CrossMark

Microbial nitrogen mining affects spatio-temporal patterns of substrate-induced respiration during seven years of bare fallow

Nele Meyer [a, *], Gerhard Welp [a], Ludger Bornemann [a], Wulf Amelung [a, b]

[a] University of Bonn, Institute of Crop Science and Resource Conservation - Soil Science and Soil Ecology, Nussallee 13, 53115 Bonn, Germany
[b] Forschungszentrum Jülich GmbH, Agrosphere Institute (IBG-3), Wilhelm-Johnen-Straße, 52428 Jülich, Germany

ABSTRACT

Decomposition of soil organic matter (SOM) is regulated by microbial activity, which strongly depends on the availability of carbon (C) and nitrogen (N). Yet, the special role of N on soil organic carbon (SOC) mineralization is still under discussion. The recent concept of microbial N mining predicts increasing SOC mineralization under N-deficiency, which is in contrast to the generally accepted stoichiometric decomposition theory.

Following this concept we hypothesized that spatio-temporal patterns of microbial activity are controlled by SOC and N contents, but that microorganisms maintain their functionality to mineralize C under conditions of N deficiency because of microbial N mining.

To test this hypothesis, we added glucose to an arable soil that had experienced increasing losses of C_3-derived SOM after one, three, and seven years of bare fallow and measured spatio-temporal patterns of substrate-induced respiration (SIR). The SIR measurements were performed with and without additions of mineral N. Selected samples were treated with C_4 sugar in order to trace the source of CO_2 emissions (sugar vs. SOC-derived) by natural ^{13}C abundance measurements. Sugar additions were repeated after the first SIR experiment to derive information on changing N availability.

The results showed that spatial patterns of SIR were not consistently regulated by SOC and N. On a temporal scale, the maximum microbial growth peak declined by 47% from one year bare fallow to seven years bare fallow but soils often developed a second growth phase in the 7th year of fallow. Intriguingly, the maximum microbial growth peak increased again when N was added together with the glucose and no second growth peak occurred. A similar effect was observed after repeated sugar additions but without N additions. The ^{13}C experiment revealed a slightly higher contribution of SOC-derived CO_2 in N-deficient samples (16.7%) than in N-fertilized samples (14.6%).

We conclude that the first SIR peak was related to the supply of immediately available N while the second growth phase indicated a delayed release of N, due to N mining from SOM. Hence, microbes were able to compensate for initial N limitation and there was no significant change in the overall substrate-induced CO_2 release with proceeding time under fallow.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The input of anthropogenic derived nitrogen (N) to soils has increased globally (Galloway et al., 2008). Nevertheless, several soils also exhibit N losses due to progressive soil degradation and organic matter decomposition (Oldeman, 1994). Hence, fundamental questions raised on the role of N and other nutrients for microbial activity and related soil organic matter (SOM) decomposition. It has long been accepted that microbial activity and soil organic carbon (SOC) mineralization is most effective when nutrient contents match microbial demands (Hessen et al., 2004). This observation is in line with the so-called stoichiometric decomposition theory, which requires that maximum microbial growth is not limited by a single nutrient, according to Liebig's law of the minimum. Indeed, a positive influence of nutrient contents on microbial activity has been reported in numerous studies (e.g., Nordgren, 1992; Wang and Bakken, 1997; Allen and Schlesinger, 2004). In contrast, other studies reported elevated C mineralization in N-deficient soils (Hagedorn et al., 2003; Craine et al., 2007;

Ramirez et al., 2010). These findings may be explained by the so-called microbial N mining theory. It assumes that microbes may use labile C as an energy source for the decomposition of recalcitrant SOM, which contains the required N (Moorhead and Sinsabaugh, 2006; Craine et al., 2007). In this regard, the decomposition of recalcitrant SOM is enhanced at low N supply, or, in turn, additional N availability might inhibit the mineralization of recalcitrant SOC. To date, only a few papers deal with microbial N mining strategies and there is still large uncertainty about the underlying processes and preconditions (Craine et al., 2007; Chen et al., 2014; Spohn, 2015).

In order to characterize microbial biomass and its potential activity, measurements of substrate-induced respiration (SIR) combined with kinetic respiration analyses have been widely suggested (Anderson and Domsch, 1978; Panikov and Sizova, 1996; Stenström et al., 1998). Substrate-induced respiration bases on the principle that glucose addition stimulates microorganisms, resulting in increased respiration and subsequent growth until glucose or other nutrients become limiting (Anderson and Domsch, 1978; Nordgren, 1992). This method has originally been developed to derive a proxy for the amount of microbial biomass C (Anderson and Domsch, 1978). It was additionally used to prove the existence of nutrient effects on SOC turnover (e.g., Nordgren, 1992; Teklay et al., 2006; Gnankambary et al., 2008) as well as of priming effects (e.g. Blagodatskaya et al., 2007; Chen et al., 2014). Hence, this method should also be suitable to derive SIR parameters indicative for nutrient deficiency and for N acquisition strategies. Thus, it might help to understand the response of SOM decomposition to N additions as well as to decreasing N supply.

