Soil Biology & Biochemistry 78 (2014) 109–117

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

The direct response of the external mycelium of arbuscular mycorrhizal fungi to temperature and the implications for nutrient transfer

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ARTICLE INFO

Article history:
Received 21 April 2014
Received in revised form
16 July 2014
Accepted 18 July 2014
Available online 11 August 2014

Keywords:
Arbuscular mycorrhizal fungi
Global climate change
Extra-radical mycelium (ERM)
Nitrogen
Phosphorus
Organic material

ABSTRACT

In this study we investigated the direct effects of temperature on the extra-radical mycelium (ERM) of arbuscular mycorrhizal fungi (AMF) and the resulting impact on the host plant nutrition and biomass production. *Plantago lanceolata* L. plants colonized by *Glomus hoi* (experiment 1) and either *G. hoi* or *Glomus intraradices* (experiment 2) were grown in compartmented microcosm units. AMF hyphae, but not roots, were permitted access to a second compartment containing a ^{15}N:^{13}C dual-labelled organic patch maintained at different temperature treatments. All plants were maintained at ambient temperature. AMF hyphal growth in the patch compartments was relatively insensitive to temperate but results were variable. *G. hoi* hyphal length density was 5 times higher at ambient (*c.* 24 °C) than cooled (*c.* 11 °C) temperatures but only at the end of the first experiment (105 d after patch addition). In contrast, in the second experiment (86 d after patch addition) AMF hyphal growth was unaffected by temperature in the patch compartment. These differences between experiments are likely due to large variation among replicates in the ERM produced and differences in how the organic patch was applied. In experiment 2, plant biomass and phosphate content differed according to the temperature at which the hyphae of both AMF species grew. Plant biomass was greater when the AMF were grown at c. 18 °C than c. 11 °C but was no different at c. 21 °C. These data show that direct temperature responses by the external hyphae of AMF can independently influence associated host plant growth. However, there were also important differences between the two AMF studied both in the amount of nutrients transferred and the distribution of the nutrients.

1. Introduction

Most plants form arbuscular mycorrhizal (AM) associations with fungi from the phylum Glomeromycota (Smith and Read, 2008). Whilst uptake of phosphorus (P) is thought to be the most important benefit derived from AM association (Mosse et al., 1973; Smith and Read, 2008), AM fungi (AMF) can also take up inorganic nitrogen (N) from organic material and transfer this to their associated plant host (Hodge et al., 2001; Atul-Nayyar et al., 2009; Leigh et al., 2009). As soil organisms, AMF likely show strong responses to a range of edaphic factors including temperature (Fitter et al., 2000; Tibbett and Cairney, 2007) yet, while the effects of temperature and other environmental global change factors on plant physiology have been well studied, few plant studies have quantified the impact directly upon the AMF symbiont (see Fitter et al., 2004; Hughes et al., 2008), even though it is known that AMF can alter their host plant responses to, for example, temperature (Atkin et al., 2009). Given the near ubiquity of the AM association, and the fact that they are likely to be increasingly important in future sustainable agricultural systems (Gosling et al., 2006; Rooney et al., 2009; Verbruggen et al., 2010; Fitter et al., 2011) this knowledge gap represents a significant problem when it comes to predicting the way in which plants will respond to predicted temperature rises.

Investigating AMF responses to temperature directly, however, is complicated by the fact that as these fungi are obligate biotrophs and so rely entirely on their host plant for their carbon (C) supply, they cannot be grown separately. Therefore, not only will the AMF respond directly to the physiological effects of a change in

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http://dx.doi.org/10.1016/j.soilbio.2014.07.025
0038-0717/

temperature, but, are also subject to indirect effects driven by the concurrent response of their host plant which may include an altered carbon allocation belowground, which, in turn, will impact upon the growth of the fungal symbiont (Fitter et al., 2000; Heinemeyer and Fitter, 2004). AMF comprise an intra-radical mycelium (IRM) growing within the plant root itself and an extra-radical mycelium (ERM) extending from the root into the surrounding soil. Most studies to date have investigated AMF temperature responses by either exposing a whole colonised plant (Hetrick and Bloom, 1984; Kytoviita and Ruotsalainen, 2007) or just the colonized roots (Smith and Roncadori, 1986; Wang et al., 2002; Ruotsalainen and Kytoviita, 2004) to different temperature regimes. Thus, these studies report the combined effects of temperature on both symbiont partners and not the direct impact upon the fungus itself. Furthermore, the response of the ERM to temperature is seldom considered.

In general, internal colonisation increases with temperature between 10 °C and 30 °C (Smith and Roncadori, 1986; Matsubara and Harada, 1996; Wang et al., 2002) and reduced colonisation is consistently reported below 15 °C (Hetrick and Bloom, 1984; Zhang et al., 1995). Moreover, AMF colonisation lead to enhanced plant P capture compared to non-AM plants, but only at temperatures of 15 °C and above (Wang et al., 2002; Kytoviita and Ruotsalainen, 2007; Karasawa et al., 2012). Similarly, Ruotsalainen and Kytoviita (2004) reported that AMF colonisation leads to enhanced shoot N content at 17 °C but not at 12 °C. Several other studies also report that AM plants grow more poorly than non-AM plants at temperatures below c. 15 °C but better above c. 15 °C (Baon et al., 1994; Liu et al., 2004; but see; Rooney et al., 2011). This suggests that the benefit of being AMF to a host plant is temperature-dependent. Such temperature driven changes in mycorrhizal benefit might reflect either a direct physiological response of the fungus or changes in host C allocation.

