line with a lower microbial biomass. Although a high specific surface area (SSA) provides a high sequestration capacity for C and N, smaller amounts were sequestered in the MT soil with a higher SSA compared to the soil IL. We suppose that a more intensive decomposition is associated with a higher microbial biomass and thus leads to higher amounts of microbial products sequestered in the clay-sized fraction. Charcoal and ferrihydrite had no additional effect in this experiment.

Keywords Clay minerals · Turnover · Organo-mineral associations · Incubation · Stable isotopes · Artificial soils

Electronic supplementary material The online version of this article (doi:10.1007/s00374-014-0987-7) contains supplementary material, which is available to authorized users.

C. Vogel (✉) · K. Heister · I. Kögel-Knabner
Lehrstuhl für Bodenkunde, Department Ecology and Ecosystem Management, Center of Life and Food Sciences Weihenstephan, Technische Universität München, 85350 Freising-Weihenstephan, Germany
e-mail: cordula.vogel@wzw.tum.de

F. Buegger
Institute of Soil Ecology, Research Center for Environmental Health, Helmholtz Zentrum München (GmbH), 85764 Neuherberg, Germany

I. Tanuwidjaja · M. Schloter
Research Unit of Environmental Genomics, Research Center for Environmental Health, Helmholtz Zentrum München (GmbH), 85764 Neuherberg, Germany

S. Haug
TUM|Stat, Lehrstuhl für Mathematische Statistik, Technische Universität München, 85748 Garching, Germany

I. Kögel-Knabner
Institute for Advanced Study, Technische Universität München, Lichtenbergstrasse 2a, 85748 Garching, Germany

Introduction

The decomposition of organic matter (OM) and the sequestration of organic carbon (C) and nitrogen (N) in soils received a high interest during the last decades due to its relevance for the global carbon cycle. The frequently discussed mechanisms involved in the protection of OM against decomposition in soils are the following: (i) selective preservation due to recalcitrance of OM, (ii) spatial inaccessibility of OM against decomposer organisms, and (iii) stabilisation by interaction with mineral surfaces (Sollins et al. 1996; von Lützow et al. 2006). In early studies, it was found that small mineral particles are important for the sequestration of residues during decomposition (Jenkinson 1977; Sørensen 1981). Sørensen

(1981) supposed that clay particles increase the content of OM in soils by forming organo-mineral associations which partly protect OM against biodegradation. The protection of OM against microbial degradation was often referred to the availability of mineral surfaces capable for adsorbing OM and the formation of a soil matrix influencing the substrate availability (Baldock and Skjemstad 2000; Oades 1984). Although the sequestration of C and N by small particles is well established, the effect of different minerals on the OM turnover and the formation of organo-mineral associations remain an open question.

Soil mineralogy has been shown to be important for the determination of the amount of organic C stored in soils and its turnover time (Barré et al. 2014; Torn et al. 1997). The interaction between small mineral particles and OM is likely affected by the soil mineralogy due to the different specific surface areas (SSAs) and the surface charge of the various minerals in soils (Feng et al. 2013; Six et al. 2002). Six et al. (2002) found out by regression analyses that soils dominated by 1:1 clay minerals (kaolinite) exhibited a lower sequestration of organic C compared to soils dominated by 2:1 clay minerals (illite, montmorillonite, vermiculite). On the contrary, Wattel-Koekkoek et al. (2001) did not find differences in organic C concentrations between soils containing 2:1 and 1:1 clay minerals. Saidy et al. (2012) revealed by analysing sand-clay mixtures supplemented with an OM solution that the organic C mineralisation was significantly affected by clay mineralogy, as they observed a higher organic C mineralisation in the presence of kaolinite than in the presence of illite or smectite. Bruun et al. (2010) concluded from analysing the basal soil respiration in tropical soils of different mineralogy that smectitic soils had a lower stabilising effect on soil organic C than allophanic and kaolinitic soils. Thus, our current knowledge about the effect of soil mineralogy on the storage of OM is based on limited and conflicting data. However, it is usually accepted that the soil OM sequestration by minerals decreases in the following order: allophane > smectite > illite > kaolinite (Bruun et al. 2010; von Lützow et al. 2006). But, direct studies on the effect of clay mineralogy on soil OM dynamics are rare (Feng et al. 2013). Investigations especially considering differences between montmorillonite and illite are even neglected, although these clay minerals provide different characteristics, e.g. cation exchange capacity (CEC) and SSA.

One difficulty in analysing the relation between mineralogy and OM stabilisation in natural soils is the association of specific mineral species with different climatic conditions, which alter the soil OM dynamics (Feng et al. 2013). Another impediment is the complexity of natural soil systems, which hampers finding a direct link between the clay mineralogy to OM characteristics and the comparability of natural soils with different clay mineralogy. Therefore, artificial soils provide an effective tool for studying the interaction between minerals, OM and microorganisms, as they serve as simplified systems of known composition and initial conditions (Babin et al. 2013; Guenet et al. 2011; Pronk et al. 2013). Pronk et al. (2012) produced artificial soils from well-known compounds under defined conditions during an incubation time of 18 months. The authors showed that macro-aggregates and organo-mineral associations were formed quickly (90 days) from clean model materials. Using these soils, Pronk et al. (2013) observed no differences neither in the mineralisation nor in the organic C quantity and quality between artificial soils with different mineralogical compositions after a single manure application and short development times of the artificial soils. Vogel et al. (2014a) found evidence for higher organic C contents in artificial soils containing montmorillonite compared to artificial soils formed from illite after an additional manure application and longer development time. Accordingly, when incubating pure minerals with an organic substrate, no effect of the different mineralogy on the organic C dynamics could be found (Pronk et al. 2013), but after additional OM input and a longer incubation time, a trend to higher organic C values in the artificial soils with montmorillonite became apparent (Vogel et al. 2014a). This raised the question whether the clay mineralogy affects the amount, composition and dynamics of soil OM in pre-produced artificial soils with additional OM input and longer development times.

A general problem in many studies analysing the effect of clay mineralogy on soil organic C and N dynamics is the fact that the mineralisation of added litter is not differentiated from the stabilisation of OM in the fine fractions. Thus, confounded effects of mineralogy on both processes are often observed and cannot be discussed independently. The combination of density and particle size fractionation with isotopic labelling allows to obtain a process-oriented understanding about the fate of litter-derived C and N through various soil fractions and the development of organo-mineral associations. In this study, we determined the fate of OM during fresh litter decomposition in a short-term incubation by following the labelled litter and the distribution of organic C and N in fine-sized fractions. By using pre-produced artificial soils with defined mineral compositions for an incubation experiment with ^{13}C- and ^{15}N-labelled plant litter in combination with a fractionation approach, we were able to differentiate the complex processes affecting C dynamics, i.e. OM mineralisation and concurrent processes, leading to stabilisation of organic C and N in the fine fractions.

The main objectives of our study were the following:

1. To study the decomposition of added labelled litter regarding the mineral composition in pre-incubated artificial soils
2. To investigate the sequestration of organic C and N dependent on the composition of the artificial soils

3. To assess the response pattern of the artificial soils concerning the decomposition of the freshly added litter material with regard to processes occurring in a natural soil

Material and methods

Material characteristics and experimental set-up

Four artificial soil compositions and a natural soil were used for our short-term incubation experiment. We used pre-incubated artificial soils, produced and described in detail by Vogel et al. (2014a), with compositions varying in the presence of illite, montmorillonite, ferrihydrite and charcoal. Detailed information on the model materials (Table S1) and the design of the artificial soils incubation were previously described by Pronk et al. (2012) and Vogel et al. (2014a). In the following, the different compositions are indicated by montmorillonite (MT), illite (IL), montmorillonite + charcoal (MT+CH) and illite + ferrihydrite (IL+FH). The artificial soils have been produced with a microbial inoculum derived from the Ap horizon of a Luvisol under agricultural use (Scheyern, Germany) and sterile manure under well-defined conditions. These artificial soils have been pre-incubated for 842 days with a second manure addition at day 562. All artificial soils have been treated in the same way. The artificial soils were restricted compared to those occurring in nature, and several factors important for soil formation and aggregation like wetting/drying and freezing/thawing cycles and the effect of plants and soil animals were excluded from this experiment to simplify the system. After 842 days, the soils were homogenised, sieved to <2 mm and subsequently incubated with labelled plant litter (Fig. 1). The artificial soils showed pH values between 7.2 and 7.6 after the pre-incubation of 842 days. Additionally, material from the uppermost 5 cm of a Luvisol Ap horizon (<2 mm) utilised to generate the microbial inoculum was used as natural soil material (Fig. 1). The Ap horizon was characterised by organic C contents of 16.0± 0.8 mg (g soil)$^{-1}$, N contents of 1.8±0.1 mg (g soil)$^{-1}$, a C/N

