Soil Biology & Biochemistry 110 (2017) 1–7

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Release of phosphorus from soil bacterial and fungal biomass following drying/rewetting

Mai-Van Dinh [a], Alexander Guhr [a, *], Marie Spohn [b], Egbert Matzner [a]

[a] Soil Ecology, University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Dr. Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany

[b] Soil Biogeochemistry, University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Dr. Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany

ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form
6 February 2017
Accepted 9 February 2017
Available online 1 March 2017

Keywords:
Drying–rewetting
Dissolved phosphorus
Soil microbial biomass
Saprotrophic fungi
Gram-positive bacteria
Gram-negative bacteria

ABSTRACT

Previous work has shown that the drying/rewetting (D/W) of soils mobilizes phosphorus (P), and that the effect of D/W on P release likely depends on the soil microbial community composition. We tested the hypotheses that (i) P release after D/W from fungi is lower than from bacteria and that (ii) gram-positive bacteria are less susceptible to D/W than gram-negative bacteria. We investigated the release of dissolved organic (DOP) and inorganic phosphorus (DIP) from bacterial and fungal biomass after rewetting of an artificial soil that was desiccated to different degrees. For this purpose, sterilized soil amended with growth medium was inoculated separately with one of two bacterial strains (*Pseudomonas fluorescens*, gram-negative and *Micrococcus luteus*, gram-positive) or with one fungal strain (*Penicillium chrysogenum*). The bacterial strains were grown for 7 days, the fungus for 25 days at 50% soil water holding capacity. After the pre-incubation period, microbial biomass P (Pmic) was determined by chloroform fumigation extraction, and soils were desiccated at 20 °C for 5–8 days until pF 6 (−100 MPa) was reached, while the controls were kept permanently at 50% water holding capacity. At different degrees of desiccation, samples were destructively harvested and soils were extracted with water to measure the release of DIP and DOP. The net release of total dissolved P per unit Pmic following D/W was in the order *P. fluorescens* » *M. luteus* = *P. chrysogenum*. In case of *P. fluorescens*, net release started already after desiccation to pF 4 (−1.0 MPa) and increased with further desiccation. For *M. luteus* and *P. chrysogenum*, a tendency for net release was only observed after severe desiccation up to pF 6. Our results suggest that the effect of D/W on P release from microbial biomass depends largely on the microbial community composition, with fungi and gram-positive bacteria being less susceptible to D/W than gram-negative bacteria.

1. Introduction

Previous studies have shown that the drying-and-rewetting of soils (in the following called D/W) leads to the lyses of microbial cells, resulting in the release of C, N and P (Birch, 1958; Blackwell et al., 2010; Halverson et al., 2000; Schimel et al., 1999; Turner et al., 2003). However, it is still not well understood how different groups of soil microorganisms respond to D/W of soil.

The change in soil water potential during drying-and-rewetting (D/W) of soils exerts physiological stress and energetic challenges to microbial communities (Kakumanu et al., 2013; Schimel et al., 2007). More than 50% of the microbial biomass may become necromass after soil D/W (Van Gestel et al., 1993; Wu and Brookes, 2005). Desiccation of soil exposes soil microorganisms to low soil water potentials, since water can cross microbial cell membranes. Thus, the cell internal osmotic potential of unicellular organisms without effective protective structures has to be equilibrated with the external soil water potential to maintain cell integrity. The sudden change in the water potential after rewetting of a dry soil requires the release of organic solutes (Halverson et al., 2000; Kieft et al., 1987) or those solutes are released after the lysis of cells (Blackwell et al., 2010; Fierer et al., 2003).

As a consequence, rewetting of dry soil often increased C and N

* Corresponding author. Soil Ecology, University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Dr.-Hans-Frisch-Straße 1-3, 95448 Bayreuth, Germany.
E-mail address: alexander.guhr@uni-bayreuth.de (A. Guhr).

http://dx.doi.org/10.1016/j.soilbio.2017.02.014
0038-0717/

mineralization (Gordon et al., 2008; Schimel et al., 1999) and led to an increase in soluble P in the soil solution (Achat et al., 2012; Bünemann et al., 2013; Dinh et al., 2016; Turner et al., 2003; Turner and Haygarth, 2001). The impact of D/W on the release of C, N and P in soils increased with decreasing soil water content prior to rewetting (Bünemann et al., 2013; Kakumanu et al., 2013). Several authors proposed that the release of P from microbial biomass is the main cause for the pulse of P after D/W of soils (Turner et al., 2003; Turner and Haygarth, 2001). However, Butterly et al. (2009) suggested a major contribution of abiotic sources.

The effect of D/W on the release of elements in soils seems to be influenced by the soil microbial community composition with fungi often being less sensitive and better adapted to D/W than bacteria (Bapiri et al., 2010; Yuste et al., 2011). One reason for the better adaptation of fungi compared to bacteria at low soil water potentials is seen in their thick cell walls with crosslinked polymers, preventing water losses (Gordon et al., 2008; Holland and Coleman, 1987). Under drought stress, fungal cell walls can be further stabilized by thickening and crosslinking of polymers (Kollár et al., 1997; Sietsma and Wessels, 1981). Fungi also release hydrophobic substances that prevent them from desiccation during drought (Spohn and Rillig, 2012). Further, filamentous fungi can extent their hyphal networks over long distances and across soil pores to access nutrient and water and counteract water stress (Guhr et al., 2015). With regard to procaryotes, gram-positive bacteria seem to be more resistant to D/W than gram-negative bacteria because of their specific cell wall properties. Gram-positive bacteria have a strong, thick cell wall with interlinked peptidoglycans to reduce water losses, while the cell wall of gram-negative bacteria consist of a single layer and an outer membrane (Madigan, 2012).

