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Comparison of fungal and bacterial growth after alleviating induced N-limitation in soil

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ABSTRACT

The extent and type of nutrient limitation will affect soil microorganism activity and may change the balance between organism groups, like fungi and bacteria. Limiting nutrients have traditionally been measured as increased respiration after adding nutrients, but this will not differentiate between fungal or bacterial responses. We compared respiration, bacterial and fungal growth after alleviating limitation in soils originally being C-limited, but experimentally altered to N-limitation. Three soils of similar pH and organic matter but with different N availability were used. We amended the soils with C-rich substrates, starch (40 mg g^{-1}) and straw (80 mg g^{-1}), followed by a 4 weeks incubation at 22 °C to induce N-limitation. Starch amendment resulted in increased respiration and bacterial growth, while straw amendment increased all three variables (respiration, bacterial and fungal growth), with only minor differences between soils. Alleviating C- and N-limitation was then tested in a short-term assay after adding C (glucose) and NH_4NO_3 in a full factorial design. In non-amended, C-limited soils, adding C resulted in increased respiration and especially bacterial growth, while fungal growth only increased in the High N soil. Straw amendment resulted in N-limitation, since adding N increased respiration and especially fungal growth. N-limitation for bacterial growth was evident in all starch amended soils, with similar effects for respiration, although adding C also increased respiration. Fungal growth was not affected by C- or N-additions in starch-amended soils. Thus, which microbial group that responded to alleviating N-limitation depended on the C-source in the soil. Furthermore, we found no indication of growth and respiration reacting differently to alleviating N-limitation indicating altered C-use efficiency.

1. Introduction

The activity of fungi and bacteria in soil is regulated by substrate flow and constrained by environmental factors, like temperature and moisture. There are many environmental factors affecting these two groups in a similar way, for example, cardinal temperatures for growth were similar for bacteria and fungi (Pietikäinen et al., 2005) and increasing temperature resulted in similar altered temperature relationships of growth for both groups (Bárcenas-Moreno et al., 2009). However, other environmental factors affect fungi and bacteria very differently. For example, low pH favor fungal growth, while high pH favor bacterial growth in soil (Rousk et al., 2009).

One factor that seldom has been compared for fungi and bacteria is effects of nutrient limitations. The most common approach to measure nutrient limitation has involved respiration methods, assessing if adding C, N and P alone or in combinations increase the respiration response (Nordgren, 1992; Duah-Yentumi et al., 1998; Tiunov and Scheu, 1999; Gnankambary et al., 2008; Garland et al., 2012). Respiration measurements, however, do not differentiate between fungal and bacterial activity. Furthermore, interpretations of respiration responses are often complicated in that adding easily available C always increases respiration, irrespective of type of nutrient limiting growth. Thus, limitation is often measured after adding C as glucose, that is, limitation is measured under excess of C to ensure growth (Garland et al., 2012).

Direct estimates of nutrient limitation for microbial growth in soil have mainly been studied for bacteria. There have been several studies indicating that bacterial growth in soils often are limited by lack of carbon (C) (Aldén et al., 2001; Ekblad and Nordgren, 2002;

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Demoling et al., 2007, 2008; Kamble et al., 2013; Kamble and Bååth, 2014), although some evidence of both N and P limitation has been reported (Aldén et al., 2001; Rinnan et al., 2007; Schmidt et al., in press), especially after amendments of high concentrations of C-rich substances (Tenney and Waksman, 1929; Fog, 1988; Recous et al., 1995; Kamble and Bååth, 2014). However, less information on nutrient limitation for fungal growth in soil is available.

To what extent a limitation and the subsequent release of a limitation by adding the limiting nutrient affect the balance of fungal and bacterial growth is little known, although it is often stated that fungi should be favored by N-limitation, especially at conditions of high C/N ratio of the substrate (Thiet et al., 2006). Thus, we would expect that when alleviating N-limitation in soil, bacterial growth will be more positively affected than fungal growth. However, Henriksen and Breland (1999) found that adding straw together with increasing amounts of N, thereby alleviating the N-limitation, increased the amount of ergosterol (a proxy of fungal biomass) with increasing levels of N, while little effect was found on the bacterial biomass. Rousk and Bååth (2007) found similar results, where adding increasing amounts of N to straw-amended soil increased fungal growth, but tended to decrease bacterial growth.

Schimel and Weintraub (2003), using a theoretical model, argued that adding N in an N-limited soil system could decrease respiration, while adding C in an N-limited system could increase respiration. These results were due to shifts in the partitioning between respiration and growth in the model (altered C use efficiency), where a large part of C added in the N-limited system would be respired as waste metabolism, since no growth would be possible. On the other hand, adding N in an N-limited situation was modeled to decrease respiration, since more of the soil organic matter was shunted into growth when the N-limitation was alleviated. One way of testing these predictions is to measure microbial growth in parallel with respiration, where an increase in both respiration and growth after alleviating N-limitation would not be in accordance with the model of Schimel and Weintraub (2003).

