



Bioavailability and isotopic composition of CO₂ released from incubated soil organic matter fractions



Carsten W. Mueller^{a,*}, Martin Gutsch^b, Katja Kothieringer^{c,1}, Jens Leifeld^d, Janet Rethemeyer^e, Nicolas Brueggemann^{c,2}, Ingrid Kögel-Knabner^a

^a Lehrstuhl für Bodenkunde, TU München, Freising, Weihenstephan, Germany

^b Potsdam Institute for Climate Impact Research (PIK), Potsdam, Germany

^c Karlsruhe Institute of Technology (KIT), Institute for Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Garmisch-Partenkirchen, Germany

^d Agroscope Reckenholz-Tänikon ART, Zürich, Switzerland

^e Institute of Geology and Mineralogy, University of Cologne, Cologne, Germany

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ABSTRACT

The stabilization of soil organic matter (SOM) is triggered by three main mechanisms: (i) low bioavailability due to aggregation, (ii) recalcitrance due to the chemical structure, and (iii) association of the SOM with mineral surfaces. In the present study we used particle size SOM fractions (sand, silt and clay), derived from the Ah soil horizon from a Norway spruce forest in Southern Germany, to study the effects of different stabilization mechanisms on the bioavailability of soil organic carbon (SOC) in a one year incubation experiment. The respired CO₂ was hourly recorded, additionally ¹³CO₂ was analysed 20 times and ¹⁴CO₂ three times during the incubation experiment. To better differentiate between particulate OM (POM) and mineral associated OM (MIN), the incubated fractions and bulk soil were separated according to density (1.8 g cm⁻³) after the incubation experiment. ¹³C-CPMAS NMR spectroscopy was used to study the chemical composition of the incubated samples. We demonstrate a clear increase in SOM bioavailability due to aggregate disruption, as the calculated theoretical CO₂ evolution of the SOM fractions recombined by calculation was 43.8% higher in relation to the intact bulk soil. The incubated sand fraction, dominated by POM rich in O/N-alkyl C, showed a prolonged bioavailability of SOC moieties with mean residence times (MRT) of 78 years. Interestingly, the silt fraction, dominated by highly aliphatic, more recalcitrant POM, showed low mineralization rates and slow MRT's (192 years) close to values for the clay fraction (171 years), which contained a large amount of mineral-associated SOM. The recorded ¹³/¹²CO₂ signatures showed a high depletion in ¹³C during the initial stage of the incubation, but an enrichment of the respired ¹³CO₂ of up to 3.4‰ relative to the incubated SOM was observed over longer time periods (after 3 and 4 days for bulk soil and sand, respectively, and after 14 days for silt and clay). Therefore, we found no evidence for a ¹³C enrichment of SOM as driven by metabolic isotopic fractionation during microbial SOM mineralization, but an indication of a change in the isotopic composition of the C-source over time.

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1. Introduction

Soil organic matter (SOM) comprises a continuum of differently stabilized components. Whereas carbohydrate monomers in the

soil solution may be mineralized by microorganisms within minutes to hours, aliphatic plant residues and mineral bound SOM can reside untouched in the soil for centuries. The turnover of soil organic matter and thus its stabilization is driven by three main mechanisms: (i) low bioavailability due to aggregation, (ii) recalcitrance due to the chemical structure, and (iii) association of the SOM with mineral surfaces, e.g. clay minerals and pedogenic oxides (von Lützwow et al., 2008). These mechanisms also divide the SOM into pools of different bioavailability and therefore different turnover times. To study the effects of these mechanisms on SOM sequestration, physical fractionation procedures are used to

* Corresponding author. Tel.: +49 (0)8161 71 4423; fax: +49 (0)8161 71 4466.

E-mail address: carsten.mueller@wzw.tum.de (C.W. Mueller).

¹ Present address: Informationsverarbeitung in der Geoarchäologie, Otto-Friedrich Universität Bamberg, Germany.

² Present address: Forschungszentrum Jülich GmbH, Institute of Bio- and Geo-sciences, Agrosphere (IBG-3), Jülich, Germany.

separate chemically and physically different SOM compartments from the bulk soil. These stabilization mechanisms also divide SOM into pools of different bioavailability and therefore different turnover times, which can be estimated by ^{14}C analysis. A broad variety of physical fractionation procedures was developed to meet different soil properties and to minimize the production of artefacts (Amelung and Zech, 1999; Balesdent et al., 1998; Christensen, 1992; Hassink et al., 1997; Magid et al., 1996; Trumbore and Zheng, 1996). Across all different fractionation schemes the obtained SOM fractions mostly represent soil compartments of different chemical composition. Whereas larger fractions, like sand-sized particles or free particulate organic matter fractions (POM, light OM particles), are mostly comprised of material with high carbohydrate contents directly linked to the fresh OM input. Smaller fractions such as fine silt and clay sized particles, but also small POM, tend to show higher proportions of aliphatic and/or aromatic soil organic carbon (SOC) (Wagai et al., 2009). At the same time the stabilization of normally highly bioavailable carbohydrates was shown to be driven by the formation of organo-mineral associations in fine sized mineral fractions (Schöning et al., 2005).

