available at www.sciencedirect.com

ScienceDirect

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

Nitrogen availability affects saprotrophic basidiomycetes decomposing pine needles in a long term laboratory study

Johanna B. BOBERG[a,*], *Torgny NÄSHOLM*[b], *Roger D. FINLAY*[a], *Jan STENLID*[a], *Björn D. LINDAHL*[a]

[a]*Uppsala BioCenter, Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, SE-750 07 Uppsala, Sweden*

[b]*Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden*

ARTICLE INFO

Article history:
Received 6 November 2010
Revision received 3 March 2011
Accepted 24 March 2011
Available online 12 May 2011
Corresponding editor:
Petr Baldrian

Keywords:
C-use efficiency
Fungal biomass
Fungal translocation
Litter decomposition
N-mineralization
Nutrient cycling
Pinus sylvestris

ABSTRACT

Fungi, especially basidiomycetous litter decomposers, are pivotal to the turnover of soil organic matter in forest soils. Many litter decomposing fungi have a well-developed capacity to translocate resources in their mycelia, a feature that may significantly affect carbon (C) and nitrogen (N) dynamics in decomposing litter. In an eight-month long laboratory study we investigated how the external availability of N affected the decomposition of Scots pine needles, fungal biomass production, N retention and N-mineralization by two litter decomposing fungi – *Marasmius androsaceus* and *Mycena epipterygia*. Glycine additions had a general, positive effect on fungal biomass production and increased accumulated needle mass loss after 8 months, suggesting that low N availability may limit fungal growth and activity in decomposing pine litter. Changes in the needle N pool reflected the dynamics of the fungal mycelium. During late decomposition stages, redistribution of mycelium and N out from the decomposed needles was observed for *M. epipterygia*, suggesting autophagous self degradation.

Introduction

Fungi are the major decomposers of forest litter (Rayner & Boddy 1988). Basidiomycetous litter decomposing fungi are considered especially important, due to their production of ligninolytic enzymes essential for the full decomposition of plant material (Steffen *et al.* 2000; Osono & Takeda 2002; Boberg *et al.* 2010b), and thereby pivotal to the turnover of carbon (C) in forest ecosystems. The turnover of organic C is connected to the nitrogen (N) cycle in a complex relationship, but exactly how the availability of N affects C storage and release to the atmosphere is not completely understood (Neff *et al.* 2002). Effects of N additions on microbial growth, activity and organic matter decomposition have been studied extensively in field experiments, but with complex and inconsistent results. The contradictory responses may be due to the vast variety of ecosystems studied, but also appear to depend on a range of different factors, such as the initial content of N and lignin in the litter (Berg & McClaugherty 2003; Knorr *et al.* 2005), the amounts applied and the ambient level of N deposition (Knorr *et al.* 2005). The use of simple laboratory systems to study basic physiological properties of litter decomposer fungi, such as C-use efficiency, N-uptake, N-mineralization and resource translocation, can help to provide a better understanding of

* Corresponding author. Tel.: +46 18 671806; fax: +46 18 673599.
E-mail address: Johanna.Boberg@slu.se (J.B. Boberg).
1754-5048/$ – see front matter
doi:10.1016/j.funeco.2011.03.004

how litter decomposition is regulated and can help to formulate hypotheses that may be tested in more complex and realistic environments.

The C allocation pattern of the decomposer organisms, i.e. the distribution of assimilated C between energy yielding metabolism and biomass production, determines the proportion of C that leaves the soil system as CO_2 in relation to the amounts that are retained in the soil organic matter pool. The C allocation may be expressed as the C-use efficiency, defined as the proportion of total assimilated C that is allocated to biomass production. Altered C-use efficiencies may be attributed to shifts in microbial species composition (Ågren *et al.* 2001) as well as to phenotypic changes within species (Boyle 1998). Increased C-use efficiency due to increased N availability has previously been observed in a wood decomposing fungus (Boyle 1998), in a litter decomposing fungus (Boberg *et al.* 2008) as well as in a fungal dominated microbial community in an agro-ecosystem soil (Thiet *et al.* 2006). Decreased C-use efficiency is due to increased respiration without a parallel increase in biomass and has been observed in a litter decomposing fungus after addition of an external supply of glucose (Boberg *et al.* 2008).

Soils receive N mainly in organic form as plant residues (Tamm 1991). The organically bound N may be mineralized into mineral N, a process mediated by saprotrophic organisms. Saprotrophic microorganisms assimilate C from the organic material to produce biomass and energy, and N-mineralization is the result of C-limitation during decomposition (Paul 2007). Although the N content of litter may initially be low, C is progressively respired during decomposition. The C:N ratio of the plant residues thus decreases and finally reaches a point at which C becomes the limiting resource. Microorganisms are then forced to use the N enriched substrate primarily as a C source and exude excess N into the environment as ammonium (Paul 2007).