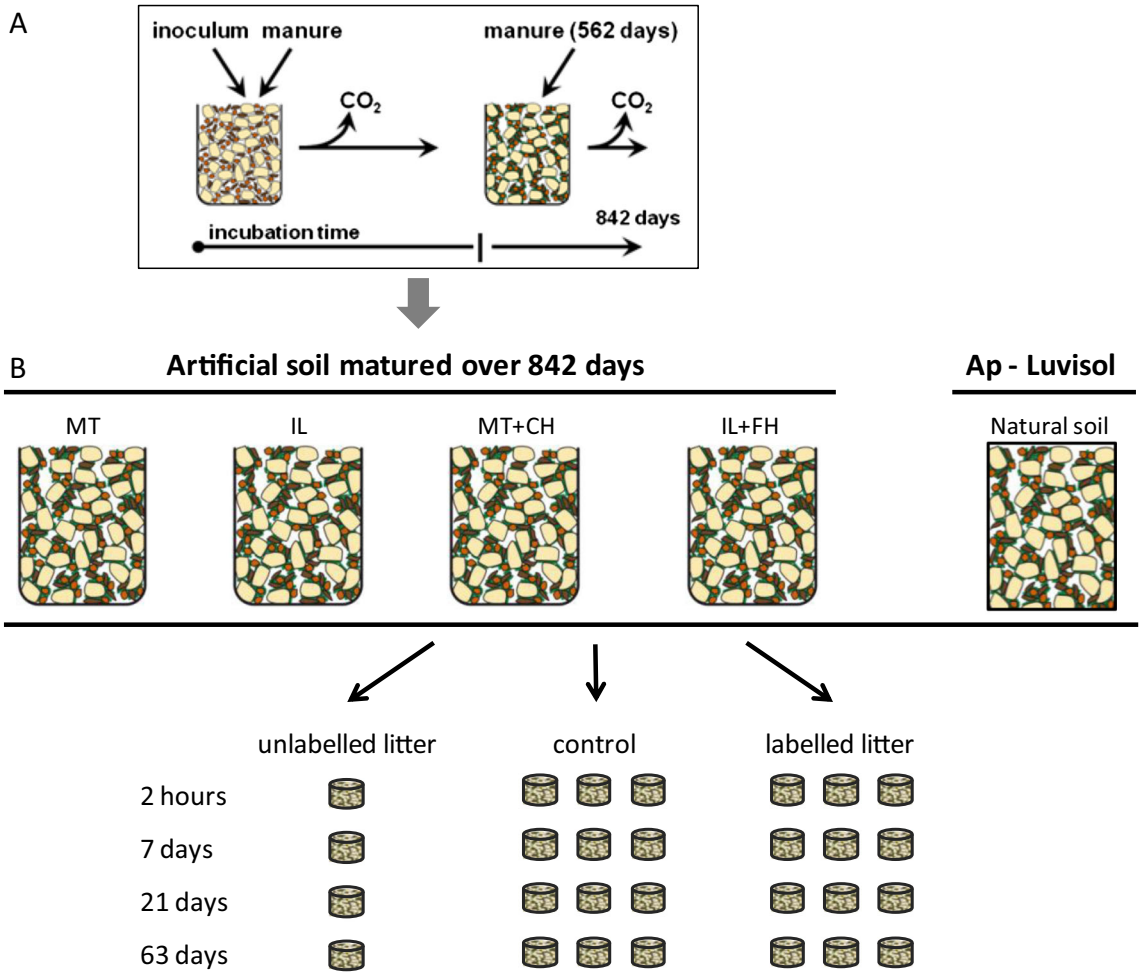

Fig. 1 Scheme of the experimental set-ups. **a** Development of artificial soils over 842 days (Vogel et al. 2014a). **b** Design of the experiment with litter addition. Four pre-incubated artificial soils with montmorillonite (*MT*), illite (*IL*), montmorillonite + charcoal (*MT+CH*), illite + ferrihydrite (*IL+FH*) and an Ap horizon of a Luvisol as a natural soil were used

ratio of 9.0±0.4 and a pH value ($CaCl_2$) of 6.6±0.1. The soil exhibited clay and silt contents of 16.3 and 58.7 %, respectively, and a sand content of 24.9 %. The Ap horizon showed an oxalate extractable iron content of 3.3±0.0 mg (g soil)$^{-1}$ and dithionite extractable iron content of 7.9±0.2 mg (g soil)$^{-1}$. The mineral composition of the clay fraction as revealed by X-ray diffraction (XRD) is dominated by illite, quartz, chlorite and mixed layer minerals. The litter material derived from a 1:1 mixture of maize (*Zea mays*) and potato (*Solanum tuberosum*) leaves and had an organic C content of 404.2±1.8 mg (g soil)$^{-1}$, an N content of 23.3±0.0 mg (g soil)$^{-1}$ and a C/N-ratio of 17.3±0.1. The plant litter material was labelled homogeneously. Therefore, plants were grown in a green house setting using tents made out of airtight transparent plastic (Esperschütz et al. 2011). Air recirculation was achieved using six fans, which were located in the tent corners and in the middle of the longitudinal sides of the tent. The CO_2 in the tent's atmosphere was subsequently replaced with enriched $^{13}CO_2$ (δ13C+170‰ V-PDB, Air Liquide, Düsseldorf, Germany). The CO_2 within the tent was maintained at 350 and 400 μmol mol^{-1} (monitored by a photoacoustic CO_2-controller, calibration at 300 to 600 μmol mol^{-1} ±2 %). Using this experimental set-up, an enriched ^{13}C-atmosphere of +140‰ V-PDB was established during the whole plant growth period. The ^{15}N label was introduced into the plant biomass by adding ^{15}N ammonium nitrate to the soil as fertiliser which corresponded to a fertiliser amount of 100 kg ha^{-1}, before plant growth started. The labelled plants were harvested before flowering after a total growth time of 6 to 8 weeks. The ^{15}N and ^{13}C concentrations of the labelled plant litter material were 6.1 atom-% and 4.9 atom-%. In addition, unlabelled litter material was used.

Three treatments were investigated in this study (Fig. 1): (i) soil with labelled litter, (ii) control treatments without litter addition, and (iii) a treatment with unlabelled litter was incubated to receive the natural abundance of ^{13}C and ^{15}N. For the experiment, we homogenised 35 g of the sieved soil (<2 mm) with 0.350-g litter (<200 μm). The control treatments were set up in the same way without litter supply. After litter addition, the soils were incubated for 63 days at 14 °C in the dark and 60 % maximum water-holding capacity. To maintain the water content, the soil columns were moistened in a 2-day interval using distilled water. Three replicated samples were taken at four time points, directly after litter addition (after 2 h), after 7, 21 and 63 days. Only for the natural abundance treatment, one column per time point was used. Samples were stored air-dried at room temperature.

Salt extractable organic C and microbial biomass

For the determination of the salt extractable organic C (SEOC), an aliquot of 5 g of fresh soil was shaken with 20 ml 0.01 M $CaCl_2$ for 45 min. Afterwards, the supernatant was filtered (4–7 μm, Whatman 595 ½, Bruchsal, Germany). SEOC was determined on a Total Carbon Analyzer (Shimadzu TOC 5050, Tokyo, Japan) by catalytic high-temperature oxidation. Microbial biomass (C_{mic}) was determined according to the fumigation-extraction method of Vance et al. (1987). An aliquot of 5 g of fresh soil was fumigated for 24 h in a chloroform atmosphere followed by an extraction as described above. C_{mic} was calculated as the difference between C_{total} in fumigated (fum) and non-fumigated (n-fum) samples using a k_{EC} value of 0.45 (Joergensen 1995).

Atom-% ^{13}C in the extracts was measured via online liquid chromatography coupled with stable isotope ratio mass spectrometry (MAT 253, Thermo Fisher Scientific, Dreieich, Germany) according to Krummen et al. (2004). The atom-% ^{13}C in microbial biomass (atom-% $^{13}C_{mic}$) was calculated as described by Marx et al. (Marx et al. 2007):

$$^{13}C_{mic}(\text{atom}-\%) = \left[\left(\text{atom}-\%^{13}C_{fum} \times C_{fum}\right) - \left(\text{atom}-\%^{13}C_{n-fum} \times C_{n-fum}\right)\right]/C_{mic} \quad (1)$$

where $^{13}C_{fum}$ and $^{13}C_{n\text{-}fum}$ are atom-% ^{13}C values of the fumigated and non-fumigated extracts, respectively, and C_{fum} and $C_{n\text{-}fum}$ are the respective C concentrations in milligrams per litre.

C and N analyses and isotopic measurements

For further analyses, the bulk soil was dried at 40 °C. Aliquots were ground and homogenised using a vibrating ball mill with zircon-grinding tools (Fritsch, Pulverisette 23, Idar-Oberstein, Germany). The samples were free of carbonates; thus, the total C concentration equals the organic C concentration. Determination of C, ^{13}C, N and ^{15}N was realised in duplicate, using an isotope ratio mass spectrometer (IRMS) (Delta V Advantage, Thermo Fisher Scientific, Dreieich, Germany) coupled with an elemental analyser (Euro EA, Eurovector, Milan, Italy). Appropriate standards were included for normalisation correction, instrument linearity and precision purposes (Werner and Brand 2001). Values of the ^{15}N and ^{13}C atom-% excess were calculated by subtracting the ^{13}C and ^{15}N enrichment of the respective unlabelled soil (treatments with unlabelled litter) from the enrichments obtained from the labelled soil. All calculated concentrations were determined from the atom-% excess values.

Physical soil fractionation

In order to remove all free-labelled litter and to isolate the organo-mineral associations, a combined density and particle

size fractionation was conducted. Approximately 10 g of dry soil material was saturated with 100 ml of sodium polytungstate solution with a density of 1.6 g cm^{-3} and allowed to settle over night. The floating particulate organic matter (POM) fraction was extracted using a water jet pump. The remaining slurry was ultrasonically dispersed (Bandelin, Sonopuls HD 2200, Berlin, Germany; VS 70 T Sonotrode Ø 13 mm with a liquid coverage of 1.5 cm) with an energy input of 200 J ml^{-1} to disrupt soil aggregates. To remove the Na polytungstate from the POM, the fractions were washed with deionised water on a pressure filtration unit. The remaining organo-mineral fraction was centrifuged several times with deionised water until an electrical conductivity of <50 μS cm^{-3} was reached. The mineral residues were wet-sieved at 63- and 20-μm mesh size to separate the sand- (>63 μm) and coarse silt-sized fraction (20–63 μm). The soil material <20 μm was separated into medium and fine silt (20–2 μm) as well as in clay (<2 μm)-sized fractions via sedimentation. All fractions were dried (40 °C) and weighed to obtain the mass proportion of each fraction to the bulk soil.

In particular, the POM and the smaller mineral fractions (medium and fine silt- and clay-sized fraction) were analysed in more detail for C, ^{13}C, N and ^{15}N as described above for the bulk soil samples. Values of the ^{13}C atom-% and ^{15}N atom-% excess were calculated by subtracting the natural abundance of the respective fractions of unlabelled soil (fractions of soil with unlabelled litter) from the enrichments obtained from the labelled fractions for each time step.

^{13}C CPMAS nuclear magnetic resonance spectroscopy

Clay fractions of each labelled treatment were analysed for their chemical composition by solid-state ^{13}C CPMAS NMR spectroscopy (Bruker DSX 200 NMR spectrometer, Rheinstetten, Germany) using the cross-polarisation magic angle spinning (CPMAS) technique. A resonance frequency of 50.33 MHz, a spinning frequency of 6.8 kHz and a contact time of 1 ms were used. The clay fractions obtained after 2 h and 63-day incubation were analysed from two independent replicates, additionally also the labelled litter and a clay fraction from the control treatments. Pulse delays of 0.8 s were used for all spectra. Depending on the C contents of samples, 505 scans for the litter and between 10,532 and 163, 545 scans for the clay fractions were accumulated. A line broadening of 50 Hz was applied. The ^{13}C chemical shifts were calibrated against tetramethylsilane (0 ppm). The relative contributions of the various C groups were determined by integration of the signal intensity in their respective chemical shift regions and were divided into four major chemical shift regions (0 to 45 ppm: alkyl-C, 45 to 110 ppm: O-alkyl-C, 110 to 160 ppm: aromatic C, 160 to 220 ppm: carboxyl-C).

