Soil Biology & Biochemistry 42 (2010) 1614–1623

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Carbon partitioning in ectomycorrhizal Scots pine seedlings

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ARTICLE INFO

Article history:
Received 4 March 2010
Received in revised form
2 June 2010
Accepted 8 June 2010
Available online 20 June 2010

Keywords:
^{14}C
Carbon allocation
Carbon budget
Ectomycorrhiza
Humus
Suillus variegatus

ABSTRACT

The complete carbon budget and the turnover rate of assimilated carbon of ectomycorrhizal Scots pine seedlings growing on natural humus were determined in microcosm conditions. The main aim was to improve understanding of the partitioning of the assimilated carbohydrates within seedlings associated with multiple ectomycorrhizal fungi, and to discover carbon dynamics of the mycorrhizosphere.

Plant photosynthesis and below-ground respiration were measured in order to obtain the actual carbon assimilation and respiration rates at the time of measurements. Soon after the photosynthesis and respiration rate measurements the seedlings were pulse-labeled with $^{14}CO_2$ to follow carbon allocation to different plant, fungal and soil compartments and rhizosphere respiration. Long-term carbon allocation during the entire life span of the seedlings was estimated by measuring plant and mycorrhizal root-tip biomass. The ectomycorrhizal community was analyzed using morphotyping and ITS-sequencing.

The ^{14}C label was detected in rhizosphere respiration after 12 h and it peaked between 36 and 60 h after labeling. More than half of the assimilated carbon was allocated below-ground as biomass or respiration and higher mycorrhizal biomass increased the below-ground carbon turnover. The presence of *Suillus variegatus* affected the plant carbon balance in several ways. When *S. variegatus* was present, the below-ground respiration increased and this carbon loss was compensated by higher photosynthetic activity. Other fungal species did not differ between each other in their effects on carbon balance. Our findings indicate that some root-associated mycorrhizal fungal symbionts can significantly alter plant CO_2 exchange, biomass distribution, and the allocation of recently photosynthesized plant-derived carbon.

1. Introduction

Trees associated with ectomycorrhizal fungi (ECM) form a symbiotic relationship where the host plant invests carbon for maintaining mycelial networks which has been shown to play a key role in enhancing water and nutrient uptake of the host plant (Smith and Read, 1997, 2008). These fungal symbionts can constitute one-third of the total microbial biomass in the soil (Högberg and Högberg, 2002) and are a very important sink for photosynthates in boreal and temperate forests (Högberg and Read, 2006). Maintaining such a large fungal biomass requires substantial amount of energy in the form of carbohydrates, which clearly plays an important role in the carbon balance of the plant. The relationship between photosynthesis (carbon input) and plant as well as plant-dependent symbiotic respiration (carbon output) determines the CO_2 accumulation capacity of a plant. The complete carbon budget of ectomycorrhizal seedlings was determined by Rygiewicz and Andersen (1994) in axenic laboratory conditions but such accurate balance data are not available for seedlings growing on natural humus and associated with multiple ectomycorrhizal fungi.

ECM communities are species-rich in boreal forests where plant species diversity is relatively low (Dahlberg, 2001). Different natural or man-made disturbances or changes in environmental conditions in forest ecosystems, including pollution (Cairney and Meharg, 1999), clear-cutting (Jones et al., 2003; Heinonsalo et al., 2007), changes in soil temperature (Buée et al., 2005), or drought (Shi et al., 2002), have been shown to cause changes in ECM community structure. The ECM species can differ in their demand for plant-assimilated carbon and, further on, respiration due to their variable ability to form extraradical mycelium in soil

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doi:10.1016/j.soilbio.2010.06.003

(Agerer, 2001). Different ECM have variable effects on the net plant photosynthesis which means that fungal species can cause differences in the amount of carbon fixed from the atmosphere. Dosskey et al. (1990) showed that *Rhizopogon vinicolor* increased the rate of photosynthesis probably because abundant hyphal growth developed a substantial carbon sink and created a demand for photosynthates. Different fungal species may also transfer nitrogen differently to plant shoot (nitrotolerant vs. nitrophobic fungi) (Gorissen and Kuyper, 2000) and leaf nitrogen content is related to photosynthetic capacity (Evans, 1989). In addition to direct sink–source relationship, this might be one mechanism how fungal species affect photosynthesis.

In addition to direct use of photosynthates by ECM, recently assimilated carbon can be transferred to the soil from hyphae or root tips as exudates consisting of various organic compounds. Plant-derived carbon is also transferred to the soil when animals feed on fungal hyphae and fine roots (Killham, 1995). The amount of these exudates and grazing losses are extremely difficult to quantify accurately in the field because of the complex and rapid consumption and transformation processes involved.

