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Association of fresh low-molecular-weight organic compounds with clay-sized mineral fraction in soils of different organic carbon loading

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ABSTRACT

Although the association of minerals and organic matter (OM) in soil plays an important role in the sequestration of C, the factors driving the initial formation of mineral-associated OM (MAOM), and thus the retention of new C input in soils are not yet fully understood. In this study, we investigated how the soil C loading and the differences in the N content of low-molecular-weight organic compound (LMWOC) input foster the rapid C retention in the soil's fine mineral fractions (clay and fine silt-sized fraction). Two topsoils (0–10 cm) with different C loading due to different long-term management (direct seeding vs. bare fallow) derived from an agricultural research trial were used for the short-term incubation experiment. In a 24-hour incubation experiment, we used two labeled substrates (without N, glucose, > 99% ^{13}C and with N, amino acid mixture, > 98% ^{13}C, > 98% ^{15}N) to investigate how the different N content delivered by the LMWOC input determine the fate of newly formed OM in the MAOM pool. Our results show that the soil with low C loading and thus a low C saturation level retained more freshly added LMWOC in the fine MAOM pool compared to the high C-loading soil, demonstrating that the soil C loading is a major factor controlling the retention of freshly added OM at the early stage of MAOM formation. The LMW OM containing N significantly enhanced the recovery of freshly added LMWOC in the low C-loading soil but not in the high C-loading soil. This points to the great importance of the N availability for the retention of freshly added OM in soils. Our study showed that the level of the native OM content affects the fast retention of freshly added OM in the clay-sized fraction to a greater extent than the N availability of the OM substrate.

1. Introduction

Soils store large amounts of organic matter (OM) and represent the largest terrestrial carbon (C) pool (Scharlemann et al., 2014; Schlesinger, 2005). Considerable work has been done to understand soil organic carbon (SOC) stabilization mechanisms (Basile-Doelsch et al., 2020), as the persistence of C in soils is of great significance concerning global climate change and agricultural services (Lal, 2004). The amount of more persistent soil C is mainly regulated by the interaction of OM with mineral surfaces and the reduced bioaccessibility within soil aggregates, namely as mineral-associated organic matter (MAOM) and as OM occluded within soil aggregates (Christensen, 1992; JASTROW, 1996; von Lützow et al., 2007). The MAOM was demonstrated to have rather longer turnover times and thus sustain the persistence of C in soils (von Lützow et al., 2007). Therefore, the interaction of OM and mineral surfaces (organo-mineral interaction) is considered as one of the main factors that control soil C storage. Accordingly, a major focus was put on the study of the cycling and fate of MAOM in recent years (Kleber et al.,

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Table 1
Basic soil characteristics of low-C and high-C soil before incubation, including C and N contents, C to N ratio, pH-values and electrical conductivity (EC) measured in H_2O.

Soil	Total C content (mg g^{-1})	Total N content (mg g^{-1})	C/N	pH	EC	Fe content (mg g^{-1})
Low-C	6.1 ± 0.0	0.7 ± 0.0	8.7 ± 0.0	6.4	54.2	9.8 ± 0.2
High-C	17.4 ± 0.3	1.8 ± 0.0	9.9 ± 0.0	7.6	67.2	14.2 ± 0.2

2015; Lehmann and Kleber, 2015).

To better understand the fate and the properties of MAOM, physical fractionation using density and particle size is commonly applied to differentiate between different SOM fractions (Kögel-Knabner et al., 2008; Poeplau et al., 2018). It was shown that the amount of persistent SOM correlates with the amount of fine mineral particles (<20 μm) (Feller and Beare, 1997) and the type of clay minerals (Denef et al., 2004), for instance, 2:1 layer silicate sequestrated a higher amount of organic compounds than 1:1 layer silicate (Saidy et al., 2013; Wang and Xing, 2005). As more than half of the total OM in temperate arable soils is concentrated in the clay-sized fraction (<2 μm) (Christensen, 2001), it is crucial to better understand the OM cycling in the fine mineral fractions. In many soils, the fine-sized mineral fraction contributes considerably more to SOM preservation compared to particulate OM (Kleber et al., 2015; Leifeld et al., 2009; Torn et al., 2013). Partly this is due to the high specific surface area (SSA) of the fine-sized mineral fraction that was demonstrated to positively correlate with the amount of stored SOC (Hassink, 1997; Six et al., 2002).

Although the SSA plays an important role in SOM storage via organo-mineral associations, the OM input determines the accumulation of SOM in general (Kaiser and Guggenberger, 2003). Soil OM content and its composition is mainly driven by the quantity and also N content of the plant-derived OM input (Dungait et al., 2012; Grandy and Neff, 2008). The mass ratio of C to N of most plant litter is above 20 (Paul, 2016; Zhang et al., 2019), while that of MAOM is between 8 and 13 (Hassink et al., 1993; von Lützow et al., 2007), which resembles the C/N ratio of microorganisms of around 8 and thus the increased amount of microbial transformed OM, including microbial biomass and necromass (microbial residues) (Buckeridge et al., 2020; Liang et al., 2019; Miltner et al., 2012). Experiments studying short-term effects of OM substrate often focus more on the low-molecular-weight organic compounds (LMWOC) absorbed within the soils and the direct microbial uptake and mineralization (Creamer et al., 2014; Fischer et al., 2010). The rapid microbial transformation of plant-derived OM and its fate as MAOM in fine-sized soil mineral fractions is less understood.

The soil OC content was suggested to be directly linked to the N content of the OM input material (Jilling et al., 2020; Kleber et al., 2007; Kopittke et al., 2020). Studies on the relation of N addition and alterations of SOC stocks demonstrated that in both short-term incubation and long-term field experiments SOC stocks increased when additional N was applied (Cotrufo et al., 2013; Kirchmann et al., 2004; Wang et al., 2019). In contrast, other studies showed that the SOC content is not always significantly related to N addition (Brown et al., 2014; Campbell et al., 1991; Reicosky et al., 2002). Other authors also pointed out that although the N content of OM input is an important factor, the overall accumulation of SOC seems to be more controlled by the quantity of the OM input (Gentile et al., 2011; Lu et al., 2011).

