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Plant species identities and fertilization influence on arbuscular mycorrhizal fungal colonisation and soil bacterial activities

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ABSTRACT

Plant species influence soil microbial communities, mainly through their functional traits. However, mechanisms underlying these effects are not well understood, and in particular how plant/microorganism interactions are affected by plant identities and/or environmental conditions. Here, we performed a greenhouse experiment to assess the effects of three plant species on arbuscular mycorrhizal fungal (AMF) colonization, bacterial potential nitrification (PNA) and denitrification activities (PDA) through their functional traits related to nitrogen acquisition and turnover. Three species with contrasting functional traits and strategies (from exploitative to conservative), *Dactylis glomerata* (L.), *Bromus erectus* (Hudson) and *Festuca paniculata* (Schinz and Tellung), were cultivated in monocultures on soil grassland with or without N fertilization. Fertilization impacted some plant traits related to nutrient cycling (leaf and root N concentration, root C:N) but did not affect directly microbial parameters. The highest PDA and PNA were observed in *D. glomerata* and *F. paniculata* monocultures, respectively. The highest AMF colonization was obtained for *F. paniculata*, while *B. erectus* exhibited both the lowest AMF colonization and bacterial activities. Bacterial activities were influenced by specific above-ground plant traits across fertilization treatments: above-ground biomass for PDA, shoot:root ratio and leaf C:N ratio for PNA. Mycorrhizal colonization was influenced by below-ground traits either root dry matter content or root C:N. Hence, AMF colonization and bacterial activities were impacted differently by species-specific plant biomass allocation, root traits and nutrient requirement. We suggest that such effects may be linked to distinct root exudation patterns and plant abilities for nutrient acquisition and/or nutrient competition.

1. Introduction

Soil microbial communities by their activities and diversity are involved in a wide range of ecosystem processes, such as carbon and nitrogen cycling (Kowalchuk and Stephen, 2001; van der Heijden et al., 2008), and influence plant growth through nutrient availability (van der Heijden et al., 2008). Identifying the drivers of the diversity and activity of soil microbial communities is therefore crucial to understand ecosystem functioning and to anticipate ecosystem responses to global changes (Allison and Martiny, 2008). At the individual plant level, previous studies have reported the importance of species identity on both fungal (Kardol et al., 2007; Rooney and Clipson, 2009) and bacterial communities (Patra et al., 2006). For example, Orwin et al. (2010) demonstrated that leaf traits and litter quality of fast growing species favoured bacteria over fungi in the rhizosphere. At the community and ecosystem levels, the diversity and structure of plant communities can affect soil microbial community size and composition as well as their enzymatic activities (Grayston et al., 1998; Hedlund et al., 2003; Harrison and Bardgett, 2010; De Deyn et al., 2011; Le Roux et al., 2013). The activity of soil microbial communities partly depends on resource availability, notably N and C (Attard et al., 2011; Falcão Salles et al., 2012; Cantarel et al., 2015). Therefore, plant influence on soil microbial communities can be mediated

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through their functional traits related to soil nutrients cycling (Mouhamadou et al., 2013; Legay et al., 2014a), and more specifically through: (i) the amount and quality of litter and root exudates as a provider of nutrient for soil microbial communities (Wardle et al., 1998; Cesco et al., 2012) and (ii) their ability to compete for nutrient uptake and thus depleting the nutrient available for microbial communities (Cantarel et al., 2015). These plant effects have been related to plant traits such as plant growth rate and size (Wardle et al., 1998), specific leaf area and leaf dry matter content (Cornelissen et al., 1999; Kazakou et al., 2006), and below-ground traits including root turnover, root morphology, exudation (Innes et al., 2004).

Plant functional traits are an intrinsic property of species reflecting their environmental niche (Grime et al., 1997), determining their response to environmental changes (Lavorel and Garnier, 2002; Amatangelo et al., 2014), and also varying within a given species in response to environmental variations (Albert et al., 2010; Kichenin et al., 2013). For example, a management practice such as nitrogen fertilization influences plant leaf traits such as vegetative height, leaf dry matter content and nitrogen concentration (Lavorel et al., 2011; Gardarin et al., 2014), and thereby ecosystem functioning (Wardle et al., 1998; Pakeman, 2011; Lienin and Kleyer, 2012). Consequently, the effects of fertilization on soil microorganisms may also be indirectly mediated through plant trait changes (De Deyn et al., 2011).

Hence, plant influences on soil microbiota may result directly from intrinsic functional traits i.e. the morphological, phenological and physiological traits and/or indirectly through modified traits by environmental conditions. The underlying mechanisms are not well understood, in particular uncertainties remain about which plant traits, (specific to plant species or mediated by environment), are involved in the response of soil microorganisms to plant species.