Here, we repeatedly investigated a heterogeneous field site under continuous bare fallow management, i.e. under conditions of increased SOM degradation and associated N losses. We hypothesized that (1) spatio-temporal patterns of basal respiration and SIR are controlled by C and N contents, but that (2) microorganisms maintain their functionality to mineralize C under conditions of increased SOM loss and N deficiency, because of microbial N mining. We additionally hypothesized that (3) SIR is a useful tool to provide information on N deficiency and acquisition strategies. To investigate these hypotheses, we determined the response of several SIR parameters to spatial and temporal patterns of SOC and N contents, with and without addition of mineral N. Selected samples were treated with C_4 sugar in order to trace the source of CO_2 emissions (sugar vs. SOC-derived) by natural ^{13}C abundance measurements. Sugar additions were repeated after the first SIR experiment to derive information on N acquisition which should deliver further hints for the occurrence of microbial N mining.

2. Material and methods

2.1. Study area

The study was conducted at a 60×190 m field site near the village Selhausen (50°52′09.34″N; 6°27′00.58″E), located in the Lower Rhine Embayment, Germany. Mean annual temperature was 9.8 °C, mean annual precipitation was 694 mm. The underlying sediments were fluvial deposits from Rhine and Meuse River and the Rur River system, which have been covered by aeolian sediments during the Pleistocene. The field was weakly inclined (<4°) over ~180 m in east-west direction. Due to its genesis (see Bornemann et al., 2011), soil properties were highly heterogeneous throughout the field site and mainly followed a gradient from northeastern upslope positions to southwestern downslope positions. Downslope, the texture represented a slightly gravelly silt loam (<10% rock fragments (>2 mm), >70% silt within the fine earth). Towards northeastern upslope parts, contents of sand and

rock fragments increased gradually towards a very gravelly loam (>45% rock fragments, <60% silt within the fine earth) (Bornemann et al., 2011). Soils ranged from Stagnic Luvisol on downslope, Orthic Luvisol on slope to Dystric Leptosol on upslope parts (WRB, 2007; Bornemann et al., 2011). Also contents of SOC revealed a distinct gradient from high contents at upslope to low contents on downslope positions (Bornemann et al., 2011). The field was under arable management for at least 100 years and was converted to continuous bare fallow in 2005. The plot was kept bare by mechanical tillage as well as by application of herbicides.

2.2. Experimental design and soil sampling

For the investigation of spatial patterns and in order to cover the full range of soil properties, we set up a geo-referenced regular grid of 10×10 m (n = 64 sampling points). For the investigation of temporal patterns, one soil sample was collected from each sampling point in 2006, 2008, and 2012, corresponding to one, three, and seven years after conversion to fallow, respectively (see Fig. S1 for a figure of the sampling design). In the 7th year of fallow, two sampling points had to be excluded for reasons of disturbance, so that the overall sample size in 2012 was 62. The homogeneous Ap horizon was sampled from 0 to 25 cm depth using a spade. Samples were sieved to 2 mm and subsequently dried at 40 °C to obtain standardized storing conditions for all samples.

2.3. Soil chemical and physical analysis

Gravimetric amounts of rock fragments were determined by sieving to 2 mm. For further chemical and physical analyses sieved soil was used and all the result refer to fine earth (<2 mm). Texture was determined by a combination of wet sieving (sand fractions) and sedimentation (silt and clay fraction) after Köhn (ISO 11277, 2002) (available from Bornemann et al., 2011). The C and N contents of the sieved and milled soils were determined by elemental analysis (ISO 10694, 1995). Water holding capacity was determined on air-dried samples, the procedure mainly following Alef and Nannipieri (1995).

2.4. Respiration measurements

2.4.1. Preparation
The dried soil samples were rewetted to 40% of water holding capacity in order to guarantee a sufficient and standardized water supply for microorganisms (ISO/DIS 17155, 2001). Water was homogenized with the soil using a mixer. Thirty gram (dry weight) of soil was filled into plastic vessels, three analytical replications each, and slightly compressed to a bulk density of 1.3 g cm^{-3} to create standardized conditions. The samples were then pre-incubated at 22 °C for 120 h to level effects of mixing and water addition and to stabilize the respiration rate (Blagodatsky et al., 2000).

2.4.2. Measurement
Measurement of CO_2 release was conducted using an automated respirometer that allows incubating 95 samples in parallel (Respicond VIII, Nordgren Innovations AB, Sweden). The system provides a continuous measurement of CO_2 evolution by trapping CO_2 in potassium hydroxide (KOH) (Nordgren, 1988). The decrease in electrical conductivity in this solution caused by CO_2 entrapment was automatically measured every 30 min per sample by platinum electrodes, and the changes in conductivity were automatically transformed to CO_2 evolution rates. Incubations were carried out at a constant temperature of 22 °C.