Soil Biology & Biochemistry 61 (2013) 69–75

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

Phosphorus mineralization can be driven by microbial need for carbon

Marie Spohn*, Yakov Kuzyakov

Department of Soil Science of Temperate Ecosystems, Georg-August-University, Göttingen, Germany

ARTICLE INFO

Article history:
Received 29 September 2012
Received in revised form
12 February 2013
Accepted 13 February 2013
Available online 4 March 2013

Keywords:
Phosphorus recycling
Organic phosphorus
Soil microorganisms
Carbon limitation
Phosphorus mineralization

ABSTRACT

Despite the importance of phosphorus (P) mineralization to maintain soil fertility, little is known about the mechanisms that regulate microbial P mineralization. We tested the hypothesis that microbial P mineralization can be driven by microbial need for carbon (C). For this purpose, net microbial uptake kinetics of ^{14}C and ^{33}P from glucose-6-phosphate were studied in a Leptosol depending on availability of C, nitrogen (N), and P. After 60 h of incubation, 16.4% of the ^{14}C from glucose-6-phosphate was recovered in the microbial biomass, while ^{33}P incorporation into the microbial biomass was a third less. The higher net uptake of ^{14}C than of ^{33}P from the glucose-6-phosphate indicates that soil microorganisms use the organic moiety of phosphorylated organic compounds as a C source, but only use a small proportion of the P. Hence, they mineralize P without incorporating it. Our finding that the net uptake of ^{14}C and ^{33}P in the soils amended with inorganic P did not differ from the control treatment indicates that P mineralization was not driven by microbial need for P but rather for C. In a second experiment with three temperate forest soils we found that the activity of ^{14}C from glucose-6-phosphate in soil solution decreased faster than the activity of ^{33}P from glucose-6-phosphate. This might suggest that higher net uptake of C than of P from glucose-6-phosphate can also be observed in other temperate forest soils differing in C, N, and P contents from the Leptosol of the main experiment. In conclusion, the experiments show that microbial P mineralization can be a side-effect of microbial C acquisition from which plants potentially can benefit.

1. Introduction

One important measure to mitigate decreasing rock phosphate resources suitable for fertilizer production is to remobilize phosphorus (P) stocks already present in soil (Cordell et al., 2009). Total P contents in top soils (0–15 cm) typically range from 50 to 500 mg kg^{-1} (Sims and Pierzynski, 2005). However, only a small percentage of this P is bioavailable due to the high sorption capacity of the phosphate ions. The chemical forms of P in soils differ with parent material, soil pH and vegetation cover, and pedogenesis. The organic P pool increases with soil development, but tends to decline again in highly weathered soils (Walker and Syers, 1976). A large proportion of P in soils of the temperate zone is present in organic forms, and has to be mineralized to become available for plants (Stutter et al., 2012). Despite the importance of P mineralization to maintain soil fertility, little is known about the factors that drive microbial P mineralization.

Microorganisms can mineralize organic P inside and outside their cells. Many genera of bacteria are able to take up intact phosphorylated organic compounds (Heath, 2005; Winkler, 1973). Hexose phosphates can be taken up via a transport system called Uhp, which is induced exclusively by glucose-6-phosphate, but transports various hexose phosphates (Dietz, 1976). It has been pointed out that this transport system might become important for bacterial energy and carbon (C) metabolism once C becomes limited and hexose phosphates are abundant (Dietz, 1976; Kadner et al., 1994). While bacterial transport systems for phosphorylated compounds have been described at a molecular scale and in physiological studies with defined culture media (Dietz, 1976; Winkler, 1973; Kadner et al., 1994), little is known about microbial uptake of phosphorylated compounds in soils. However, it has been reported that C-limited microorganisms in deep strata of the ocean and in marine sediments use the organic moiety of phosphorylated compounds as a C source (Hoppe and Sören, 1999; Hoppe, 2003; Steenbergh et al., 2011). In soils, microbes are commonly C limited because organic matter is stabilized against decomposition by various mechanisms such as sorption, spatial inaccessibility or recalcitrance (De Nobili et al., 2001; Demoling et al., 2007; Blagodatskaya et al., 2009). Yet, according to a conceptual model proposed by McGill and Cole (1981) P mineralization in soils is decoupled from C mineralization. The model states that while nitrogen (N) is mineralized during SOM

* Corresponding author. Tel.: +49 (0)551 393507; fax: +49 (0)551 393310.
E-mail address: mspohn1@gwdg.de (M. Spohn).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.02.013

decomposition driven by bacterial need for C or energy, P mineralization is strongly controlled by the organisms' need for this element.