Data presentation and statistical analysis

Several statistical models were used to analyse, if our treatments were depending on the soil composition. The models were fitted using the software environment R (2014). The other influence factor in our analysis was time. We mainly applied two approaches to model our measured parameters. In our first approach, we fitted a linear model to the parameters. Besides the soil type, we used time as a categorical covariate. For some of the parameters, we further included an interaction effect between soil type and time. Since the distribution of the residuals was not symmetric for all of the linear models under consideration, we had to transform some of the parameters to obtain the desired properties of the residuals. Hence, we applied a Box-Cox transformation to some of the parameters. In the most general case, our model fitted to the different data sets was the following:

$$Y = \beta_0 + \beta_1 \cdot D_{IL} + \cdots + \beta_4 \cdot D_{naturalsoil} + \beta_5 \cdot D_{t_7} + \cdots + \beta_7 \cdot D_{t_{63}} + \beta_8 \cdot D_{IL} \cdot D_{t_7} \cdots + \beta_{10} \cdot D_{IL} \cdot D_{t_{63}} + \cdots + \beta_{19} \cdot D_{naturalsoil} \cdot D_{t_{63}} + \varepsilon, \quad (2)$$

in which Y is either the parameter of interest itself or the Box-Cox transformed parameter, D_k is a dummy variable indicating the soil type k, $k \in \{IL, MT+CH, IL+FH, naturalsoil\}$, (MT) was chosen as reference category) and D_{t_j} is a dummy variable indicating the time level $j \in \{7, 21, 63\}$. To compare the influence of different soil types and time levels, we conducted a Tukey's test using the R package multcomp (Bretz 2011). In case the model included an interaction term, we compared different soil types within one time level and the other way round.

In a second approach, we used time as a continuous covariate. More precisely, we fitted the following time-dependent model to the different ^{13}C values, remaining in the bulk soils over incubation time under consideration

$$Y = c_{0,k} \cdot \left(1 - \exp\left(-c_{1,k} \cdot t\right)\right), \quad (3)$$

in which Y is the parameter of interest, $c_{i,k}$, $i=0,1$ are unknown coefficients depending on the soil type $k \in \{MT, IL, MT+CH, IL+FH, naturalsoil\}$ and t denotes time. The coefficients were estimated by non-linear least square optimisation using the function gnls from the R package nlme. In a post hoc test procedure, the influence of different soil types was analysed.

In a third approach, we tried to illustrate the differences between the different soil types and time levels taking simultaneously all the 12 recorded parameter values into account. A single observation is now given by 36 dimensional vectors consisting of the 12 parameter values for each of the three replications. The aim was to analyse the potential structure of

these data sets. We therefore applied the classical multidimensional scaling technique to the Euclidean distance matrix D computed from the data matrix X. Roughly speaking, the method computes an m -dimensional representation of our 36 -dimensional observations with m being smaller as 36, such that

$$\sum_{i=1}^{20}\sum_{j=1}^{20}\left(d_{ij}^{2}-\left(d_{ij}^{(m)}\right)^{2}\right) \quad (4)$$

is minimised, where d_{ij} is the Euclidean distance between the soil type time combinations i and j based on all 36 variables and $d_{ij}^{(m)}$ is the corresponding distance calculated from a m -dimensional representation. For more details, we refer to Everitt and Hothorn (2011). The larger the observed dissimilarity between two observations, the further apart the corresponding points in the m -dimensional model will be. Figures were prepared with SigmaPlot software (ver. 11.0, Systat Software, Erkrath, Germany).

Results

SEOC and microbial biomass (C_{mic})

For the artificial soil samples of the control treatments without litter addition, the SEOC concentrations were in the range of 119.6 to 146.0 μg (g soil)$^{-1}$ (Table S2), with slightly higher values in IL soils than in MT. SEOC concentrations in the natural soil were lower (21.5 ± 8.9 μg (g soil)$^{-1}$). The highest SEOC concentrations in all treatments where litter has been added were measured at the beginning of the incubation experiment (Table 1). The absolute highest values were found in soil samples derived from IL and MT+CH, whereas SEOC concentrations for MT and the natural soil were significantly lower at this sampling time point (Table 1). The SEOC concentrations declined over time. At the last sampling time point, concentrations were in the range of 14–31 % of the initial values. The SEOC-^{13}C concentrations followed the same trend (Fig. S1). The microbial biomass in the control treatments, without litter addition, ranged between 173.9 and

Table 1 Bulk properties of the artificial soils and the natural soil in the treatments with labelled litter

Bulk soil	Incubation time	MT				IL				MT+CH				IL+FH				Natural soil			
Organic C mg (g soil)$^{-1}$	2 h	18.7	±0.4	A	a	16.1	±0.6	A	b	30.4	±0.6	A	c	16.9	±0.6	A	b	19.6	±0.1	A	a
	7 days	16.6	±1.1	B	a	16.8	±0.3	A	a	29.5	±0.4	AB	b	16.2	±0.5	AB	a	18.7	±0.0	AB	c
	21 days	17.1	±1.0	B	a	14.2	±0.5	B	b	28.8	±1.1	B	c	15.6	±0.4	AB	ab	18.7	±0.5	AB	d
	63 days	16.7	±0.1	B	ad	15.3	±0.5	AB	ac	28.8	±0.4	B	b	15.1	±0.1	B	c	17.5	±0.1	B	d
N mg (g soil)$^{-1}$	2 h	1.8	±0.0	A	ac	1.6	±0.1	AB	bc	1.9	±0.1	A	a	1.7	±0.1	A	c	1.9	±0.0	A	a
	7 days	1.7	±0.1	B	a	1.7	±0.0	A	a	1.8	±0.0	A	ab	1.7	±0.0	A	a	1.9	±0.0	A	b
	21 days	1.8	±0.1	AB	ac	1.5	±0.0	B	b	1.8	±0.1	A	a	1.7	±0.0	A	a	1.9	±0.0	A	c
	63 days	1.8	±0.0	AB	a	1.6	±0.1	AB	b	1.8	±0.0	A	ac	1.7	±0.0	A	ab	1.9	±0.0	A	c
C/N-ratio	2 h	10.2	±0.0	A	a	10.0	±0.0	A	ac	16.3	±0.4	A	b	9.7	±0.1	A	c	10.1	±0.0	A	ac
	7 days	9.8	±0.1	B	a	9.9	±0.1	A	a	16.2	±0.1	A	b	9.3	±0.1	B	c	9.9	±0.1	A	a
	21 days	9.5	±0.1	BC	ac	9.5	±0.0	B	ac	16.4	±0.2	A	b	9.3	±0.1	B	a	9.8	±0.1	A	c
	63 days	9.5	±0.0	C	ad	9.5	±0.1	B	a	16.1	±0.1	A	b	9.1	±0.1	B	c	9.2	±0.1	B	cd
SEOC μg (g soil)$^{-1}$	2 h	520.7	±8.5	A	a	725.3	±36.8	A	b	736.0	±47.1	A	b	624.8	±7.9	A	c	164.6	±3.1	A	d
	7 days	312.4	±59.7	B	a	303.2	±25.3	B	a	226.7	±8.9	B	b	284.6	±39.1	B	ab	48.8	±2.7	B	c
	21 days	201.2	±12.2	C	a	225.8	±34.8	C	a	161.1	±3.2	BC	a	189.3	±13.5	C	a	20.3	±3.5	B	b
	63 days	161.1	±23.5	C	ab	213.5	±7.4	C	a	127.9	±16.7	C	b	189.5	±14.0	C	ab	23.8	±7.1	B	c
^{13}C μg (g soil)$^{-1}$	2 h	120.1	±15.3	A	a	142.1	±8.1	A	ab	146.8	±12.4	A	b	131.4	±12.2	A	ab	145.2	±8.6	A	b
	7 days	119.9	±15.4	A	a	108.0	±14.6	B	ab	95.4	±7.6	B	b	117.2	±3.3	A	ab	109.2	±4.7	B	ab
	21 days	98.5	±5.7	AB	ab	76.2	±9.5	C	a	83.6	±3.4	BC	ab	84.8	±4.3	B	ab	100.4	±6.2	B	b
	63 days	80.9	±5.0	B	a	60.5	±3.7	C	a	71.9	±3.6	C	a	68.6	±2.3	B	a	68.2	±1.2	C	a
^{15}N μg (g soil)$^{-1}$	2 h	12.6	±0.7	A	a	13.9	±0.9	A	a	14.5	±0.5	A	a	13.9	±0.8	A	a	14.7	±0.7	A	a
	7 days	13.2	±1.7	A	ab	12.5	±1.0	A	ab	11.4	±1.1	B	a	14.1	±0.1	A	b	12.1	±0.8	B	ab
	21 days	13.9	±0.8	A	a	10.3	±1.2	B	b	11.0	±0.3	B	b	12.9	±0.6	A	a	13.0	±1.2	AB	a
	63 days	13.7	±0.2	A	a	10.2	±0.1	B	b	12.7	±0.5	AB	a	12.2	±0.2	A	ab	11.8	±0.6	B	ab

The determined organic C, N, SEOC, ^{13}C and ^{15}N concentrations as well as C/N ratios of the artificial soils and the natural soil at the different incubation times. Values are shown as mean with standard deviation of three independent replicates. Capital letters point to significant differences between time points (n=3; p<0.05). Lowercase letters point to significant differences between soils at this time point (n=3; p<0.05)

342.1 μg (g soil)$^{-1}$ (Fig. 2), whereas the highest values were measured in the natural soil (Fig. 2, n=12, p<0.01). Concentrations in the control treatment did not differ significantly in the artificial soils (Fig. 2, n=12, p<0.01). Due to the litter addition, a two to four times increase in microbial biomass was observed in all samples because of the litter addition reaching concentrations of 598.3 to 818.4 μg (g soil)$^{-1}$ (Fig. 2) and thereby accounted for 2 to 5 % of the total organic C in the respective soils. The amounts of microbial biomass were significantly affected by the different soil mixtures (Fig. 2). Highest concentrations were found in soil samples from IL, which were significantly different to the artificial soils MT and IL+FH (Fig. 2, n=12, p<0.01), where the lowest values were measured. Interestingly, the litter application did not increase the microbial biomass in the natural soil to the same extent as in the artificial soils. The ^{13}C label in the microbial biomass decreased in all artificial soils over the incubation time (Fig. S2a) from 15 to 3 %. For the natural soil, higher values were only found at the early incubation phase (Table 1; Fig. S2b).

Decomposition of added labelled litter

The amounts of organic C in the different soil mixtures varied between 14.2 and 30.4 mg (g soil)$^{-1}$ and decreased slightly during the incubation in all soils. After 63 days, 13 % of the initial organic C was mineralised in the natural soil. In the artificial soils MT, IL, MT+CH and IL+FH, 10, 11, 9 and 14 % of the initial organic C were mineralised. Overall highest organic C values were found in the artificial soils containing charcoal. The total N content did not differ between the various soils (1.5 to 1.9 mg (g soil)$^{-1}$) and was not affected by the incubation time of the respective soil (Table 1). The litter decomposition with simultaneous mineralisation of organic C and storage of N was also reflected by decreasing C/N ratios in all soils over the incubation time (Table 1), except for the soil MT+CH.

The added labelled litter was rapidly mineralised in all soils, which is indicated by a much faster decrease of the ^{13}C, which remained in the bulk soil compared to the total organic C (Table 1) and followed an exponential decay over the incubation time in all soils (Fig. 3). A significant decrease in the ^{13}C residues was found between the first and second time sampling time point mainly for the artificial soils IL, also for MT+CH as well as the natural soil (n=3; p<0.001). In contrast, the artificial soil MT showed a significant difference in ^{13}C concentration only after 63 days, compared to the first time step (n=3; p<0.001). Overall, after 63 days, 60 % of the initial ^{13}C was mineralised in the artificial soil IL, whereas in the artificial soil MT, only 47 % was mineralised. For the other soils after 63 days of incubation, 53, 55 and 55 % of the initial ^{13}C were mineralised in the artificial soils MT+CH, IL+FH and in the natural soil, respectively.

