



Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil



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ABSTRACT

To better understand how carbon and nitrogen mineralization are linked in soils, we conducted a long-term incubation experiment and compared carbon and nitrogen dynamics in the bulk soil and in soil fractions. Topsoil of a Rendzic Leptosol from a beech forest site near Tuttingen, Germany, was separated into three particle size classes: sand (2000–20 μm), silt (20–2 μm), and clay (<2 μm). Bulk soil and particle size fractions were incubated in replicate, allowing periodic destructive sampling of triplicates at day 0, 14, 42, 84, 140, 210, and 280. We monitored CO₂–C respiration, NH₃–N emissions, nitrogen mineralization, pool sizes of total and salt extractable (0.01 M CaCl₂) organic carbon and nitrogen, and microbial biomass carbon and nitrogen. The chemical composition of selected samples was further characterized by ¹³C-NMR spectroscopy. Fractionation did not influence carbon mineralization (Σ fractions ≈ bulk soil), which decreased in the order sand > clay > silt. The fractions respired between 10.4% (sand fraction), 8.8% (clay fraction) and 4.4% (silt fraction) of total soil organic carbon. However, nitrogen mineralization was affected by the fractionation procedure (Σ fractions < bulk soil) and followed the order clay > silt > sand. Fractionation increased the surface area and hence provided accessory mineral surfaces, which allowed new binding of especially nitrogen-rich compounds, in addition to ammonium fixation via cation exchange. As indicated by lower metabolic quotients, microbial carbon mineralization was more efficient in the bulk soil compared to the calculated sum of fractions. In the clay fraction, carbon mineralization rates, salt extractable organic carbon contents, and microbial biomass carbon and nitrogen contents declined strongly towards the end of the incubation. This indicates that in the clay fraction, organic carbon was not available for microbial degradation and that microorganisms were strongly carbon-limited causing a subsequent inhibition of nitrogen immobilization. Density fractionation revealed that organic matter in the sand fraction consisted mainly of particulate organic matter present as light material containing partly decomposed plant remnants. The organic matter in the clay fraction was mostly adsorbed on mineral surfaces. Organic matter in the sand and in the clay fraction was dominated by O/N-alkyl C indicating low recalcitrance, but the C/N ratio of organic matter narrowed with decreasing particle size. Our results suggest that carbon and nitrogen mineralization are decoupled in the mineral-associated fractions of the soil. The specific interactions of both carbon and nitrogen containing components with the mineral matrix strongly modulate the mineralization dynamics. Therefore, isolated considerations of C/N or alkyl C to O/N-alkyl C ratios of organic matter are insufficient as indicators for decomposition in plant residues. The combined consideration of C/N and alkyl C to O/N-alkyl C ratios provides a first impression about the degree of decomposition in plant residues. However, bioavailability in fractions where organic matter is mainly stabilized by spatial inaccessibility and by organo-mineral interactions cannot be explained by these ratios, but can be examined by an incubation approach.

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1. Introduction

Soil organic matter (SOM) can be subdivided in three functional pools that are stabilized by specific mechanisms, leading to specific turnover times (Collins et al., 2000; von Lützow et al., 2007): (1) the active pool consisting of microbial biomass and metabolites (turnover <10 years); (2) the intermediate pool built up by partially stabilized organic matter (turnover 10–100 years); and (3) the passive pool (turnover >100 years) (von Lützow et al., 2008). Three stabilization mechanisms protect SOM from decomposition: (1) selective preservation due to recalcitrance; (2) protection by occlusion; and (3) interaction with minerals and metal ions (Sollins et al., 1996; von Lützow et al., 2006). Selective preservation of recalcitrant compounds is relevant for the active pool but does not explain long-term stabilization. Occlusion of SOM by biogenic aggregation operates in the intermediate pool while occlusion of SOM in clay microstructures by abiotic aggregation and the formation of organo-mineral interactions were found to be important mechanisms in the passive pool (von Lützow et al., 2008).

Assuming that sorption is a major stabilization mechanism, SOM within the sand fraction is assigned to the active pool, and SOM in silt and clay fractions to the intermediate and passive pool (von Lützow et al., 2007). Although fractionation procedures can create slight redistributions of SOM (Cambardella and Elliott, 1994; Gregorich et al., 2006), the main advantage is the analytical characterization and comparison of individual soil fractions and their comparison with and contribution to the bulk soil, which is otherwise not accessible. Studying decomposition and biodegradability, such as mineralization, in the different particle size fractions allows evaluation of the stability of SOM associated with these fractions (Christensen, 1987). Therefore, particle size separation with subsequent incubation experiments provides a useful approach to describe the SOM stability within discrete fractions obtained from a complex matrix (Crow et al., 2007).

