Soil Biology & Biochemistry 43 (2011) 2012–2022

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Seasons differently impact the structure of mineral weathering bacterial communities in beech and spruce stands

C. Collignon [a,b], S. Uroz [a,b,*], M-P. Turpault [a], P. Frey-Klett [b]

[a] INRA, UMR1136 INRA-Nancy Université "Interactions Arbres-Microorganismes", IFR 110, Centre INRA de Nancy, 54280 Champenoux, France
[b] INRA UR 1138 "Biogéochimie des Ecosystèmes Forestiers", Centre INRA de Nancy, 54280 Champenoux, France

ARTICLE INFO

Article history:
Received 18 January 2011
Received in revised form
25 April 2011
Accepted 13 May 2011
Available online 31 May 2011

Keywords:
Seasonal variations
Bacterial communities
Mineral weathering
Rhizosphere
Nutrient-poor soil
Beech
Spruce

ABSTRACT

Bacterial communities play an essential role in the sustainability of forest ecosystems by releasing from soil minerals the nutritive cations required not only for their own nutrition but also for that of trees. If it is admitted that the nutritional needs of trees vary during seasons, the seasonal dynamics of the mineral weathering bacterial communities colonizing the tree rhizosphere remain unknown. In this study, we characterized the mineral weathering efficacy of bacterial strains, from the rhizosphere and the adjacent bulk soil at four different seasons under two different tree species, the evergreen spruce and the deciduous beech, using a microplate assay that measures the quantity of iron released from biotite. We showed that the functional and taxonomic structures of the mineral weathering bacterial communities varied significantly with the tree species as well as with the season. Notably, the *Burkholderia* strains from the beech stand appeared more efficient to weather biotite that the one from the spruce stand. The mineral weathering efficacy of the bulk soil isolates did not vary during seasons under the beech stand whereas it was significantly higher for the spring and summer isolates from the spruce stand. The weathering efficacy of the rhizosphere isolates was significantly higher for the autumn isolates compared to the isolates sampled in the other seasons under the beech stand and in summer compared to the other seasons under spruce. These results suggest that seasonal differences do occur in forest soil bacterial communities and that evergreen and deciduous trees do not follow the same dynamic.

1. Introduction

In temperate forest ecosystems, which are generally developed on nutrient-poor soils, many organisms and microorganisms such as plants, lichens, fungi and bacteria are expected to play an essential role in soil fertility, especially by releasing key nutrients from soil minerals (Balogh-Brunstad et al., 2008; Boyle and Voigt, 1973; Kalinowski et al., 2000; Marschner,1995; Uroz et al., 2009a, in press). However, their relative impact on the global mineral weathering process is difficult to discriminate from purely abiotic processes. Although the mineral weathering potential of ectomycorrhizal fungi have been deeply studied (Arocena et al., 2004; Gadd, 2007; van Breemen et al., 2000; Wallander, 2000), the identification and the relative contribution of forest soil bacterial communities have just begun to be investigated. Mineral weathering bacteria have been isolated in various environments, and particularly from the tree rhizosphere and ectomycorrhizosphere environments, which constitute the tree root–soil interfaces where nutrient exchanges take place (Leyval and Berthelin, 1991; Puente et al., 2004; Calvaruso et al., 2007). Recent studies demonstrated that the mineral weathering efficacy of bacterial isolates colonizing the oak-*Scleroderma citrinum* ectomycorrhizosphere was significantly higher than those living in the surrounding bulk soil, suggesting that the bacterial communities selected in the ectomycorrhizosphere could provide nutritive cations to the ectomycorrhizal fungi through mineral dissolution, thus improving tree nutrition (Uroz et al., 2007). Notably, this plant growth-promoting effect was demonstrated in microcosms experiments for some of these efficient mineral weathering bacterial strains (Calvaruso et al., 2006; Koele et al., 2009).