A concept of soil C saturation was proposed, which suggested that an upper limit of C content exists for the soil C pool and this capacity limits the further increase of more persistent SOC with OM input (Six et al., 2002). The research of C saturation effects on agricultural soils indicated that the C input by aboveground crop residues (e.g. litter, straw) roots and rhizodeposition is usually too low to show a considerable saturation effect, although it was demonstrated that the total SOC pool has an upper limit (Stewart et al., 2007; Stewart et al., 2008). Soil OC in agricultural soils is mainly stored in mineral fractions < 20 μm and thus the capacity to store OC is mainly determined by the soil texture (Hassink, 1997). According to the author, the maximal soil C storage is dependent on climate, topography, parent material, organisms and soil properties, which can be expressed in various linear regressions of the maximum SOC content (Wiesmeier et al., 2019). Thus, for a specific soil texture the SOC storage can be estimated using the appropriate equation according to the climate, land use and clay type (Eq. (5) in this case). Each soil has a typical OC storage capacity, the amount of which generally remains static. However, the soil C and N contents are constantly circulating out into the environment, to be replaced by new C and N. In this way, they maintain a dynamic equilibrium (Six et al., 2004). This idea of the dynamic behavior of SOM addresses the point that the overall SOC storage is different from SOC retention (Chenu et al., 2018) since C retention is a process (Lal et al., 2015). Soil C retention depends on both C stabilization and destabilization. Recent studies on SOC retention focusing on MAOM demonstrated a more efficient C retention with the input of higher N availability compared to low N available OM (Cotrufo et al., 2013). However, this occurs only when the soil is unsaturated with OC in the MAOM fraction (Castellano et al., 2015; Lavallee et al., 2020). Studies on the destabilization of native SOC due to the addition of LMWOC demonstrated that the amount of soil C lost correlated with the N content of the added LMWOC substrate (Blagodatskaya et al., 2007; Keiluweit et al., 2015).

Yet, it is not well understood how the N content of OM input controls the rates of the rapid C retention in the MAOM pool, and how the SOC loading affects the short-term retention of freshly added C. Many studies focused on long-term experimental field trials but only a few pieces of information exist about the SOC stock on a very short timescale. Short-term incubation studies have been primarily concerned with microbial activities involving the LMWOC, and consequently missed the rapid LMWOC retention as MAOM on soil fine fractions.

Here, we performed a short-term experiment applying two LMWOC substrates on agricultural soils with different SOC saturation levels as a result of different long-term management. The objectives were to investigate how soil C loading and the N availability of the LMWOC impact the initial retention of C in the MAOM pool. We hypothesized that (1) the soil carbon saturation level influences the short-term retention of LMWOCs, and (2) the soil C loading and the N content of the substrate control the recovery of newly added OM in MAOM.

2. Materials and methods

2.1. Soil samples

To compare soils with different degrees of C loading, we sampled agricultural soils from long-term field experiments maintained by the Bavarian State Research Center for Agriculture (LfL). The experimental sites are located in Puch (southern Germany, 48°11′37.0″N, 11°12′57.4″E) at an elevation of 550 m a.s.l. with a mean annual temperature of 8.8 °C and mean annual precipitation of 872 mm. The experiments comprised sites under different crop rotations and tillage systems with a wide SOC gradient as a result of different C inputs over several decades. For this study we selected a site (10 × 15 m) with a typical rotation including winter wheat (Triticum aestivum), grain maize (Zea mays), winter rapeseed (Brassica napus) and spring barley (Hordeum vulgare) under direct seeding started in 1992 and a bare fallow (5 × 50 m) experiment started in 1953, where no crops were cultivated and potential C input by germinating plants was prevented by monthly plowing. The two experimental sites are in close vicinity and comparable in terms of soil mineralogy (see Fig. A.1 in appendices). Soils were characterized as Luvisols derived from Loess deposits (IUSS Working Group WRB, 2015). Soil samples were taken from the upper 10 cm of the Ap horizon from 20 random locations of each plot and thoroughly mixed to form composite samples. The fresh soil material was

Table 2
Soil texture of low-C and high-C soil before incubation, demonstrating as distribution of clay, silt and sand.

Fractions	Clay (%)	Fine silt (%)	Medium silt (%)	Coarse silt (%)	Fine sand (%)	Medium sand (%)	Coarse sand (%)
Particle size (μm)	< 2	2–6.3	6.3–20	20–63	63–200	200–630	630–2000
Low-C soil	28.6	11.1	22.8	25.4	7.0	3.8	1.3
High-C soil	38.8	10.1	18.5	17.4	8.1	5.3	1.8

air-dried at room temperature and subsequently sieved to 2 mm and macroscopic plant residues were picked out by hand. Basic soil characteristics and texture of the two soils are given in Table 1 and Table 2.

Soil pH and electrical conductivity were measured in the soil suspension (soil to water ratio of 1:5 (w/v)) after shaking for 1 h and sitting for 1 h. The Fe was extracted by citrate bicarbonate dithionite procedure according to Holmgren (1967), and measured by using inductively coupled plasma optical emission spectrometry (ICP-OES, Vista Pro CCD Simultaneous, Varian, Darmstadt, Germany).

For soil texture analyses, all soils were treated with 30%–H_2O_2 to remove OC, and subsequently with 0.0025 M sodium pyrophosphate ($Na_4P_2O_7$) to ensure dispersion of primary particles. Subsequently, ultrasonic dispersion was performed in 0.0025 M $Na_4P_2O_7$ suspension at 450 J ml^{-1}. Wet sieving was used to separate the particle size classes 630–2000 μm, 200 – 630 μm, 63 – 200 μm and < 63 μm. The suspensions < 63 μm were freeze-dried and an aliquot was analyzed after resuspending in 0.0025 M $Na_4P_2O_7$ using x-ray absorption (Sedigraph III Plus (Micromeritics, Aachen, Germany).

Carbon and nitrogen contents of both bulk soil materials were determined before the experiment in duplicate by dry combustion using a CN elemental analyzer (HEKAtech, Wegberg, Germany). The soil under bare fallow revealed C and N contents of 6.4 mg g^{-1} and 0.8 mg g^{-1}, respectively, and the soil managed by direct seeding revealed C and N contents of 17.9 mg g^{-1} and 1.9 mg g^{-1}, respectively. In the following we refer to bare fallow as low-carbon (low-C) and to direct seeding as high-carbon (high-C) soil. As the studied soils show no differences in clay mineralogy (Fig. A1, in appendix), it can be assumed that the formation processes of mineral-associated OM are comparable between the soils.

2.2. Low-molecular-weight organic substrate experiment

To elucidate the direct incorporation of freshly added substrates with differing C/N ratios we used two low-molecular-weight (LMW) substrates, both enriched in ^{13}C and one enriched in ^{15}N (D-Glucose-$^{13}C_6$, Sigma-Aldrich, ≥ 99 atom% ^{13}C and algal amino acid mixture, Sigma-Aldrich, ≥ 98 atom-% ^{13}C and ≥ 98 atom-% ^{15}N). This approach allowed us to trace the fate of C and N into soil microstructures of the two soil materials with different C loading. The glucose was chosen as an extreme substrate that contained no N. The amino acid mixture was chosen due to its N being delivered together with C, whilst the mixture of 16 amino acids weakened the impact of a certain group of amino acids.