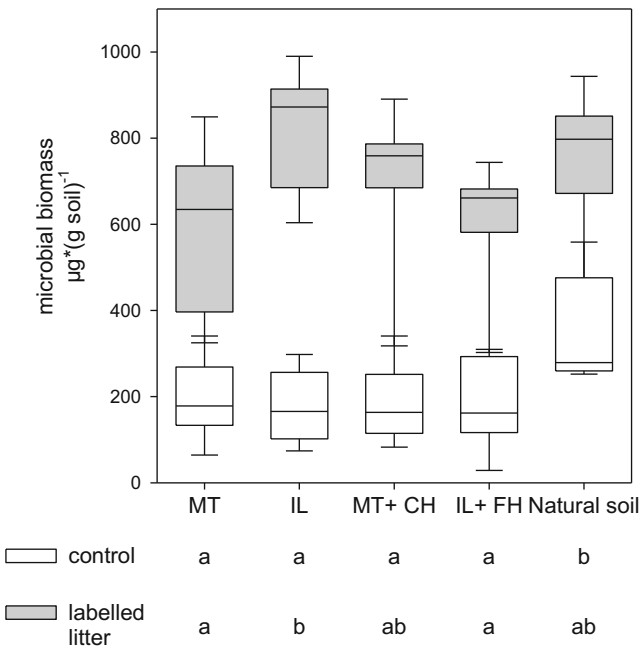

Fig. 2 Microbial biomass in the different soils. *Box plots* (medians, *error bars* indicate standard error (SE), n=12) indicate the microbial biomass in the control treatment (*white box plots*) and the treatment with labelled litter (*grey box plots*). *Lowercase letters* point to significant mean differences between the different soils (n=12; p<0.01)

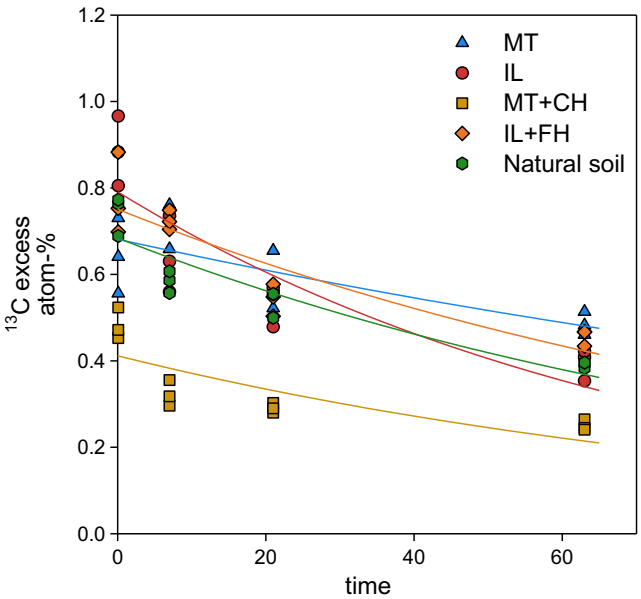

Fig. 3 Curve progression of the ^{13}C label in bulk soil. The development of the ^{13}C label (atom-% excess) in the bulk soil for the different soils is indicated by markers for three independent replicates at each time step. The lines demonstrate the fitted time-depending exponential decay curves of the ^{13}C label for each soil

Distribution of total organic C and N in soil fractions

The total mass recovery of the soil fractionation was 97 ± 2 %. The recovery of organic C in the soil fractions in relation to the organic C of the bulk soil samples was 91 ± 5 %. Organic C concentrations in the POM fraction were in the range of 0.3 to 5.5 mg (g soil)$^{-1}$. The medium and fine silt-sized fractions contained 1.9 to 7.8 mg organic C (g soil)$^{-1}$ and 0.20 to 0.42 mg N (g soil)$^{-1}$, respectively (Table S3). The organic C concentrations in the clay-sized fraction ranged from 4.1 to 9.4 mg (g soil)$^{-1}$. The N contents in the clay-sized fraction showed values of 0.60 to 1.20 mg (g soil)$^{-1}$ (Table 3). Throughout all fractions, the clay-sized fractions showed the highest organic C and N concentrations, while exhibiting the lowest C/N ratios (Fig. 4a, b, Table 3). With percentages of 49 to 61 % of the bulk soil organic C and N, these fractions stored the highest amounts of organic C and N of all fractions in the natural soil. In the artificial soils, the organic C and N concentrations in the clay-sized fractions accounted for 21 to 38 % of the bulk organic C and 34 to 44 % of the bulk N, respectively. The distribution of organic C and N within the soil fractions was influenced by the different soil compositions (Fig. 4a, b). The artificial soil MT showed the significant highest organic C and N concentrations in the POM fraction compared to the other soils, except for the organic C concentrations in the soil MT+CH (Fig. 4a, b, n=12; p<0.05). The amounts of organic C and N contained in the POM fractions were lowest in the soil IL compared to all artificial soils. The natural soil had the smallest concentrations of organic C and N in the POM fraction, as this soil exhibited all in all a low mass-% contribution of POM. Overall, the artificial soils IL and MT exhibited significant differences in the concentration of organic C and N accumulated in the clay-sized fraction (Fig. 4a, b, n=12; p<0.05). The artificial soils IL and MT+CH showed the highest amounts of stored organic C and N in the clay-sized fraction. However, the natural soil revealed highest organic C and N concentrations compared to all other soils (Fig. 4a, b).

Litter-derived organic C and N in the soil fractions

The amounts of ^{13}C in the POM fraction showed a decrease over the incubation time (Table 2). The highest ^{13}C and ^{15}N amounts in the POM fraction were observed in the artificial soil MT after 63 days. The lowest ^{13}C and ^{15}N amounts in this fraction were found in the soil IL and the natural soil at this time. The ^{13}C and ^{15}N label was already detectable in medium and fine silt as well as in the clay-sized fractions in the earliest samples. During the incubation, the ^{13}C declined significantly in the medium and fine silt-sized fraction in all soils (Table S3), except for the soil MT. The ^{15}N increased significantly (n=3; p<0.01) from the first to the second sampling time step (except for composition IL+FH). At a later time, the ^{15}N amounts decreased in the soils IL, MT+CH, IL+FH and in the natural soil (Table S3). For the artificial soil MT, no significant decrease was observed. The ^{13}C amounts in the clay-sized fractions were between 15.5 and 50.4 μg (g soil)$^{-1}$ (Table 3). The highest amounts of ^{13}C were found between 7 and 21 days after incubation start. The ^{15}N got enriched in all soils in the clay-sized fraction over the incubation time

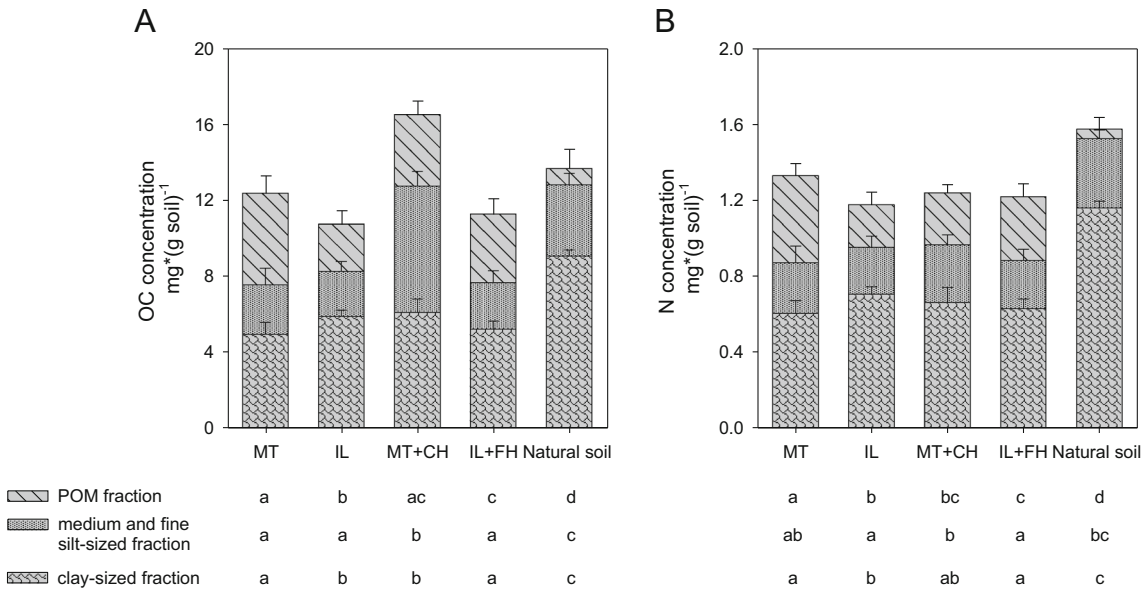

Fig. 4 Distribution of organic C and N in the soil fractions depending on the soil composition. **a** Data indicate the organic C concentrations (mean, *error bars* indicate standard deviation (SD), n=12) in the different soil fractions (POM fraction, medium and fine silt-sized fraction, clay-sized fraction) per gram of soil. **b** *Bars* represent the N concentrations (mean, *error bars* indicate SD, n=12) in the different soil fractions. *Lowercase letters* point to significant mean differences between the different soils (n=12; p<0.05)

Table 2 Properties of the POM fraction in the artificial soils and the natural soil