All the studies performed on the mineral weathering bacterial communities were performed at a unique season. However, seasons are expected to directly and on indirectly impact the distribution and the functioning of the soil microbial communities. Firstly, the environmental fluctuations related to temperature, light and moisture, directly influence on the soil bacterial communities as well as the tree physiology (Brant et al., 2006; Waldrop and

* Corresponding author. INRA, UMR1136 INRA-Nancy Université "Interactions Arbres-Microorganismes", IFR 110, Centre INRA de Nancy, 54280 Champenoux, France. Tel.: +33 (0)3 83 39 40 81; fax: 33 (0)3 83 39 40 69.
E-mail address: uroz@nancy.inra.fr (S. Uroz).

0038-0717/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2011.05.008

Firestone, 2006; Williams and Rice, 2007). Besides, the seasonal quantitative and qualitative variations in the root exudation, also impact the rhizosphere bacterial communities (Allen and Schlesinger, 2004; Esperschütz et al., 2009a; Kaiser et al., 2010). So far, most of the studies dealing with seasonal fluctuations in soils focused on the taxonomic diversity of soil bacterial communities (Burke and Chan, 2010; Krave et al., 2002; Ruiz Palomino et al., 2005) and few have evaluated the relative impact of these seasonal fluctuations on the functional diversity of these bacterial communities (Cho et al., 2008; Mergel et al., 2001).

In our study, we addressed the question of the seasonal dynamics of the rhizosphere bacterial communities involved in the mineral weathering process, based on the hypothesis that trees select efficient mineral weathering bacterial communities to promote their growth in function of their nutrition needs during the seasons. We tested this hypothesis under two contrasted forest tree species, beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* Karst.), which are abundant and economically important in Europe. As no genes involved in mineral weathering have been identified to date, we focused our analyses on the culturable bacterial communities originating from the rhizosphere and the surrounding bulk soil under beech and Norway spruce stands collected during autumn 2007 and winter, spring, summer 2008. A total of 315 bacterial isolates was tested for their mineral weathering ability using an *in vitro* microplate assay. For each season showing significant differences in term of functional diversity (spring and autumn), the related bacterial isolates were genotypically characterized by amplifying and sequencing a portion of the 16S rRNA gene. Data were statistically analyzed to determine whether the environment of origin, the physicochemical characteristics of the soil, the tree species and the seasons altered the distribution and the efficacy of the soil mineral weathering bacterial communities.

2. Materials and methods

2.1. Study site and soil properties

This study was conducted in the Breuil-Chenue experimental forest site located in the Morvan (47°18′N, 4°5′E, France). This forest is situated on a plateau at an altitude of 638 m. The native forest was clear-felled and replaced in 1976 by mono-specific plantations distributed in plots of 0.1 ha of different species such as beech (*F. sylvatica* L.) and Norway spruce (*P. abies* Karst.). The soil is acidic, well-drained, nutrient-poor and classified as a Typic Dystrochrept (Giovagnotti and Giovagnotti, 1999). It is developed on the "Pierre qui Vire" granite (Seddoh, 1973). This granite is characterized by quartz, K-feldspar, plagioclase (albite), muscovite, black mica (biotite), chlorite and the main accessory minerals of granite (apatite, zircon). Its composition is in % SiO_2, 75.42; Al_2O_3, 14.13; Fe_2O_3, 1.22; MnO, 0.05; MgO, 0.21; CaO, 0.37; Na_2O, 3.32; K_2O, 4.72; TiO_2, 0.10; P_2O_5, 0.13; LI, 1.13 (LI, loss on ignition). The bulk soil has a sandy-loam texture (55% sand and less than 20% clay) and is acidic (pH_{KCl} 3.1–4.3). The cation exchange capacity (CEC) is small and mainly occupied by aluminium. Base saturation is less than 10% (Mareschal et al., 2010). Humus is present as a moder (Brethes et al., 1995) in the native forest. The climatic conditions (average values of air temperature and rainfall) in the region of Breuil-Chenue site registered during the period studied are presented in Figure S1. Minimum and maximum temperatures were recorded in December 2007 (−3.2 °C) and July 2008 (23.6 °C). The annual precipitation was of 1300 mm.

