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Evidence for the coupling of extraradical mycorrhizal hyphae production to plant C assimilation in Japanese warm-temperate forest of arbuscular mycorrhizal and ectomycorrhizal tree species

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ABSTRACT

In temperate and boreal forest, the production of extraradical mycorrhizal hyphae (EMH) is a major pathway for the plant – soil carbon (C) flux. For warm-temperate forest, however, scarcely available field data provide inconclusive evidence for the drivers of EMH production dynamics. In this study, we measured the seasonal variation in EMH production in Japanese warm-temperate *Chamaecyparis obtusa* (arbuscular mycorrhizal evergreen) and *Quercus serrata* forest (ectomycorrhizal broad-leaf), and assessed the relationship of EMH production to air temperature, global solar radiation, and soil water content. EMH productions of six consecutive two-month periods were estimated from hyphal lengths (HLs) and hyphal carbon masses (HCs) in 360 hyphal in-growth mesh bags and corrected for non-mycorrhizal HLs and HCs in 72 control bags from root-trenched areas. Seasonal variations in HL and HC productions of EMH were significant in both forest types. HL and HC productions of EMH were significantly related to air temperature which drives plant C assimilation in both forest types. We, thus, present evidence for coupling of EMH production to plant C assimilation in warm-temperate forest, regardless of the dominating mycorrhizal type.

1. Introduction

In forest ecosystems, roots and associated mycorrhizal fungi – ubiquitous plant symbionts that benefit plant nutrient and water uptake [1] – are allocated 25–63% of total plant-assimilated carbon (C) [2]. In a survey of five field studies in temperate and boreal forest stands, up to 22% of plant-assimilated C was allocated to mycorrhizal fungi [3]. In the same stands, extraradical mycorrhizal hyphae (EMH) alone accounted for 13% on average of the below-ground net primary production (NPP) [3,4]. In 14 laboratory studies with tree and shrub species of temperate or boreal forests, EMH accounted for 9% on average of the below-ground NPP [3,4]. Additionally, the respiration of EMH contributed up to a third of total soil CO_2 efflux in temperate forest [5]. EMH production is, therefore, an important driver of the plant – soil C flux in temperate and boreal forest and understanding of EMH production dynamics in field soils is essential for assessing and predicting forest ecosystem C fluxes [4,6–8].

The majority of past research on EMH production dynamics has been conducted within boreal and cold-temperate forest of the ectomycorrhizal (ECM) tree species *Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), or *Quercus robur* (European oak; see Ekblad et al. [4] for a review of approximately 140 research sites). In the studied boreal and cold-temperate forest, EMH production in the upper 10 cm of the soil was 0.8–22.5 $g\,C\,m^{-2}$ growth $season^{-1}$ [4,9,10] (here and hereafter a C content of 40–45% is assumed for EMH of ECM fungi [11,12]). Strong seasonal variation in EMH production was observed with maximum EMH production in late summer to autumn and little EMH production in winter [4,10,13]. Seasonal EMH production was suggested to be driven by the variation in plant C assimilation, or current photosynthate production, throughout the year and the competition for plant assimilated C with plant organs, especially fine roots [4].

For warm-temperate forest, however, field studies on EMH production are largely limited to three sites with evergreen conifer (*Pinus* sp.) forest [8,12,14–16] and two sites with deciduous broad-leaved forest, all dominated by ECM tree species [12]. This scarcity of field studies is in contrast with the finding that the, to our knowledge,

Abbreviations: AM, arbuscular mycorrhizal; ECM, ectomycorrhizal; EMH, extraradical mycorrhizal hyphae; HC, hyphal carbon mass; HL, hyphal length; SHL, specific hyphal length

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highest EMH production (40–45 g C m^{-2} 4 $months^{-1}$) has been observed in warm-temperate forest [4,14]. Warm-temperate forests also frequently have high proportions of arbuscular mycorrhizal (AM) tree species [17–20]. In forests of the warm-temperate Southeastern United States, AM tree species account for 37% of the forests' basal area [17]. In Japan, part of the East-Asian warm-temperate zone, AM conifers of the cypress family (*Cupressaceae*) comprise 44% of the forest plantation area [20,21]. The dominant mycorrhizal type was shown to affect the rate of C cycling in forest ecosystems [17,22] and, hence, may also influence EMH production dynamics.

