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Microbial biogeography at the soil pore scale

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ABSTRACT

Microbial communities exist and are active in a complex 3-D physical framework which can cause a variety of micro-environments to develop that are more or less suitable for microbial growth, activity and survival. If there is a significant microbial biogeography at the pore scale in soil, then the relationship between microbial diversity and ecosystem function is likely to be affected by micro-environmental variations at the pore scale. In this laboratory study we show that there is a significant pore-scale microbial biogeography by labelling microbial communities in different pore size classes of undisturbed soil cores with ^{13}C-labelled fructose (a soluble, labile substrate). This was achieved by adding the substrate solution to the samples at different matric potentials (−100 kPa, −3.15 kPa and −1 kPa; placing the substrate in pores with maximum diameter of 0.97, 9.7 and 97 μm, respectively) and incubating the samples for two weeks. The mineralisation of soil organic carbon and fructose was measured as CO_2 and $^{13}C-CO_2$, respectively, in the jar headspace throughout the incubation. At the end of incubation we analysed the total microbial community structure using PLFA. The structure of microbial communities in different pore size classes was measured by PLFA stable isotope probing. Total PLFA profiles suggested that there was little effect of the incubation conditions on microbial community structure. However, labelled PLFA profiles showed that microbial community structure differed significantly among pore size classes, the differences being due primarily to variations in the abundance of mono-unsaturated lipids (Gram-biomarkers) and of the fungal biomarker (C18:2(9,12)). This is the first evidence for a significant microbial biogeography at the pore scale in undisturbed soil cores.

1. Introduction

Soil is a highly complex material which harbours among the most important reservoirs of biodiversity on the planet (Curtis et al., 2002; Roesch et al., 2007). Despite the fact that the wealth of functions carried out by soil is a result of the biological diversity, no clear relationship between diversity and function has been observed. Theoretical advances in ecosystem functioning have emphasized the importance of the environmental context in shaping the relationship between biodiversity and function (Cardinale et al., 2000; Mouquet et al., 2002). The mechanism through which environmental factors are hypothesised to change the relationship between diversity and ecosystem function is by altering community structure. There are no *a priori* reasons to believe that the relationship between microbial community function and microbial diversity should be any different, in particular as microbial community structure is known to be significantly influenced by environmental factors: studies of microbial biogeography suggest that microbial community composition is primarily influenced by contemporary environmental properties (Martiny et al., 2006; Remenant et al., 2009).

Soil microbial communities exist and are active in a complex 3-D physical framework which can cause a variety of niches, or micro-habitats, to develop that are more or less suitable for microbial growth, activity and survival (Sexstone et al., 1985; Chenu et al., 2001; Nunan et al., 2006; Young et al., 2008). The structure of solid and pore space results in a complex distribution of oxygen, water films and gradients of solutes spanning distances as small as a few micrometers and therefore in radically different local conditions at very fine scales. If the local environment affects the makeup of microbial communities, then it can be expected that microbial function will also be affected by the local environmental properties. This means that if microbial community structure is non-random at the micro-habitat scale, then one can expect interactions at this scale to affect the relationship between microbial diversity and function. However, little is known about how microbial community structure varies at these scales as most studies have searched for patterns in microbial community structure at coarser scales (e.g. landscape (Lauber et al., 2008); region (Wu et al., 2009); continent (Fierer et al., 2009)). A few notable exceptions have shown that microbial community structure is related to their location within or

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at the surface of soil aggregates (Ranjard and Richaume, 2001; Mummey and Stahl, 2004), but these studies altered the structure of the soil and therefore the micro-environment of the microbial communities, possibly biasing the results.

The aim of the study therefore, was to determine whether microbial community structure was related to pore size class. Pore size class was taken as a proxy for habitat. The view was taken that pore size integrated many habitat properties such as oxygen levels, water content, substrate accessibility and protection from predation (Postma et al., 1989; Kuikman et al., 1990; Young et al., 1998). A number of these properties are also likely to affect the chemistry of the habitat. Although variation in properties within each size class can be expected, it was felt that this was the most appropriate way to tackle the problem, as identifying individual habitat properties in a non-destructive manner was not possible. Furthermore, it is known that the rate of C turnover is dependent upon location within the soil pore network, C turnover being faster in pores with neck diameters >6 μm than in pores with neck diameters <6 μm (Killham et al., 1993; Strong et al., 2004) providing further justification for this approach.

The moisture characteristic was used to introduce substrate into targeted pore size classes as was done by Killham et al. (1993) in order to measure the C turnover in different locations in soil. A ^{13}C-labelled substrate was introduced into pores of three different size classes and, after a-two week incubation, the community structure in each pore class was estimated by measuring ^{13}C-labelled phopholipid fatty acid (PLFA) profiles to targeted pores would be consumed primarily by local microbial communities and the labelled C integrated into local microbial PLFA. Total PLFA profiles were measured to ensure that microbial community structure was not affected by the experimental conditions. The mineralisation of both the soil organic matter and the added substrate in each pore size class was also measured to determine the dependence of C decomposition on location within the pore network.

