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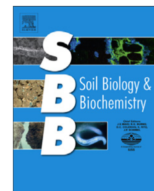

Extraradical arbuscular mycorrhizal fungal hyphae in an organic tropical montane forest soil

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ABSTRACT

Previous research from the tropics indicates that AMF may be well adapted to organic soils and even represent the dominant mycorrhizal form, though the extraradical part of the symbiosis was omitted as in most other tropical studies. Our study aims at characterizing the extraradical part of arbuscular mycorrhizal fungi (AMF) in a highly organic tropical montane forest soil in Southern Ecuador. Based on recent studies on the interaction of AM fungal hyphae and litter we hypothesized that within the organic layer AM hyphae grow in close contact with decomposing material. To test this idea, AM fungal hyphal distribution in the organic layer was determined by directly staining roots and decomposing leaves and extracting hyphae from the remaining particulate organic material. AM and non-AM fungal hyphae were analyzed, as well as root colonization patterns. Our results showed that AMF indeed represented the dominant mycorrhizal form with an average root colonization of 43%. The extraradical AM hyphal length ranged from 2 to 34 m g^{-1} soil with a mean of 10.4 m g^{-1} soil (equals 3.1 m cm^{-3} soil), and therefore exceeded root length about 13-fold. As hypothesized, 29% of AM extraradical hyphae were closely attached to decomposing leaves. These hyphae were mainly located at the leaf surface, though in some parts leaf veins and inner leaf tissues were colonized. More than half of AM hyphal biomass was detected on the root surface, a pattern potentially driven by the predominant Paris-type AMF. Non-AM fungal hyphae colonized decomposing material to a significantly greater extent, though hyphal length attached to roots was equal. This study supports the adaptation of AMF to highly organic soils in the tropics and the existence of a widespread extraradical mycelium, which is not readily detectable by standard methods. The close association with decomposing leaves most likely improves direct nutrient uptake from decomposed material and points to a potential indirect contribution of AMF to the decomposition process.

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) explore the soil with their extraradical mycelium, transferring nutrients to the plant via specialized intraradical structures (Smith and Read, 2008). Generally, beside other functions, such as pathogen protection and soil stabilization, the uptake of mineral nutrients as phosphorus (P), nitrogen (N) and various micro-nutrients is regarded the main benefit to the plant (Johnson, 2010).

AMF are associated with more than 80% of land plants and occur globally in most terrestrial ecosystems (Treseder and Cross, 2006). However, in contrast to the hypothesis proposed by Read (1991) – limiting the dominance of AMF to ecosystems characterized by "mineral soils with lower altitude" – several studies performed in nutrient-poor organic soils in the tropics have shown that AMF represent in fact the dominant mycorrhizal form (Bereau et al., 1997; Moyersoen et al., 2001; Kottke et al., 2004), even up to high altitudes of the Páramo vegetation (Barnola and Montilla, 1997; Aristizabal et al., 2004; Camenzind, unpublished data). Likewise, a direct competition with ectomycorrhiza for the same niche was reported (Moyersoen et al., 1998). Apart from the work of Aristizabal et al. (2004), these studies employed AMF root colonization as a measure of abundance, neglecting the extraradical phase and thus an important functional part of these fungi and the symbiosis (Leake et al., 2004). This omission is likely related to methodological constraints. Only some early studies attempted a functional appreciation of mycorrhizal hyphae in tropical soils: Went and Stark (1968) proposed "direct nutrient cycling" based on

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the simple observation that in tropical forests a dense network of hyphae covers the decomposing litter layer, which is in close contact to roots colonized by "endotrophic mycorrhiza". They hypothesized that a hyphal connection between decomposing leaves and roots directly cycles mineralized nutrients back to the plant. Herrera et al. (1978) supported the idea by morphological analyses of root and litter samples collected in the Amazon basin. They described "hyphae bridges" as a direct connection between decomposing leaves and roots and even reported a direct transfer of labeled ^{32}P. However, the respective fungal group was simply specified as "numerous septate fungal hyphae bridges", and thus not necessarily restricted to AMF. Only recently, this idea was picked up and decomposing leaves from the litter layer collected at different sites in Colombian tropical forests were stained and analyzed (Aristizabal et al., 2004; Posada et al., 2012). These authors reported extensive litter colonization by AM fungal hyphae comparable to the amount of saprobic fungi (specified as aseptate hyphae), especially in highly decomposed material with easily accessible nutrients, located in close contact to roots.

