Soil Biology & Biochemistry 113 (2017) 53–59

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Turnover of carbon and phosphorus in the microbial biomass depending on phosphorus availability

Marie Spohn*, Meike Widdig

Soil Biogeochemistry, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany

ARTICLE INFO

Article history:
Received 23 March 2017
Received in revised form
16 May 2017
Accepted 17 May 2017
Available online 6 June 2017

Keywords:
Element turnover time
Soil nutrient cycling
Soil microbial community
Phosphorus uptake
Isotopic labeling of DNA
Stoichiometry

ABSTRACT

Adjustment of the mean residence time (MRT) of elements in the soil microbial biomass (SMB) might be an important process by which microbial communities adapt to nutrient-poor environments and maintain their element biomass ratio. Yet, little is known about the turnover of elements such as carbon (C) and phosphorus (P) in the SMB, which is partly due to methodological challenges. This is the first study to determine the MRT of C and P in SMB based on labeling of microbial DNA with ^{14}C and ^{33}P. We studied the MRT of C and P in the SMB of topsoil from a temperate, mesic grassland fertilized with five different amounts of P. For this purpose, soil was labeled with ^{14}C-glucose and ^{33}P-phosphate and incubated for 24 h, and then, ^{14}C and ^{33}P in the soil microbial DNA were determined. The MRT of C and P was calculated based on the C and P content of DNA, and the observation that soil microbial biomass C and microbial DNA are linearly correlated. The SMB-C and the MRT of C in the SMB were not affected by P availability, whereas the microbial community significantly increased the MRT of P in its biomass from 18.1 to 39.0 days with decreasing P availability. Our results indicate that the adjustment of the MRT of individual elements in the SMB is an important process by which microbes adapt to nutrient poor environments and are able to maintain their biomass element ratio when decomposing substrate with a very high C-to-nutrient ratio.

1. Introduction

Microorganisms can immobilize large amounts of nutrients in their biomass, which strongly affects the concentrations of plant available nutrients (Van der Heijden et al., 2008; Richardson et al., 2009). While there are many publications dealing with soil microbial biomass C, N and P, and biomass stoichiometry (for reviews see Cleveland and Liptzin, 2007; Xu et al., 2013), very little is known about the mean residence time (=turnover time) of individual elements in the microbial biomass, and how it changes with element availability (Spohn, 2016).

The mean residence time (MRT) of an element in the microbial biomass likely increases with decreasing availability of the element (Oberson and Joner, 2005; Kaiser et al., 2014; Spohn, 2016), similar to the MRT of nutrients in plants (Aerts and Chapin, 2000). The reason for this seems to be that the efficiency of internal element recycling by microorganisms increases with decreasing element availability. This might be important especially during the decomposition of nutrient-poor substrates as for example dead wood and forest litter, in which the C-to-nutrient ratios exceed by far the C-to-nutrient ratios of the microbial biomass (Spohn, 2016). In an arable soil of the temperate zone, C in the microbial biomass had a MRT of 82–95 days, while P had a MRT of 37–42 days (Kouno et al., 2002), indicating that C was more efficiently retained in the microbial biomass than P. This finding is in accordance with the generally observed C limitation of microorganisms in mineral soil (De Nobili et al., 2001; Demoling et al., 2007). Cheng (2009) reported MRTs of the microbial biomass C of 48 and 35 days in planted soils, and 97 days in unplanted soils, suggesting that the presence of roots stimulated the turnover of microbial biomass C, likely due to the release of exudates, i.e. easily available C. Other studies on P in the microbial biomass determined P based on labeling of soil with ^{33}P in the field (McLaughlin et al., 1988; Oehl et al., 2001a). However, in these studies the period of time between labeling and determination of the isotope in the microbial biomass was very long, which likely led to an overestimation of the MRT.

So far, there is little reliable data on the MRT of individual elements in the microbial biomass pool because of several

* Corresponding author. Dr. Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany.
E-mail address: marie.spohn@uni-bayreuth.de (M. Spohn).

http://dx.doi.org/10.1016/j.soilbio.2017.05.017
0038-0717/

methodological difficulties. Most studies that investigated MRT of elements in the microbial biomass pool did so by tracing isotopes in the microbial biomass based on the chloroform-fumigation-extraction method (Kouno et al., 2002; Oberson and Joner, 2005). However, this method has the serious limitation that it is a differential method (based on the difference between two samples), which leads to relatively large errors because errors from the extraction of the two samples sum up. Kouno et al. (2002) determined the turnover of C and P based on isotope dilution. For this purpose, they labeled soil with ^{14}C and ^{33}P and determined the decrease in recovery of the tracer in the SMB over several months. Besides the previously mentioned shortcomings, this approach likely leads to an overestimation of the MRTs of elements in the SMB because once the tracer is in the soil, it might be constantly used for the build-up of new SMB. Achat et al. (2010) determined the turnover of microbial biomass P in forest soils combining an isotopic dilution method with a modelling approach. They found that a large part of the microbial P turned over in just a few days. In contrast, when the turnover time of microbial P was estimated from the seasonal fluctuations of microbial P, it was found to range between 224 and 293 days (Liebisch et al., 2014). This comparatively longer time is most likely due to the approach used, which likely overestimates the MRT.

The aim of this study was to overcome these methodological difficulties by determining the MRT of C and P in the SMB based on short-term incubation and isotopic labeling of microbial DNA. We hypothesized that microbes recycle an element more efficiently internally when little of the element is available than when it is highly available. Therefore, the MRT of P but not of C in the SMB increases with decreasing P availability.