We tested the hypothesis that microbial P mineralization can be driven by microbial demand for C. More precisely, we tested the hypothesis that microorganisms use the organic moiety of phosphorylated organic compounds, but do not incorporate the P. This would mean that P mineralization can be a side effect of microbial C acquisition. To test this hypothesis we studied net microbial uptake kinetics of C and P from glucose-6-phosphate either uniformly labeled with ^{14}C or with ^{33}P. Phosphate monoesters represent a significant part of P in soils (Stutter et al., 2012). Glucose-6-phosphate is a common form of P in microorganisms, and therefore part of the highly cycled P pool in soils (Kadner et al., 1994; Oberson and Joner, 2005). Both ^{33}P- and ^{14}C-labeled glucose-6-phosphate was incubated with a Leptosol, and net microbial uptake kinetics of ^{33}P and ^{14}C were analyzed. In order to decrease potential limitations and allow microbial growth, the Leptosol was amended with C, N or P. To estimate whether we can find indications that the obtained results also hold true for temperate soils that differ in terms of C, N, and P contents, a further incubation experiment was carried out. In this experiment, the decreases in the contents of C and P of glucose-6-phosphate in the soil solution were studied.

2. Material and methods

2.1. Soils and sampling

The soils chosen for the experiments differ in C and P contents (Table 1). The soils are located in central Germany, in forests around the city of Göttingen. The mean annual temperature in the study area is 8.7 °C and the mean annual precipitation is approximately 640 mm. The Leptosol is located in the east of Göttingen (51°33′23 N, 9°58′25 E) in the Göttinger Wald. The soils in Göttinger Wald are largely developed from shell limestone and a mixed deciduous forest can be found featuring *Fagus sylvatica*, *Acer* sp., and *Sorbus aucuparia*. The Podzol and the Cambisol are located in the Bramwald in the southwest of Göttingen. The Podzol (51°31′01 N, 9°39′15 E) was formed on tertiary sands, while the Cambisol (51°30′51 N, 9°39′08 E) has developed from basalt. The vegetation on the Podzol is strongly dominated by *Picea abies*, while *F. sylvatica* is the dominant species on the Cambisol.

Soils were sampled in May 2012. Three profiles were dug in each soil. From each soil profile, we collected one sample from the subsoil below the A horizon at a depth of 21–25 cm. Subsoil was chosen because it is assumed that here C limitation of microorganisms is especially severe. The sampled horizons were classified as rendzic (Leptosol), spodic (Podzol), and cambic (Cambisol) according to WRB. One sample per horizon was taken from each profile. The soils were sieved (2 mm) and pre-incubated at 20 °C and 40% water holding capacity for 4 weeks prior to the incubation experiments in order to let them reach equilibrium under laboratory conditions after sampling and sieving disturbance.