The amount of C input with these substrates was based off of the mean C input (3.2 t ha^{-1} yr^{-1}) at the research site Puch (Wiesmeier et al., 2014a). This was done under the assumption that the majority of the C input localizes in the top 10 cm of the soil with a bulk density of 1.3 g cm^{-3}, thus accordingly the calculated C input is 0.82 mg g^{-1} soil. In brief, five grams (dry weight) of sieved fresh soil were filled into a 100 mL centrifuge tube (Ultra-High Performance, VWR). Each treatment including the control was set up with 3 replicates. To allow homogeneous mixing of the glucose and amino acid with the soils, the LMW substrates were added as solutions. The LMW substrates were weighed into beakers and dissolved into 25 mL deionized water to obtain the solution of 2.183 mg glucose mL^{-1} and 2.169 mg amino acid mL^{-1}, respectively. For the treatment setup, 25.2 μL solution, containing 0.273 mg ^{13}C, was added into the tube for each gram of soil. Furthermore, the amino acid treated soil obtained 0.18 mg ^{15}N g^{-1} soil via the substrate addition. All substrate-amended soil samples were brought to the moisture level of 50% field capacity. The same volume of deionized water was added to the control group to adjust the moisture to the same level as the LMWOC amended soils. In each setup, a glass rod was used to stir and homogenize the mixture. The tubes were sealed subsequently with lids and were kept in dark at 20 °C for 24 h. After 24 h all samples were freeze-dried to directly disrupt any biological activity and yield dry soil material.

2.3. Density fractionation

To obtain the clay-sized mineral-associated OM fraction, a density fractionation procedure adapted from Golchin et al. (1994) was used. The procedure ensured a good separation of particulate organic matter (POM) and mineral-associated organic matter (MAOM), to avoid any bias from translocated POM in the later-to-be analyzed microaggregate MAOM samples.

In brief, approximately three grams of freeze-dried soil sample were used for the soil fractionation. The soil samples were submerged in 50 mL sodium polytungstate (NaPWO4) with a density of 1.8 g cm^{-3} and dispersed using an ultrasonicator with an energy input of 480 J mL^{-1}. After centrifugation (3500 rpm, 30 min, 20 °C), the floating light particulate OM (POM) fraction was collected via a vacuum system. Both light fraction and heavy fraction (MAOM) were washed with deionized water using a pressure filtration (0.45 μm filter pore size) to remove excess salt until the electric conductivity dropped below 50 μS cm^{-1}. The heavy fraction was separated into three size fractions - clay (<2 μm), fine silt (2–6.3 μm) and silt and sand (>6.3 μm) - by sedimentation and subsequently freeze-dried.

2.4. Chemical and mineralogical analysis

The ^{13}C and ^{15}N enrichment, as well as the total carbon (C) and nitrogen (N) concentrations of bulk soils and soil fractions after the adsorption experiment, were measured by an Isotope ratio mass spectrometer (IRMS, Delta V Advantage, Thermo Fisher, Dreieich, Germany), coupled to an elemental analyzer (EuroEA, Eurovector, Pavia, Italy). The enrichment is given in atom-% (at%).

The multi-point BET method (Chiou et al., 1993; Heister, 2014) was used to determine the specific surface area (SSA) of clay and fine silt-sized MAOM fractions. Nitrogen was employed as an adsorbate. The samples were outgassed at 40 °C for 23 h and then measured at 77 K.

X-ray powder diffraction (XRD) was carried out on a Philips PW 1820 diffractometer, using Co Kα radiation (λ = 1.7902 Å) for all clay and fine silt-sized mineral fractions. The freeze-dried powder samples (random powder preparation) were measured from 2° to 26° 2θ in steps of 0.02° 2θ with a counting time of 5 s for each step. The mineralogy was assigned according to Moore and Reynolds Jr (1989). Each mineral phase was determined by semiquantitative evaluation in accordance with Niederbudde (1973) and Stanjek and Häusler (2000).

2.5. Calculations and statistical analysis

In the clay and fine silt-sized fractions, the ^{13}C excess and ^{15}N excess were calculated using Eq. (1):

$$Excess_x(at\%) = At_{fraction}(at\%) - At_{control}(at\%) \quad (1)$$

The x stands for the isotopes ^{13}C or ^{15}N. The $Excess_x$ represents the ^{13}C excess or ^{15}N excess. $At_{fraction}$ and $At_{control}$ represent the ^{13}C at% or ^{15}N

Table 3
Total C and N contents and C to N ratios of bulk soil, clay and fine silt-sized fractions of low-C soil and high-C soil after 24 h of incubation.

		Total C content (mg *g^{-1})			Total N content (mg *g^{-1})			C to N ratio		
Soil		Control	Glucose	Amino acid	Control	Glucose	Amino acid	Control	Glucose	Amino acid
Low-C	Bulk soil	6.1 ± 0.0	6.6 ± 0.1	6.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	8.7 ± 0.0	8.9 ± 0.1	8.1 ± 0.0
	Clay	23.8 ± 0.6	23.6 ± 0.3	23.1 ± 0.1	3.4 ± 0.1	3.3 ± 0.0	3.4 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	6.8 ± 0.1
	Fine silt	7.2 ± 1.2	8.4 ± 0.7	9.2 ± 0.9	0.9 ± 0.2	1.0 ± 0.1	1.1 ± 0.2	8.4 ± 0.5	8.2 ± 0.3	8.3 ± 0.4
High-C	Bulk soil	17.4 ± 0.3	18.7 ± 0.5	19.0 ± 0.1	1.8 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	9.9 ± 0.0	10.0 ± 0.2	9.7 ± 0.1
	Clay	39.8 ± 4.6	38.7 ± 1.7	39.3 ± 0.4	4.9 ± 0.4	4.8 ± 0.1	4.7 ± 0.1	8.0 ± 0.4	8.3 ± 0.1	8.3 ± 0.2
	Fine silt	24.4 ± 0.6	27.3 ± 1.4	30.0 ± 0.2	2.1 ± 0.1	2.3 ± 0.1	2.6 ± 0.2	11.6 ± 0.4	11.3 ± 0.4	11.6 ± 0.2

Table 4
The SOC saturation level of the low-C and high-C soil used for the incubation experiment. The calculation of the SOC saturation level was adapted from Wiesmeier et al. (2019).

	Fraction < 6.3 μm (%)	SOC capacity (mg *g^{-1})	SOC content of fraction < 6.3 μm (mg *g^{-1})	SOC saturation level
Low-C soil	40	14	4.8	33%
High-C soil	49	17	12.9	75%

at% values of the size fraction and control, respectively.