In the last decade a number of laboratory incubation studies were carried out trying to elucidate the different SOM mineralization mechanisms within different soil fractions, also with respect to their susceptibility to temperature changes (Bol et al., 2003; Fang et al., 2005; Leifeld and Fuhrer, 2005). However, in most studies bulk soils were incubated, and the separation into differently stabilized SOM fractions was done afterwards. Thus, a continuous passage of OM from one pool to another was possible, for instance the release of labile dissolved organic carbon from fresh large sized POM (sand fraction/free POM according to particle size or density fractionation), leading to the priming of SOM mineralization in smaller fractions (small POM or mineral associated OM). The approach followed here, i.e. to directly incubate SOM fractions, has the main advantage to study the effect of different stabilization mechanisms on the potential mineralization rates of these differently stabilized SOM fractions separately, which was shown by the few studies carried out so far (Crow et al., 2007; Haile-Mariam et al., 2008; Plante et al., 2010). At the same time it is possible to directly study the isotopic composition of the respired CO_2 of the different fractions, in contrast to indirect assumptions derived from bulk soil CO_2 isotopic measurements. Furthermore, by the parallel incubation of natural aggregated bulk soil and the SOM fractions derived from this soil the effect of aggregation on SOM stabilization can be studied. Thus, the main objective of the present work was to determine the effect of accessibility/aggregation, recalcitrance and the association with minerals on the stability of SOC within different SOM fractions. For a detailed understanding of these processes and the sources of respired soil CO_2 we combined the measurement of heterotrophic respiration with $^{13}\text{CO}_2$ and $^{14}\text{CO}_2$ analyses in a long-term laboratory mineralization experiment. Especially the use of ^{14}C analyses allowed for the determination of mean residence times (MRT) which provides a good measure accounting for long term SOC stability.

2. Materials and methods

2.1. Soil material

The sampling site is located in Southern Germany (Höglwald, 48°17'24"N, 11°04'24"E) and stocked with an even aged Norway spruce forest (*Picea abies* (L.) Karst). To account for the natural variability at the sampling site, the soil material was randomly taken at 10 subplots (within a 20 m × 20 m area) from the Ah horizon of an acidic Albic Luvisol (IUSS Working Group, 2006) in 2005, air dried and sieved (<2 mm). For all further analysis a

composite sample of these 10 subsamples was used. As the study focused on the main characteristics of differently stabilized SOM fractions concerning their potential heterotrophic respiration, isotopic respired CO_2 composition and chemical composition, the composite sample provided a good proxy for widely distributed acid forest soils. However, prior to mixing the subsamples at equal mass and thus producing the composite sample, the 10 subsamples were analysed for C/N ratio (21.1 ± 1.2) and pH (pH in H_2O : 3.5 ± 0.1 , pH in CaCl_2 : 2.8 ± 0.1) to check natural variability. The coefficients of variation were 5.7% for the C/N ratio, 2.2% for the pH in H_2O and 2.4% for the pH in CaCl_2 , indicating a good representativeness of the used subsamples.

2.2. Physical fractionation

To obtain SOM fractions for the incubation experiment a particle size fractionation scheme (Fig. 1) was applied in order to avoid any use of density agents (e.g. sodium-polytungstate), which may negatively affect microbial activity (Crow et al., 2007; Swanston et al., 2002). In order to break up soil aggregates, the air dried bulk soil (<2 mm) was subjected to a two-step ultrasonication (Fig. 1), with the first step (60 J ml^{-1}) to break up macro-aggregates, and the second step (440 J ml^{-1}) to break up micro-aggregates (Amelung and Zech, 1999). The coarse and medium sand fraction, containing fresh POM, was separated after the first ultrasonication step with a sieve of 200 μm mesh size. The fine sand fraction was separated after the second ultrasonication step, using a sieve of 63 μm mesh size. The sand fractions were subsequently recombined according to their percentage of weight in the bulk soil. This 2-step approach was chosen to avoid the destruction and translocation of larger fresh OM particles of medium and coarse sand size into finer fractions. The fine silt and clay (<6.3 μm) was separated from the coarse and medium silt fraction (>6.3 μm –63 μm) by sedimentation at 20 °C. The fine silt and clay fraction was combined, as their chemical composition and percentage of organo-mineral associations is similar, while the time needed for sedimentation of the fine silt/clay mixture is almost 10 times shorter than for pure clay (<2 μm). Thus, in the following the fractions are called sand (63 μm –2000 μm), silt (6.3 μm –63 μm) and clay (<6.3 μm). During the wet sieving and sedimentation a continuous extraction of water extractable OC takes place, which ends up in the clay fraction. The loss of this highly bioavailable OC from the sand and silt fraction, however, is suggested to have only an effect during the first 2–3 days of the incubation experiment as shown by Mueller et al. (2012) for sequentially extracted soil samples (see Discussion).

To evaluate the content and chemical composition of POM versus mineral associated OM (MIN) of the three incubated particle size fractions (sand, silt and clay) a subsequent density fractionation was applied to aliquots of the particle size fractions. In brief, aliquots (replicated) of 5 g of every particle size fraction ($n = 3$) before and after the incubation were suspended in a density solution (sodium–polytungstate, 1.8 g cm^{-3}), shaken and centrifuged at 3074 g for 20 min. The floating POM was collected using a water jet pump. The procedure was repeated until no additional POM was floating in the density solution. In order to remove excessive salt, the POM and MIN (heavy residues) fractions were repeatedly rinsed with deionized water via pressure filtration until electric conductivity dropped below $5 \mu\text{S cm}^{-1}$.

2.3. Incubation experiment

A modified automated system according to Heinemeyer et al. (1989) as shown in Fig. 2, was used for continuous soil respiration measurements, allowing for the hourly recording of the

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