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The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics

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ABSTRACT

Bacterial communities living in forest soils contribute to the decomposition of organic matter and the recycling of nutrients in these ecosystems and form one of the most diverse habitats on Earth. Unfortunately, due to difficulty in culturing soil bacteria, the understanding of their ecology is still limited. In the case of temperate deciduous forests, soil microbial communities face large seasonal variations in environmental conditions, such as temperature or moisture. Moreover, the supply of nutrients also differs due to seasonal processes, such as the allocation of photosynthates into soil by the roots of primary producers or the seasonal input of fresh litter. The aim of this study was to reveal how the bacterial community responds to these seasonal processes in the litter and soil of a *Quercus petraea* forest. Bacterial communities from litter and from the organic and mineral horizons of soil were analyzed during the four seasons of the year by 16S rRNA gene pyrosequencing. The results revealed that the composition of the bacterial community is horizon specific. The litter horizon had a higher relative abundance of *Proteobacteria* and *Bacteroidetes* than soil, while the organic and mineral horizons had a higher abundance of *Acidobacteria*, *Firmicutes* and *Actinobacteria* than litter. Moreover, the bacterial community was significantly affected by seasonality in all horizons. Bacterial communities in the litter showed significant differences between the vegetation season (May and July) and the autumn and winter seasons (October, February). In mineral soil, bacterial community composition was specific in the summer, when it was significantly different from all other seasons, with a larger number of taxa described as rhizosphere and mycorrhizosphere inhabitants. The results indicate that litter decomposition is the main driver of bacterial community composition in litter horizon. In contrast to reports on fungal communities, bacterial community composition in mineral soil responds to the seasonal peaks of rhizodeposition in the summer.

1. Introduction

Temperate deciduous forests represent one of the most important carbon (C) sinks in Europe, Asia and Northern America and contribute to the health of the planet (Janssens et al., 2003). The flux of C into these ecosystems is mainly mediated by the photosynthetic fixation of CO_2 by broadleaved trees, its allocation belowground via tree roots in the form of root exudates and root litter and the accumulation of the aboveground litter on the forest floor due to yearly seasonal litterfall. This pool of C-rich organic matter (OM) is used as a carbon and energy source by a diverse community of microorganisms, responsible for its transformation and mineralization throughout the year (Uroz et al., 2011; Rasche et al., 2011; Voříšková et al., 2014). Microbial communities thus play a critical role in soil biogeochemical processes, as the main drivers of C efflux from the ecosystem and its transformation, resulting in the formation of a forest soil profile, one of the most diverse habitats on Earth (Roesch et al., 2007). The yearly input of litter and the ongoing decomposition process in forests result in three distinct topsoil compartments: the litter, the organic (or humic) horizon and the mineral soil horizon. This vertical stratification is characterized by a decrease in the content and quality of OM with soil depth, which is accompanied by changes in the activity of extracellular enzymes and in the size and composition of the microbial community (Sinsabaugh et al., 2002; Šnajdr et al., 2008a).

In addition to the organic matter in the soil, microbial activity is also influenced by environmental factors, with the variations in temperature and moisture across different seasons being the most

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notable (Brockett et al., 2012). In a temperate forest, temperature variation due to seasonality directly influences C fluxes in the ecosystem (Baldrian et al., 2013). Most importantly, the photosynthetic production of plants is limited to the vegetation period. As a consequence, the increasing rate of photosynthesis during the spring and summer is associated with the deposition of easily decomposable compounds, such as amino acids, sugars and organic acids, into plant roots and the deeper soil horizons either directly or in a process mediated by root-associated mycorrhizal fungi (Ekblad and Högberg, 2001). The annual period of litterfall restricted to autumn and early winter leads to the seasonal accumulation of large amounts of nutrients on the soil surface. The continuous decomposition of litter results in gradual changes in its quality until the next litterfall (Šnajdr et al., 2011; Baldrian et al., 2013). Microbial communities are subject to these profound changes in nutrient content and composition during the year, which results in seasonal changes in their abundance and activity (Kaiser et al., 2010; Voříšková et al., 2014). Importantly, the understanding of the seasonal dynamics of microbial communities is necessary for the prediction of the future response of temperate deciduous forests to global climate change and its effects on the C balance in the ecosystem (Schindlbacher et al., 2012; Rousk et al., 2012).