The substrate-derived isotope in each fraction was calculated using Eq. (2):

$$f_x = Excess_x(at\%)/At_{substrate}(at\%) - At_{control}(at\%) \quad (2)$$

The fx represents the proportion ^{13}C or ^{15}N derived from the substrate. $At_{substrate}$ and $At_{control}$ represent the ^{13}C at% or ^{15}N at% values of the substrate and control, respectively.

The amount of substrate derived ^{13}C ($Substrate_x$) in each fraction was calculated using Eq. (3):

$$Substrate_x(mg*g^{-1}) = f_x*c\ (mg*g^{-1}) \quad (3)$$

where the c is the C content of the fraction, using mg *g^{-1} as the unit.

The recovery of ^{13}C (R_x) of the bulk soil in each fraction was calculated using Eq. (4):

$$R_x(\%) = Substrate_x(mg*g^{-1})*f(\%)/C_{input}(mg*g^{-1}) \quad (4)$$

where the f is the content of the size fraction and C_{input} is the C input of the bulk soil, expressed in mg *g^{-1}.

The recovery of ^{15}N is calculated with the same principle.

The estimation of the SOC storage capacity was adapted from Feng et al. (2014) and Wiesmeier et al. (2019) using Eq. (5):

$$Capacity_{SOC} = 1.68 + 0.32*ff < 6.3\mu m \quad (5)$$

The $ff < 6.3\mu m$ represents the content of the mineral fractions smaller than 6.3 μm, determined by soil texture analysis.

The C content in the fraction smaller than 6.3 μm in the soil samples was calculated according to Eq. (6):

$$C\%_{<6.3\mu m}(mg*g^{-1}) = C\%_{Clay}(mg*g^{-1})*f_{Clay}\% + C\%_{Finesilt}(mg*g^{-1})*f_{Finesilt}\% \quad (6)$$

Where $C\%_{Clay}$ and $C\%_{Finesilt}$ stand for the C content of clay and fine silt-sized fractions, using mg *g^{-1} as the unit. The $f_{Clay}\%$ and $f_{Finesilt}\%$ represent the clay and fine silt-sized fraction content of the soil.

The SOC saturation level ($L_{Saturation}$) of the soils was calculated using Eq. (7):

$$L_{Saturation}(\%) = C\%_{<6.3\mu m}\ (mg*g^{-1})/Capacity_{SOC}(mg*g^{-1})*100(\%) \quad (7)$$

A one-way analysis of variance (ANOVA) with Tukey test was carried out using Origin 2020 (OriginLab) for statistical analysis of the IRMS and BET datasets. Tow treatments in two soils were treated as 4 samples in the statistical analysis. Differences between subsamples within each fraction were tested separately. The differences were considered significant when $p < 0.05$. Other data, except the soil texture, are presented as mean values of the three replicates with standard deviation. The soil texture was measured using a larger quantity of the homogenized bulk soil that was used for the incubation experiments without replicates and thus no standard deviation is given.

3. Results

3.1. Total carbon and nitrogen content

After 24 h of incubation, total C and N contents were measured for the recovered fine mineral fractions. Since the amount of the LMW substrates (0.27 mg C *g^{-1} dry soil) was low compared to the native SOM, all incubated soils showed no change in their total C and N content (Table 3), due to the OM addition. The C-to-N ratio of the incubated soils was 8.7 (low-C soil) and 9.9 (high-C soil). In both soils, the clay fractions showed higher C contents than the fine silt fractions. The clay fractions ($n = 3$) of the low-C soil contained 23.5 ± 0.5 mg *g^{-1}C and 3.4 ± 0.1 mg *g^{-1}N (Table 3) after the 24 h incubation. The C and N contents of the fine silt-sized fraction of the low-C soil were 8.3 ± 1.24 mg *g^{-1} and 1.0 ± 0.2 mg *g^{-1}, respectively. The high-C soil contained 39.2 ± 2.3 mg *g^{-1}C and 4.8 ± 0.3 mg *g^{-1}N in the clay-sized fraction while it contained 27.2 ± 2.8 mg *g^{-1}C and 2.4 ± 0.3 mg *g^{-1}N in the fine silt-sized fraction.

3.2. SOC saturation level

The bulk soil capacity to store SOC was estimated according to Feng et al. (2014) using Eq. (5). The calculated capacity for low-C soil and high-C soils is 14 mg C and 17 mg C of each gram of soil (Table 4), respectively. The SOC content in the fine mineral fraction (<6.3 μm) of the low-C soil was 4.8 mg *g^{-1}, while the high-C soil contained 12.9 mg *g^{-1} SOC. The calculation of the SOC saturation level (Eqs. (6) and (7)) demonstrates that the low-C soil has a low saturation of 33%, while the SOC saturation of the high-C soil reaches 75% of the calculated potential SOC storage capacity.

3.3. Recovery of substrate-derived ^{13}C and ^{15}N in MAOM fractions

The ^{13}C excess and ^{15}N excess were calculated based on the ^{13}C and ^{15}N contents in the clay and fine silt-sized OM fractions according to Eq. (1) (Fig. 1, A, B). For both clay and fine silt-sized MAOM of low-C soil and high-C soil, it could be demonstrated that the glucose and amino acid addition lead to a significant enrichment in ^{13}C (Fig. 1, A). For the ^{13}C excess of fine silt-sized MAOM of the low-C soil, no significant differences were detectable both for glucose and the amino acid treatment, with a ^{13}C excess of around 0.7%. For the high-C soil a lower ^{13}C excess compared to the low-C soil was demonstrated for all MAOM fractions (Fig. 1, A). Except for the glucose treatment of the low-C soil, the clay-sized fraction showed a greater ^{13}C excess and ^{15}N excess (Fig. 1, B) than the fine silt-sized fraction. For the low-C soil MAOM fractions, the clay-