Based on these findings we here aimed at identifying and quantifying the extraradical mycorrhizal fungal phase in a tropical forest soil characterized by a thick organic layer, typical of tropical montane forests (Grieve et al., 1990; Wilcke et al., 2008). The study area was previously characterized to be dominated by arbuscular myorrhizae (Kottke et al., 2004) and high rates of AM root colonization (Homeier et al., 2012) suggesting a high contribution of AM hyphae to soil fungal biomass (Leake et al., 2004). However, this hyphal biomass is only detectable by methods adapted to leaf litter samples, whereas standard hyphal extraction protocols designed for mineral soils are ineffective. These findings lead us to the hypothesis that within the thick organic soil layer the majority of extraradical AM hyphae is closely attached to decomposing leaves, similar to previous findings in litter of certain plant species (Aristizabal et al., 2004; Posada et al., 2012). To test this hypothesis, hyphal length was quantified across soil depths by a direct observation of hyphae attached to every single soil fraction (leaf litter, root surfaces, and remaining particulate organic material). Additionally, the distribution of non-AM fungal hyphae in the substrate was evaluated, which represent other fungal groups, such as saprobes.

2. Materials and methods

2.1. Study area

The study site of the Reserva Biologica San Francisco, bordering the Podocarpus National Park, is located in the Cordillera Real, an eastern range of the South Ecuadorian Andes (Beck et al., 2008). This area represents a hotspot of biodiversity, with more than 280 tree species described, with Lauraceae, Melastomaceae and Rubiaceae as the dominant plant families. *Graffenrieda emarginata* Triana (Melastomaceae) is the most abundant tree species at the site. The sampling site is located at 2030–2120 m a.s.l. (3°59′S, 79°05′W). The vegetation type is an evergreen lower montane forest (Homeier et al., 2008). The climate is warm humid with an average annual temperature of 15.2 °C at 1950 m and an annual precipitation of approx. 2600 mm. Precipitation is particularly high from April to September without a pronounced dry season. The soil is a Stagnic Cambisol (IUSS Working Group WRB 2007) with a thick organic layer up to 35 cm (Wullaert et al., 2010). The organic layer is divided into three horizons: The Oi horizon consisting of fresh litter, the Oe horizon of fragmented litter and the Oa horizon of humified material of un-recognizable plant origin. The pH of the organic layer ranges from 3.8 to 5.0, C/N ratio from 23.6 ± 1.3 in the Oa horizon to 34.0 ± 3.2 in the Oi horizon (Wullaert et al., 2010).

2.2. Sampling design

In October 2010, six soil samples were taken with a soil corer (3 cm diameter, 15 cm in depth). Three sampling sites were randomly chosen at 2050 m, the other half at 2100 m, at both altitudes within an area of approx. 800 m^2 in the undisturbed forest. Every soil core was divided into three soil samples from different depth layers (upper: 0–5 cm; middle: 5–10 cm; lower: 10–15 cm). These layers do not correspond to O-horizons of the organic layer. Soil samples were oven-dried at 40 °C.

2.3. Determination of soil fractions

The proportion of leaf and root weight as well as the respective surface area in the soil profile was determined (see below). The remaining material of non-recognizable plant origin was hereafter referred to as "particulate organic material". Using forceps we picked all recognizable pieces of leaves and roots (regardless of size) out of each 5 g soil sample. The dry weight of roots and leaves was determined and the respective surface area (cm^2 g^{-1} soil) as well as root length (cm g^{-1} soil) analyzed with WinRhizo (version 2007, Regent Instrument Inc., Quebec, Canada). Results were expressed on a soil weight basis. The (dry) bulk density of the organic layer at the study site was 0.31 ± 0.03 (mean ± standard error) g cm^{-3} soil.

2.4. Method optimization

In the course of optimizing morphological methods suitable for the quantification of extraradical hyphal biomass, two different protocols were tested. Results and detailed methodological descriptions are provided in the Supplementary materials. AMF hyphae were identified by the following criteria: non-regularly septate hyphae with characteristic unilateral angular projections, that are stained dark- to light-blue by Trypan Blue (Mosse, 1959). All other fungal hyphae were referred to as non-AMF, since there is no reliable further morphological differentiation of fungal groups.

The first protocol tested was an aqueous filtration extraction method slightly modified from Bardgett (1991). The second protocol was based on a method originally described for hyphal quantification in tropical leaf litter (Herrera et al., 1986; Aristizabal et al., 2004). Considerable modifications were applied in order to meet the requirements of an accepted morphological differentiation of AM and non-AM hyphae: besides the prevention of severe hyphal losses through the 40 μm sieve, a staining step with Trypan Blue was inserted and hyphae were quantified at 200× magnification instead of 100×.

2.5. Hyphal quantification in single soil fractions

Testing the hypothesis that extraradical AMF hyphae are attached to decomposing leaves, the amount of hyphae attached to every soil fraction (leaf litter, root surfaces and particulate organic material) was determined separately.

2.5.1. Roots

Roots were stained with a modified staining protocol of Phillips and Hayman (1970). 1–2 cm root pieces were cleared in 10% KOH at 60 °C for 1–2 days, bleached in 20% H_2O_2 for 20–30 min at RT, acidified in 1 M HCl and stained for 1.5–2.5 h in 0.05% Trypan Blue at 60 °C. The duration of staining depended upon the respective root diameter. Root colonization was counted at 200× magnification using the line-intersect method described by McGonigle et al. (1990). For the calculation of extraradical hyphae attached to the root surface, half of one line incorporated into the microscopic lens

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