POM fraction	Incubation time	MT			IL			MT+CH			IL+FH			Natural soil		
Organic C mg (g soil)$^{-1}$	2 h	5.5	±1.1	a	2.7	±0.6	a	3.3	±0.7	a	3.8	±0.3	a	0.9	±0.2	a
	7 days	4.8	±1.9	a	2.7	±0.3	a	3.3	±1.4	a	3.8	±0.9	a	1.8	±1.5	a
	21 days	4.0	±0.4	a	2.3	±0.6	a	4.7	±0.6	a	3.6	±1.1	a	0.4	±0.2	a
	63 days	5.1	±0.1	a	2.2	±0.3	a	3.7	±0.9	a	3.3	±0.1	a	0.3	±0.1	a
N mg (g soil)$^{-1}$	2 h	0.47	±0.08	a	0.24	±0.05	a	0.26	±0.04	a	0.33	±0.03	a	0.04	±0.01	a
	7 days	0.46	±0.20	a	0.25	±0.03	a	0.24	±0.08	a	0.36	±0.09	a	0.11	±0.09	a
	21 days	0.40	±0.04	a	0.20	±0.05	a	0.33	±0.02	a	0.35	±0.11	a	0.03	±0.01	a
	63 days	0.51	±0.01	a	0.21	±0.03	a	0.26	±0.05	a	0.31	±0.01	a	0.02	±0.00	a
C/N ratio	2 h	11.5	±0.9	a	11.6	±0.2	a	12.6	±0.8	a	11.7	±0.2	a	21.3	±1.4	a
	7 days	10.7	±0.5	ab	11.0	±0.5	ab	13.9	±1.1	a	10.5	±0.2	b	17.0	±4.8	a
	21 days	10.0	±0.0	b	11.2	±0.3	ab	14.1	±1.0	a	10.4	±0.3	b	15.2	±4.2	a
	63 days	10.1	±0.2	b	10.5	±0.2	b	14.1	±0.8	a	10.7	±0.2	b	16.7	±4.6	a
^{13}C µg (g soil)$^{-1}$	2 h	36.9	±26.5	a	16.4	±1.7	a	20.3	±7.0	a	27.7	±2.6	a	10.8	±1.9	a
	7 days	21.9	±8.2	a	10.2	±3.2	b	9.3	±3.8	ab	16.1	±5.3	b	5.3	±1.7	b
	21 days	11.4	±2.1	a	4.2	±2.4	c	7.8	±2.8	b	10.2	±5.7	b	1.1	±0.3	c
	63 days	18.3	±1.8	a	0.8	±0.1	c	3.5	±1.6	b	6.6	±2.1	b	0.4	±0.1	c
^{15}N µg (g soil)$^{-1}$	2 h	3.3	±2.7	a	0.7	±0.3	a	0.7	±0.8	a	1.6	±0.3	a	0.4	±0.1	ab
	7 days	2.5	±1.5	a	2.0	±0.6	b	3.1	±2.1	a	1.6	±0.5	a	1.2	±0.7	a
	21 days	1.2	±0.2	a	0.9	±0.4	a	1.7	±0.5	a	1.6	±1.0	a	0.2	±0.0	b
	63 days	2.6	±0.1	a	0.2	±0.0	a	0.8	±0.4	a	0.9	±0.3	a	0.2	±0.0	b

The determined organic C, N, ^{13}C and ^{15}N concentrations as well as C/N ratios are shown for the artificial soils and the natural soil at the different incubation times. Values are given as mean with standard deviation of three independent replicates. Lowercase letters point to significant differences between time points (n=3; p<0.05)

(Fig. S3). After 63 days, the soil MT showed the highest ^{13}C and ^{15}N amounts in the POM fraction. The ^{13}C amounts in the POM fractions with regard to the soil composition showed the highest values in the artificial soils MT and IL+FH (Fig. 5a). The soil MT revealed also the highest ^{15}N amounts in the POM fraction (Fig. 5b). Overall, the most litter-derived ^{13}C and ^{15}N were found in the clay-sized fraction (Fig. 5a, b). The soil MT and IL+FH accumulated the lowest ^{13}C amounts in the clay-sized fraction (Fig. 5a). In contrast, IL and MT+CH revealed significantly higher ^{13}C amounts. The highest amounts of ^{13}C and ^{15}N were stored in the clay-sized fraction of the natural soil (Fig. 5a, b).

Chemical composition of organic C in the clay-sized fractions observed by solid-state ^{13}C NMR spectroscopy

In Table 4, the chemical composition of organic C as relative intensities of the chemical shift areas in the litter and the clay-sized fractions of all soils at the beginning of the incubation and after 63 days of incubation are summarised from two independent replicates. Representative spectra of the soils after 63 days are demonstrated in Fig. S4. The integration of the chemical shift regions showed high relative intensities of O/N-alkyl-C (66 %) in the litter and alkyl-C (22–30 %) in the clay-sized fractions. Generally, lower intensities were found for carboxyl-C (11–15 %) and aryl-C (7–20 %) (Table 4). The contribution of O/N-alkyl-C to the chemical composition of organic C decreased from 66 % in the litter to 38–42 % in the clay-sized fractions at the end of the incubation. The relative intensity of alkyl-C increased in this order from 16 % in the litter over 22–26 % in clay-sized fractions after 2 h, to 27–30 % in clay-sized fractions after 63 days. Compared to the litter, the clay-sized fractions had less O/N-alkyl-C (polysaccharides) and more aryl- and alkyl-C. This is also reflected by the higher alkyl-C to O/N-alkyl C ratio in the clay-sized fractions (Table 4). Overall, no differences between the chemical composition of organic C in the artificial soil compositions could be seen, except for the aryl-C intensity of the artificial soil MT+CH generated by the charcoal in this soil composition.

Differences between artificial soils and the natural soil

The multidimensional scaling plot of the control treatment (five measured values) visualised that due to the higher organic C concentrations and higher C/N ratios, the soil MT+CH did not cluster together with the other artificial soils. Here, the dissimilarities between the soils were higher than the

Table 3 Properties of the clay-sized fraction in the artificial soils and the natural soil

Clay-sized fraction	Incubation time	MT			IL			MT+CH			IL+FH			Natural soil		
Organic C mg (g soil)$^{-1}$	2 h	5.5	±0.5	a	5.9	±0.2	a	5.2	±0.5	a	5.0	±0.5	a	9.0	±0.3	a
	7 days	4.1	±0.5	b	5.5	±0.2	a	6.7	±0.4	b	5.3	±0.2	a	9.4	±0.2	a
	21 days	5.2	±0.4	a	6.0	±0.3	a	6.1	±0.2	ab	5.4	±0.5	a	8.9	±0.4	a
	63 days	4.8	±0.1	ab	6.1	±0.2	a	6.4	±0.7	ab	5.1	±0.5	a	8.8	±0.1	a
N mg (g soil)$^{-1}$	2 h	0.66	±0.05	a	0.69	±0.05	a	0.56	±0.05	a	0.59	±0.05	a	1.15	±0.04	a
	7 days	0.52	±0.06	b	0.67	±0.03	a	0.72	±0.04	b	0.64	±0.02	a	1.20	±0.03	a
	21 days	0.64	±0.03	a	0.72	±0.04	a	0.66	±0.01	ab	0.66	±0.06	a	1.14	±0.03	a
	63 days	0.60	±0.01	ab	0.73	±0.03	a	0.71	±0.08	b	0.63	±0.06	a	1.15	±0.01	a
C/N ratio	2 h	8.3	±0.1	a	8.6	±0.5	a	9.3	±0.3	a	8.4	±0.1	a	7.9	±0.1	a
	7 days	8.0	±0.1	b	8.1	±0.1	a	9.3	±0.1	a	8.3	±0.1	a	7.9	±0.0	a
	21 days	8.2	±0.2	b	8.4	±0.0	a	9.2	±0.2	a	8.3	±0.1	a	7.8	±0.1	a
	63 days	8.1	±0.1	b	8.3	±0.1	a	8.9	±0.1	a	8.1	±0.1	a	7.6	±0.1	a
^{13}C µg (g soil)$^{-1}$	2 h	21.2	±2.4	a	28.1	±2.6	a	25.3	±3.6	a	20.8	±2.4	a	32.7	±2.3	a
	7 days	15.5	±2.5	b	31.5	±1.7	a	39.6	±4.9	b	28.2	±2.0	b	50.4	±5.7	b
	21 days	26.0	±1.1	a	32.1	±2.3	a	33.5	±0.5	ab	28.7	±2.1	b	45.4	±2.7	b
	63 days	22.6	±1.0	a	30.7	±1.2	a	35.2	±6.1	ab	27.1	±1.8	b	35.8	±1.3	a
^{15}N µg (g soil)$^{-1}$	2 h	2.0	±0.2	a	2.7	±0.3	a	2.6	±0.3	a	2.1	±0.2	a	3.3	±0.3	a
	7 days	1.9	±0.3	b	3.3	±0.1	a	4.3	±0.7	b	3.9	±0.3	b	5.3	±0.9	b
	21 days	4.3	±0.2	c	4.5	±0.3	b	4.4	±0.2	b	5.0	±0.3	c	6.2	±0.2	b
	63 days	4.2	±0.2	c	4.7	±0.1	b	5.2	±0.9	b	5.0	±0.3	c	5.8	±0.2	b

The determined organic C, N, ^{13}C and ^{15}N concentrations as well as C/N ratios are shown for the artificial soils and the natural soil at the different incubation times. Values are given as mean with standard deviation of three independent replicates. Lowercase letters point to significant differences between time points (n=3; p<0.05)

sampling time point. Thus, the soil MT+CH and the natural soil exhibited the highest dissimilarities compared to the soils MT, IL and IL+FH (Fig. 6a). The multidimensional scaling plot of the treatment with labelled litter (34 measured values) indicated a higher effect of the sampling time points than the influence of different soil compositions (Fig. 6b). Thus, the

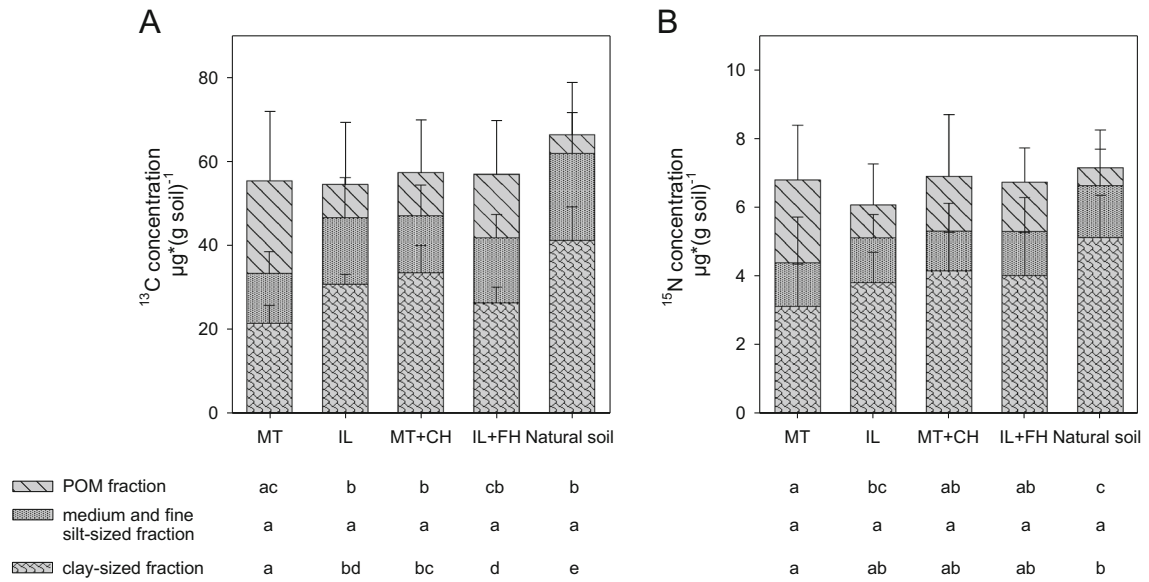

Fig. 5 Distribution of litter-derived organic C and N in the soil fractions depending on the soil composition. **a** Data indicate the ^{13}C concentrations (mean, *error bars* indicate SD, n=12) in the different soil fractions (POM fraction, medium and fine silt-sized fraction, clay-sized fraction) per gram of soil. **b** *Bars* represent the ^{15}N concentrations (mean, *error bars* indicate SD, n=12) in the different soil fractions. *Lowercase letters* point to significant mean differences between the different soils (n=12; p<0.05)