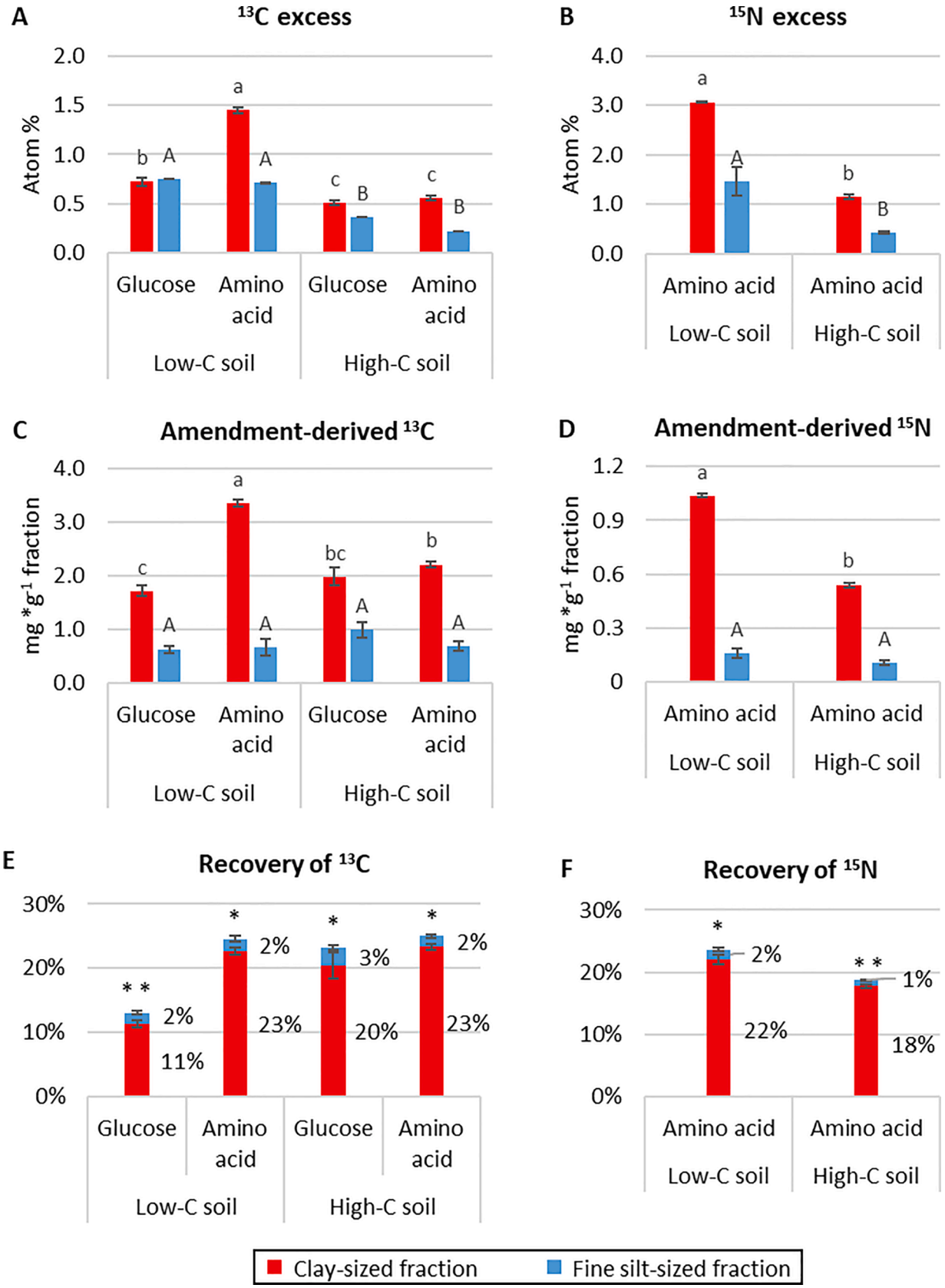

Fig. 1. ^{13}C excess (A), ^{15}N excess (B), substrate-derived ^{13}C (C) and ^{15}N (D) of clay and fine silt-sized MAOM of low-C and high-C soils after 24 h incubation with glucose or amino acid addition. The recovery of ^{13}C (E) and ^{15}N (F) of clay and fine silt-sized fractions of low-C and high-C soils after 24 h incubation with glucose or amino acid addition. Lowercase letters, capital letters and asterisks (*) represent significant differences ($P < 0.05$) between clay-sized MAOM fractions, fine silt sized MAOM and clay plus fine silt-sized MAOM, respectively.

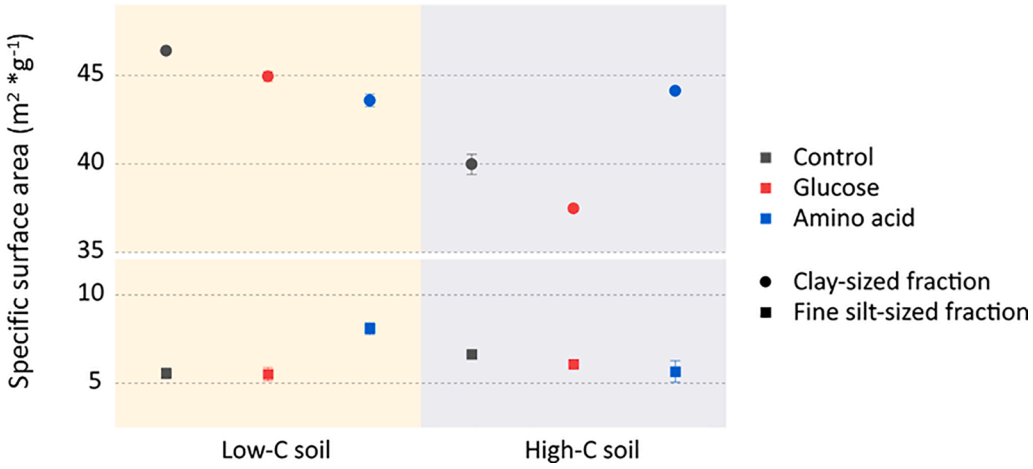

Fig. 2. BET analysis on clay and fine silt-sized fractions of each treatment.

sized fraction amended with amino acids showed a ^{13}C excess of 1.4%, which was twice the glucose-amended soil fractions (0.7%). For both soils, a two-times higher ^{15}N excess was demonstrated for the clay-sized MAOM fractions (3.1% for low-C soil, 1.1% for high-C soil) compared to the fine silt-sized MAOM fractions (1.5% for low-C soil, 0.4% for high-C soil).

The substrate-derived ^{13}C and ^{15}N demonstrated a higher amount for the clay-sized MAOM than the fine silt-sized MAOM (Fig. 1, C, D). In both low-C and high-C soils amended with glucose and amino acid, the substrate-derived ^{13}C ranged between 1.7 and 3.3 mg $*g^{-1}$ on clay-sized MAOM fractions but that of fine silt MAOM fractions were below 1 mg $*g^{-1}$ (Fig. 1, C). In the amino acid amended soils, 1.0 mg $*g^{-1}$ (low-C soil) and 0.5 mg $*g^{-1}$ (high-C soil) of the substrate-derived ^{15}N retained on the clay-sized MAOM, 5 times higher than the fine-silt MAOM (0.2 mg $*g^{-1}$ for low-C soil, 0.1 mg $*g^{-1}$ for high-C soil).