2.2. Microbial uptake kinetics of C and P

Microbial net uptake kinetics of C and P from D-glucose-6-phosphate in a Leptosol amended with C, N or P were studied in an incubation experiment. The experiment was comprised of 120 experimental units. Each experimental unit consisted of 8.55 g of pre-incubated dry mass equivalent soil in a glass jar. One quarter of them received C, the second quarter N, the third quarter P, and the last quarter did not receive any amendment and served as a control. The C, N, and P amendments were designed according to Aldén et al. (2001) and Demoling et al. (2007). The soils received 2 mg g^{-1} C as glucose, 0.1 mg g^{-1} N as NH_4NO_3 or 0.1 mg g^{-1} P as KH_2PO_4 in 1 ml distilled H_2O. The control units received 1 ml distilled H_2O. The addition of KH_2PO_4 increased the content of $NaHCO_3$-extractable P to 8.1 (±0.1) μg g^{-1}, while the other treatments did not affect the content of $NaHCO_3$-extractable P. The soils were incubated at 20 °C in the dark for 48 h. Subsequently, 64 units were labeled with 20 kBq ^{14}C(U)-D-glucose-6-phosphate (specific ^{14}C activity: 9.0 GBq $mmol^{-1}$), and 60 units were labeled with 90 kBq ^{33}P-D-glucose-6-phospahate (specific ^{33}P activity: 111.0 GBq $mmol^{-1}$) in 1 ml distilled H_2O. The labeling led to an addition of 0.1 nmol glucose-6-phosphate per gram soil in the units labeled with ^{14}C and to 2.9 nmol glucose-6-phosphate per gram soil in the units labeled with ^{33}P. All experimental units were incubated at 20 °C in the dark throughout the experiment. Four parallel units of every treatment labeled with ^{14}C-glucose-6-phosphate were equipped with small glass vessels with 2 ml 0.2 M NaOH in order to trap the respired CO_2. The NaOH was renewed every day and ^{14}C activity in the NaOH was determined with a multi-purpose scintillation counter (Beckman–Coulter) using the scintillation cocktail RotiszintEcoplus (Roth).

At regular intervals three parallel units of each treatment were destructively harvested and the fumigation extraction method was applied to determine the uptake of ^{14}C and ^{33}P into the microbial biomass (Brookes et al., 1982; Wu et al., 1990). The units labeled with ^{14}C-glucose-6-phosphate were harvested 2, 7, 27, and 64 h after the labeling. The samples labeled with ^{33}P-glucose-6-phosphate were harvested 7, 26, 53, 168, and 240 h after the labeling. Each sample was split into two equal parts. One half was directly extracted. The other half was fumigated with chloroform for 24 h, and subsequently extracted. In order to analyze C uptake, 4.25 g soil labeled with ^{14}C-glucose-6-phosphate were extracted with 25 ml 0.5 M K_2SO_4 according to Wu et al. (1990). To analyze the P uptake 4.25 g soil labeled with ^{33}P-glucose-6-phosphate were extracted with 80 ml 0.5 M $NaHCO_3$ according to Brookes et al. (1982). The extracts of the non-fumigated soils are called soil extracts in the following. In order to determine the recovery efficiency of inorganic P, the 4.25 g of the same soil used in the experiment soil was spiked with 25 μg g^{-1} P and extracted with 0.5 M $NaHCO_3$

Table 1
Texture, pH, total organic carbon (C), total nitrogen (N), total phosphorus (P), C/P ratio, and $r_{1\ min}/R$ in the Leptosol, Podzol, and Cambisol. TOC and N were determined with an element analyzer. Total P was measured with ICP-AES (Spectroflame, Spectro). $r_{1\ min}/R$ is the percentage of ^{33}P orthophosphate that can be recovered from the soil 1 min after its addition to the soil. The values depict means that were calculated from independent analyses of three soil profiles per soil type. Values in brackets depict standard deviations.

Soil type	Texture [%]			pH_{H_2O}	C [g kg^{-1}]	N [g kg^{-1}]	P [g kg^{-1}]	C/P [mol mol^{-1}]	$r_{1\ min}/R$ [%]
	Sand	Silt	Clay						
Leptosol	1.1 (±0.1)	53.9 (±1.9)	45.0 (±1.9)	4.8 (±0.0)	15.6 (±3.9)	1.2 (±0.1)	0.32 (±0.01)	126	6
Podzol	65.0 (±1.3)	22.4 (±5.2)	12.6 (±6.1)	4.0 (±0.0)	28.1 (±3.5)	0.9 (±0.2)	0.13 (±0.01)	558	64
Cambisol	24.8 (±0.9)	55.1 (±1.1)	20.1 (±2.0)	5.0 (±0.0)	7.9 (±2.3)	0.6 (±0.1)	0.20 (±0.03)	102	3