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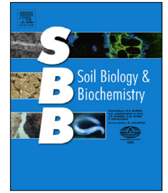

The spatial distribution of exoenzyme activities across the soil micro-landscape, as measured in micro- and macro-aggregates, and ecosystem processes☆

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The spatial ecology of soil microbial communities and their functioning is an understudied aspect of soil microbial ecology. Much of our understanding of the spatial organisation of microbial communities has been obtained at scales that are inappropriate for identifying how microbial functioning and spatial patterns are related. In order to reveal the spatial strategies of soil microorganisms, we measured the microscale spatial distribution of 6 exoenzyme activities (EEA) and related them to the catalytic potential of three soils. The relationship between EEA profiles and microbial community structure was also measured in soil aggregates. All the EEA exhibited scale-invariant spatial clustering. The extent of spatial clustering varied significantly among EEA, suggesting that microbial communities employ different spatial strategies when foraging for different elements. The dispersed distribution of alkaline phosphatase suggests that microorganisms invest more heavily in the acquisition of P. The EEA associated with the C and N cycles, but not the P cycle, were significantly affected by management practices in the loamy soil. A significant negative relationship between the extent of spatial clustering of EEA and the overall intensity of the EEA was identified in the two loamy soils, indicating that the microscale spatial ecology of microbial activity may have a significant impact on biogeochemical cycles. No relationship was found between microbial community structure and EEA profiles in aggregates. However, a number of negative relationships between the relative abundance of certain taxa and the most dispersed EEA (alkaline phosphatase and β-glucosidase) were found, suggesting that these taxa make the EEA products available by means other than the production of exoenzymes (e.g. solubilisation of phosphate through the production of organic acids).

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1. Introduction

It is well established that exoenzyme activities (EEA) catalyse rate-limiting steps in organic matter decomposition and therefore have significant influence on global energy and nutrient cycles (Sinsabaugh et al., 2008, 2009). EEA in soil mediate microbial nutrient and energy acquisition from soil organic matter (Burns et al., 2013). They are excreted or released through cell lysis into the soil environment where their activities catalyse the fundamental ecosystem processes of humification and organic matter decomposition (e.g. lignin or xenobiotic degradation, proteolysis etc.). Soil is a spatially structured environment in which the distributions of organic matter and microorganisms are very heterogeneous over spatial scales of several orders of magnitude (Robertson et al., 1997; Stoyan et al., 2000; Franklin and Mills, 2003; Ritz et al., 2004; Lehmann et al., 2008). Furthermore, the spatial

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separation between the sites of enzyme production and the location of the target substrate may have a significant effect on the effective rate at which the specific reactions proceed. The heterogeneous nature of the microbial environment raises questions about the spatial strategies that are adopted in the production of enzymes in soil.

The study of spatial pattern is an integral part of ecology, be it to understand the mechanisms underlying the diversity of life (Hughes Martiny et al., 2006) or in an effort to establish links between ecological patterns and ecosystem function (Gustafson, 1998). Community processes, such as competition, which affect microbial functioning, occur at fine scales (Ettema and Wardle, 2002). However, most studies of spatial pattern in soil microbial ecology have been carried out at scales that are inappropriately large for gaining insight into the processes that regulate microbial diversity or function (Vos et al., 2013). Soil is made up of a huge diversity of microenvironments in which the microbial component of soil exists and is active (Young and Ritz, 1998). The relationships among microorganisms and between microbial communities and the resources available to them are altered or lost completely during sampling and subsequent processing (sieving and mixing) because the spatial organisation of the habitats is altered. This may partly explain why relationships between microbial communities and the functions they carry out have remained so elusive.

Geostatistical analyses have indicated that bacterial abundance and activity are spatially autocorrelated to distances of up to 2 mm (Grundmann and Debouzie, 2000; Nunan et al., 2003). These patterns suggest that individual bacteria or activities separated by distances of up to 2 mm are subject to similar environmental constraints or are connected in some way. The analysis of EEA associated with the C, N and P cycles and the analysis of bacterial community structure in aggregates of 2 mm or less may reveal the relationships between bacterial communities and their extracellular activity as well as the spatial strategies adopted for acquiring extracellular nutrients.

