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Dynamics of bacterial communities in relation to soil aggregate formation during the decomposition of ^{13}C-labelled rice straw

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ABSTRACT

The addition of fresh organic matter is known to modify both microbial community structure and soil aggregation. The objective of this study was to understand the relationship between the dynamics of the soil microbial community structure in relation to that of their habitats during the decomposition of straw. Soil samples, ground (<200 μm) to remove macroaggregates, were amended with uniformly ^{13}C-labelled powdered rice straw (<500 μm) and incubated for 21 days. Unamended control samples were also incubated under the same conditions. Total C and rice straw C (C_{Straw}) mineralised or remaining in different soil fractions (0–50, 50–200, 200–2000 and >2000 μm) were measured. Fatty acid methyl ester (FAME) profiling was used to determine total bacterial community structure and FAME based stable isotope probing (FAME-SIP) was used to characterise the straw degrader communities. The mineralisation rate of the native C and the C_{Straw} was high. The formation of macroaggregates (>2000 μm) occurred within 2 days in amended and unamended samples but did so to a greater extent in the amended samples. The C_{Straw} was mainly located in fractions >200 μm, where degraders were the most abundant. The ^{13}C-FAME profiles followed the same trends as total FAME profiles through time and within soil fractions, suggesting common dynamics between straw degraders and total bacterial communities: Gram-negative were more important in fraction >200 μm and during the early stages of the incubation while Gram-positive and actinobacteria dominated in fine fractions and at the end of the incubation. Bacterial community structure changed rapidly (within 2 days) in conjunction with the formation of new microbial habitats, suggesting that the relationship between the two is very close.

1. Introduction

The soil environment is made up of a huge diversity of microenvironments in which the biological component of soil exists and is active (Young and Ritz, 1998). The physical architecture 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. These microenvironments form the microbial habitats in soil. It is known that the different microenvironments select for different microbial communities. Community structure is dependent on the size of soil aggregates and on the location within soil aggregates (Ranjard et al., 2000; Poly et al., 2001; Mummey and Stahl, 2004), as are a number of physiological characteristics of the communities (Hattori, 1988). Aggregate size classes differ in their potential denitrification (Lensi et al., 1995; Sey et al., 2008), mineralisation of organic matter (Franzluebbers and Arshad, 1997; Sey et al., 2008) and enzymatic activities (Drażkiewicz, 1995). Microbial communities and activities are also known to vary with pore size class (Strong et al., 2004; Ruamps et al., 2011). However, the microbial habitat in soil exists in a highly dynamic state as microbial activity (Dorioz et al., 1993; Feeney et al., 2006), plant root growth or alterations in the water status can all affect the physicochemical properties of the microenvironments. Changes in the microbial habitat can also occur due to soil management practices such as tillage (Six et al., 2004; Young et al., 2006). Soils consist of mineral and organic materials bound together to form soil aggregates. Soil aggregates are defined by their sizes and their stability in water (Tisdall and Oades, 1982). Microaggregates (<200 μm) and macroaggregates (>200 μm) regulate key factors such gas and liquid diffusion (Sexstone et al., 1985; Horn and Smucker, 2005) and soil organic matter (SOM) turnover (Puget et al., 2000; Six et al., 2004). These changes are all likely to affect the abundance, the structure, and therefore the functioning, of the resident microbial communities (Young and Crawford, 2004).

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The process of aggregation occurs firstly by the formation of macroaggregates (>200 μm), which are formed by mineral associations with particulate organic matter (POM) via temporary binding agents (i.e. fungal hyphae and plant roots). Microaggregates (<200 μm) are formed within macroaggregates around POM (Oades, 1984; Six et al., 2000). The decomposition of POM within macroaggregates by microorganisms produces exopolysaccharides and other metabolites which act as persistent binding agents for the formation of microaggregates (as reviewed by Degens, 1997). Thus, the dynamics of soil aggregates create different ecological niches. There is a particularly strong niche differentiation during the formation of macroaggregates around POM, as there are large amounts of readily available substrate. Regions of the soil surrounding POM (extending no further than a few millimeters) have been termed the 'detritusphere' and are described as microbial activity hotspots (Gaillard et al., 1999; Poll et al., 2006). Macroaggregates contain more C, and more labile young C, than microaggregates (Puget et al., 2000) and the C turnover is also higher in macroaggregates (Buyanovsky et al., 1994; Monreal et al., 1997). In contrast, microaggregates are more stable and persistent than macroaggregates (Jastrow, 1996; Bossuyt et al., 2002) and are characterised by low predation rates, low nutrient availability and low gas diffusion rates as reviewed by Ranjard and Richaume (2001).

