Soil Biology & Biochemistry 43 (2011) 2457–2460

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

Short communication

Plant regulation of microbial enzyme production *in situ*

Colin Averill[*,1], Adrien Finzi

Boston University, Biology Department, 5 Cummington Street, Boston MA 02215, USA

ARTICLE INFO

Article history:
Received 25 June 2011
Received in revised form
23 August 2011
Accepted 4 September 2011
Available online 20 September 2011

Keywords:
Enzymes
Soil microbial community composition
Mycorrhizal fungi
Root–soil interactions
Organic nitrogen
Forest ecosystems
Biogeochemistry

ABSTRACT

Soil extracellular enzymes regulate the rate at which complex organic forms of nitrogen (N) become bio-available. Much research has focused on the limitations to heterotrophic enzyme production via lab incubations, but little has been done to understand the limitations to enzyme production *in situ*. We created root and symbiotic mycelia exclusion treatments using mesh in-growth bags in the field to isolate the effect of roots and other portions of the microbial community on enzyme production. When fertilized with complex protein N we found increases in N-degrading enzyme concentrations only when root in-growth was allowed. No response was observed when complex N was added to root-free treatments. Expanding on economic rules of microbial element limitation theory developed from lab incubation data, we suggest this is due to active transport of labile carbon (C) from roots to associated microbial communities in root bags. Roots alleviate C limitation of microbial enzyme synthesis, representing a tradeoff between plants and microbes–plant C for microbially-derived N.

The depolymerization of nitrogen (N) from soil organic matter (SOM) is the rate-limiting step in converting complex organic N into bio-available forms (Schimel and Bennett, 2004). Free-living, heterotrophic soil microbes and symbiotic mycorrhizal fungi mediate this process through the release of extracellular enzymes (Ratledge, 1994; Read and Perez-Moreno, 2003). Our current understanding of microbial enzyme production emphasizes the importance of carbon (C) and N availability to enzyme synthesis (Schimel and Weintraub, 2003). Allison and Vitousek (2005) demonstrated that heterotrophic microbes increase N-degrading enzyme production in response to complex, organic N addition (collagen protein) only when added in combination with labile C and other limiting micronutrients. Based on these findings, Allison and Vitousek (2005) suggested that enzyme production by soil microbes is regulated by economic rules; microbes only increase enzyme production in response to addition of a complex limiting nutrient when they are relieved from co-limitation by carbon and other limiting micronutrients.

Most of the work on limitations to microbial extracellular enzyme activity occurs in the lab in the absence of plant roots. It is well known however that plant roots act as an active conduit for labile C inputs to the soil either through C allocation to mycorrhizal symbionts (Jennings, 1995) or exudation of labile C compounds (Kuzyakov, 2002). Hence, even though soil microbes may be limited by C supply in the lab, in the field C subsidies from roots may alleviate this C limitation.

In this study we hypothesized that plant roots ameliorated microbial-C limitation to the point where the production of extracellular enzymes involved in the acquisition of N from complex sources was N-substrate limited, rather than co-limited by C and N as it is in the lab. On this basis, we made two explicit predictions: **P1** – *Addition of complex organic N (i.e. collagen) in the presence of roots increases the production of N-degrading enzymes; and* **P2** – *In the absence of roots and mycorrhizae, complex organic N addition does not stimulate enzyme production due to C limitation of enzyme synthesis.*

We constructed three types of mesh in-growth bags to test this hypothesis, isolating the effects of roots, mycorrhizas and heterotrophic microbes. Each type of in-growth bag was amended with factorial additions of complex N and inorganic phosphorus (P), an often equally important limiting nutrient.

Study plots (10 × 10 m) were established on Mount Eisenhower, in the White Mountain National Forest in NH USA. Site details can be found in Averill and Finzi (2011). Three replicate plots at 4 elevation sites were established across a steep elevation-climate gradient, from 636 to 1356 m above sea level for a total of 12 study plots. By sampling across a steep gradient in temperature and precipitation we intended to capture a wide range of environmental conditions, and thus increase the generality of our findings.

* Corresponding author. Tel.: +1 203 444 8925; fax: +1 512 471 3878.
E-mail address: colin.averill@mail.utexas.edu (C. Averill).
[1] Present address: The University of Texas at Austin, Patterson Lab Building, 2401 Speedway Blvd, Austin TX 78712, USA.

0038-0717/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2011.09.002

To examine the effect of plant C allocation on microbial extracellular enzyme production, we built in-growth bags with varying mesh size to allow root plus hyphal growth (2 mm nylon mesh) or hyphal growth only (50 μm nylon mesh). In both treatments free-living heterotrophic components of the microbial community were present, so a third set of in-growth bags were placed in 60 × 60 cm trenches to sever all root and mycorrhizal connections making for a plant C input-free treatment. These treatments are referred to herein as root, mycelia, and trench bags, respectively. Bags were filled with 150 g of sieved organic horizon material from the field, then fitted with pieces of Tygon tubing so nutrient additions could be delivered *in situ* throughout the growing season without disrupting the soil.

