



Plant species influence microbial diversity and carbon allocation in the rhizosphere

Natalia Ladygina, Katarina Hedlund*

Department of Ecology, Lund University, Sölvegatan 37, SE-223 62 Lund, Sweden

ARTICLE INFO

Article history:

Received 13 January 2009

Received in revised form

7 October 2009

Accepted 11 October 2009

Available online 22 October 2009

Keywords:

Stable isotopes

PLFA markers

Microbial communities

Carbon partitioning

Plant mixture

ABSTRACT

Plant species effects on microbial communities are attributed to changes in microbial community composition and biomass, and may depend on plant species specific differences in the quality of resources (carbon) inputs. We examined the idea that plant–soil feedbacks can be explained by a chance effect, which is the probability of a highly productive or keystone plant species is present in the community and will influence the functions more than the number of species per se. A ^{13}C pulse labelling technique was applied to three plant species and a species mixture in a greenhouse experiment to examine the carbon flow from plants to soil microbial communities. The ^{13}C label was given as CO_2 to shoots of a legume (*Lotus corniculatus*), a forb (*Plantago lanceolata*), a grass (*Holcus lanatus*) and a mixture of the three species. Microbial phospholipid fatty acids (PLFA) was analysed in order to determine the biomass and composition of the soil microbial community. The incorporation of the stable isotope into soil microorganisms was determined through GC–IRMS analyses of the microbial PLFAs. Plant species identity did not influence the microbial biomass when determined as total carbon of microbial phospholipid fatty acids. However, the labelled carbon showed that the grass monoculture (*H. lanatus*) and the plant mixture allocated more ^{13}C into bacteria and actinomycete biomass than the other plant species. *H. lanatus* monocultures had also the highest amounts of ^{13}C allocated to AM-fungi and saprophytic fungi. The carbon allocation from plants to soil microorganisms in a plant species mixture can thus be explained by the presence of a highly productive species that influence soil functions.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

The diversity and composition of soil microbial communities and plant communities are generally attributed to feedback interactions between the above and below-ground communities (Bever et al., 1997). The feedback is based on the quality and quantity of organic matter from the plants to the soil organisms, that in return influence the nutrient turnover and biotic interactions of symbionts or pests in the soil (Chapman et al., 2005; Ehrenfeld et al., 2005). Plant species differ in what quantity and quality of carbon resources they produce which influence the composition of the microbial community in the rhizosphere (Grayston et al., 1998; Bardgett et al., 1999; Berg and Smalla, 2009).

In natural ecosystems such as grasslands, different levels of plant species diversity can influence composition and biomass of microbial communities (Wardle and Nicholson, 1996; Kowalchuk et al., 2002; Wardle et al., 2003). However, interactions between plant species and soil organisms have also been reported to be

context dependent (Bezemer et al., 2006a) or even lack such relationships (Nunan et al., 2005; Singh et al., 2007; Kielak et al., 2008). The different responses by microbial communities to plant carbon resources can partly be explained by coarse sampling techniques in root systems with a high number of plant species overlapping and only the net effect of carbon exudates can be detected (Bezemer et al., 2006b).

A more general explanation can be extracted from ideas of the relation between diversity and functioning of plant communities where functions in an ecosystem can be a consequence of the prevailing traits of the species in the community (Lepš et al., 2001; Huston, 1997). This is in contrast to the idea that a higher diversity will have higher functioning by complementarity of species traits (Tilman et al., 1997; Loreau, 1998). Here we attempt to examine the idea that plant–soil feedbacks can be explained by a chance effect, where a highly productive or keystone plant species present in the community results in greater influence over soil functions than the contribution of additional diversity per se (Tilman et al., 1997; Lepš et al., 2001). Thus, we tested whether allocation of carbon by plants is determined by species identities and that the outcome of the plant–soil feedback is influenced by the presence of a highly productive species.

* Corresponding author. Tel.: +46 46 222 44 50; fax: +46 46 222 47 16.
E-mail address: katarina.hedlund@ekol.lu.se (K. Hedlund).

Plant carbon partitioning into rhizosphere microbial communities is commonly evaluated by examining the decomposition dynamics of ^{13}C labelled plant material (Williams et al., 2006; Moore-Kucera and Dick, 2008). Carbon allocation by living plants into the belowground community can now be estimated after foliar exposure to $^{13}\text{CO}_2$ that is allocated to roots and subsequently to the soil microbial community (Cheng, 1996; Ostle et al., 2000; Gavito and Olsson, 2003; Butler et al., 2003; Fan et al., 2008). A combination of pulse labelling and analyses of ^{13}C ratios of signature fatty acids can yield quantitative as well as qualitative data of the partitioning of plant carbon that is useful in studies of natural communities (Treonis et al., 2004; Elfstrand et al., 2008).

We designed a greenhouse experiment with monocultures and plant communities of three plant species and tested whether allocation of plant carbon to the soil microbial community is determined by species identities. The allocation of carbon from plants into the soil microbial community was determined by pulse labelling plant shoots with $^{13}\text{CO}_2$. The partitioning of carbon into plant roots and the soil microbial community was determined by analyses of phospholipid fatty acids (PLFA) that were used as markers for biomass of the microbial community.

2. Material and methods

2.1. Experimental design

Soil was collected in February 2005 from a grassland in the Weluwe area in the eastern part of the Netherlands, that was used as field site of a diversity experiment described in Van der Putten et al. (2000). The area was taken out of agricultural production in 1995 and was colonized by naturally colonising plant species from the surrounding grassland. The soil was a sandy loam with a pH of 6.3 and had 4.5% organic content. The nutrient content of the soil was 13 mg total N/g soil and 11 mg total P/g soil (Van der Putten et al., 2000). The soil was collected as several large bulk samples from 0 to 5 cm depth and hand sorted to remove plant residues and stones. The soil was mixed by hand to adjust for the effect of local heterogeneity in the field. The soil was transported fresh to Sweden in large plastic boxes and stored at +4 °C until it was used in the microcosms.