The purpose of our study was: (i) to investigate the effects of three plant species on soil bacterial activities and mycorrhizal status, (ii) to determine how plant/microorganism interactions are affected by plant identities through their functional traits, and (iii) how fertilization could impact these interactions. We focused on AMF colonization and N-cycling bacteria representing key soil microbial groups regulating nitrogen cycling in terrestrial ecosystems (Veresoglou et al., 2011). Three dominant subalpine grass species from the Central French Alps with contrasting functional traits were cultivated under similar soil nutrient availabilities with and without nitrogen addition in greenhouse conditions. *Festuca paniculata* is a conservative species with traits indicative of slow nutrient turnover while *Dactylis glomerata* is a more exploitative species with traits indicative of a faster nutrient turnover and *Bromus erectus* has intermediate characteristics (Grassein et al., 2010). Above- and below-ground traits, bacterial enzymatic activities (nitrification and denitrification) and mycorrhizal colonization (frequency and intensity) were measured for each plant species under low and high nutrient availability. We hypothesized that (i) AMF colonization and bacterial activities are impacted differently by the three plant species with contrasting nutrient turnover and that (ii) fertilization impacts bacterial activities and AMF colonization directly through change in nutrient availability and indirectly through change of plant traits (root biomass and nutrient status).

2. Materials and methods

2.1. Soil and plant species

Soil and three co-occurring grasses, *D. glomerata* (L.), *B. erectus* (Hudson) and *F. paniculata* (Schinz and Tellung), differing in their relative growth rates and in their resource use strategy (Grassein et al., 2010), were sampled in the upper Romanche valley of the central French Alps (45.041°N 6.341°E, 1650–2000 m a.s.l.). During the autumn 2010, three mother tussocks for each species were collected in grassland which was lightly grazed. Plants and especially roots were carefully washed to remove soil particles prior to vegetative multiplication in a nutritive solution and perlite as described in (Grassein et al., 2015). After one month, each replicate of plant species was clipped at 6-cm for the above-ground part and 4-cm for the below-ground part prior to the plantation in order to standardize plant size and to reduce the carryover of fungi from the field soil.

2.2. Growing conditions

Clipped tillers were transplanted in an air-dried and sieved (5.6-mm mesh) grassland soil collected in autumn 2010 at a depth of 5–20 cm, in an area of 2 m in diameter in the same grassland (45.041°N 6.341°E, 1650–2000 m a.s.l.). Perlite (25 g per pot) was added to limit soil compaction since all gravels and stones were removed. Initial physical and chemical soil properties were as follows: clay, 30%; silt 46%; sand 24%; total carbon content: 44.4 g kg^{-1}; total nitrogen content, 4.14 g kg^{-1}; total phosphorus content, 1.79 g kg^{-1}; pH (H_2O), 5.5–6. Plants were cultivated in mesocosms constituted by cylindrical PVC boxes (7 cm of diameter and 16 cm of height; 617-cm^3). In each mesocosm, two individuals per plant species were grown in 500 g of the perlite/soil mix. Mesocosms were placed in a glasshouse with air temperature kept at 20/16 ± 2 °C (day/night), additional light from artificial lighting (400-W high-pressure sodium lamps, Philips Son-T-pia Agro) provided 450-μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at plant height with a 16/8 h photoperiod cycle. Soils were watered daily to keep humidity at 20 g water 100 g^{-1} dry soil, and two treatments were used: no fertilized or fertilized with 50 kg N ha^{-1} (14 mg N kg^{-1} of dry soil) in the form of a urea-based slow release N fertilizer (Osmocote®) applied at the beginning of the experiment. This type of fertilization was chosen to simulate organic manure fertilization applied in mountain grassland. A total of 18 pots (3 plant species × 2 treatments × 3 replicates) were set up and moved twice a week to avoid any positional effect.

2.3. Harvest and plant trait measurements

After three months, rhizospheric soil, aerial and root parts were harvested separately. We gently washed the roots with water on a 0.5-mm sieve to avoid any loss of fine roots. The root biomass was split into three aliquots, one aliquot was dried at 60 °C, the two others were kept in an alcoholic solution (ethanol 10%, acetic acid 5% v: v) until (i) arbuscular mycorrhizal determination (see below) and (ii) root trait analyses. Plant functional traits for leaf and root were measured following standardised protocols (Cornelissen et al., 2003). For leaves, we measured fresh leaf area (LICOR Li-3100), fresh biomass and dry biomass after 72 h at 60 °C, in order to calculate specific leaf area (SLA), leaf dry matter content (LDMC) and above-ground biomass (ABM). For roots, we measured root length (WINRHIZO software (Regent Instruments Inc., Canada)), fresh biomass and dry biomass after 72 h at 60 °C, in order to calculate specific root length area (SRL), root dry matter content (RDMC) and root biomass (RBM). Above- and below-ground biomasses were used to compute the shoot root ratio (SRR). Leaf and root dried masses were finely ground (5-μm diameter) for analysis of N, C using an isotope ratio mass spectrometer (IRMS, Isoprime, GV Instrument) for leaf N and C content (LNC and leaf C:N ratio) and root N and C content (RNC, root C:N ratio) determination.

2.4. Soil analysis

At the harvest, the root density was so high after three months of growth that all the soils in the PVC box were considered as under