Table 4 The chemical composition of the organic C in the clay-sized fraction of each artificial soil and the natural soil after the incubation start and at the end of the incubation (63 days) analysed by solid-state ^{13}C CPMAS NMR spectroscopy, as well as of the litter added in the beginning

Chemical composition of organic C	Integrated area (ppm)		Litter	Incubation time	MT		IL		MT+CH		IL+FH		Natural soil	
Carboxyl-C	160-220	%	11	2 h	13	±0	13	±1	13	±1	12	±2	13	±1
				63 days	15	±0	15	±0	15	±1	13	±0	14	±1
Aryl-C	110-160		7	2 h	18	±2	15	±1	21	±3	17	±3	18	±1
				63 days	15	±0	15	±1	20	±3	15	±0	17	±1
O/N-Alkyl-C	45-110		66	2 h	45	±0	46	±2	42	±3	47	±3	46	±0
				63 days	41	±0	41	±1	38	±2	42	±0	42	±0
Alkyl-C	0-45		16	2 h	24	±2	26	±0	23	±1	24	±2	22	±1
				63 days	29	±0	30	±0	28	±2	30	±0	27	±1
Alkyl-C/			0.2	2 h	0.5	±0.0	0.6	±0.0	0.5	±0.0	0.5	±0.0	0.5	±0.0
O-Alkyl-C ratio				63 days	0.7	±0.0	0.7	±0.0	0.7	±0.0	0.7	±0.0	0.6	±0.0

The values of the soils are presented as mean with a standard deviation of two independent replicates

natural soil and the artificial soils did not differ greatly with respect to the litter decomposition and the distribution of the label in the soil fractions.

Discussion

The decomposition of fresh litter affected by soil mineralogy

In our experiments, the added ^{13}C- and ^{15}N-labelled litter was rapidly mineralised in all soils, which was revealed by the decrease of ^{13}C in the POM fraction and the bulk soil. The mineralisation of the added labelled litter was affected by the mineralogy, as we found a significant difference in amount as well as curve progression of the ^{13}C label remaining in the MT and IL soil (Table 1, Fig. 3). Significantly higher ^{13}C and ^{15}N amounts in the POM fraction of the soil MT were in line with this observation (Fig. 5a, b). At the end of the incubation, 60 % of the initial ^{13}C was mineralised in the IL soil and only 47 % in the MT soil. The natural soil as well as the artificial soils MT+CH and IL+FH showed no differences in the decrease of the ^{13}C label over the incubation time (Fig. 3) and exhibited almost the same intermediate portion (55, 53, 55 %) of mineralised ^{13}C at the end of the incubation time. On the whole, we found a faster decomposition of the added litter in the artificial soil with illite compared to the artificial soil with montmorillonite, whereas the other soils showed no difference. Several mechanisms on how mineralogy affects the mineralisation of organic materials in soils are discussed. For instance, it is assumed that clay minerals may directly interact with microbes, thereby altering the rate of the microbial metabolism, modifying the solution environment and binding extracellular enzymes, and thus modify their activity

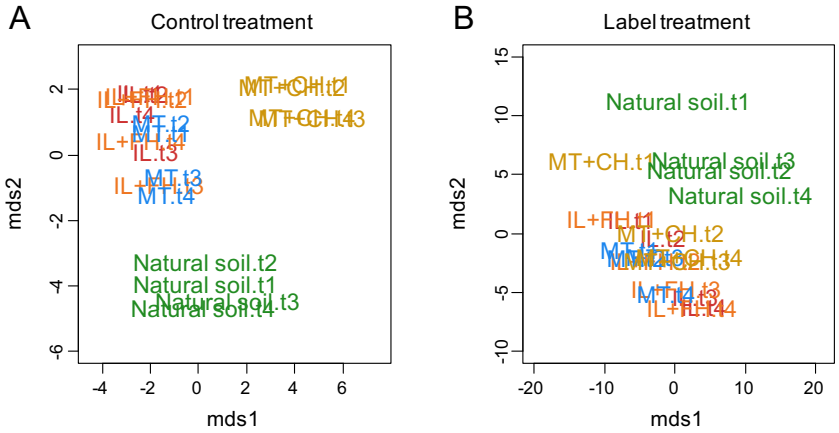

Fig. 6 Multidimensional scaling plot of the control treatment and the treatment with labelled litter. **a** Multidimensional scaling (*mds*) plot of the control treatment (five measured values). The different soils are given in *different colours*. The different time points are indicated by *t1* (2 h), *t2* (7 days), *t3* (21 days) and *t4* (63 days). **b** Multidimensional scaling plot of the treatment with labelled litter addition (34 measured values). The different soils are given in *different colours*. The different time points are indicated by *t1* (2 h), *t2* (7 days), *t3* (21 days) and *t4* (63 days)

and consequently the degradation of OM (Sollins et al. 1996; Stotzky 1967, 1986). Physical protection of OM by the interaction with minerals also plays an important role for the decomposition of OM, as this results in physical or chemical inaccessibility of OM to the decomposer organisms (Baldock and Skjemstad 2000; Oades 1984). In our study, the SEOC followed a fast decrease over the incubation time and was probably used as easily available substrate by microorganisms (Table 1, Fig. S1). Directly after the incubation start, the soil MT exhibited the lowest litter-derived ^{13}C-SEOC concentrations (Fig. S1). Differences in the sorption capacity of the diverse clay minerals may have an effect on the decomposition of OM. Thus, the observed lower SEOC values directly after the incubation start could indicate that the easily available organic C was more and/or stronger bound to the soil MT and therefore protected from microorganisms. Saggar et al. (1996) assumed that stabilisation of OM by adsorption on soil surfaces is the principal mechanism controlling the OM decomposition. The authors attributed a more rapid decomposition to lower surface areas, as in their experiment, and the ^{14}C decomposition was lower in smectitic soils (Saggar et al. 1996). But although the montmorillonite had a higher SSA and smaller SEOC concentrations, we did not find higher amounts of the label in the fine-sized fractions of the artificial soils with montmorillonite compared to illite (Fig. 5a). We can thus conclude that the size of SSA and the sorption of the labelled OM on those are not decisive for the mineralisation, as we further did not find a reduced mineralisation in the IL+FH soil with a high SSA due to the ferrihydrite in this mixture.

The microbial biomass in the artificial soil MT was significantly lower than in the artificial soil IL (Fig. 2). As the mineralisation of OM is carried out by the soil microflora, it was assumed that this process is at least partly regulated by the size, activity and diversity of the microbial population (Fontaine and Barot 2005). The supply of fresh organic substrate increased the size of the decomposer population, which was shown by the increase in microbial biomass of the labelled treatment compared to the control without litter addition (Fig. 2). As the amount of microbial biomass in the control treatments was comparable for all artificial soils, it seems that the growth of soil microorganisms induced by the substrate addition was less in the soil MT (Fig. 2). Recently, Wei et al. (2014) found out that higher clay contents promote the OM decomposition by sustaining a greater microbial biomass. The lower microbial biomass is in agreement with the slower mineralisation rates in MT soil and hence seems to be the reason for the slower decomposition of the added litter. In the literature, contradictory effects of montmorillonite on the degradation of OM are reported (Filip 1973; Haider et al. 1970). An inhibiting effect of higher montmorillonite concentrations due to limitations in O_2 diffusion was reported by Stotzky (1986). Filip (1973) stated in a review that clay minerals of the smectite group were usually found to reduce the rate of decomposition and promote the C accumulation in soils. On the other hand, it was also indicated in the literature that montmorillonite stimulates microbial activity and therefore the decomposition of OM, but this was mainly attributed to the buffering capacity of montmorillonite (Haider et al. 1970; Kunc and Stotzky 1974; Stotzky and Rem 1966). Although an effect of the mineral compositions on the establishment of different microbial communities was observed (Babin et al. 2013; Ding et al. 2013), we were not able to detect an effect of mineralogy on the OM decomposition in previous studies (Pronk et al. 2013; Vogel et al. 2014a). For this reason, it is unlikely that direct effects of the clay mineral surfaces, either directly on the activity of the microorganisms or indirectly by sorption on the mineral surfaces, are responsible for the difference in decomposition. Moreover, the use of stable isotope tracing allowed us to observe the difference in decomposition between IL and MT soils. However, we assume that the structural development in the artificial soils after several OM additions may provide a potential reason for the reduced decomposition in the artificial soil MT. As the aggregation of soils is supposed to affect OM dynamics by influencing the microbial activity (Sollins et al. 1996), the structural development of the artificial soils could serve as possible explanation for the differences in decomposition. Aggregates physically protect soil OM by forming physical barriers between microbes, their enzymes and the substrates which altogether controls the microbial turnover (Six et al. 2002). Vogel et al. (2014a) showed that artificial soils with montmorillonite exhibited more macro-aggregates than the artificial soils with illite after two OM additions and a development of more than 2 years. As the litter was mixed with the soils at the beginning of the experiment, the reduced growth of the microbes in the soil MT might be explained by higher aggregation resulting in an inaccessibility of substrate to the microorganisms.

Although we regarded charcoal and ferrihydrite as important constituents, the partly substitution of the clay minerals by charcoal and ferrihydrite had no additional effect on the decomposition in this experiment. Charcoal particles were considered as important constituents by providing surfaces supporting microbial growth and activity, leading to enhanced decomposition (Lehmann et al. 2011; Wardle et al. 2008). However, contradictory data have been published about the effect of charcoal on the decomposition of OM, and the mechanisms as well as the effects behind this remain to be determined (Sohi et al. 2010). In our experiment, the charcoal showed no effect on the decomposition, possibly due to the fact that the charcoal used in the experiment is highly aromatic (Pronk et al. 2012). Sorption of organic substrates to mineral particles like ferrihydrite could provide an important mechanism by which the bioavailability and capability for biodegradation through soil microflora are reduced (Jones and Edwards 1998). However, we did not find an effect of the iron oxide on the decomposition of the added labelled litter. This

could be due to the relatively high crystallinity of the ferrihydrite and the pH of around 7, which probably made the ferrihydrite less reactive than respective minerals occurring in natural soils. We can conclude that the surfaces of the additional charcoal and ferrihydrite had no additional effect on the decomposition of the freshly added litter material in this experiment.

To sum up, the OM decomposition was significantly affected by the clay mineral composition in the artificial soils, as the soil MT showed a slower mineralisation rate than the soil IL. The SSA of the respective minerals seems not to be decisive for the decomposition of OM in this experiment. The slower decomposition of OM in the artificial soil MT is supposedly caused by its lower microbial biomass. Nevertheless, it has to be kept in mind that the differences in decomposition between the two clay minerals are small.

