Microbial utilization of mineral-associated nitrogen in soils

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ABSTRACT

In soils, a large portion of organic nitrogen (ON) is associated with minerals and thus, possibly stabilized against biological decay. We therefore tested if mineral-associated N is an important N source for soil microorganisms, and which soil parameters control its bioavailability. Microcosm experiments with mineral-associated organic matter, obtained as heavy fraction (HF) via density fractionation, and bulk soil from mineral topsoil of the Franz Josef chronosequence were conducted for 125 days. We examined the effects of O_2 status, soil age (differences in mineralogical properties), as well as cellulose and phosphate additions on the turnover of mineral-associated N. Using a combination of activity measurements and quantitative PCR, microbial N transformation rates and abundances of N-related functional genes (amoA, narG, chiA) were determined. Similar or higher values for microbial N cycling rates and N-related functional abundances in the HF compared to bulk soil indicated that mineral-associated N provides an important bioavailable N source for soil microorganism. The turnover of mineral-associated N was mainly controlled by the O_2 status. Besides, soil mineralogical properties significantly affected microbial N cycling and related gene abundances with the effect depending on the N substrate type (ON, NH_4^+ or NO_3^-). In contrast, cellulose or phosphate addition hardly enhanced microbial utilization of mineral-associated N. The results of our microcosm study indicate that mineral-associated N is highly bioavailable in mineral topsoils, but effects of the mineral phase differ between N cycling processes.

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1. Introduction

In soil, the availability of N as a major nutrient element and limiting growth factor determines fertility and biomass production to a large extent (Knicker, 2011). Nitrogen can be found in eight oxidation states and most of the N transformations are mediated by soil microorganisms that act as key players of N cycling (Robertson and Groffman, 2015). Present ecological concepts in N cycling and ecosystem dynamics largely ignore the role of mineral—organic associations, thereby organic N (ON) is considered as one pool without further differentiation into ON contained in functionally different organic matter (OM) fractions, e.g. particulate and mineral-associated OM. However, the interaction between OM and soil minerals determines the turnover of soil OM (SOM) (Schmidt et al., 2011). Competing with plants and microorganism for available N, soil minerals are relevant for ecosystem productivity by directly and indirectly influencing microbial C and nutrient cycling.

Several studies showed that soil minerals can indirectly affect microbial nutrient cycling by stabilizing OM via adsorption to mineral surfaces or coprecipitation reactions (summarized by Kleber et al., 2015). These interactions mostly result in lower substrate bioavailability and can therefore impair microbial activity (Schneider et al., 2010; Kleber et al., 2015). Similarly, activities of microbial extracellular enzymes can be inhibited by soil minerals

such as poorly crystalline Fe and Al oxide phases and other clay-sized minerals, thus slowing down microbial OM degradation and mineralization (Bayan and Eivazi, 1999; Nannipieri and Smalla, 2006; Turner et al., 2014).

The bioavailability of SOM depends, among other factors, strongly on properties of the mineral phase such as specific surface area and surface charge (Kleber et al., 2015). Mineralization rates of organic carbon (OC) and ON in the sand fraction were shown to be higher than in the silt or clay fraction during an incubation experiment with soil particle size fractions (Bimüller et al., 2014). Vogel et al. (2015) revealed in an incubation experiment with artificial soils that the clay mineral type affected plant litter decomposition with slower mineralization in montmorillonite-rich soil than in illite-rich soil. Also Saidy et al. (2012) observed in a similar experiment a significant effect of the clay mineral type with higher decomposition rates in the presence of kaolinite than illite or smectite. Colman and Schimel (2013) identified in an analysis of 84 distinct mineral soils the clay content as an important predictor for microbial respiration as well as net N mineralization with a negative effect on both. Besides soil particle size and clay mineral type, the content of Fe and Al (hydr)oxides was reported to impact microbial activity. While Achat et al. (2012) detected a negative relationship between basal soil respiration and the content of Al (hydr)oxides, other studies reported either no, minor or strong effects of Fe (hydr)oxide type on C mineralization (Mikutta et al., 2007; Saidy et al., 2012; Heckman et al., 2013; Vogel et al., 2015).

Overall, there are several studies focusing on the effect of mineralogical properties on C cycling, whereas the impact of minerals on N cycling is still poorly understood. Although C and N cycling are linked to each other via biomass production and degradation (Knicker, 2011), a few previous studies indicated that OC and ON mineralization respond differently to the mineral phase pointing to a decoupling of OC and ON mineralization in the mineral-associated soil fraction (Heckman et al., 2013; Bimüller et al., 2014). Additionally, our previous results obtained from a soil chronosequence showed that profile-based OC stocks of mineral soils were positively correlated to the content of Al originating from poorly crystalline Al minerals and Al-humus complexes, whereas the ON stocks were positively correlated to the stocks of crystalline Fe oxides (Turner et al., 2014). Nevertheless, recent evidence suggests that mineral-associated ON can be utilized by soil microorganisms. For example, Dippold et al. (2014) reported that alanine, sorbed to different Fe oxides or clay minerals in an incubation experiment, was quickly mineralized at variable rates, thus also highlighting the effect of the soil mineral type.

