Unravelling the effects of plant species diversity and aboveground litter input on soil bacterial communities

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ABSTRACT

In order to differentiate the effects of root functioning and aboveground litter inputs on soil bacterial communities, a pot experiment was designed using different combinations of three plant species with contrasting chemical characteristics (0, 1, 2 or 3 species per plot) grown with or without aboveground litter inputs from the same plant species (no litter, litter from 1 of the species, or litter from the 3 species). Bacterial community structure (ITS diversity-ARISA), as well as total bacteria and denitrifier abundances (qPCR targeting the $16S$ rDNA and $nirK$ or $nirS$ genes) and denitrifying enzyme activity (DEA) were determined. No clear effects of the plant and litter identities were revealed over the incubation time. Moreover, differences in litter C:N values did not influence the bacteria or denitrifier abundances nor DEA. Interestingly, litter diversity modified the bacterial community structure, while plant richness altered the total bacteria and denitrifier abundances as well as DEA. Soil moisture appeared to be the major driver of plant and litter richness effects in our experiment.

1. Introduction

In terrestrial ecosystems, plants and soil microbial communities interact through the release of carbon and nutrient coming from the root system and plant litter (Dehlin et al., 2006; Nilsson et al., 2008; Wardle et al., 2006) These interactions are a major driver of nutrient cycling. Microbial communities act as a sink or source for C- and N-containing compounds and can provide up to 80% of the plant mineral needs, principally ammonium and nitrate. They can, indirectly, regulate plant growth and plant community composition by determining the residence time of available nutrients (Nelson and Mele, 2007; Smith, 2002; Wardle, 2004). Plants, in turn, provide organic matter as bioavailable exudates (10 to 30% of their photosynthetic products) or aboveground litter (Kuzyakov and Schneckenberger, 2004; Paterson et al., 2008). These two organic matter pools are characterized by different chemical compositions (simple C molecules, polysaccharides, proteins versus cellulose and lignin, respectively) and are consumed at different rates (Haichar et al., 2008). It is therefore likely that the interactions between plants and soil microbial communities that are mediated by root functioning or aboveground litter input differ both in intensity and in the microbial communities that are involved. For example, the litter C:N ratio has been shown to influence the microbial decomposition of organic matter: high C:N ratios have often been associated with low decomposition rates (Liu et al., 2009; Melillo, 2002). Moreover, the organic matter differs in both quantity and composition between plant species (El Moujahid et al., 2017) and, therefore, plant diversity may be a major factor determining the community structures, abundances and activities of soil microorganisms involved in C and N cycling (Carney and Matson, 2006; Kowalchuk et al., 2002; Lange et al., 2014; Schlatter et al., 2015; Thompson and Kao-Kniffin, 2016). However, it is not clear how these diversity effects are mediated. Effectively, individual plant species are known to select specific microbial communities (Berg and Smalla, 2009; Haichar et al., 2008; Patra et al., 2006). However, this relationship has generally been shown for plants that are grown in isolation or in monoculture (Kowalchuk et al., 2002; Marschner et al., 2001). The relationships between plants and microbial communities are not always very strong and, in grasslands, where plant density is high and roots are highly intermingled, such relationships are less clear (Kielak et al., 2008; Ritz et al., 2004). It is more likely that microbial communities in grassland soils are influenced by differences in plant communities, such as differences in diversity or composition (Kowalchuk et al., 2002). Although many studies have investigated the

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relationships between plants and soil microbial communities in grass-lands, the differentiation between the effects of root functioning, in particular rhizodeposition, and aboveground litter inputs is still un-clear. In most experiments and in particular in field studies, both effects are confounded due to the fact that the presence of particular plant species and the inputs of their aboveground litter vary together. In order to overcome this confounding effect, a microcosm experiment was set up in which plant influences on soil through root functioning and through aboveground litter inputs were separated. The main pur-poses of the study were to investigate whether bacterial community structure, abundance and activity were influenced by (i) the identity of the plant species present and the type of aboveground litter input, and/or (ii) the levels of plant diversity and litter diversity. The total bacterial community structure and abundance were determined via molecular techniques. Because plant diversity can have an impact on bacterial functional groups like denitrifiers (Le Roux et al., 2013; McGill et al., 2010; Niklaus et al., 2016), the effects of plant diversity and litter di-versity on denitrifier abundances and DEA were also determined.

2. Material and methods

2.1. Experimental set-up

A pot experiment was set up in a full factorial design with different plant associations and different litter amendments. The *plant interaction* treatment consisted in different assemblages of three plants species in monocultures, two- and three-species mixtures (seven combinations in total). The *litter* treatment consisted of five combinations of above-ground biomass of the same plant species: no litter, litters from single species and a mixture of litters of the three species. The full factorial design thus comprised 35 combinations (7 plant associations × 5 litter inputs) in four replicates. Three European grassland plant species were selected according to their morphological, ecological and chemical characteristics (Baude et al., 2011): *Mimulus guttatus* (MG, Scrophular-iaceae), *Lamium amplexicaule* (LA, Lamiaceae) and *Medicago sativa* (MS, Fabaceae). The litter amendment corresponded to the addition of 2.36 g C kg^{-1} soil per pot (approx. 1.1 kg of soil) filled with a loamy sand soil (sieved < 2 mm) (CEREEP Foljuif, Saint-Pierre-les-Nemours, Seine-et-Marne, France). Litter preparation, litter amendment and plant growth is presented in Baude et al. (2011). The soil had a C:N ratio of 10.40 and a C:P ratio of 26.03. The C:N ratio of the species litters was 13.95, 18.17 and 23.98, and the C:P ratio was 9.01, 12.74 and 27.49 for *M. guttatus*, *L. amplexicaule* and *M. sativa*, respectively. Soil was sampled with a small corer (1 cm diameter and 10 cm length) after the addition of litter at the beginning (T = 0) and the end (T = 72 days) of the experiment. Soil sub-samples were stored at 4 °C for chemical analysis and at − 20 °C for molecular analysis.