After 24 h of incubation, the total recovery of the substrate-derived ^{13}C in the bulk soils of all treatments ranged between 45% – 53% (data not shown). The MAOM of the clay-sized fraction retained a larger amount of substrate-derived C and N than the fine silt fraction. In the low-C soil, 11% of the glucose-derived C was retained in the clay-sized fraction, while 20% glucose-derived C was retained in the clay-sized fraction of the high-C soil. The recovery of fresh amino acid derived ^{13}C in the clay fraction of both soils was 23%. However, only 2–3% of substrate-derived C was recovered in the fine silt-sized fractions (2–6.3 μm) for both glucose and amino acid substrates.

3.4. The specific surface area of fine-sized mineral fractions

The specific surface area (SSA) of clay and fine silt-sized fractions of each treatment (Fig. 2) were determined by BET analyses (Mayer, 1994b). For the clay-sized fractions of the control treatments, the SSA of the low-C soil (46.4 $m^2 *g^{-1}$) was 6.4 $m^2 *g^{-1}$ larger than the SSA of the high-C soil. However, the SSA of the fine silt-sized fractions of the low-C soil was 1 $m^2 *g^{-1}$ lower than that of the high-C soil (6.6 $m^2 *g^{-1}$). The SSA of the clay-sized fractions ranged between 35 and 47 $m^2 *g^{-1}$, and the SSA of fine silt-sized fractions are considerably lower, ranging between 5 and 10 $m^2 *g^{-1}$. The SSA for both clay and fine silt-sized fractions of both treatments of the low-C soil are significantly different ($P < 0.05$). For the high-C soil, significant differences ($P < 0.01$) in the SSA were found for the clay-sized fractions between both treatments, whereas no significant differences were found for the fine silt-sized fractions between treatments. The addition of glucose led to a decrease of the SSA compared to the control in the clay and fine silt-sized fractions of both soils. For the clay-sized fractions of the low-C soil, the SSA follows the order, $SSA_{Control} > SSA_{Glucose} > SSA_{A.A.}$. However, the amino acid substrate resulted in an increased SSA of the fine silt-sized fraction of the low-C soil and the clay-sized fraction of the high-C soil.

4. Discussion

4.1. Rapid MAOM formation after addition of low molecular weight OC

The freshly added LMWOC was recovered in both analyzed MAOM fractions, associated with fine silt and clay-sized minerals. Due to the very short timescale of the experiment, we assumed that the OM recovered as new MAOM-associated C was mainly due to the direct association of the added LMWOC with the mineral surfaces and to a lower extent by microbially transformed OM. If this assumption holds true, the amino acid derived OM $^{13}C/^{15}N$ ratios post-incubation would be close to the original amino acid $^{13}C/^{15}N$ value (2.91). We assumed clay minerals as the main mineral factor that determines MAOM formation due to the rather low contents of dithionite extractable Fe in the soils (9.8 mg$*g^{-1}$ for low-C soil and 14.2 mg$*g^{-1}$ for high-C soil, respectively, Table 1) (McKeague and Day, 1966).

However, we demonstrated that the substrate-derived $^{13}C/^{15}N$ ratio (between 3.28 and 6.29) (Table A2, in appendices) of the MAOM was higher than the $^{13}C/^{15}N$ ratio of the substrate itself (2.91), thus clearly pointing to the fact that after 24 h of substrate addition, part of the new MAOM is already microbially transformed. This assumption is supported by the results of a short-term glucose substrate experiment by Geyer et al. (2020), who found 30% of amended C was recovered as microbial residues in bulk soils after only six hours. Moreover, this assumed rapid microbial transformation is more pronounced in the soil with a high C loading. This is shown by $^{13}C/^{15}N$ ratios of the amino acid derived OM in the low-C soil (3.28) being lower (closer to the original amino acid $^{13}C/^{15}N$ value) than its counterpart in the high-C soil (4.16). Our finding is in accordance with other studies, that demonstrated such rapid microbial transformation. For instance, in an incubation experiment amending ^{14}C glucose and ^{15}N-NH_4 to a Vertisol and Alfisol Ladd et al. (1996) demonstrated that after three days of incubation the organic $^{14}C/^{15}N$ ratio was higher in the soil that contained more C. Poirier et al. (2013) also reported similar results for a 51-day incubation experiment by studying the effects of maize residue on the fate of the fresh OM in soils with differences in stored C (topsoil vs. subsoil). The authors demonstrated that the residue-derived C/N ratios were closer to the original residue's C/N ratio in the soil with lower C content compared to the soil with higher C content.

This supports our assumption that the retention of fresh OM as MAOM in the fine-sized fractions of soils with different C loadings is driven by different pathways, which can be due to the microbial activity. In addition, differences in the microbial community structure between the two soils might have fostered the reported differences in the fate of

the freshly added OM. The C-rich soils sustain a higher microbial activity, which also may lead to higher amounts of microbial-derived MAOM. This is in accordance with studies that used glucose addition to study the correlations between glucose-derived C and microbial biomass. For instance, Van Veen et al. (1985) found that native biomass C was highly correlated with glucose-derived biomass C after 44 and 66 weeks of glucose addition. In a four-week incubation experiment by Creamer et al. (2016), the authors demonstrated that the initial amount of soil microbial biomass was one of the main drivers for the formation of new SOC.

As the low-C soil and high-C soil have differences in their pH (6.4 and 7.6, respectively), we have to consider the potential role of soil pH on the retention of the amino acid derived OM. Decreasing soil pH promotes the adsorption of basic amino acids over acidic and neutral ones in the soil (Gao et al., 2018; Henrichs and Sugai, 1993; Vinolas et al., 2001), as negatively charged mineral surfaces enhance the sorption of positively charged molecules. However, we demonstrated that the amino acid derived OM was more efficiently retained in clay-sized fractions of the soil with lower pH than the soil with higher pH, whereas the amount of the amino acid derived OM retained on the fine silt-sized fraction of two soils did not show a significant difference. It can be assumed that soil pH is highly unlikely a dominant controlling factor for the retention of the added amino acids. Otherwise, the reduced amount of retained amino acid derived OM should have been demonstrated in both fine-sized fractions between the two soils. As stated above, we also assume that the added amino acid has been rapidly transformed by microorganisms and thus the retention of microbial-derived OM might be less affected by differences in soil pH. We thus demonstrate that even on very short timescales, the contribution of microbial transformed OM to the formation of MAOM should not be overlooked.