Previous researchers have proposed mechanisms of SOM stabilization based on spatial orientation and interactions with the organo-mineral phase (Ellerbrock et al., 2005; Kleber et al., 2007), but there have been few attempts to demonstrate this by incubation experiments focusing on both carbon and nitrogen mineralization, despite the fact that carbon and nitrogen dynamics are closely associated (McGill et al., 1975; Gärdenäs et al., 2011). Few studies have investigated either carbon or nitrogen in SOM fractions (Sollins et al., 1984; Christensen, 1987; Swanston et al., 2002; Mueller et al., 2014), but, instead, investigations combining carbon and nitrogen dynamics have been mainly carried out on bulk soil (Robertson et al., 1988; Joergensen et al., 1990; Raubuch and Joergensen, 2002; Weintraub and Schimel, 2003). The simultaneous evaluation of carbon and nitrogen dynamics in individual SOM fractions has been achieved only in two studies until present. Swanston et al. (2004) applied this approach in a nitrogen fertilization experiment. They found higher carbon and nitrogen mineralizations from loamy and sandy bulk soils compared to those obtained from recombined and incubated density fractions. Relationships between carbon and nitrogen mineralization were negative in the light fraction with particulate organic matter (POM), indicating net nitrogen immobilization, and were positive in the heavy fraction with mainly mineral-associated SOM. They identified the latter, more stable fractions as sources for nitrogen release. Parfitt and Salt (2001) incubated particle size fractions in an agricultural loamy soil and considered the clay fraction as a major site of nitrogen mineralization. They found that the carbon mineralization in soil fractions was related to the availability of substrates rather than to the C/N ratio, because SOM in the different particle size fractions behaved differently according to its specific

decomposition status. However, a detailed understanding of the processes that impact turnover of carbon and nitrogen is still missing. Specifically it is not clear how the stabilization of SOM in the fine soil fractions affects the interactive mineralization of C and N. Thus, in this study we aimed to achieve a more complete understanding of the control of carbon and nitrogen turnover with respect to its different SOM pools by analyzing net mineralizable carbon and nitrogen pools, microbial biomass carbon and nitrogen, and total carbon and nitrogen in specific soil fractions.

We separated a clay-rich topsoil of a Rendzic Leptosol from a beech forest site near Tuttlingen, Germany, into particle size classes at cut-offs of 20 µm and 2 µm, and we termed the fractions sand (2000–20 µm), silt (20–2 µm), and clay (<2 µm). We used extended laboratory incubation (280 d) of bulk soil and the sand, silt, and clay fractions to analyze the SOM in physical soil fractions. We measured mineralized nitrogen pools (N_{\min}), microbial biomass carbon and nitrogen, and their $\text{CO}_2\text{-C}$ and $\text{NH}_3\text{-N}$ release at several time points during incubation. Essential information on the composition of SOM in the different fractions is obtained from solid-state ^{13}C -NMR spectroscopy. Our study includes data on microbial biomass showing the efficiency and limitations of the microorganisms during mineralization. These data are vital to understanding the mineralization behavior of the different SOM pools. Differences between the artificial fractions and the bulk soil illustrate the relative importance and function of the fractions in the soil as a whole at this site. This approach allows quantifying the potential bioavailability of carbon and nitrogen in the different particle size fractions in order to improve our understanding of soil carbon and nitrogen stabilization patterns in specific SOM fractions and to develop predictive parameters for carbon and nitrogen cycling.

2. Materials and methods

2.1. Site characteristics and collection and storage of soil material

On October 16, 2010, 10 kg of soil were sampled randomly from three spots on the northwest slope at the experimental site in a beech dominated forest near Tuttlingen (Swabian Jura, Baden-Württemberg, Germany, 47°59'N, 8°45'E), derived from the upper 15-cm horizon of a Rendzic Leptosol after removing the O-layer. The site was selected because it is well characterized concerning the ecosystematic N dynamics (Dannenmann et al., 2006, 2007, 2009; Bimüller et al., 2014). Furthermore, this mull site is known to have a high aggregation with a slow aggregate turnover (Bimüller et al., 2013). The studied Ah horizon material had a texture classified as heavy clay and a pH of 6 (FAO, 2006; Bimüller et al., 2014). The material was composited and stored at 4 °C in tightly sealed bags until sieving with a mesh size of 2 mm. Bulk soil had a mean organic carbon concentration of 64.8 mg g⁻¹ and a mean nitrogen concentration of 4.6 mg g⁻¹ (Table 1).

Bulk mineralogy by X-ray diffraction showed a relative enrichment of the clay fractions in phyllosilicates, especially irregular mixed-layer illite-smectite and kaolinite. Quartz dominates in the larger grain sizes (Bimüller et al., 2013).

2.2. Physical fractionation

To isolate SOM fractions for the incubation experiment, a particle size fractionation scheme (Appendix S1) was used for the subsequent decomposition study rather than density fractionation (Magid et al., 1996), thus avoiding the latter's repressive effects on microbial activity. Air-dried soil was dry-sieved by hand to <2 mm. A two-step ultrasonic disruption (Amelung and Zech, 1999) was performed according to Mueller et al. (2012), breaking up macro-

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