2.2. Soil sampling and soil solution analyses

Soil samples were collected under beech and Norway spruce stands in November 2007 (autumn), February 2008 (winter), May 2008 (spring) and August 2008 (summer). Soil samplings were carried systematically at 3–10 cm (organo-mineral horizon) for each season and both tree species in four replicates in independent plots (*c.* 10 m distance between each soil sample): i) cores of (120 × 80 cm) to perform soil solution analyses and ii) cores of (18.5 × 14 cm) to perform the bacterial collections. For the soil solution analyses, separation of soil samples into bulk (BS) and rhizosphere (R) fractions was conducted in the field. Living roots with diameters <2 mm were carefully recovered by hand in clean bags and lightly shaken to separate the rhizospheric soil strongly associated to the roots. The soil remaining after picking out the roots was considered as bulk soil. Both bulk soil and rhizosphere were sieved (2 mm mesh) and homogenized. The soil solutions from these two soil fractions were then extracted by centrifugation (15 °C, 20 min, 3000 rpm; JOUAN KR422) and filtered at 0.45 μm. The pH of the soil solutions was determined (pHmeter SENTRON, Argus X). Total carbon and nitrogen in soil fractions were estimated using a TOC analyzer (TOC-5050, Shimadzu). Table 1 represents the chemical characteristics of soil solution collected under beech and Norway spruce stands in November 2007, February 2008, May 2008 and August 2008. In both stands, the quantity of carbon and nitrogen in rhizospheric soil solutions tended to be higher in February and May compared to November.

2.3. Bacterial strains and growth media

The separation of soil samples into bulk and rhizosphere fractions was conducted in the laboratory. The bacterial strains were isolated from two environments: (1) bulk soil (called "BS"), *i.e.* soil remaining after picking out the roots, 9 g of fresh soil was

Table 1
Soil solution characteristics at the different sampling seasons.

Tree species	Compartment	November			February			May			August		
		mg/kg of soil		pH	mg/kg of soil		pH	mg/kg of soil		pH	mg/kg of soil		pH
		C	N		C	N		C	N		C	N	
Beech	BS	2.41^{aX}	0.27^{aX}	4.05^{abX}	2.79^{aX}	0.39^{aX}	4.36^{bY}	2.13^{aX}	0.58^{aX}	3.68^{aX}	0.49^{1}	0.06^{1}	4.30^{1}
	R	2.19^{aX}	0.33^{aX}	4.00^{bX}	4.92^{aY}	0.60^{abY}	3.80^{aX}	4.65^{aX}	0.88^{bX}	4.12^{bY}	0.56^{2}	0.14^{2}	5.60^{2}
Spruce	BS	1.09^{aX}	0.30^{aX}	3.82^{aX}	1.90^{aX}	0.70^{bX}	4.08^{aY}	1.32^{aX}	0.36^{bX}	3.91^{aX}	0.49^{2}	0.44^{2}	4.21^{2}
	R	1.57^{aX}	0.21^{aX}	3.69^{aX}	9.17^{bX}	1.09^{bX}	3.69^{aX}	4.36^{abY}	0.44^{abX}	3.82^{aX}	3.82^{2}	1.23^{2}	5.67^{2}

Each value is a mean value of four replicates, except for August. In August, the values presented are the mean value of two replicates (1) or an unique value (2).
For legibility reason, the standard deviation was not presented.
For each soil compartment (in line) different letters (a,b,c) indicate that the amount of C, N or the pH are significantly different according to a one-factor (season) ANOVA ($p < 0.05$).
For each sampling season (in column) different letters (X,Y) indicate that the amount of C, N or the pH are significantly different according to a one-factor (compartment) ANOVA ($p < 0.05$).