For warm-temperate forest, scarcely available data do not provide a clear picture of the drivers of EMH production dynamics. In *Pinus palustris* (longleaf pine) forest, seasonal variation of EMH production was insignificant [23], despite limitation of the local forest annual growth season to 200–280 days [24]. Even the reduction in foliar leaf area and, hence, plant C assimilation capacity, via scorching treatments did not affect EMH production, which led to the conclusion that plant C storage may decouple EMH production from plant C assimilation [23]. In a *Pinus pinaster* (maritime pine) and two deciduous broad-leaved forests, low soil water content was suggested to seasonally limit the rate of plant C assimilation and EMH growth itself, with an effect on the seasonal EMH production that overruled effects of other environmental factors or competition for plant assimilated C with plant organs [12].

The objectives of this study were to (1) assess the seasonal variation in EMH production in *Chamaecyparis obtusa* (hinoki cypress, evergreen AM conifer) and *Quercus serrata* (konara oak, deciduous ECM broad-leaved species) forest in the warm-temperate zone of Japan and (2) test the relationship of the seasonal EMH production to seasonal means of air temperature, daily global solar radiation, and soil water content with mixed-effect modeling. EMH productions of six consecutive 2-month periods were determined from hyphal lengths and C masses in hyphal in-growth mesh bags. Air temperature and solar radiation are the main drivers of plant C assimilation in nearby forests of the same type [25,26]. The lack of dry seasons at the study site made limitations of EMH production through low soil water contents unlikely. We, hence, hypothesized that EMH production in both forest types is (I) significantly related to the mean air temperature and mean daily global solar radiation but (II) not to the mean soil water content in each of the six consecutive 2-month periods.

2. Materials and methods

2.1. Site description

The study was conducted in plantation forest of *C. obtusa* Endl. and secondary forest dominated by *Q. serrata* Murray in Ryukoku Forest (38 ha), Otsu City, Shiga Prefecture, Japan (34°58′ N, 135°56′ E). The subcanopy layers of both forest types are dominated by evergreen species, including *Eurya japonica* Thunb., *Ilex penduncolosa* Miq., *Symplocos prunifolia* Sieb. et Zucc., and *Clethra barbinervis* Sieb. et Zucc. Local mean air temperature and mean annual precipitation over a period of 20 years (1997–2016) were 15.3 °C and 1570 mm, respectively [27]. Daily global solar radiation typically peaks in May (Fig. 1a; retrieved from Ref. [27]). Daily mean air temperature typically peaks in August (Fig. 1a; measured 1.5 m above the forest soil surface at the study site; TR-52, T&D, Matsumoto, Japan). Daily accumulated precipitation is present in all seasons (Fig. 1c; measured at the study site; RT-5, Ikeda Keiki, Tokyo, Japan; Y Taketo, personal communication) and sustains a similar daily mean soil water content throughout the year (Fig. 1c; measured 0.1 m below the forest soil surface at the study site; EC-5, Decagon Devices, Pullman, USA). Local soils are cambisols [28,29] derived from lacustrine sediments of Cenozoic origin, and are characterized by weathered granite sand and small round gravel stones [30]. Experiments were conducted in two plots in *C. obtusa* forest (0.06 and 0.11 ha) and two plots in *Q. serrata* forest (0.08 and 0.12 ha). *Q. serrata* was the main ECM tree species in plots, *C. obtusa* and most of the subcanopy species were AM according to published mycorrhizal types [19,20,31,32]. Annual stem production was 310 and 330 g biomass m^{-2} $year^{-1}$ in *C. obtusa* forest and *Q. serrata* forest, respectively, of which AM tree species contributed 87 and 41% (tree censuses 2013–2015).