4.2. C loading impacts short-term fresh organic matter retention in fine-sized fractions

Our results indicated that the fine-sized mineral fractions (<6.3 μm) of the low-C soil are clearly depleted in SOC (Table 4, with a much lower C loading of 33% compared to the mean saturation level of Bavarian cropland soils of 50% (Wiesmeier et al., 2014b). In contrast to the low-C soil, the saturation level of the high-C soil of 75% is well above this average SOC saturation level reported by Wiesmeier et al. (2014b). This demonstrates the significant differences in cropland SOC storage dominated by MAOM, as regulated by long-term differences in C input. Based on the two soils in our study, it was shown that soils deficient in SOC are more efficient in retaining fresh OM compared to already well-saturated soils. This was indicated by the higher isotopic enrichment of the OM in the fine mineral fractions of the low-C soil, compared to those of the high-C soil (Fig. 2, A, B). Our findings on SOC saturation level and fresh OM retention potential are in agreement with Stewart et al. (2007), who showed that soils far below their specific SOC storage capacity show a greater efficiency in sequestering new C. The difference in the SOC loading of the low-C and high-C soil resulted in clear differences in the retention of freshly added OM, thus the initial soil C loading is decisive for the retention of newly added OM also in the short-term. Thus, as pointed out in the long-term perspective (Chenu et al., 2018; Lavallee et al., 2020), we can now demonstrate that the OC saturation level of the MAOM is also important for the overall SOC sequestration rate at an early stage.

We recovered around 50% of fresh OM in the bulk soil for all samples. These amounts of recovered freshly added OM are in the range of values (~60% for glucose and ~80% for amino acid in bulk soil) reported for other short-term experiments (24 h), in which glucose was amended to temperate forest soils (Typic Dystrudepts of the Gloucester series) (Geyer et al., 2020) and amino acids were added to forest soils (Jones and Kielland, 2002). The newly added OM that retained in the bulk soil but was not recovered as MAOM in the analyzed fine-sized mineral fractions were probably retained as MAOM in the fraction > 6.3 μm. Besides that, a considerable amount might also be still in the dissolved OM phase or be associated with particulate OM. The retention of fresh OM occurred primarily in the clay-sized fraction (up to 23% of the added OM) (Fig. 1, E, F), which illustrates the high importance of the fine mineral fractions (clay-sized minerals) for the sequestration of rather labile OM inputs in soils (Christensen and Sørensen, 1985; Kaiser and Zech, 2000). The retention of substrate-derived OM in the clay-sized fraction was substantially above the level recovered in the fine silt-sized fraction. This distinct difference can be suggested to be due to the differences in the specific surface area (SSA) of both fractions. The mineral surface area was previously shown to closely correlate with the amount of associated OM (Mayer, 1994a; Saggar et al., 1996; Sarkar et al., 2018) and the SSA decreases with the increasing particle size (Keil et al., 1994). The clay-sized fractions with greater SSA provide a larger number of reactive sites available for the sorption of OM, and therefore, retained more fresh OM than the fine silt-sized fractions. We assume that the freshly added LMWOC in the low-C soil is likely to bind with free mineral surfaces via organo-mineral interactions rather than with existing MAOM via organo-organo interactions (Possinger et al., 2020). This is supported by Gao et al. (2018), who demonstrated that minerals with dissolved organic matter coating suppressed the further adsorption of acidic amino acid. The higher C content of the MAOM can thus be the reason for the high-C soil being less efficient in retaining amino acid than the low-C soil. A higher soil C loading results in fewer possible interactions of free mineral surfaces with OM, with this type of interaction being considered as the main factor in the persistence of SOM (Lützow et al., 2006; Sollins et al., 1996).

4.3. Does N content of substrate determine short-term fresh OM retention in clay fractions?

In the low-C soil, the N-rich substrate led to twice as much retained freshly added LMWOM as the substrate without N, thereby indicating the importance of N content of freshly added OM for soil C sequestration. With the addition of glucose (N-free substrate), the high-C soil, which also contained more N, retained more substrate-derived OM than the low-C soil (Fig. 1, C, E). This suggests the initial soil N content might be a limiting factor in the sequestration of fresh OM inputs in the MAOM pool. The fact that a high N content enhanced fresh OM sequestration is in accordance with studies that tested the effects of the addition of N on the SOC stock. In a glucose tracing study with and without N addition using soils from a temperate forest, Wang et al. (2019) demonstrated a higher increase of SOC when glucose was added together with N. The positive effect of higher N availability on SOM formation was not only demonstrated using LMWOM but also with more complex natural plant litter. A positive relation was observed between the N content of added plant litter and C accumulation in the soil MAOM pool (Córdova et al., 2018). This is nicely corroborated by a *meta*-analysis spanning over 257 soils under various land uses, in which Lu et al. (2011) showed a significant increase of the soil C pool (3.5% for agricultural soils) due to N fertilization.

Depending on the soil C loading, the recovery of fresh OM differed considerably between the two LMW substrates (Fig. 1, C to F). We show that the N content of the substrate determines the retention of fresh OM in the low-C soil (Fig. 2, C), which leads to the assumption that, for degraded soils with low C saturation, the addition of OM with higher N contents supports the build-up of mineral-associated OM. However, the substrate with high N content (amino acids) did not enhance the recovery of fresh OM in the clay-sized MAOM fraction (Fig. 1, E). Although the high-C soil contains more clay-sized minerals, which was previously shown to be positively related to the retention of OM (Guillaume et al., 2022; Hassink, 1997), in the present study this did not lead to a higher retention of fresh LMWOC, which demonstrates a decoupling of the retention of fresh LMWOC in the form of MAOM in C saturated soils. In accordance with our results, Creamer et al. (2014) observed no

enhancement in glucose-derived SOC with N addition. The authors used an agricultural soil with very low clay content (1.7%) and thus a higher saturation level of OC in the MAOM pool. Based on our findings for the retention of fresh OM in a highly saturated soil, we assume that as soon as soils reach a C storage level that is close to the saturation level (Stewart et al., 2007), the N content of the input is no longer decisive for the build-up of new MAOM. Although studies reported the addition of N increased the C stock in the MAOM pool (Diekow et al., 2005), in soil microaggregates (Kirkby et al., 2014) and at the bulk soil scale (Hyvönen et al., 2008), we question this relationship between the substrate N content and the amount of stored freshly added OM at the scale of functional SOM pools. The importance of the N content of the organic substrate for the sequestration of freshly added OM can be assumed to be lower at higher N availability in native MAOM. Mineral-associated OM as an important microbial N source is also suggested by Guillaume et al. (2022). The authors demonstrated that cropland soils with lower MAOM C saturation level showed a stronger N depletion in their particulate OM than grassland soils. In our study, the N-rich substrate did not significantly impact the storage of fresh OM in the high-C soil, indicating the soil C loading; and thus the soil C saturation level, as an important regulator in retaining freshly added OM. We suggest that C retention in the MAOM pool of soils with high C contents is primarily controlled by the soil C saturation level rather than by the N content of the OM input. This is supported by a model study on agricultural and forest soil (Castellano et al., 2015), demonstrating that the N availability of litter input affected SOC storage based on the SOC saturation level.

