Soil Biology & Biochemistry 42 (2010) 926–934

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil

Johannes Rousk [a,*], Philip C. Brookes [b], Erland Bååth [a]

[a] Department of Microbial Ecology, Lund University, Ecology Building, SE-223 62 Lund, Sweden
[b] Soil Science Department, Rothamsted Research, Harpenden, Herts ALS 2JQ, UK

ARTICLE INFO

Article history:
Received 26 October 2009
Received in revised form
6 January 2010
Accepted 11 February 2010
Available online 24 February 2010

Keywords:
Fungal-to-bacteria ratio
Fungal growth
Bacterial growth
Soil pH
Decomposer interaction
Acetate incorporation into ergosterol
Leucine incorporation

ABSTRACT

Soil pH is one of the most influential variables in soil, and is a powerful factor in influencing the size, activity and community structure of the soil microbial community. It was previously shown in a century old artificial pH gradient in an arable soil (pH 4.0–8.3) that bacterial growth is positively related to pH, while fungal growth increases with decreasing pH. In an attempt to elucidate some of the mechanisms for this, plant material that especially promotes fungal growth (straw) or bacterial growth (alfalfa) was added to soil samples of the pH gradient in 5-day laboratory incubation experiments. Also, bacterial growth was specifically inhibited by applying a selective bacterial growth inhibitor (bronopol) along the entire pH gradient to investigate if competitive interaction caused the shift in the decomposer community along the gradient. Straw benefited fungal growth relatively more than bacterial, and vice versa for alfalfa. The general pattern of a shift in fungal:bacterial growth with pH was, however, unaffected by substrate additions, indicating that lack of a suitable substrate was not the cause of the pH effect on the microbial community. In response to the bacterial growth inhibition by bronopol, there was stimulation of fungal growth up to pH 7, but not beyond, both for alfalfa and straw addition. However, the accumulation of ergosterol (an indicator of fungal biomass) during the incubation period after adding alfalfa increased at all pHs, indicating that fungal growth had been high at some time during the 5-day incubation following joint addition of alfalfa and bronopol. This was corroborated in a time-series experiment. In conclusion, the low fungal growth at high pH in an arable soil was caused to a large extent by bacterial competition, and not substrate limitation.

1. Introduction

Fungi and bacteria dominate the decomposition of organic matter in soils. There are important differences between these microbial groups, however, and it has been shown that they are differently affected by such factors as nutrient status (De Vries et al., 2006, 2007; Demoling et al., 2008), metal toxicity (Rajapaksha et al., 2004) and substrate quality (Meidute et al., 2008; Rousk and Bååth, 2007b; Güsewell and Gessner, 2009; Strickland et al., 2009a,b). Changes in the relative importance of fungi and bacteria may have significant effects on the soil ecosystem. For instance, a fungal-dominated system has been suggested to contribute more to carbon (C) sequestration due to higher growth efficiency (Six et al., 2006), although this has been questioned (Thiet et al., 2006), and to increase biomass turnover time (Bardgett and McAlister, 1999; Van Groenigen et al., 2007).

Soil pH is one of the most influential factors in soil, and strongly influences the biomass, activity and composition of the microbial community (e.g. Matthies et al., 1997; Blagodatskaya and Anderson, 1998; Bååth and Anderson, 2003; Högberg et al., 2007; Nilsson et al., 2007; Lauber et al., 2008; Jones et al., 2009; Rousk et al., 2009). In a long-term experimental field at Rothamsted Research, UK, an artificial pH gradient was initiated in the mid-19th century that resulted in a pH gradient ranging from 4.0 to 8.3 within 200 m in the same agricultural field. No fertiliser amendments have been applied, and the same cropping history has been used since its establishment. This experiment, the Hoosfield acid strip, thus presented a soil where the variable soil pH was uniquely isolated from confounding variables (Aciego Pietri and Brookes, 2007a,b, 2009). Recently, fungal and bacterial growth was estimated along the Hoosfield acid strip in an attempt to estimate how soil pH influenced the relative importance of fungi and bacteria (Rousk et al., 2009). There was more than a five-fold increase in fungal growth between pH 8.3 and 4.5, while bacterial growth decreased more than five-fold in the same interval. This resulted in an almost 30-fold increase in the

* Corresponding author. Tel.: +46 70 7507522.
E-mail address: johannes.rousk@mbioekol.lu.se (J. Rousk).

0038-0717/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2010.02.009

relative importance of fungi, indicated by the growth ratio, from the high pH soils to pH 4.5.

In this study we wanted to investigate potential mechanisms for the different pH relationships of fungal and bacterial growth, especially focusing on the competitive interaction between the decomposer groups. It was previously demonstrated that additions of straw especially promoted fungal growth, while additions of alfalfa especially promoted bacterial growth (Rousk and Bååth, 2007b). Thus, the addition of straw and alfalfa along the pH gradient would remove any confounding influence that different substrate availabilities may have on the pH influence on fungal and bacterial growth (Rousk et al., 2009), and also create a situation where either fungi (straw addition) or bacteria (alfalfa addition) would be relatively more benefited. Recently, a framework to estimate the competitive influence that bacteria exercise on fungi was developed using the combination of selective bacterial inhibitors and measurements of growth (Rousk et al., 2008). Unfortunately, the lack of efficient yet specific fungal inhibitors prevented the direct investigation of reciprocal relationship, the fungal competitive influence on bacteria. Thus, to partially test if competitive interaction between fungi and bacteria could explain their different pH relationships, we selectively inhibited bacterial growth along the entire pH gradient and monitored the fungal response to this in unamended soil, in soil with an added fungi-promoting substrate (straw), and in soil with an added bacteria-promoting substrate (alfalfa). In addition, we also investigated the functional consequence of the fungal:bacterial dynamics along the pH gradient by measuring basal respiration as well as the total microbial biomass. Consequently, our hypotheses were: (i) Addition of straw will especially promote fungal growth, while addition of alfalfa will especially promote bacterial growth, irrespective of pH. (ii) The bacterial growth inhibition (by bronopol) will stimulate fungal growth, and the stimulation will be proportional to the decline in bacteria, and will thus also be higher combined with bacteria-promoting alfalfa compared with fungi-promoting straw. (iii) When the increasing competitive pressure exerted by bacteria with increasing pH is removed (bacterial growth is inhibited), the negative correlation between fungal growth and increasing pH will cease.