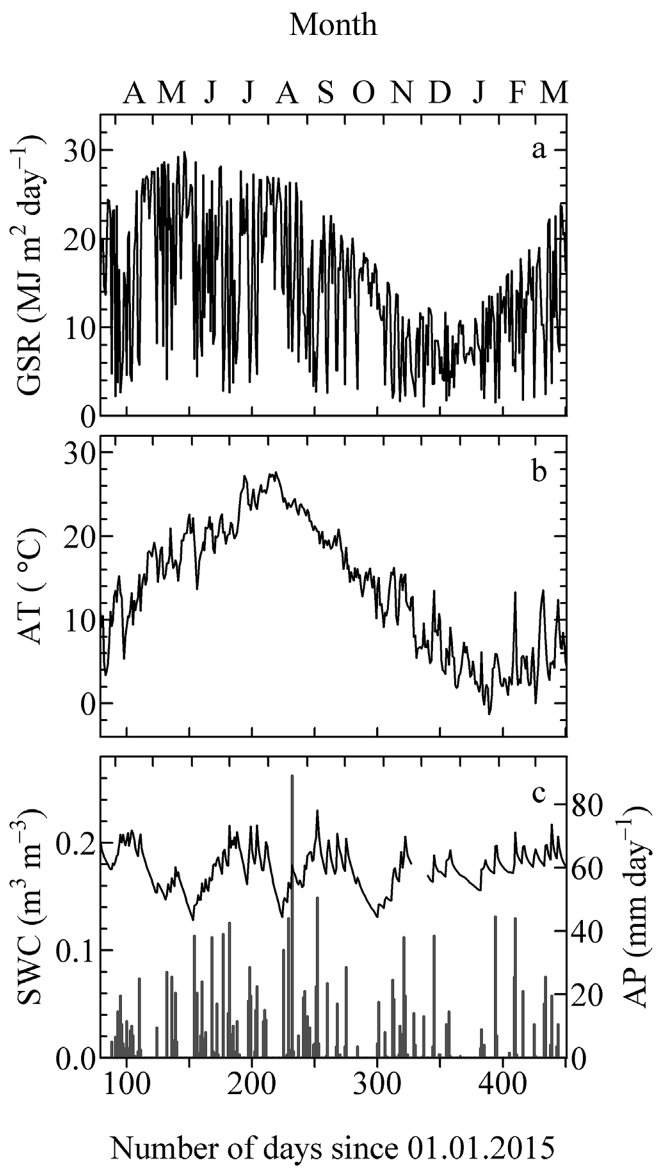

Fig. 1. Seasonal variation during the study period from March 20, 2015 to March 25, 2016 in (a) daily global solar radiation (GSR; retrieved from Ref. [27]), (b) daily mean air temperature (AT; measured 1.5 m above the forest soil surface), and (c) daily mean soil water content (SWC; volumetric, measured 0.1 m below the forest soil surface) and daily accumulated precipitation (AP; Y Taketo, personal communication) in warm-temperate forest of *Chamaecyparis obtusa* and *Quercus serrata* in Otsu City, Japan.

2.2. Setup of hyphal in-growth mesh bags and control bags

Hyphal in-growth mesh bags (referred to as “in-growth bags” hereafter) – proposed by Wallander et al. [13] and used in numerous studies for estimation of EMH production [4,11] – were made in cylindrical shape (10 cm length × 3 cm diameter) from nylon mesh cuttings (50 µm mesh size) with a hot glue pistol. The small mesh size enabled hyphal in-growth, while root in-growth was prevented. To limit colonization of saprotrophs, in-growth bags were filled with ca. 115 g of granite sand (grain size mostly between 0.01 and 2.0 mm, Daiki, Japan;