2.2. Abiotic soil variables

Soil total C and N contents were measured using an CHN Elemental Analyzer (NA1500 Series 2, Fisons, Manchester, UK). Mineral N was extracted from the soil by shaking in a 2 M KCl solution (1:5 soil:so-lution). Nitrate was reduced to nitrite, and then nitrite and ammonium concentrations were measured with a continuous-flow nitrogen ana-lyzer (SKALAR, San Plus System, Breda, the Netherlands). Dissolved organic carbon was measured on a Shimadzu 5000 TOC analyzer (Shimadzu, Kyoto, Japan), after water extraction from soils (1:10 soil:solution).

2.3. Total bacterial community structure

The total bacterial community structure was determined by B-ARISA analysis at the end of the experiment (T = 72 days), and were performed at the Genosol platform (http://www2.dijon.inra.fr/plateforme_genosol). The bacterial ribosomal intergenic spacer (IGS)

of the DNA fractions was amplified by PCR using the primers L-D-Bact-132-a-A-18/S-D-Bact-1522-b-S-20. The S-D-Bact-1522-b-S-20 was la-beled at its 5′-end with the IRD800 fluorochrome for the detection of the resulted amplicons by a LiCor DNA sequencer (ScienceTec, Les Ulis, France) (Ranjard et al., 2003).

2.4. Quantification of total bacteria and denitrifiers abundances

Total DNA was extracted from 0.5 g fresh soil samples (Guenet et al., 2011). Total bacteria and denitrifier abundances were quantified by qPCR using primers targeting the *16S* rRNA, *nirK* and *nirS* genes, as described previously (Bru et al., 2011; Leloup et al., 2009). Reactions were carried out in an CFX96 Real Time PCR detection system (Bio-Rad, Marnes la Coquette, France), and carried out in a 20 mL reaction vo-lume containing the SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, Marne la Coquette, France), 1 mM of each primer, 2 μM bo-vine serum albumin, and 0.4 ng and 4 ng of DNA (in order to detect amplification inhibition). Two quantitative PCR assays were performed for each gene. Standard curves were obtained using serial dilutions of linearized plasmids containing the studied genes (respectively ampli-fied from *Pseudomonas fluorescens*, *Sinorhizobium meliloti*, and *Pseudo-monas stutzeri*) Gene abundances were determined in duplicate for two treatment replicates. Calculated gene copy numbers were expressed per g dry soil, with the assumption that use of a unique soil limited the bias related to DNA extraction yield between samples.

2.5. Denitrifying enzyme activity

Denitrifying enzyme activity (DEA) was measured in fresh samples at the end of the experiment, as previously described by Patra et al. (2006). Briefly, samples (10 g dry soil equivalent) were placed in 150 mL plasma flasks, and 6 mL of distilled water containing KNO$_3$ (200 μg NO$_3$ – N g^{-1} dry soil), glucose (0.5 mg C g^{-1} dry soil) and glutamic acid (0.5 mg C g^{-1} dry soil) was added. Additional water was provided to bring the soil to 100% water holding capacity and flasks were sealed. The atmosphere of each flask was replaced by a 90:10 He–C$_2$H$_2$ mixture, to ensure that the conditions were anaerobic and to inhibit N$_2$O reductase activity. During incubation at 28 °C, gas samples were taken at 2, 4 and 6 h (linearity during the first 6 h was checked for all samples) and immediately analyzed for N$_2$O using a gas chromato-graph (Varian STAR 3400 CX, walnut Creek, CA, USA). DEA was measured on all the treatment replicates.

2.6. Statistical analysis

All statistical analyses were carried out using R statistical software (R Core Team, 2014). After log-transformation of the data when ne-cessary, the effects of litter identity, plant species identity, litter species richness and plant species richness were determined by two-way ana-lysis of variance. Tukey post hoc tests were used to determine the sig-nificant differences among treatments. Multiple comparison between groups were calculated using the multcomp package (Hothorn et al., 2008). The B-ARISA data were first converted using the PrepRISA program (Ranjard et al., 2003). Between-class analysis (BCA) was car-ried out using the ade4 package (Dray and Dufour, 2007) on B-ARISA profiles in order to determine how microbial community structure varied as a function of plant or litter identities, and diversity richness.

In order to explore possible relationships between variables, we used Structural Equation Modelling and developed a simple model taking into account known biogeochemical relations between variables. The structural model was constructed as follow: 1) the diversity treat-ment can influence soil C and N content, NH$_4^+$ and NO$_3^-$ concentra-tions, soil moisture and denitrifier abundances (NirK, NirS) as well as soil moisture, 2) soil N content can influence NH$_4^+$ and NO$_3^-$ con-centrations and NH$_4^+$ influences NO$_3^-$ concentrations, 3) NH$_4^+$ and NO$_3^-$ concentrations, as well as soil water content can influence