2. Material and methods

2.1. Soil and experimental setup

We sampled the A horizon of a mesic grassland in the Botanical Garden of the University of Bayreuth (49°55′18 N, 11°35′03 E). The soil is a Cambisol and the A horizon has the following features: 14.1 g kg^{-1} total organic C, 1.0 g kg^{-1} total N, pH 4.5, and 0.32 mg g^{-1} total P (extractable in 0.5 M H_2SO_5, determined by ICP-OES).

The soil was sieved (<2000 μm), and all fine roots visible with the naked eye were removed using a tweezer. In total 15 PE bottles received each 30.0 g of fresh soil, and each represented one experimental unit. The water content was adjusted to 50% of the water holding capacity in all bottles. Simultaneously, five different levels of P fertilizer were added in the form of dissolved NaH_2PO_4 to reach P fertilization levels of 0, 20, 40, 60 and 80 μg P per g dry soil, each three times replicated. The fertilized soils were pre-incubated for 19 days at 20 °C in order to allow the microbial biomass to adjust to the altered P availability. Subsequently, two subsamples of each experimental unit (400 mg of fresh soil corresponding to 337 mg dry mass) were each placed in a 2 ml screw caps and were labeled with either ^{33}P or ^{14}C. For this purpose, 4 kBq ^{14}C-U-glucose (Hartmann Analytics) was added to one subsample (corresponding to 7.53 nmol C per g dry soil) and 20 kBq ^{33}P-orthophosphate (Hartmann Analytics) to the other subsample (corresponding to 0.52 pmol P per g dry soil), both dissolved in 50 μl millipore water. It should be noted that the amounts of P and C added were negligible, and ^{33}P and ^{14}C served only as a tracer without affecting the C or P availability. The soils were further incubated for 24 h at 20 °C, and subsequently they were frozen at −25 °C. This short incubation time after addition of the label was chosen in order to prevent an underestimation of the rate of DNA formation due to the decomposition of labeled DNA (see Discussion).

2.2. Analyses

The DNA was extracted from labeled samples using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals) which, compared to other protocols, has been shown to extract microbial DNA from soil with a high efficiency (Vishnivetskaya et al., 2014). The cells in the soil samples were lysed by bead beating in the FastPrep Instrument for 40 s at a speed setting of 6.0. Soil particles, colloids and cell debris was sedimented by centrifugation for 15 min at 14,000×*g*. Subsequently, the supernatant was collected and proteins were denatured by adding the protein precipitation solution, and were sedimented by centrifugation for 5 min at 14,000×*g*. The resulting supernatant was gently shaken for 2 min together with the binding matrix in order to allow the DNA to bind, and subsequently, the total volume of the mix was loaded on a spin filter that was inserted into a 2 ml cup. The DNA bound onto the matrix was washed with the washing solution containing mostly ethanol. The matrix on the filter was centrifuged to remove residues of the washing solution and air-dried. At last, the DNA was eluted from the binding matrix in 200 μl DNase-free water. Our DNA extracts were transparent, indicating that no humic substances were co-extracted. However, this might be different when studying soils that contain more humic substances, which might require washing the samples previous to DNA extraction according to He et al. (2005). The weight of the DNA extract was determined gravimetrically, and the DNA concentration in each extract was then determined fluorimetrically on a 5 μl aliquot of each extract by the picogreen assay (Sandaa et al., 1998) using a kit (Quant-iT™ PicoGreen® dsDNA Reagent, Life Technologies). The ^{14}C activity and the ^{33}P activity were determined by a Perkin-Elmer scintillation counter (Tri-Carb TR/SL 3180, Waltham, MA, USA) using scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany), and the signal was corrected for radioactive decay.

Inorganic P (P_i) and organic C, as well as the ^{33}P activity in the soil solution were determined using a different subsample from each experimental unit. For this purpose, 400 mg of fresh soil (corresponding to 337 mg dry mass) of each experimental unit were labeled with the same amount of ^{33}P as the samples used for DNA-extraction (20 kBq) and incubated at 20 °C. After 24 h, the soils were extracted in 20 ml distilled water by placing them on a horizontal shaker for 20 min. Subsequently, the samples were filtered through whatman filters. The concentration of P_i in the extracts was determined by the molybdenum blue assay using a multiplate reader (Infinite® M200 PRO, Tecan). The concentration of organic C in the water extracts was determined using a TOC/TN analyzer (Multi N/C 2100S, Analytik Jena AG, Jena, Germany), and the ^{33}P activity was determined by a Perkin-Elmer scintillation counter (Tri-Carb TR/SL 3180, Waltham, MA, USA) using scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany), and the signal was corrected for radioactive decay. We assumed that all glucose added to the soil was available for the microorganisms because glucose does not sorb to the soil matrix (Fransson and Jones, 2007) and is quickly taken up by microorganisms. The uptake of ^{14}C glucose into the SMB is so fast (Gunina and Kuzyakov, 2015) that one would strongly underestimate the bioavailability of ^{14}C-glucose if assessed by ^{14}C activity in water extracts.

Soil microbial biomass C and P (SMB-C and SMB-P) concentrations were determined by the chloroform fumigation extraction method (Brookes et al., 1982; Vance et al., 1987). Exactly 4.0 g of fresh soil were fumigated with chloroform for 24 h. Dissolved P was extracted from the fumigated and the non-fumigated soil in Bray-1 solution (0.03 M NH_4F - 0.025 M HCl) following Khan and Joergensen (2012), and quantified by the molybdenum blue assay using a multiplate reader (Infinite® M200 PRO, Tecan). A conversion factor of 2.5 was used to calculate SMB-P (Brookes et al., 1982).