Several previous studies reported that seasonality may affect the structure of microbial communities and the functional properties of soil microorganisms in the temperate forest, suggesting that microbial dynamics is mainly influenced by changing temperature, moisture and nutrient availability (Collignon et al., 2011; Chemidlin Prevost-Boure et al., 2011; Rasche et al., 2011; Koranda et al., 2013). However, the resolution of most of this work was limited by the use of methods based on DNA fingerprinting or qPCR, which are insufficient for the characterization of the community composition (Osborn et al., 2000; Zhang et al., 2008). High-throughput DNA pyrosequencing provides more detailed information on microbial communities inhabiting soils (Roesch et al., 2007) and has been successfully used to explore the ecology and diversity of soil microorganisms in a wide variety of types of ecosystems (Lauber et al., 2009; Eilers et al., 2012; Uroz et al., 2013; Williams et al., 2013). Recently, Voříšková et al. (2014) applied pyrosequencing for the in-depth description of the seasonal development of the structure and functioning of fungal communities and demonstrated that the fungal abundance and community composition undergoes seasonal changes. The effects of seasonality on bacterial communities using pyrosequencing was highlighted by Kuffner et al. (2012), who found no substantial differences in a mountainous forest between these communities across the seasons. Unfortunately, that study only compared summer and was performed in a forest with prevailing evergreen coniferous trees where the litter input is not seasonally restricted. Moreover, the limitation of the study to soil makes any inferences on carbon balance difficult because the bulk of the organic matter loss and microbial activity occurs in litter (Šnajdr et al., 2011, 2008a). The analysis of a deciduous forest ecosystem considering both litter and soil are unavailable.

The aim of this work was to fill this knowledge gap by describing the bacterial community living in the litter and upper soil and determining how it changes throughout the year. We hypothesized that phenomena such as litter input, temperature and moisture variation and the seasonal pattern of C rhizodeposition would affect the bacterial community composition across the year. The data describing the bacterial community composition were obtained from the same study that analyzed the seasonality of fungi (Voříšková et al., 2014). That study showed that the fungal community is stratified in the soil profile and its composition in litter undergoes profound seasonal changes as a consequence of changing litter chemistry. In contrast, despite the fact that root-associated fungi dominated deeper soil horizons where they could have theoretically responded to changes in root exudation, the seasonal changes in fungal community composition were minor. Based on that observation, we suggest that the seasonality of bacterial communities is horizon specific and most pronounced in the litter because there are many bacterial taxa that are involved in decomposition in forest ecosystems (Šťursová et al., 2012). The parallel exploration of the fungal and bacterial community dynamics should also complete the picture of microbial response to ecosystem seasonality and reveal possible differences among these ecophysiologically distinct groups of microorganisms (Boer et al., 2005).

2. Materials and methods

2.1. Site description and sample collection

The experimental study site was situated in a sessile oak (*Quercus petraea*) forest in the Xaverovský Háj Natural Reserve in the Czech Republic (50°5′38″N, 14°36′48″E). Soil and litter chemistry and decomposition processes have been studied in the area previously (Šnajdr et al., 2008a, 2011; Baldrian et al., 2013), as well as the structure and functioning of fungal communities associated with litter and soil (Voříšková and Baldrian, 2013; Voříšková et al., 2014). The soil was an acidic cambisol with developed litter (L), organic (humic, H), and mineral (Ah and A) horizons. Vegetation season defined as the presence of living leaves on trees starts in mid-April and lasts until the end of September. This study used the material collected for the study of Voříšková et al. (2014). Sampling of the topsoil was performed in the spring (9 May, approximately two weeks after the emergence of leaves), summer (29 July), autumn (28 October, during the late phase of litterfall) and winter (19 February). Soil samples were collected in four defined plots (10 m^2, approximately 100 m from each other) at the sampling site. Six soil cores were collected at each sampling plot using sampling tubes with a 4.5-cm diameter and were divided into litter (L horizon, ~0.5–1 cm), H horizon (~1–3 cm) and Ah horizon (upper portion, up to a depth of 5 cm). Samples of the L horizon were cut into approx. 0.25 cm^2 pieces, while the soil samples were sieved using a 2-mm sieve. The resulting material was combined to yield a composite sample from each horizon and plot. Subsamples for chemical analyses, quantification of microbial biomass and DNA extraction were frozen and stored at −45 °C, and subsamples for enzyme analysis were stored at 4 °C. In each sample, soil moisture content, pH, C and N content and the activity of selected extracellular enzymes was determined following the methods of Voříšková et al. (2014).

2.2. DNA extraction and 454-pyrosequencing of 16S rRNA

Total DNA was isolated from 0.3 g of soil or litter material using the modified Miller-SK method (Sagova-Mareckova et al., 2008). Bacterial 16S rRNA genes were PCR-amplified with the primer pair eub530f and eub1110br modified from Dowd et al. (2008) and Baldrian et al. (2012). Amplification was performed in two steps as described previously (Baldrian et al., 2012). In the first step, each of three independent 50 μl reactions per DNA sample contained 1 U of OmniTaq PCR buffer, 0.4 μM of each primer, 0.2 μM dNTPs mix, and 1.5 U of polymerase (Pfu DNA polymerase: OmniTaq DNA polymerase, 1:24). The amplification was carried out using the following program: initial denaturation at 94 °C for 5 min, 35 cycles of at 94 °C for 1 min, 62 °C for 1 min and 70 °C for 1 min, with a final extension step at 72 °C for 10 min. PCR products from triplicates were pooled and purified using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany). Products were used as the template for