



# Carbon and nitrogen mineralization in hierarchically structured aggregates of different size



Carolin Bimüller<sup>a,\*</sup>, Olivia Kreyling<sup>a</sup>, Angelika Kölbl<sup>a</sup>, Margit von Lützwow<sup>a</sup>,  
Ingrid Kögel-Knabner<sup>a,b</sup>

<sup>a</sup> Chair of Soil Science, Research Department Ecology and Ecosystem Management, TUM School of Life Sciences Weihenstephan, Technical University of Munich, 85350 Freising-Weihenstephan, Germany

<sup>b</sup> Institute for Advanced Study, Technical University of Munich, Lichtenbergstraße 2a, 85748 Garching, Germany

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## ABSTRACT

Soil organic matter pools are turned over at different rates, but uncertainty persists regarding how far the hierarchically organized soil structure controls the mineralization dynamics. To better understand carbon and nitrogen mineralization in undisturbed aggregate classes (coarse aggregates: 2–6.3 mm, fine aggregates: <2 mm), we conducted a long-term (224-days) laboratory incubation experiment. A grassland soil (Haplic Cambisol) was chosen since its aggregates were not disturbed by tillage. The field-moist aggregate size classes were separated by a gentle dry-sieving method. We monitored the CO<sub>2</sub>-C and NH<sub>3</sub>-N emissions, nitrogen mineralization, pool sizes of total and salt extractable (0.5 M K<sub>2</sub>SO<sub>4</sub>) organic carbon and nitrogen, and microbial biomass carbon and nitrogen. By this approach, we could distinguish between the carbon and nitrogen mineralization processes of two soil aggregate size classes, relative to undisturbed bulk soil. The classes showed different aggregate architectures with variously sized subunits, but confirmed the aggregate hierarchy. For both aggregate classes, the recombinant sum of respired CO<sub>2</sub>-C per unit of soil organic carbon equaled the bulk soil, proving that our aggregate separation preserved the original aggregates as intact functional units. Both aggregate size classes and the bulk soil respired only 4% soil organic carbon throughout the incubation period. The coarse aggregates, which mostly comprised small macroaggregates, mineralized more carbon per unit soil organic carbon than the fine aggregates (composed of microaggregates), indicating a higher bioavailability of soil organic matter in the coarse aggregates. Accordingly microbial metabolic efficiency was higher in coarse than in fine aggregates. Nitrogen mineralization was higher in fine aggregates than in coarse aggregates, but was impaired by carbon limitation as the incubation experiment proceeded. We conclude that bioavailability of soil organic matter was affected by different aggregate architectures in the different aggregate size classes. The lower bioavailability of soil organic matter in fine aggregates is due to an enhanced stabilization through cation bridging of the dolomitic soil material at the microaggregate level, whereas the higher soil organic matter bioavailability in coarse aggregates is explained by labile organic components at the macroaggregate level.

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## 1. Introduction

Soil aggregation depends on the soil fauna, microorganisms, roots, organic and inorganic binding agents, and environmental and physical forces (Oades, 1993; Six et al., 2004). Neutral to alkaline soils with C/N ratios <15, derived from calcareous parent material with mull humus form, are characterized by low proportions of organic matter present as plant debris (Oades,

1984, 1988). Under these slightly alkaline conditions, calcium and magnesium cations preferentially form bridges between negatively charged clay colloids and negatively charged soil organic matter (SOM), enhancing the stability of the aggregate system (Baldock et al., 1997; Baldock and Skjemstad, 2000; Bockenhoff et al., 1997; Oades, 1988; Rashad et al., 2010). These polyvalent metal ions ensure the integrity of microaggregates and other persistent organic materials (Elliott, 1986; Tisdall and Oades, 1982).

Previous studies on aggregate carbon mineralization have focused on land use (Akinsete and Nortcliff, 2014; Gupta and Germida, 1988; Rabbi et al., 2015) and tillage systems (Beare et al., 1994; Fernandez et al., 2010; Nyamadzawo et al., 2009; Six et al.,

\* Corresponding author.

E-mail address: [carolin.bimueller@wzw.tum.de](mailto:carolin.bimueller@wzw.tum.de) (C. Bimüller).

2000). Carbon mineralization has been altered by management practices. In a 100-day incubation study, the carbon mineralization per unit of soil organic carbon (SOC) in pastures (0.005%) was double that of arable cropping systems (0.002%) (Curtin et al., 2014). This difference is attributed to a continuous supply of labile carbon in the form of coarse organic matter from perennial vegetation, which is greatly reduced under arable cropping (Baker et al., 2007; Curtin et al., 2014). However, the stability of SOC with undisturbed natural aggregates has been little investigated (Álvarez et al., 2007). The simultaneous evaluation of carbon and nitrogen mineralization behavior in aggregates has been undertaken only recently (Curtin et al., 2014; Oorts et al., 2006), and reports of SOC and nitrogen in undisturbed aggregates remain rare. The carbon and nitrogen mineralization of aggregates is commonly evaluated in complementary laboratory studies, wherein the soils are segregated into aggregate size classes and incubated (Six and Paustian, 2014). In the laboratory experiment of Curtin et al. (2014), carbon and nitrogen mineralization was calculated by the increased surface area with decreasing aggregate size.