2. Materials and methods

2.1. Soil, sampling and preparatory experiments

Undisturbed soil cores were collected with a soil corer (internal diameter of 5 cm) from the surface 25 cm of control plots in the "Closeaux" field experiment at the INRA research centre in Versailles, France, in May 2008. The field experiment is a wheat–maize (C3–C4) chronosequence, established in 1992, in which 3 randomly selected plots that had previously been cropped to C3 plants for at least 50 years were sown to maize each year. The control plots have remained under wheat throughout the experiment and therefore the isotopic signature of the organic matter in these plots was that of C3 plants ($\delta^{13}C = -26.1‰$). The soil is classified as a Eutric Cambisol (17.4% clay, 53% silt and 29.6% sand) with a pH of 6.8, a CEC of 12 cmolc kg^{-1} and a bulk density of 1.5 Mg m^{-3}. Organic C and total N contents were 13.5 and 1.23 mg g^{-1} soil, respectively. Forty undisturbed samples, 3 cm in height, were subsequently excised from the cores at a depth of 3–6 cm.

2.2. Soil water retention and pore size distribution

Ten of the samples were used to establish the moisture characteristic of the soil using pressure plates in order to estimate the pore neck diameter of the water-filled pores in the different treatments (see below) and to estimate the relative abundance of the different pore size classes. The drying curve was determined by measuring the water content at different water potentials after dehydration of saturated samples. Samples then were equilibrated at −31.5 kPa, −316 kPa or −1600 kPa and wetted to reach water potentials of −1 kPa, −3.15 kPa or −100 kPa, respectively and the water content was determined (Fig. 1). As hysteresis was observed, the water content after rewetting was used to estimate the volumetric relative abundance of the different pore size classes using Young–Laplace's law.

2.3. Addition of ^{13}C-labelled fructose and incubation

The remaining 30 cores were used to introduce ^{13}C-labelled substrate into different pore size classes. ^{13}C-labelled fructose was chosen because it is soluble and labile and both these properties were necessary for the experiment to be successful. Samples were first equilibrated at given water potentials (−31.5 kPa, −316 kPa or −1600 kPa) meaning that there were 10 samples with maximum water-filled pore neck diameters of 10, 0.9 and 0.2 μm, respectively (Fig. 1). Subsequently, either a solution of ^{13}C-labelled fructose ($\delta^{13}C = 3449‰$ and at a concentration equivalent to 13 μg fructose C mg^{-1} soil C) or water was added to each of the samples bringing samples at −31.5 kPa to a water potential of −1 kPa, those at −316 kPa to −3.15 kPa and those at −1600 kPa to −100 kPa. In order to ensure an even distribution of the fructose, the solution was added via a glass fibre filter (Whatman GF/D) placed on the surface of the sample. Care was taken to account for the solution remaining in the filter after the addition. Preliminary experiments carried out under the same conditions showed that the aqueous addition equilibrated rapidly in each treatment (within 6 h of the addition) and that there were no discernable vertical or horizontal fructose gradients in the samples (determined 6 h after addition by measuring the distribution of ^{13}C; data not shown).

The labelled substrate was therefore introduced predominately into pores with neck diameters between 10 and 290 μm, between 0.9 and 97 μm and between 0.2 and 3 μm, respectively (Table 1). Those pore size classes represent respectively 18.42%, 45.36% and 32.51% of the total porosity. This means that the proportion of porosity filled by water and fructose solution was 51.2, 82.3 and 96.1%, respectively. Although it was presumed that the majority of the labelled fructose would be consumed in the pores to which it was introduced, fructose diffusion and therefore consumption in other water-filled pores was probable. The carbon fructose concentration "experienced" by the micro-organisms varied between 5.4 and 21.9 mg mL^{-1} assuming there was no diffusion and between 1.4 and 2.3 mg mL^{-1} if the fructose diffused into all the soil solution immediately. These values were calculated using the fructose concentration of the solution added to the samples and the volume of soil solution in each sample.

Immediately after the addition of labelled substrate solution or water, the samples were placed on sample holders in 1 L air-tight

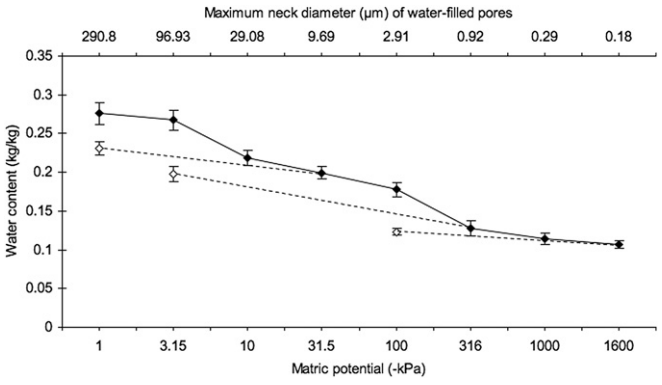

Fig. 1. Drying (◆) – wetting (◇) curve. The dashed lines link the matric potential of the soils prior to water or fructose addition to the incubation matric potential.