Therefore the aims of our study were to elucidate (i) if mineral-associated N is an important bioavailable N source for soil microorganisms, and (ii) which soil properties control the bioavailability of mineral-associated N and the abundances of N cycling microorganism. We used soils of different ages of the 120-kyr old Franz Josef soil chronosequence (New Zealand) which is characterized by a nutrient and mineralogical gradient (Stevens, 1968; Turner et al., 2014). High precipitation regularly creates water-saturated conditions in soils of the chronosequence resulting in changes of the redox regime which may affect microbial N cycling by generally slowing down microbial activity. Due to leaching and weathering of P-containing minerals the soil P content drastically decreases with increasing soil age, possibly limiting the microbial utilization of mineral-associated N in older soils. The use of mineral-associated N may also require the supply of easily available additional C sources, such as cellulose derived from decomposing root litter, which has been shown to stimulate microbial activity and SOM decomposition in subsoil environments ("priming effect", Fontaine et al., 2007).

Hence, we hypothesized that mineral-associated N is an important bioavailable N source and explored the effect of soil mineralogical composition, O_2 status (redox regime), and the additions of phosphate and cellulose (priming) on its mineralization. To address this, we set up a microcosm experiment comparing microbial N cycling in the mineral-associated OM fraction with bulk soil of four soil ages under oxic and anoxic conditions. Thereby, we tested the effect of P and C additions (phosphate, ^{13}C-labeled cellulose, and phosphate + ^{13}C-labeled cellulose). During the 125-days incubation period, N_2, N_2O and CO_2 production as parameters for microbial activity were measured regularly, and at the end of the experiment net N mineralization and gene copy numbers of N cycle-related marker genes were determined by quantitative PCR (qPCR).

2. Material and methods

2.1. Site description and soil sampling

The Franz Josef chronosequence is located on the West Coast of the South Island of New Zealand (~43° S, 170° E). The soils developed due to repeated glacial advance and retreat from greywacke and mica schist spanning a time scale from present to 120 000 years (Stevens, 1968; Almond et al., 2001). The climate is wet and temperate and the soils are covered by rainforest (Richardson et al., 2004). The chronosequence is characterized by a steep nutrient and mineralogical gradient (Turner et al., 2014, Table 1, Table 2). Soil samples were taken from A horizons of four sites with different soil ages (0.5k, 5k, 12k, and 120k years) in February 2014 and were kept at <8 °C prior to incubation. Using A horizons guaranteed relatively high microbial activities that facilitated the detection of changes during the incubation period and concomitantly a high proportion of mineral-bound N. Contrary to whole soil profiles that were characterized by an increasing content of Fe and Al (hydr)oxides and clay with soil age, the A horizons separately showed an inverse trend for the content of these soil mineral phases (Table 2).

2.2. Microcosm incubation experiment

To assess the bioavailability of mineral-associated N and its controls we set up a laboratory incubation experiment in microcosms with either the mineral-associated OM fraction or bulk soil (Fig. S1). We used A horizons of four soil ages (0.5k, 5k, 12k, and 120k years). For soil fractions, soil samples were separated by density fractionation using sodium polytungstate solution ($\rho = 1.6$ g cm^{-3}; Cerli et al., 2012). Twenty-five g of soil were dispersed in 125 mL sodium polytungstate solution and sonicated for 9 min 38 s with 60 J mL^{-1} with an ultrasonic device (LABSONIC®, Sartorius Stedim Biotech GmbH, Göttingen, Germany). The floating light fraction (LF, $\rho < 1.6$ g cm^{-3}, representing mostly particulate OM) was removed from the heavy fraction (HF, containing mineral-associated OM) by decantation after deposition for 1 h and centrifugation for 10 min at 3500g. To minimize possible negative effects of the sodium polytungstate solution on microbial activities (Blackwood and Paul, 2003; Crow et al., 2007), both fractions were washed with deionized water until the electrical conductivity was <50 μS cm^{-1}. Thereafter, samples were shock-frozen in liquid N_2 and freeze dried resulting in approximately 95 wt% of the HF and 2 wt% of the LF. Toxic effects caused by tungsten residues in the fractionated samples could be ruled out because of similar mineralization rates of bulk soil compared to density fractions (Gentsch et al., 2015).

To test the influence of P and C addition on C and N turnover, we tested different treatments: without any addition (wo), with NaH$_2$PO$_4$ solution (P; 500 μg PO$_4$-P g^{-1} soil fraction), with ^{13}C-labeled cellulose powder (C; 40 mg cellulose-C g^{-1} soil fraction C)