Nevertheless, despite some studies (Gupta and Germida, 1988; Sainju et al., 2009; Zhang et al., 2012) researching the distribution of microbial biomass carbon (MBC) and nitrogen (MBN) in different aggregate classes, and how this biomass relates to SOM mineralization in each aggregate class, this topic still remains poorly understood. Miller and Dick (1995) found a qualitative difference in the microbial communities occupying macro- and micro-aggregates. Gupta and Germida (1988, 2015,) emphasized the importance of microbial biomass in the formation of macro-aggregates and as a primary source of SOM for carbon mineralization. They identified a crushing effect of the macroaggregates, which enhances carbon mineralization. This disruption releases the microbial biomass mucilage that binds the macroaggregates, which contributes to the mineralizing SOM. Therefore, by collecting data on MBC and MBN and the community structure across aggregate size classes, we can begin to elucidate carbon and nitrogen cycling (Six and Paustian, 2014) and their coupling as determined by the soil structure.

To better understand the coupled regulation of carbon and nitrogen mineralization in undisturbed aggregates, we conducted an incubation experiment. We studied mineralization in two aggregate size classes of a dolomitic grassland soil (Haplic Cambisol), composed of different hierarchically organized substructures. Specifically, we asked the following questions: (1) Do different aggregate classes mineralize at similar rates when their

water and air availabilities are comparable? (2) Do aggregate size classes differ in their substructures, external surface areas, and bioavailability of SOM? (3) Do aggregate classes of different sizes exhibit diverse heterotrophic respiration efficiencies? Thus, the present study seeks to identify the role of different intact structural units to the overall soil mineralization of carbon and nitrogen as controlled by bioavailability in aggregate substructures.

## 2. Materials and methods

### 2.1. Site characteristics and origin of soil material

The sampling site Graswang is located near Garmisch-Partenkirchen in a foothill valley (877 m above sea level) of the German limestone Alps in southern Bavaria. This area is designated as permanent grassland (Unteregelsbacher et al., 2013). Soil material for the incubation study was collected from the Ah horizon (10–15 cm) of a Haplic Cambisol (Calcaric, Humic, Siltic) (IUSS Working Group WRB, 2014) underneath the main rooting zone in the fall of 2009. The parent material is dolomitic alluvial gravel; the climate is characterized by a mean annual air temperature of 6.7 °C and a precipitation of 1757 mm (Kreyling et al., 2013). Undisturbed soil samples were taken under field-moist conditions. To preserve their microbial communities, the samples were stored under cooled conditions (4 °C) prior to further preparation (Petersen and Klug, 1994). Material was well-aggregated by the abiotic dolomitic parent material and free particulate organic material was almost absent (Kreyling et al., 2013). The texture was dominated by silt (68%), with minor quantities of clay (22%) and sand (10%) (Unteregelsbacher et al., 2013). The pH was 7.1 and the organic carbon and total nitrogen concentrations were  $57 \pm 4$  and  $6.1 \pm 2.0 \text{ mg g}^{-1}$ , respectively, corresponding to a C/N ratio of 9.

### 2.2. Soil preparation and incubation

We separated and incubated three aggregate size classes of the sampled soil material from the Ah horizon. The material in the bulk soil class was not processed, since we wanted to conserve the intact aggregation. For both aggregate classes, the complete field-moist soil samples were gently separated into macroaggregates <6.3 mm using a sieve (Kölbl et al., 2005). Fragments such as roots and stones constituted a negligible share and were removed. The resulting aggregates were subdivided into coarse (2–6.3 mm) and fine aggregates (<2 mm) by sieving

Setup	Coarse aggregates	Fine aggregates	Bulk soil	Sampling days	Vessels	Analyses
(1)	○○○○	○○○○	○○○○	every week	20 g	4 replicates for titration (CO <sub>2</sub> -C and NH <sub>3</sub> -N)
(2)	○○○	○○○	○○○	0	100 g	3 replicates for sampling (C/N, MBC, MBN, SEOC, SEON)
	○○○	○○○	○○○	28		
	○○○	○○○	○○○	56		
	○○○	○○○	○○○	84		
	○○○	○○○	○○○	112		
	○○○	○○○	○○○	154		
	○○○	○○○	○○○	224		

Fig. 1. Experimental setups: (1) for titration and (2) for sampling. MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; SEOC: salt extractable organic carbon; and SEON: salt extractable organic nitrogen.

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