Sequestration of organic C and N differs in the small-sized fractions and depends on soil mineralogy

The litter-derived ^{13}C and ^{15}N labels were found in all fine mineral fractions shortly after the start of the incubation experiment (Table 3, Table S3). Thus, our data demonstrate a fast formation of new organo-mineral associations. Right from the beginning, the labelled litter-derived OM got attached to the particles of the medium and fine silt-sized fraction, although it got lost rapidly thereafter over the incubation time. Hence, the medium and fine silt-sized fraction appeared as a transient fraction for the litter-derived organic C and N. The ^{13}C concentrations in the clay-sized fractions firstly got enriched over the incubation time, which was then followed by a small decrease, while the ^{15}N concentrations increased over the whole experimental time in the clay fraction of all soils (Table 3). This was also reflected by a slight decrease in the C/N ratios of the clay-sized fraction, indicating an enrichment of N in the accumulated OM (Table 3). The accumulation of ^{15}N in the clay-sized fraction followed a growth curve over the incubation time (Fig. S3), suggesting that the chemical composition of the OM becomes dominated to a greater extent by microbial residues and metabolites. Consequently, the development of new organo-mineral associations in our experiment can be divided into two phases. The first phase reflects an instantaneous development of organo-mineral associations, indicated by an immediate enrichment of ^{13}C and ^{15}N in the fine organo-mineral fractions, which possibly resulted from leaching of soluble litter-derived OM and sorption to the fine mineral fractions after water addition. The second phase was driven by transformation and maturation of added OM by microbial activity, as demonstrated by the specific progression of ^{13}C and ^{15}N.

Generally, the clay-sized fractions showed the highest amounts of organic C and N as well as the highest litter-derived ^{13}C and ^{15}N amounts per gram of soil (Figs. 4, 5), but with a substantial effect of the varying mineral composition on the organic C and N sequestration. The soils IL and MT+CH accumulated the highest amounts of label in the clay-sized fraction compared to the other artificial soils (Figs. 4 and 5). Significant differences in the amount of organic C and N and the litter-derived ^{13}C accumulated in the clay-sized fraction were observed between the artificial soils IL and MT (Figs. 4a, b and 5a), with the soil IL sequestering more organic C and N in the clay-sized fraction. This is in contrast to a laboratory incubation experiment by Sørensen (1972), in which different clay minerals and ^{14}C-labelled carbohydrates were added to soil-sand mixtures. The authors found a greater contribution of carbohydrate-derived amino acids in montmorillonite than in illite and kaolinite mixtures (Sørensen 1972). Even though it was supposed that clays favour more and/or stronger organic C-clay interactions due to their larger SSA (von Lützow et al. 2006; Wattel-Koekkoek et al. 2003), we found that the soil IL stored more organic C and N in the clay-sized fraction than the soil MT with a higher SSA. As we observed lower amounts of organic C and N in the clay-sized fraction of the soil MT containing montmorillonite with a higher SSA than illite, it is likely that the SSA is of minor importance for the sequestration of organic C and N in our experiment. It has to be kept in mind that over the long preparation time of the artificial soil materials of more than 2 years, organo-mineral associations have already developed, which may react differently compared to the clean mineral surfaces and controlled OM binding in soils, although there might be enough free mineral surfaces available. Vogel et al. (2014b) found that already existing OM clusters were decisive for the accumulation of new OM in the fine fraction of a structured soil, rather than the large proportion of free mineral surfaces. The differences we found in our early artificial soil experiments with the results presented here on more developed artificial soils might indicate that freshly added pure minerals react differently compared to minerals already incorporated in a soil-like structure.

Whereas the differences in the amount of sequestered organic C and N in the clay-sized fraction depended on the mineralogy, the quality of the organic C bound to the minerals' surfaces did not differ (Table 4). This is in contrast to the assumption that the composition of clay-associated OM is influenced by the type of clay minerals present, as it was found that smectite-associated OM is higher in aromatic compounds (Wattel-Koekkoek et al. 2001).

Contrary to our assumption that iron oxides and charcoal serve as important constituents affecting the sequestration of C and N, we did not find differences in C and N sequestration caused by charcoal and ferrihydrite. Sorptive protection by minerals like ferrihydrite was assumed to be a major mechanism of the organic C sequestration (Kalbitz et al. 2005). But even though the ferrihydrite in the artificial soil exhibits a high SSA (Table S1), which could serve as important sorption site,

we did not find an additional effect of the iron oxide on the OM sequestration. The pH of the artificial soil IL+FH of around 7, which is close to the point of zero charge of the ferrihydrite, could have decreased the affinity of ferrihydrite for OM.

Overall, we observed a faster mineralisation of the fresh litter in the artificial soil IL (Fig. 3) and subsequently higher amounts of organic C, N and ^{13}C in the clay-sized fraction of this soil (Figs. 4a, b and 5a), although usually, a faster mineralisation is supposed to be associated with a lower sequestration of organic C (Six et al. 2002). The lower amounts of OM sequestered within the artificial soil MT seem to be affected by the microbial processing of the fresh litter material and its necessity to transform the freshly added OM in forms that are more likely generating organo-mineral associations (Miltner et al. 2012). Consequently, we assume that the faster decomposition in the soils IL resulted in higher amounts of microbial metabolites accumulated in the clay-sized fraction and generated higher amounts of OM sequestered in the soil IL.

As Pronk et al. (2013) and Vogel et al. (2014a) did not find differences in the amounts of organic C and N in the fraction <20 μm with respect to the different mineral compositions of the artificial soils, we suppose that several OM inputs are necessary, until differences in the OM sequestration regarding soil mineralogy emerge. It is conceivable that over the longer production time of the artificial soils used in this experiment, a soil structure developed which controls the OM sequestration on the long-term.

Natural versus artificial soils: can artificial soils be used as model for natural soils?

The development of artificial soils demonstrated the potential to produce soil-like systems from clean minerals after the addition of OM and a microbial inoculum, as organo-mineral associations and aggregates were formed after several months (Pronk et al. 2012). Nevertheless, there is no doubt that the natural soil and the artificial soils used in this study differ from each other with respect to their properties and functions. However, the question if an artificially produced soil-like system can be used to study processes occurring naturally in soils remained open. In our incubation experiment, we compared four artificial soils with a natural Ap horizon (Luvisol) with a mineral composition dominated by chlorite, illite, quartz and mixed layer minerals in the clay-sized fraction. Although the natural soil exhibited the highest amount of microbial biomass compared to the artificial soils in the control treatment, the mineralisation rate of the added labelled litter showed no difference to the artificial soils. As mentioned above ('The decomposition of fresh litter affected by soil mineralogy'), 55 % of the initial ^{13}C was mineralised in the natural soil, which was almost the same percentage found for the artificial soils IL, MT, IL+FH and MT+FH (60, 47, 55, 53 %). Chotte et al. (1998) observed that 51 to 64 % of the organic C were mineralised after 66 days in natural soils amended with ^{14}C-labelled glucose, starch, legume and wheat. In a long-term study, Saggar et al. (1996) reported that 35 to 52 % of the added material was mineralised in natural soils. Thus, the mineralisation of organic C in the artificial soils was in the same range as found for natural soils. As it is likely that the natural and artificial soils have different microbial communities, the same mineralisation of the added litter material in the natural and artificial soils may also confirm the hypothesis of functional redundancy of soil (Nannipieri et al. 2003).

The multidimensional scaling plot of the control treatment illustrates the dissimilarities between the artificial soils and the natural soil caused by the higher organic C and N and microbial biomass as well as the smallest SEOC concentrations in the natural soil (Fig. 6a). In the treatments with added litter, the dissimilarities between the sampling time points were higher than between the different soils. Due to the litter addition, the

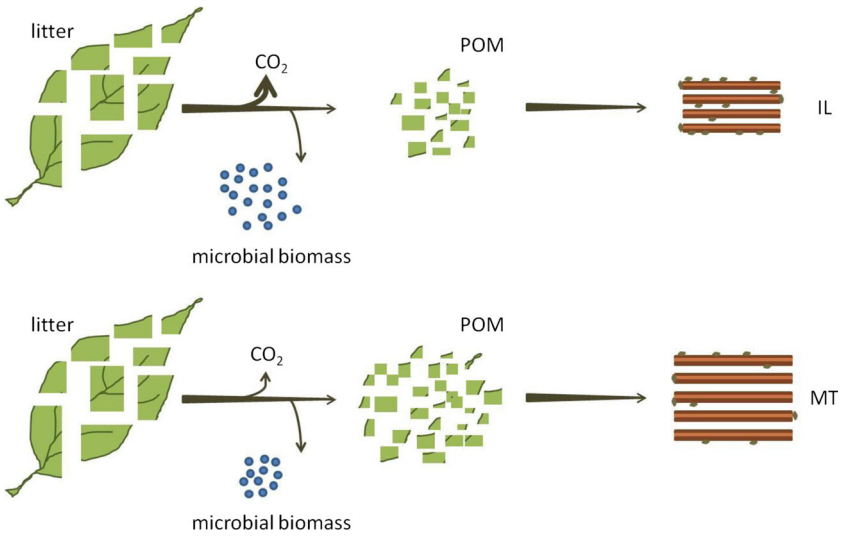

Fig. 7 Enhanced decomposition leads to higher organic C and N sequestration in fine-sized fractions modified by clay mineralogy

natural soil approximated the artificial soils (Fig. 6b); for example, the microbial biomass in the natural soil was in the same range of values found for the artificial soil after addition of the labelled litter. Consequently, the artificial soils seem to be appropriate as model systems for studying processes occurring in natural soils, as they react similarly in laboratory incubation experiments.

Conclusion

In our short-term incubation experiment, we could show that the decomposition of fresh substrate in soils is affected by the type of clay mineral present. Stable isotope tracing gave us the opportunity to observe small differences between the pre-produced artificial soils with illite or montmorillonite. In general, the artificial soil MT exhibited a slower mineralisation compared to IL, which was in line with a smaller microbial biomass in this soil. These small differences seem to remain hidden, if only total organic C is analysed. In the present study, high litter amounts were mixed into the soils, which are in natural soil ecosystems only occurring in agricultural soils if minimal tillage is used in combination with green manure application. This was mainly done due to the thresholds of the used methods. Reduced amounts of added litter may induce different lower microbial activity patterns and thus may also influence organic C turnover in bulk soil. It is an open question which fraction would be affected most by reduced amounts of applied litter. Here, future studies are needed to understand the role of litter amount and quality.