Plant monocultures were established in March 2005 with 3 grassland species *Lotus corniculatus* L. (legume), *Plantago lanceolata* L. (forb), and *Holcus lanatus* L. (grass), which were common species at the grassland site (Lepš et al., 2001). The soil was sieved (5 mm mesh) and 500 g of fresh soil per replicate was put in an open plastic box (19.5 × 14 × 5 cm height) and a total of 27 boxes were used. The soil was watered to 20% water content, which was an average of the field conditions. Seeds were obtained from Appels Wilde Samen GmbH (Darmstadt, Germany). The seeds were germinated in Petri dishes on a filter paper during one week and 9 seedlings were planted in boxes (19.5 × 14 × 5 cm) with 500 g of soil in each box. A species mixture was set up containing 3 seedlings of each of the three plant species in a similar sized box. The monocultures and the species mixture treatments were replicated 6 times giving a total of 24 boxes. One additional box of each of the plant monocultures was planted and used as controls to determine natural abundances of $\delta^{13}\text{C}$ in the microbial communities and in root and shoot tissue. All boxes were kept in the greenhouse with a 15/9 h light/dark cycle and average temperatures of 20 °C at day and 17 °C at night. The water content in the soil was adjusted to 20% by watering which was checked by weighing the boxes with the plant cultures. A nitrogen fertilizer, 1 ml of 3% NH_4NO_3 was diluted in 100 ml of tap water and added to each box at the beginning of the experiment in order to avoid nitrogen deficiency.

Thirty-six days after the seedlings were planted all 24 boxes were labelled once with $^{13}\text{CO}_2$ (99% $^{13}\text{CO}_2$; Larodan Fine Chemicals, Malmö, Sweden). Each box was closed with an air tight transparent top chamber of 4.5 dm³ and 25 ml $^{13}\text{CO}_2$ was injected with a gas-tight syringe through a septum into the chamber. The labelling period was stopped after 3 h by removing the lid of the chamber and the labelled and non fixed CO_2 was allowed to diffuse into the air of the greenhouse.

2.2. Sampling

Soil was sampled from the boxes with a small (1.0 cm diam) metal corer from five different parts of each box, from the surface of the soil to the bottom of the box. The soil was sieved (2 mm mesh) and subsamples mixed to avoid heterogeneity within the boxes thus yielding a sample of about 10 g of soil. Roots visible in the soil were taken away as they otherwise can interfere with the PLFA analyses, as roots and saprophytic fungi have the same PLFA marker, see below. The samples were collected just before labelling and at day 3, 9, 13, 20 after labelling and kept frozen at –20 °C until further analyses. The soil samples taken before labelling, and those taken from unlabelled boxes were used as unlabelled controls for natural abundance of ^{13}C of soil microbial PLFAs. Plant biomass was harvested at 20 days after labelling (56 days after planting seedlings) in all boxes both labelled and controls. Soil, shoots and roots were separated and the roots were rinsed carefully with water on a sieve (1.0 mm) before further processing. Shoots and roots were oven dried and weighed after harvest (at 105 °C for 24 h). Prior to lipid extraction the dry shoots and roots were milled with stainless steel balls in stainless steel beakers (15 s, 300 strokes min⁻¹).

2.3. PLFA analysis

The lipids from roots (0.1–1.4 g [dry mass]), shoots (0.1–2.2 g [dry mass]), and soil (3 g [wet mass]) were extracted according to Frostegård et al. (1993). The lipids were separated into neutral lipids, glycolipids, and phospholipids on prepacked silica columns (100 mg of sorbent mass, Varian Medical Systems, Palo Alto, CA, USA) by elution with 1.5 ml of chloroform, 6 ml of acetone, and 1.5 ml of methanol, respectively. The fatty acid residues in the neutral lipids and phospholipids were transformed into free fatty acid methyl esters and analysed by gas chromatography (Hewlett–Packard, Palo Alto, CA, USA). The PLFAs were analysed on GC–FID using a 30 m × 0.25 mm fused silica capillary column (HP-5) with H_2 as the carrier gas to determine amounts of the PLFAs (for details, see Hedlund (2002)). Twenty-five fatty acids were identified from their relative retention times relative to that of standards. In the GC–IRMS analyses (See description below) the detection limits of PLFAs were higher and thus reduced the number of PLFAs that could be used to 13. To compare microbial community composition based on PLFA abundances with active microbial community based on amounts of ^{13}C -PLFA the same number of fatty acids was chosen. Thus, a total of 13 PLFAs, including bacterial PLFAs, saprophytic and AM-fungi PLFA, actinomycetes as well as 16:0, 18.1 ω 7, 10Me16:0, 18:0 PLFAs were used.

The PLFAs thus chosen to represent bacterial biomass were i15:0, a15:0, i16:0, cy17:0, 18:1 ω 7, and cy19:0 (Frostegård and Bååth, 1996). The PLFA 10Me18:0 was used as a marker for actinomycete biomass (Kroppenstedt, 1985). Saprophytic fungi, plant root and shoot tissue were represented by the PLFA 18:2 ω 6 marker (Frostegård and Bååth, 1996; Olsson and Johansen, 2000). Saprophytic fungi and root tissue have the same PLFA marker which means that saprophytic fungi can not be determined in roots and care was taken before extraction to avoid roots in the soil samples that were extracted, see above. The PLFA markers for saprophytic

Download English Version:

<https://daneshyari.com/en/article/2025123>

Download Persian Version:

<https://daneshyari.com/article/2025123>

[Daneshyari.com](https://daneshyari.com)