Experimental evidence for the better adaptation of soil fungi than of bacteria to D/W is not fully consistent. Barnard et al. (2013) and Cosentino et al. (2006) showed that fungal communities were less affected by D/W than bacterial communities. Kakumanu et al. (2013) also found that the fungi-to-bacteria ratio increased with drying of the soil. Gordon et al. (2008) showed that D/W led to a more intensive leaching of nutrients from grassland soils with a low fungal abundance. However, some studies observed that the ratio of fungi-to-bacteria remained unchanged after D/W stress (Hamer et al., 2007; Schmitt et al., 2010). Bapiri et al. (2010) investigated the effect of repeated D/W on bacterial and fungal growth based on leucine and ergosterol incorporation and found that D/W decreased bacterial growth while fungal growth remained unaffected. While this method very elegantly allows to distinguish bacterial and fungal growth is does not allow to determine differences between gram-negative and gram-positive bacteria.

In a previous study, we showed that the release of P following D/W differed between the layers of the forest floor, which might be due to different microbial communities inhabiting the different layers (Dinh et al., 2016). Hence, here we investigated the release of P after D/W from a fungus, a gram-positive and a gram-negative bacterium. We hypothesized that following D/W (1) the release of phosphorus from bacterial biomass is larger than from fungal biomass, (2) that the release of phosphorus from gram-positive bacteria is lower than from gram-negative bacteria, and (3) that the release of P from the microbial biomass increases with drought stress prior to rewetting. To test these hypotheses, we conducted a laboratory experiment using an artificial soil inoculated separately with different species.

2. Materials and methods

2.1. Experimental setup

The experimental unit of this experiment was a petri dish with artificial soil amended with growth medium, both steam-sterilized, and inoculated with one out of three different soil microorganisms. The artificial soil used in this experiment was a 3:1 mixture of sand (Dorsilit Nr. 9, particle size: 0.1–0.5 mm, 97% SiO_2, Dorfner GmbH & Co. KG, Hirschau, Germany) and silt (Sikron SF300, particle size: 2–64 μm, 98% SiO_2, Quarzwerke GmbH, Frechen, Germany) from pure quartz which was cleaned by rinsing with deionized water. The bulk density of the mixture was 1.06 g cm^{-3}360 g of the artificial soil was arranged in a 1 cm layer in petri-dishes with 200 mm diameter. The P content of the artificial soil measured in Bray-1 extracts (0.025 M HCl + 0.03 M NH_4F) was less than the detection limit of ICP-OES (<2 mg P kg^{-1} soil). The soil was inoculated separately with the bacteria (*Pseudomonas fluorescens* Migula (gram-negative, DSMZ-No: 4358) or *Micrococcus luteus* (Schroeter) Cohn (gram-positive, DSMZ-No: 20030)) or the fungus (*Penicillium chrysogenum* Thom). The species were chosen since they are well studied and commonly found in soils. Further, they are fast growing and can be considered as r-strategists. Hence, they likely have a high RNA and consequently cellular P content since both are strongly linked to the growth rate and the growth strategy (Sterner and Elser, 2002; Keiblinger et al., 2010). In addition, *P. fluorescens* is considered as a phosphate-accumulating organism under conditions of excessive available P (Sidat et al., 1999). Bacterial and fungal cultures were kindly provided by the Department of Ecological Microbiology and the Department of Mycology, respectively, University of Bayreuth. 1 ml of a liquid pre-culture was mixed with 43 ml of a malt extract glucose meat extract peptone liquid medium (MGMPB: 0.3% malt extract, 0.3% meat extract, 0.5% peptone and 1% glucose, w/v). The amount of medium was chosen to reach 50% of the maximum water holding capacity of the soil. The addition of nutrients with the MGMPB solution to the artificial soil amounted to (in mg kg $soil^{-1}$): 3.1 dissolved inorganic P (DIP), 5.3 dissolved organic P (DOP), 1.070 organic carbon, and 111 total N (with 108 organic N). The pre-incubation and the experiment were conducted in a climate chamber at 20 °C. All treatments and controls were set up with 4 replicates. In total, 40 petri dishes were established for each bacterium, and 64 for the fungus.

After, inoculation petri-dishes were closed and incubated for 7 days (bacteria) and for 25 days (fungus) at 20 °C to allow growth. At the end of the pre-incubation period, the desiccation experiment was started by opening the petri-dishes of the D/W treatment to allow the soil to dry. The control petri-dishes were kept close during the whole experiment. Soil water potentials were measured daily by a dew point potentiometer (WP4C, Decagon Devices Inc. Pullman WA, U.S.A.). The experiment lasted until a water potential of about −100 MPa (pF 6) was reached. At each day, 4 petri dishes and 4 controls for each of the three microorganisms were destructively harvested. A subsample of 6 g per petri dish was extracted in deionized water (rewetting event) in a soil: water ratio of 1:10 by shaking the soil for 140 min on a horizontal shaker in order to determine DOP and DIP.

2.2. Determination of microbial biomass P and ergosterol

At day 1 of the desiccation, microbial biomass P (Pmic) was measured by chloroform fumigation-extraction (Brookes et al., 1982; Vance et al., 1987). After fumigation, soils were extracted in Bray-1 solution (0.025 M HCl + 0.03 M NH_4F) with a soil: solution ratio of 1:10 (Aponte et al., 2010; Bray and Kurtz, 1945; Heuck et al., 2015). Total P in the Bray-1 extracts was measured by ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, U.S.A.). Pmic was calculated as the difference of inorganic P in the fumigated and non-fumigated soil extracts, using a conversion factor of 2.5 (Brookes et al., 1982; Jenkinson, 2004).

Ergosterol was measured as an indicator of fungal biomass as