Often, decomposer organisms are perceived as unicellular microbes, which depend exclusively on the resources available in their immediate surroundings. Most fungi, however, exhibit filamentous growth and translocate resources, such as carbohydrates, mineral nutrients and water, within their mycelia (Boddy 1999; Lindahl & Olsson 2004). Single compounds, such as P and N, may be transported bi-directionally in fungal mycelia (Lindahl *et al.* 2001; Tlalka *et al.* 2002), and different resources, such as carbohydrates and P (Olsson & Gray 1998) or N (Olsson 1995; Frey *et al.* 2003), may also be translocated in opposite directions simultaneously. Such translocation of resources implies that the mycelium functions as a single entity in which the nutritional status of one part affects, and is affected by, physiological processes in other parts of the connected system (Lindahl & Olsson 2004). We have recently shown that fungal translocation of C from an external source may support fungal metabolism in a low C:N ratio substratum, preventing local C-limitation and restricting N losses as ammonium (Boberg *et al.* 2010a). Likewise, fungal N translocation to high C:N ratio substrata may increase the fungal activity in N limited substratum. Net increases in the amount of N in litter during early stages of decay have been observed repeatedly (Berg & Staaf 1981; Berg *et al.* 1982; Fahey *et al.* 1985; Melillo *et al.* 1989; Chadwick *et al.* 1998; Moore *et al.* 2006). Net gain of N is typically associated with more recalcitrant litter types with low initial N content, and N import has been hypothesized as a strategy of fungi to overcome N limitation in the newly shed litter (Lindahl & Boberg 2008). Net gain of N mediated through fungal translocation has been shown in decaying beech litter (Zeller *et al.* 1998) as well as in wheat straw on the soil surface in a non-tillage agricultural soil (Frey *et al.* 2000).

Previously we have demonstrated short term changes in C allocation patterns of litter decomposing fungi in response to altered external C and N availability and the implications for respiration, fungal growth and decomposition (Boberg *et al.* 2008). We have also demonstrated that fungal translocation of C may have decisive implications for N-mineralization (Boberg *et al.* 2010a). Here we integrate observations of litter decomposition, fungal growth, N dynamics and resource redistribution in a longer perspective (8 months), by manipulating the external availability of organic N in the form of glycine. The hypotheses were that: (1) colonization of needle litter by a decomposer fungus with access to an external N source would lead to a net gain of the total litter N pool; (2) increased N availability would increase both fungal growth and decomposition, i.e. both the activity and growth of the fungus would be N limited; (3) export of C from colonized needles would reduce N-mineralization of an external substratum with a low C:N ratio (glycine).

Materials and methods

Long term decomposition of Scots pine needles, and N dynamics and metabolic efficiency of two species of well known litter decomposing fungi, *Marasmius androsaceus* and *Mycena epipterygia*, were studied in axenic laboratory systems. Isolates of *M. androsaceus* (isolate JB14) and *M. epipterygia* (isolate JB13) were obtained from sporocarps collected underneath Scots pine (*Pinus sylvestris*) trees in mixed forests located near Uppsala, central Sweden, and maintained on Hagem agar (Stenlid 1985) at 20 °C. Both isolates are deposited at the Department of Forest Mycology and Pathology, Uppsala, Sweden and their rDNA region sequences can be found at NCBI (GU234007 and GU234008). Brown abscised Scots pine needles were collected on sheets in a 25-yr-old pine stand situated on a nutrient poor sediment soil in Jädraås, central Sweden (Axelsson & Bråkenhielm 1980), air dried and then stored frozen at −27 °C. The needles were dried at 80 °C for 72 hr to determine the dry weight (dw) and then autoclaved at 121 °C for 15 min before use. The pine needles contained 0.4 % N and 25 % Klason lignin (Johansson *et al.* 1995).

Split Petri dishes were used to spatially separate two substrata with different C/N-ratios; *Pinus sylvestris* needles in one half and glycine containing agarose medium in the other (Fig 1). By growing out into the different halves of the dish, fungal mycelia were able to connect the two substrata. Systems with both substrata added were compared to systems where either of the two substrata was excluded. Split Petri dishes were prepared with the compartments containing one of three different substrata: (1) 30 ml of autoclaved basal medium (1.5 % of low melting temperature agarose (Pronadista®, Conda, Madrid, Spain), 0.5 g l^{-1} KH_2PO_4 and 0.5 g l^{-1} $MgSO_4 \cdot 7H_2O$); (2) 0.6 g of Scots pine needles placed on top of 30 ml of basal agarose; or (3) 30 ml of basal agarose medium enriched with