Since the primary benefit to plants of AMF colonisation is the enhancement of plant nutrition resulting from the ability of the ERM to acquire nutrients from soil, any factor which limits the growth of the ERM might reduce that benefit (e.g. Leigh et al., 2011). The few studies of temperature responses of AMF that have considered the ERM show that its' growth is more limited by lower temperatures than that of roots (Liu et al., 2004; Kytoviita, 2005; but see; Karasawa et al., 2012). Temperatures below 15 °C often lead to a reduction (Gavito et al., 2003; Liu et al., 2004; Hawkes et al., 2008) or complete suppression of ERM growth (Gavito et al., 2005) and optimal growth temperatures vary among AMF species (Gavito et al., 2005). To date, only a few studies have measured the direct impact of temperature on ERM growth: Heinemeyer et al. (2006) found that growth of the ERM doubled when warmed by 6 °C whilst keeping the host plant at an ambient temperature of *c.* 12/23 °C (night/day), whereas Heinemeyer and Fitter (2004) found only transient effects on growth of the ERM from warming the ERM by 8 °C while the host plant remained at *c.* 12 °C. They report however, that the specific root length of the host plant increased, suggesting that direct effects of temperature on AMF growth may have indirect impacts on host plant growth. Karasawa et al. (2012) investigated the impact of a short period of soil chilling on ERM growth, respiration and ^{13}C allocation and found no differences compared to when the soil was not chilled. In contrast, respiration and ^{13}C content of the roots was reduced by chilling. Thus, if, as the majority of these studies suggest, AMF are more temperature sensitive than roots to low soil temperature this would be expected to have large implications for nutrient capture via the fungal symbiont.

In this study we conducted two experiments to investigate the direct impacts of temperature on the ability of two AMF species, *Glomus intraradices* and *Glomus hoi*, to grow in, and transfer nutrients (N and P) from, an organic nutrient patch. In the first experiment *G. hoi* alone was screened for its ability to transfer nutrients under cooled versus ambient temperature conditions. Microcosms in which AMF ERM but not plant roots were permitted access to a compartment containing an organic patch of ^{15}N:^{13}C dual-labelled grass shoots were used. The 'no AMF access' treatment was included to determine if N movement via mass flow or diffusion was an important N transfer pathway under these experimental conditions. As the latter was found not to be the case in the second experiment we omitted these 'no AMF access' controls, thus allowing two AMF species and a greater number of temperature treatments to be examined. In the second experiment, *G. intraradices* was also included because its growth has been reported to be severely repressed at temperatures below 15 °C (Smith and Roncadori, 1986; Liu et al., 2004; Gavito et al., 2005) while *G. hoi* was found to grow and transfer nutrients to its host plant even at temperatures of 10–12 °C (Barrett et al., 2011). In both experiments the temperature of the patch compartment was cooled to varying degrees whilst the host plant in the adjacent compartment always remained at ambient temperature. We tested the following hypotheses: (i) that AMF promote nutrient cycling by capturing nutrients from decomposing organic matter and (ii) that AMF effectively capture nutrients even when temperature is reduced. We further hypothesised that (iii) growth of *G. intraradices* hyphae would be more adversely affected than *G. hoi*, as *G. intraradices* is frequently reported to be temperature sensitive (Gavito et al., 2005; but see; Lekberg et al., 2011).

2. Materials and methods

2.1. Experimental set-up

Two microcosm experiments were carried out in successive years; experiment 1 (Expt 1) began on 25th May 2007, experiment 2 (Expt 2) on 23rd November 2008. In Expt 1 the growth and N capture ability of the AM fungus *G. hoi* was compared at ambient (*c.* 24 °C) or a cooled (*c.* 11 °C) temperature treatment. In Expt 2, the growth and nutrient (N and P) capture ability of two AM fungi, *G. hoi* (isolate number UY 110, University of York) and *G. intraradices* (isolate BB-E, Biorhize, Dijon, France), were compared at 4 different temperature treatments; *c.* 11, 14, 18 or 21 °C. In both experiments the plant compartment was maintained at the ambient temperature in the glasshouse. In Expt 1, destructive harvests took place at 30 and 105 d after patch addition. In Expt 2, at 36 d a non-destructive harvest took place followed by a full destructive harvest at 86 d. These sampling and harvests time points were broadly based on previous work examining temperature impacts on AMF development in similar experimental systems (e.g. Barrett et al., 2011; Karasawa et al., 2012). Although changes to the nomenclature of many AMF species have recently been proposed (e.g. Krüger et al., 2012; Redecker et al., 2013), here we retain the previous name of '*G, intraradices*' given the phylogenetic position of the particular isolate used in this study is uncertain.

AMF cultures of *G. hoi* and *G. intraradices* were established three months prior to the start of experiments, in pots with *Plantago lanceolata* in a sand:Terragreen mix (see below) with 0.25 g l^{-1} bone meal (Vitax, Leicestershire, UK), a complex P and N source to encourage AMF development. Either, 50 g fresh weight of *G. hoi* (Expt 1 and 2) or *G. intraradices* (Expt 2 only) inoculum comprising root pieces and growth medium was added to the plant compartments of the experimental microcosms.

2.2. Microcosm set-up

Microcosm units (adapted from Hodge and Fitter, 2010) were made by joining two open top plastic boxes (each