On the other hand, the chemical structure of the added LMWOC can be assumed to foster the different retention of the added OM in the soil, as the functional groups of OM influence their adsorption (Henrichs and Sugai, 1993). Glucose adsorbs to soil minerals through the hydroxyl (R-OH) groups and the sorption of amino acid occurs mainly by amino (R-NH_2) or carboxyl (R-COOH) groups (Johnston and Tombacz, 2002; Thompson and Goyne, 2012) and thus, the sorption of amino acid depends on the soil pH. The negative charge of clay minerals does not change with pH. The lower pH indicates a possible higher positive charge on mineral surfaces, which would not benefit the cation absorption. A larger amount of basic amino acid absorbed in soil compared to neutral and acidic ones, which was reported at the soil pH at 6.9 (Jones and Hodge, 1999). This indicates that acidic amino acids would be stronger sorbed in the studied low-C soil. As acidic amino acid makes up 50% of the used amino acid mixture, this might have led to the lower amount of amino acid retained in the soil with higher pH. Beyond the properties of the fine-sized soil minerals, the composition of the MAOM might also directly affect the sorption of the amino acid (Gao et al., 2018). The authors demonstrated that the amount of absorbed amino acids varied on the mineral surface coated with O horizon-derived dissolved OM and leaf litter-derived dissolved OM. As due to the different management the studied fine-sized mineral fractions showed different C/N ratios, one can assume that this is also a driver for the demonstrated differences in the retention of fresh OM.

As the specific surface area of the mineral fractions is negatively related to the sorption of OM (Kaiser and Guggenberger, 2000), we expected a decrease in SSA with adding fresh LMWOC. We also expected the high-C soil to present a lower SSA than the low-C soil due to a higher surface coverage with SOM. Although the amino acid substrate was highly retained in the clay-sized fraction of the high-C soil, demonstrated by the isotopic enrichment of the OM (Fig. 2, A), we observed an increase of the clay SSA due to the LMWOC addition. This could potentially indicate a loss of native MAOC that could be accelerated due to increased N availability due to the addition of amino acids. This assumption is supported by Blagodatskaya et al. (2007), who found a higher native C lost under C-limiting conditions but not under N-limiting conditions. This points to a possible increased microbial consumption of native C fostered by the low C/N input material.

5. Conclusions

Our short-term incubation experiment indicated that the short-term recovery of freshly added low-molecular-weight organic compounds (LMWOC) in the MAOM pool is controlled by the soil C saturation level and the N content of the added substrate. The freshly added OM retained preferentially in the smaller MAOM fractions, especially in the clay-sized fractions, pointing to the importance of the specific mineral surface area for C sequestration. We demonstrate that N-rich substrate only has a positive effect on the retention of fresh OM as MAOM in soils with low C saturation level. Therefore, we state that the initial soil organic matter content (especially N content) affects the sequestration of fresh OM to a greater extent than the N content of the OM substrate.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendices

Mass distribution

Table A1
Mass distribution of particulate organic matter (POM) and each mineral fraction using density fractionation (density of 1.8 g/cm^3).

	POM (mg *g^{-1})	Mineral fraction (mg *g^{-1})		
		< 2 µm	**2 - 6.3 µm**	**> 6.3 µm**
Low-C soil	2 ± 1.5	184 ± 5.3	69 ± 3.7	745 ± 9.3
High-C soil	14 ± 9.1	281 ± 9.1	73 ± 2.6	632 ± 14.8

Mineralogy

Using X-ray diffraction (XRD) analysis, it was possible to demonstrate similar mineralogy of the clay and fine silt fractions for both soils (Fig. A1); thus showing a high level of comparability between the fine-sized mineral fractions. The XRD spectra of all clay and fine silt fractions show major peaks that indicate four minerals: illite, chlorite, kaolinite and quartz. The overlapped peak of kaolinite and chlorite is located between 14° to 15° 2θ and a mixed layer of clay minerals is found between 7° to 10° 2θ. The content of main clay mineral phases in both soils follows the order: illite > chlorite > kaolinite. For each size fraction the proportion of every mineral phase is similar in the two soils, whereas the proportion of mineral phases between silt and clay is slightly different.

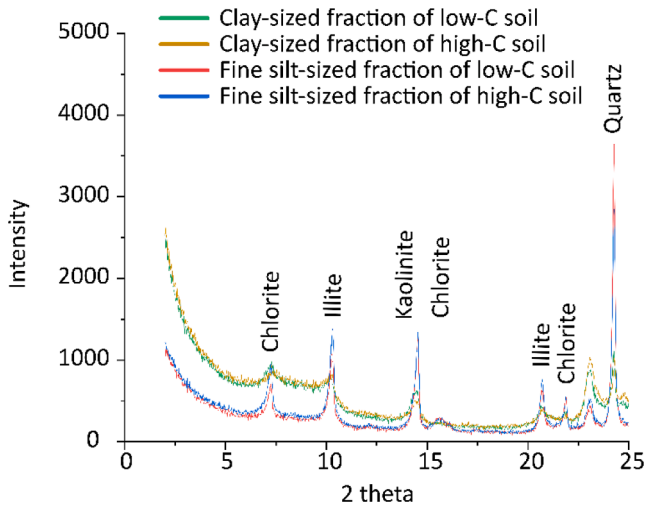

Fig. A1. XRD results of clay and fine silt-sized fractions of bare fellow (low-C) and direct seeding (high-C) soil.

Amino acid derived $^{13}C/^{15}N$ ratio

Table A2
The amino acid $^{13}C/^{15}N$ ratio and the amino acid derived $^{13}C/^{15}N$ ratio of clay-sized MAOM fractions of low-C and high-C soil after 24 hours incubation.

	Clay-sized fraction		Fine silt-sized fraction		Amino Acid
	Low-C Soil	**High-C Soil**	**Low-C Soil**	**High-C Soil**	
$^{13}C/^{15}N$ ratio	3.28 ± 0.01	4.16 ± 0.09	4.08 ± 0.21	6.29 ± 0.17	2.91

Amino acid mixture

Table A3
Relative composition of algal amino acid mixture.

Amino acid	Relative composition %	Amino acid	Relative composition %
ASP	40.6	**MET**	0.6
THR	3.9	**ILE**	1.3
SER	5.1	**LEU**	3.8
GLU	9.2	**TYR**	1.2
PRO	2.6	**PHE**	0.8
GLY	12.1	**HIS**	0
ALA	13	**LYS**	2.1
VAL	2.3	**TRP**	0

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