Furthermore, we found a differentiation in the OM sequestration with regard to the soil composition of the artificial soils. Usually, a high SSA of a clay mineral is considered to provide a high storage capacity for organic C and N. However, we observed a smaller amount of organic C and N stored in the MT soil with the higher SSA compared to the soil IL. Charcoal and ferrihydrite had no additional effect on the sequestration in this experiment. Hence, the mineral surface area seemed to be of minor importance for the storage capacity in the small-sized fractions in our experiment. Certainly, the microbial biomass was affected by the artificial soil composition. Thus, the accumulation of microbial residues and products appeared to be the most important factor influencing the sequestration of organic C and N in the small-sized fractions, depending on the microbial biomass. Consequently, the OM stabilisation in the fine fraction seemed to be determined by the microbial biomass decomposing OM, which is in turn controlled by the soil mineralogy (Fig. 7). However, it remains an open question if the clay minerals affect the microorganisms directly or indirectly, e.g. by aggregation. As the discrepancies in the decomposition of the labelled litter between the different clay minerals are small, we suppose that several OM inputs and longer development times are needed to reveal a differentiation in the amount of stored organic C and N depending on the different clay minerals, as we could not find a differentiation depending on the mineralogy in former studies.

The mineralisation process in a natural soil of such an incubation experiment was not different to the process in the artificial soils developed under several OM inputs and more than 2.5 years of incubation. Thus, it seems possible to produce soil-like systems with OM dynamics comparable to natural soils, from secondary minerals incubated with soil microorganisms and repeated substrate addition over sufficient development time. Overall, the artificial soils serve as valuable model, where the interactions of soil minerals and OM decomposition processes can be studied under well-defined conditions.

Acknowledgments We thank Lilli Zeh, Maria Greiner, Bärbel Angres and Geertje Pronk for their technical assistance during preparation and characterisation of the soils. This project was carried out within the framework of the priority program 1315 'Biogeochemical Interfaces in Soil' funded by the Deutsche Forschungsgemeinschaft (DFG): SM59/8-2 and KO1035/33-2. Cordula Vogel was further supported by the Deutsche Forschungsgemeinschaft (DFG) through the TUM International Graduate School of Science and Engineering (IGSSE).

References

Babin D, Ding G-C, Pronk GJ, Heister K, Kögel-Knabner I, Smalla K (2013) Metal oxides, clay minerals and charcoal determine the composition of microbial communities in matured artificial soils and their response to phenanthrene. FEMS Microbiol Ecol 86:3–14. doi:10.1111/1574-6941.12058

Barré P, Fernandez-Ugalde O, Virto I, Velde B, Chenu C (2014) Impact of phyllosilicate mineralogy on organic carbon stabilization in soils: incomplete knowledge and exciting prospects. Geoderma 235–236: 382–395. doi:10.1016/j.geoderma.2014.07.029

Bretz F (2011) Multiple comparisons using R. CRC Press, Boca Raton

Bruun TB, Elberling B, Christensen BT (2010) Lability of soil organic carbon in tropical soils with different clay minerals. Soil Biol Biochem 42:888–895. doi:10.1016/j.soilbio.2010.01.009

Chotte JL, Ladd JN, Amato M (1998) Sites of microbial assimilation, and turnover of soluble and particulate 14C-labelled substrates decomposing in a clay soil. Soil Biol Biochem 30:205–218. doi:10.1016/S0038-0717(97)00115-6

Ding G-C, Pronk GJ, Babin D, Heuer H, Heister K, Kögel-Knabner I, Smalla K (2013) Mineral composition and charcoal determine the bacterial community structure in artificial soils. FEMS Microbiol Ecol 86:15–25. doi:10.1111/1574-6941.12070

Esperschütz J, Welzl G, Schreiner K, Buegger F, Munch JC, Schloter M (2011) Incorporation of carbon from decomposing litter of two pioneer plant species into microbial communities of the detritusphere. FEMS Microbiol Lett 320:48–55. doi:10.1111/j.1574-6968.2011.02286.x

Everitt B, Hothorn T (2011) An introduction to applied multivariate analysis with R. Springer, New York. doi:10.1007/978-1-4419-9650-3

Feng W, Plante A, Six J (2013) Improving estimates of maximal organic carbon stabilization by fine soil particles. Biogeochemistry 112:81–93. doi:10.1007/s10533-011-9679-7

Filip Z (1973) Clay minerals as a factor influencing the biochemical activity of soil microorganisms. Folia Microbiol 18:56–74. doi:10.1007/bf02884250

Fontaine S, Barot S (2005) Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecol Lett 8:1075–1087. doi:10.1111/j.1461-0248.2005.00813.x

Guenet B, Leloup J, Hartmann C, Barot S, Abbadie L (2011) A new protocol for an artificial soil to analyse soil microbiological processes. Appl Soil Ecol 48:243–246. doi:10.1016/j.apsoil.2011.04.002

Haider K, Filip Z, Martin JP (1970) Einfluß von Montmorillonit auf die Bildung von Biomasse und Stoffwechselzwischenprodukten durch einige Mikroorganismen. Arch Mikrobiol 73:201–215. doi:10.1007/bf00410621

Jenkinson DS (1977) Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from 14C labelled ryegrass decomposing under field conditions. J Soil Sci 28:424–434. doi:10.1111/j.1365-2389.1977.tb02250.x

Joergensen RG (1995) The fumigation extraction method to estimate soil microbial biomass: extraction with 0.01 m CaCl2. Agribiol Res Z Agrarbiol Agrikchem Okol 48:319–324

Jones DL, Edwards AC (1998) Influence of sorption on the biological utilization of two simple carbon substrates. Soil Biol Biochem 30:1895–1902. doi:10.1016/S0038-0717(98)00060-1

Kalbitz K, Schwesig D, Rethemeyer J, Matzner E (2005) Stabilization of dissolved organic matter by sorption to the mineral soil. Soil Biol Biochem 37:1319–1331. doi:10.1016/j.soilbio.2004.11.028

Krummen M, Hilkert AW, Juchelka D, Duhr A, Schlüter H-J, Pesch R (2004) A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom 18:2260–2266. doi:10.1002/rcm.1620

Kunc F, Stotzky G (1974) Effect of clay minerals on hetrotrophic microbial activity in soil. Soil Sci 118:186–195

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota - A review. Soil Biol Biochem 43:1812–1836. doi:10.1016/j.soilbio.2011.04.022

Marx M, Buegger F, Gattinger A, Marschner B, Zsolnay Á, Munch JC (2007) Determination of the fate of 13C labelled maize and wheat rhizodeposit-C in two agricultural soils in a greenhouse experiment under 13C-CO2-enriched atmosphere. Soil Biol Biochem 39:3043–3055. doi:10.1016/j.soilbio.2007.06.016

Miltner A, Bombach P, Schmidt-Brücken B, Kästner M (2012) SOM genesis: microbial biomass as a significant source. Biogeochemistry 111:41–55. doi:10.1007/s10533-011-9658-z

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670. doi:10.1046/j.1351-0754.2003.0556.x

Oades JM (1984) Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76:319–337. doi:10.1007/bf02205590

Pronk GJ, Heister K, Ding G-C, Smalla K, Kögel-Knabner I (2012) Development of biogeochemical interfaces in an artificial soil incubation experiment; aggregation and formation of organo-mineral associations. Geoderma 189–190:585–594. doi:10.1016/j.geoderma.2012.05.020

Pronk GJ, Heister K, Kögel-Knabner I (2013) Is turnover and development of organic matter controlled by mineral composition? Soil Biol Biochem 67:235–244. doi:10.1016/j.soilbio.2013.09.006

Baldock JA, Skjemstad JO (2000) Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Org Geochem 31:697–710. doi:10.1016/S0146-6380(00)00049-8

Saggar S, Parshotam A, Sparling GP, Feltham CW, Hart PBS (1996) 14C-labelled ryegrass turnover and residence times in soils varying in clay content and mineralogy. Soil Biol Biochem 28:1677–1686. doi:10.1016/S0038-0717(96)00250-7

Saidy AR, Smernik RJ, Baldock JA, Kaiser K, Sanderman J, Macdonald LM (2012) Effects of clay mineralogy and hydrous iron oxides on labile organic carbon stabilisation. Geoderma 173–174:104–110. doi:10.1016/j.geoderma.2011.12.030

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241:155–176. doi:10.1023/a:1016125726789

Sohi SP, Krull E, Lopez-Capel E, Bol R, Donald LS (2010) Chapter 2 - A Review of Biochar and Its Use and Function in Soil. Adv Agron Vol 105:47–82. doi:10.1016/S0065-2113(10)05002-9

Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74:65–105. doi:10.1016/S0016-7061(96)00036-5

Sørensen LH (1972) Stabilization of newly formed amino acid metabolites in soil by clay minerals. Soil Sci 114:5–11

Sørensen LH (1981) Carbon-nitrogen relationships during the humification of cellulose in soils containing different amounts of clay. Soil Biol Biochem 13:313–321. doi:10.1016/0038-0717(81)90068-7

Stotzky G (1967) Clay minerals and microbial ecology. Trans N Y Acad Sci 30:11–21. doi:10.1111/j.2164-0947.1967.tb02449.x

Stotzky G (1986) Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In: Huang PM, Schnitzer M (eds) Interactions of Soil Minerals with Natural Organics and Microbes. SSSA Special Publication. Soil Science Society of America, Madison, pp 305–428. doi:10.2136/sssaspecpub17.c10

Stotzky G, Rem LT (1966) Influence of clay minerals on microorganisms: I. Montmorillonite and Kaolinite on bateria. Can J Microbiol 12:547–563. doi:10.1139/m66-078

Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. Nature 389:170–173. doi:10.1038/38260

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707. doi:10.1016/0038-0717(87)90052-6

Vogel C, Babin D, Pronk GJ, Heister K, Smalla K, Kögel-Knabner I (2014a) Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils. Soil Biol Biochem. doi:10.1016/j.soilbio.2014.1007.1012

Vogel C, Mueller CW, Höschen C, Buegger F, Heister K, Schulz S, Schloter M, Kögel-Knabner I (2014b) Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nat Commun 5:2947. doi:10.1038/ncomms3947

von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions—a review. Eur J Soil Sci 57:426–445. doi:10.1111/j.1365-2389.2006.00809.x

Wardle DA, Nilsson M-C, Zackrisson O (2008) Fire-derived charcoal causes loss of forest humus. Science 320:629. doi:10.1126/science.1154960

Wattel-Koekkoek EJW, van Genuchten PPL, Buurman P, van Lagen B (2001) Amount and composition of clay-associated soil organic matter in a range of kaolinitic and smectitic soils. Geoderma 99:27–49. doi:10.1016/S0016-7061(00)00062-8

Wattel-Koekkoek EJW, Buurman P, Van Der Plicht J, Wattel E, Van Breemen N (2003) Mean residence time of soil organic matter associated with kaolinite and smectite. Eur J Soil Sci 54:269–278. doi:10.1046/j.1365-2389.2003.00512.x

Wei H, Guenet B, Vicca S, Nunan N, Asard H, AbdElgawad H, Shen W, Janssens IA (2014) High clay content accelerates the decomposition of fresh organic matter in artificial soils. Soil Biol Biochem 77:100–108. doi:10.1016/j.soilbio.2014.06.006

Werner RA, Brand WA (2001) Referencing strategies and techniques in stable isotope ratio analysis. Rapid Commun Mass Spectrom 15:501–519. doi:10.1002/rcm.258