2. Materials and methods

2.1. Soil

The soil pH gradient was previously described in greater detail by Aciego-Pietri and Brookes (2007a,b). In April 2008, 27 samples covering the pH gradient were sampled, after which they were stored frozen until September. The samples were subsequently thawed, sieved (<2.8 mm) and water content was determined (105 °C, 24 h). The variation in moisture content of the soils samples was low, and all were close to 40% of the water holding capacity, so moisture adjustment was not needed. The samples were then stored in the dark at 5 °C for 1–2 months, until used. Background data on soils from the same sampling has been presented previously (Rousk et al., 2009).

2.2. Experimentation

2.2.1. Main experiments

Soil subsamples (5 g) were added to 50 mL polyethene tubes. These soils samples were treated with two levels (with or without) of two factors (substrate and bacterial inhibitor) in a full-factorial design (totalling 4 treatments). Two different substrates were used in two separate experiments, each with the indicated full-factorial design. The substrates were dried and ball-milled (<250 μm) straw (C:N = 75) or alfalfa (C:N = 15) and were added at 1 mg C g^{-1} soil, thus increasing the soil C-content by about 10%. The bacterial inhibitor treatment was bronopol (40 μg g^{-1} soil added with 2 μl water g^{-1} soil; all treatments received the same amount of water). Pilot experiments were used to determine inhibitor concentrations and incubation periods; a concentration of bronopol was chosen with the criterion of almost completely reducing bacterial growth (>90% reduction) without affecting fungal growth across the entire pH gradient (we tested soils at pH 4.0, 5.1, 6.7 and 8.0). The two main experiments totaled 108 microcosms for each plant material. The straw-amended series was run in November and the alfalfa-amended series in December 2008. The microcosms were incubated in the dark for 5 days at 22 °C (cf. Rousk et al., 2008), and were subsequently analysed for fungal growth, bacterial growth, ergosterol concentration, respiration and SIR-biomass.

2.2.2. Time-series experiment

Microcosms (15 g) of four soil samples from the high end of the gradient (pH 8.1 ± 0.1), one sample at pH 5.1 and one sample at pH 4 were each treated in a factorial design of straw or alfalfa addition (1 mg C g^{-1} soil) and in the presence or absence of bronopol (40 μg g^{-1} soil in 2 μl water g^{-1} soil). Subsamples were analysed for fungal growth, ergosterol concentration and bacterial growth immediately following treatment application (0 days), and after 1, 2, 4, and 7 days incubation in the dark at 22 °C.

2.2.3. Bronopol tolerance

The bacterial community tolerance of one of the soils from the high end of the pH gradient (pH 8) was investigated for bronopol tolerance following the time-series experiment according to Aldén Demoling et al. (2009). Subsamples of the bacterial suspension from the four different treatments (straw and alfalfa addition with and without bronopol) were exposed to 12 bronopol concentrations of (0–1.5 mg ml^{-1}) to determine the concentration that inhibited bacterial growth by 50% (EC_{50}). A higher EC_{50} would indicate that the bacterial community was more tolerant to bronopol. Note that the units for bronopol additions, and subsequently also bacterial growth rates, are given per volume of bacterial suspension, which are not directly translatable to the bronopol concentration administered to the soil. However, this provides an effective index that can be used to screen for changes in tolerance in differently treated soils (Aldén Demoling et al., 2009).

2.3. Microbial analyses

2.3.1. Bacterial growth

The bacterial growth was estimated using leucine (Leu; Kirchman et al., 1985) incorporation in bacteria extracted from soil using the homogenization/centrifugation technique (Bååth, 1992, 1994) with modifications (Bååth et al., 2001). We added 2 μl radio-labelled Leu ([^{3}H]Leu 37 MBq ml^{-1}, 5.74 TBq $mmol^{-1}$, Amersham) combined with non-labelled Leu to each tube, resulting in 275 nM Leu in the bacterial suspensions. The amount of Leu incorporated into extracted bacteria per h and g soil was used as a measure of bacterial growth.

2.3.2. Fungal growth and biomass

Fungal growth was assessed using the acetate into ergosterol incorporation method (Newell and Fallon, 1991) adapted for soil (Pennanen et al., 1998; Bååth, 2001) with modifications (Rousk et al., 2009), adding 1-[^{14}C]acetic acid (sodium salt, 7.4 MBq ml^{-1}, 2.04 GBq $mmol^{-1}$, Amersham) combined with unlabelled sodium acetate resulting in a final acetate concentration of 220 μM in a soil slurry and having a 5 h incubation at 22 °C without light. Ergosterol was extracted, separated and quantified using HPLC equipped with