Bags were fertilized factorially with complex protein N as collagen (11 μg N g^{-1} soil), and inorganic phosphorus as sodium phosphate (20 μg P g^{-1} soil). Nutrients were delivered as 10 mL solutions during the first weeks of June, July, and August 2009. Bags were harvested the first week of September. Unamended controls received water additions. This study aimed to understand *in situ* limitations of microbial enzyme production, with additions of nutrients intentionally low, to avoid fundamentally altering the soil environment (i.e., microbial activity, root growth, exudation, pH) and within the range of variability observed at these sites. Each factorial nutrient addition (control, +N, +P, +N + P) was performed for every mesh type and replicated once in all 12 plots, generating 144 in-growth bags.

Upon collection soil amino acid, ammonium and nitrate pools were extracted using 2 M potassium chloride. Subsamples were frozen at −80 °C for enzyme assays and soil ergosterol extraction. We measured C-degrading enzymes polyphenol oxidase (PPO) and peroxidase (PRX), N-degrading enzymes leucine aminopeptidase (LAP) and β-N acetylglucosaminidase (NAG), and the P acquisition enzyme acid phosphatase (AP). Chemical analyses were conducted using standard methods, outlined in Supplementary Information.

The response of all measurements to fertilization was determined using a linear mixed effects model using the LME function in the NLME package for R (R Development Core Team). Plot was set to be a random effect in order to keep each plot's control bag paired to its respective treatments within the same plot. Fertilization was coded as a fixed effect. Responses are reported as significant if $p < 0.05$. To control for an inflated Type-I error rate due to multiple comparisons, a Bayesian multilevel model, identical in structure to the one described above, was fitted using WinBUGS software (Thomas, 1994). Significant results were excluded if the 95% credible interval for a parameter overlapped zero.

A significant increase in the sum of N-degrading enzymes LAP and NAG was observed in root bags fertilized with complex N. Furthermore, this response was absent from trenched bags (Fig. 1). These observations match our economic-based predictions and provide strong support for our hypothesis that plant roots alleviate microbial C limitation in the field, allowing microbes to respond to complex substrate addition directly via enzyme production (*sensu* Allison and Vitousek, 2005).

Similar to LAP and NAG responses, ergosterol concentrations as well as PPO activity significantly increased in root bags fertilized with complex N (Fig. 1). Because PPO is often produced in concert with N-degrading enzymes to liberate N from humic and tannin complexes (Sinsabaugh, 2010), this result is also consistent with our hypothesis.

No change in extractable AA-N concentrations was observed (Table 1). This is also consistent with our hypothesis, as an increase in N-degrading enzyme production suggests an increase in plant and microbial N demand- it is likely that any increase in AA-N production as a result of increased N-degrading enzyme activity was matched by an increased amino acid N uptake rates of plants and fungi in the root in-growth bags.

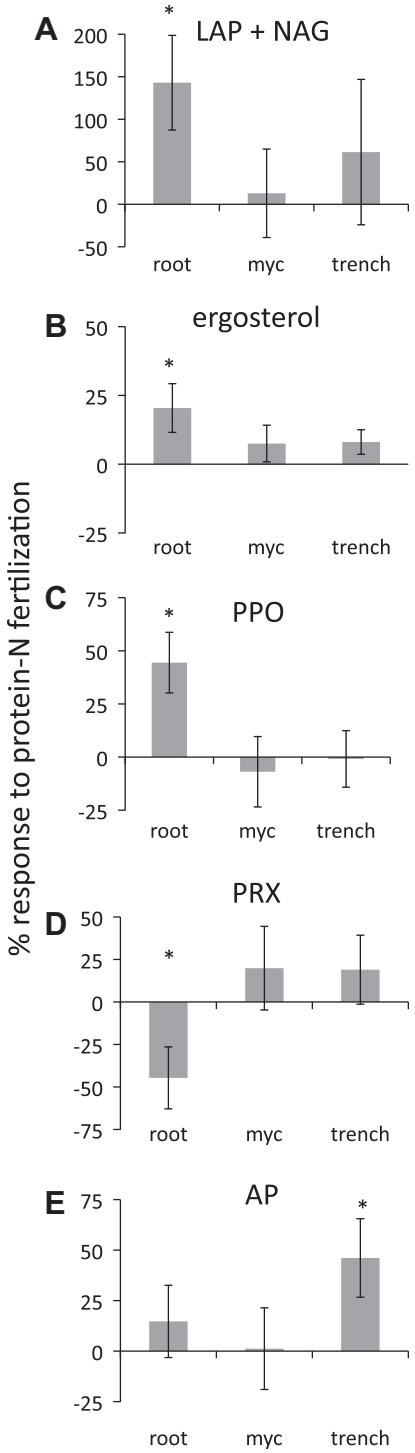

Fig. 1. Percent change of potential enzyme activities in root, mycelia and trench bags to complex organic nitrogen addition, relative to controls. Error bars show standard error of each response, asterisks are used to denote significance at the $p < 0.05$ level.

No responses to complex N additions were observed in the mycelia bags (Table 1). This is surprising as the dominant tree species on Mt Eisenhower are ectomycorrhizal (ECM) with near 100% root tip colonization at every site (Averill and Finzi, 2011), ECM fungi have broad enzymatic capability (Chalot and Brun, 1998)