In the present study we compared fungal and bacterial growth and total microbial activity (respiration) in three soils that were experimentally fertilized with 0, 50, or 150 kg N ha^{-1} for 22 years to mimic increased N pollution (the Harvard Forest Chronic Nitrogen Amendment Study). We incubated the soils for 4 weeks with two C-rich substrates, straw or starch. Cellulose rich substrates, like straw, have been shown to favor fungal growth (Hu and van Bruggen, 1997; Rousk and Bååth, 2007) and starch bacterial growth (Rinnan and Bååth, 2009). Adding these C-rich substrates was expected to alter the soil from being C- to N-limited for microbial growth (a similar experimental design as in Kamble and Bååth, 2014). We then added C and N in a short term assay to determine limiting factors (Aldén et al., 2001; Demoling et al., 2007) and how alleviating the limitation affected microbial activity. We hypothesized that i) in the non-amended soils, microbial growth would be C-limited, seen as increased respiration and microbial growth when adding C but not when adding N, ii) that substrate amendments had altered the soils to become N-limited for microbial growth, and iii) alleviating N-limitation in the substrate amended soils would induce more fungal growth in straw than in starch amended soil, and the other way around for bacterial growth. Thus, the C-source was predicted to be important for the fungal to bacterial balance even if the soil initially is N-limited. Finally, we wanted to test the prediction of Schimel and Weintraub (2003), that alleviating N-limitation could result in increased microbial growth at the same time as a decrease in respiration.

2. Material and methods

2.1. Soils

Soil samples were collected in September 2009 from the Chronic Nitrogen Amendment Study at the Harvard Forest Long Term Ecological Research (LTER) site in central Massachusetts, USA. These plots were established in 1988 to study long-term effects of N addition (Aber and Magill, 2004). The forest is dominated by black (*Quercus velutina*) and red (*Q. rubra*) oak mixed with black birch (*Betula lenta*), red maple (*Acer rubrum*) and American beech (*Fagus grandiflora*). Soils are of the Gloucester series (fine loamy, mixed, mesic, Typic Dystrochrepts, USDA). There were three N-fertilization regimes amended annually with 0 (No N), 50 (Low N) or 150 (High N) kg N ha^{-1} as NH_4NO_3.

Soil samples were collected from each of four replicate 5 × 5 m sub-plots within each treatment plot. Two 8 cm diameter and 10 cm long cores were removed from each sub plot, separated into the organic and mineral horizon, and bulked by soil horizon. Samples were sieved (2 mm) and stored field moist at 4 °C until analyzed. Only the mineral soil was used in the present study. Soil organic matter (as loss on ignition after heating to 600 °C for 6 h in a muffle furnace) was: No N 12.8%, Low N 12.7% and High N 13.2%. pH in water was 5.0, 5.2 and 5.4 in the No N, Low N and High N. Moisture content was similar in the three soils.

2.2. Microbiological measurements

2.2.1. Respiration

Respiration was measured using 1 g of soil in 20 ml glass vials. The vials were sealed with a crimp cap and incubated for 24 h at 22 °C, after which the CO_2 concentration was determined using a gas chromatograph.

2.2.2. Bacterial growth

Bacterial growth was estimated using the leucine (Leu) incorporation technique on bacteria extracted from the soil (Bååth et al., 2001). Briefly, 1 g of soil was mixed with 20 ml distilled water, vortexed for 3 min, and then centrifuged for 10 min at 1000 × *g* to obtain a bacterial suspension (the supernatant). The supernatant (1.5 ml) was transferred into 2 ml micro-centrifugation tubes. Radiolabeled Leu (2 μl L-4,5-3H-Leucine, 37 MBq ml^{-1}, 1.48–2.22 TBq $mmol^{-1}$, Perkin Elmer, USA) was added together with non-radioactive Leu (final concentration 275 nM). After a 2-h incubation period at 22 °C, growth was terminated by adding 75 μl 100% trichloroacetic acid. Washing and subsequent measurement of radioactivity of the bacteria were performed according to Bååth et al. (2001). Leu incorporation per h and g soil into extracted bacteria was calculated as a proxy of bacterial growth.

2.2.3. Fungal growth and biomass using ergosterol

Fungal growth was estimated by acetate incorporation into ergosterol (Ac-in-erg, Newell and Fallon, 1991; Bååth, 2001). 1 g of soil was put into 10 ml test tubes with 1.5 ml water, 20 μl [1^{-14}C] acetic acid (sodium salt; 7.4 MBq ml^{-1} and 2.04 GBq $mmol^{-1}$; Perkin Elmer, USA), and 480 μl 1 mM unlabeled sodium acetate, resulting in a final acetate concentration of 220 μM. The test tubes containing the soil slurry was then incubated at 22 °C for 4 h. One milliliter of 5% formalin was used to terminate the incorporation of acetate. Ergosterol in the soil was extracted in 5 ml 10% KOH in methanol, and separated and quantified using HPLC with a UV detector (282 nm) according to Rousk et al. (2009). The ergosterol peak was collected and the amount of incorporated radioactivity was determined using a scintillator counter. Ac incorporation into ergosterol per g soil was calculated as a proxy of fungal growth. The