A large proportion of carbon fixed by photosynthesis in boreal forests has been shown to return to the atmosphere within a short time through autotrophic respiration by roots or by fungal mycelia in the mycorrhizosphere (Högberg et al., 2001). Recent tree and ecosystem scale ^{13}C- and ^{14}C-labeling experiments have shown that the appearance of tracer carbon in soil CO_2 efflux takes place within only a few days and similar results have also been obtained by coupling natural $\delta^{13}C$ values in soil respiration and photosynthesis (Högberg and Read, 2006; Trumbore, 2006). In addition, latest quantitative studies with physiological manipulation or isotope labeling techniques have shown that as much as half of the carbon released in soil respiration originates from recent photosynthates (Högberg and Read, 2006; Högberg et al., 2008). Recently fixed carbon is therefore clearly an important driver of soil biological processes and measured carbon effluxes.

The differentiation of total soil respiration between autotrophic photosynthate-dependent respiration by roots and mycorrhizal fungi, and heterotrophic respiration that depends on degradation of soil organic matter is important for understanding the carbon cycling in forest soil. Increased heterotrophic respiration relative to biomass production leads to decreased carbon accumulation or even to decreased carbon storage in soil and vice versa. In addition, the formation or decomposition of recalcitrant organic compounds below-ground affects carbon accumulation in the soil ecosystem. The CO_2 concentration of the atmosphere is largely regulated by these two major fluxes, photosynthesis and respiration that are several orders of magnitude higher than anthropogenic CO_2 emissions (Trumbore, 2006). Even small changes in those fluxes could have significant consequences on CO_2 concentration in the atmosphere. In plant or forest scale carbon balance estimates it is of crucial importance to know how the below-ground allocation of plant-assimilated carbon depends on temperature, humidity, ectomycorrhizal community composition, or other related factors. Difficulties in estimating allocation of carbon to fungal symbionts have prevented so far the incorporation of mycorrhizal fungi into system-scale models of carbon and nitrogen cycling but Hobbie (2006) made an important effort in summarizing the literature and providing equations that estimate the percentage of carbon allocation to ectomycorrhizal fungi of below-ground net primary production (NPP). However, the methodological discrepancies in the reviewed studies weaken the confidence of the estimations and therefore, new studies using e.g. isotope tracer methods are still needed to confirm these results.

In this study, a complete carbon budget of Scots pine seedlings growing in natural forest humus and colonized by various ectomycorrhizal fungi (ECM) was measured in laboratory conditions. The budget was determined by measuring the amount of carbon assimilated in photosynthesis and respired in the different parts of the system, and by analyzing the turnover rate of CO_2 and the allocation of assimilated carbon in different compartments of the plant and soil. To increase the species variation of the natural ECM community prevailing in the humus used as the growth media, the seedlings were inoculated with one of the following fungal strains: *Piloderma croceum*, *Cenococcum geophilum* or *Phialocephala fortinii*, or allowed to grow without inoculation. The carbon utilization and allocation patterns of the seedlings were statistically analyzed within these inoculation groups and against the actual observed fungal community data. We hypothesized that the presence of certain individual ECM species can affect carbon utilization and allocation even if it does not occupy the whole rhizosphere, but exists as a member of a fungal community.

2. Materials and methods

2.1. Humus sampling and seedling production

The site from where the humus used as the growth media in this experiment was taken is a boreal forest located in Southern Finland (61°84′N, 24°26′E) near the Hyytiälä Forestry Field station of the University of Helsinki. The forest was clear-cut in March 1998, and was naturally regenerated with pine. The dominant tree species prior to clear-cutting was Scots pine (*Pinus sylvestris* L.) with an understorey of Norway spruce (*Picea abies* Karst.) and silver birch (*Betula pendula* Roth) (Levula et al., 2003). The soil at the site is podzolized glaciofluvial sand covered with a humus layer (see Ilvesniemi et al., 2000 for a detailed site description). The humus samples were collected in October 2004, subsamples representing the 1 ha clear-cut area were pooled from four different locations, sieved and homogenized using 4 mm mesh size and stored at +4 °C until used in the experiment.

Surface-sterilized Scots pine seeds were first germinated on glucose agar plates and transferred to test tubes containing agar-slope and Leca® (clay) beads after one week (for details, see Heinonsalo et al., 2001). The inoculation of the sterile seedlings was performed as follows: two agar plugs containing growing fungal mycelium were transferred to test tubes and the tubes were moistened with 4 mL liquid Hagem's solution (modified from Modess, 1941) after the emergence of the first lateral roots. The plants with roots visually colonized by the introduced fungus were selected and transplanted to microcosms after approximately four weeks from inoculation. Control seedlings were treated in the same way but without the addition of fungal inoculum.

2.2. Microcosm growth system and experimental design

The microcosms consisted of separate root and shoot compartments. The root compartment (200 × 300 mm) was constructed of a 20 mm polyethylene back plate in which the root growth chamber (170 × 280 mm, depth 4 mm) and separate channels allowing circulating cooling liquid were engraved. To allow the visual inspection of the rhizosphere during the experiments, a transparent perspex® sheet was used as a cover plate. The shoot compartment consisted of an aluminium back plate with internal cooling water circulation system and a transparent perspex® cover. The shoot and root compartments can be closed airtight, allowing gas exchange measurements. The root compartments of the microcosms were filled with humus. The system is described in detail in Pumpanen et al. (2009).

The treatments used were humus microcosms without seedling and with uninoculated or inoculated seedlings. Uninoculated