The general objective of this study was to describe the spatial distribution of EEA across scales that are likely to integrate the processes that may affect the spatial strategies employed by microbial communities in the production of EEA (spatial distribution of microorganisms and of substrate, diffusion of substrate and enzymes in the pore network, variations in local conditions) and to determine whether any relationship between EEA production and microbial community structure could be established at the aggregate scale. The specifics aims were threefold: 1. to determine whether the spatial distributions of EEA varied as a function of enzyme activity in a clay soil under conventional management and in a loamy soil under either conventional or low input management systems, 2. to determine whether the spatial distribution of EEA was related to the bulk enzyme activity of soil and 3. to determine whether the activity of exoenzymes was related to microbial community structure or composition measured using taxonomic microarrays.

2. Materials and methods

2.1. Site descriptions and sampling

The soils were collected from the surface 5 cm of two plots of the "La Cage" long-term field trial (established in 1998), located at the INRA (Institut National de Recherche Agronomique) research center in Versailles (+48°48′ 28.78″, +2°4′ 55.00″), and from an agricultural field in the Yvelines (+48°54′ 8.70″, +1°36′21.19″), France. The soils are classified as a silty loam (Luvisol, WRB) and a loamy clay (Eutric Cambisol, WRB), respectively. The main soil characteristics are presented in Table 1. The plots sampled at the "La Cage" field trial were under conventional and low input management whilst the loamy clay was under conventional management. Three samples from three locations were collected from each site in January 2012 and stored moist for two months, at 20 °C, until analysis. The microbial communities in the soils were characterised by terminal restriction fragment length polymorphism (t-RFLP) and phospholipid fatty acid profiling (PLFA) (Nunan et al., 2005).

2.2. Exoenzyme assays for spatial analysis

Soil core samples were mixed by hand and all litter and roots were removed. The samples were air-dried to allow sampling of aggregates of different sizes. Each air-dried soil was sieved to 0.25, 0.5, 1, 2 and 3 mm size of aggregates, which size was determined using a 0.5 mm grid paper and a microscope. Forty-eight aggregates for each size and each soil were transferred to 96-well micro-plates using a sterilized tweezers for exoenzyme assays. This approach is similar to that used for the measurement of the microscale spatial distribution of biodegradation activities (Pallud et al., 2004).

Enzyme activities involved in the C cycle (β-glucosidase, α-cellobiohydrolase and xylosidase), the N cycle (chitinase and leucine amino peptidase) and the phosphorus cycle (alkaline phosphatase) were assayed using the fluorogenic MUF (4-methylumberlliferone) and AMC (4-methylcoumarin) substrate analogues. One hundred μL of 500 μM substrate solution in ultrapure water (resulting in 50 nmoles substrate per well) were added to microplate wells containing a single aggregate and incubated for 9 h at 20 °C. The fluorescence was measured immediately and after 9 h incubation with an automated fluorimetric plate reader (SAFAS FLX-Xenius). The excitation wavelength was 360 nm for both fluorescent substrates used and emissions were measured at 460 nm in the case of the MUF substrate treatments and 450 nm in the case of the AMC substrate treatments. The different enzymatic activities were calculated as the amount of MUF or AMC released during the 9 h incubations by reference to appropriate standard curves. Different standard curves were prepared for each aggregate size in order to account for the different levels of fluorescence quenching that can occur due to the presence of soil phenolics. Two sets of blank controls were prepared to account for any autofluorescence of the aggregates and to account for the un-catalysed breakdown of the analogues. The first set of blanks contained an aggregate in ultrapure water and the second set of blanks contained the substrate analogues alone. The activity was expressed as μM MUF or AMC aggregate^{-1} hr^{-1} (Marx et al., 2001).

2.3. Bulk exoenzyme assays

Bulk exoenzyme assays were performed on three replicate samples of each of the soils following the method of Marx et al. (2001). Soil suspensions were prepared by mixing soil (1 g) with 5 ml ultrapure water and mixing vigorously for 1 min on a shaker. Fifty μL aliquots of the soil suspension were transferred to 96-well microplates using a multichannel pipette. There were 8 analytical replicates per sample. Ultrapure water (50 μl) and 100 μl of substrates were then added to each well. The 96-well microplate was incubated in the dark at 20 °C for 18 h. The standard curves and the fluorescence or absorbance readings were carried as indicated above.

2.4. Aggregate-scale enzyme profiles

Aggregate-scale enzymes profiles were established by measuring the activity of four of the most commonly measured EEAs (α-D-glucosidase, xylosidase, chitinase and alkaline phosphatase) in 2 mm aggregates of soil. These four enzymes were

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