The transient nature of soil aggregates makes it legitimate to ask how the dynamics of microbial communities are related to the evolution of their habitat but also how this affects the active communities involved in the degradation of POM. As microorganisms are active agents of aggregate formation and stabilization (Tisdall and Oades, 1982), they impact on their own habitat. The *de novo* formation of macroaggregates provides the best conditions for studying this, as habitat differentiation is likely to be the most significant. Several studies (e.g. Denef et al., 2001, 2002) have investigated the influence of nutrients addition on macroaggregates formation/stabilization on soil in which macroaggregates were crushed (<250 μm) prior to incubation. However, none of these studies assessed the response of the microbial communities to macroaggregate formation after OM addition. Accordingly, the objectives of this study, were: (i) to determine whether microbial community structure was related to habitat differentiation during soil aggregate formation (ii) to assess the influence of fresh organic matter addition on microbial dynamics during aggregation and (iii) to identify the location (macroaggregates vs microaggregates) and the structure of the bacterial communities that assimilate C from fresh organic matter into their biomass. To this end, soil without macroaggregates was amended with uniformly ^{13}C-labelled rice straw and incubated for 21 days. The ^{13}C-labelling was used in order to differentiate native from added residue C and to target bacterial communities using straw C among the total soil bacterial communities using FAME-SIP.

2. Materials and methods

2.1. Soil samples and plant residues

The experimental site was located in the Anjozorobe district (18°46′S, 47°32′E), near Antananarivo, Madagascar. The soil studied was a Ferrasol under a vegetated fallow dominated by *Aristida* sp. The soil texture was constituted by 12% of fine sand (50–200 μm), 28% of coarse sand (>200 μm), 30% of silt and 30% of clay. The soil was constituted of different soil fractions: 0.2% >2000 μm, 84.1% 200–2000 μm, 13.5% 50–200 μm and 2.3% 0–50 μm. The total organic carbon (TOC) was $23.0 \pm 0.6\ g\ kg^{-1}$, the total nitrogen was $1.7 \pm 0.1\ g\ kg^{-1}$ and the pH (H_2O) was 5.8. The bulk isotopic signature of the organic carbon was -12.3 ± 1.76‰. In March 2007, samples were collected to a depth of 10 cm from several locations at the site and bulked to form a composite sample of about 3 kg. The soil was air-dried after sampling. Particulate organic matter was removed from the soil with tweezers to reduce the amount of native POM available to microorganisms. About 48% of total POM was removed ($0.0047\ g\ POM\ g^{-1}$ soil remained). In order to destroy the macroaggregates (>200 μm), the soil was crushed and sieved (<200 μm). The sand that had been removed by the sieving (>200 μm) was added to the sieved soil to ensure that the soil texture was not altered.

Uniformly ^{13}C-labelled rice straw was obtained from the Groupement de Recherches Appliquées en Phytotechnologies (CEA/DSV/DEVM, Cadarache, France). The rice was grown in culture chambers in which the atmospheric total CO_2 concentration was kept constant at 360 ppm. The CO_2 partial pressure in the chamber was continuously monitored by Near Infrared Spectroscopy. Regulation was achieved by automatic injection of ^{13}C-enriched CO_2 (10.5 ± 0.4%), which started 20 days after sowing. Rice plants were harvested 129 days after sowing. The straw used was a mix of stems and leaves from rice plants, with C and N contents of $417.5 \pm 1.7\ g\ kg^{-1}$ and $19.7 \pm 0.3\ g\ kg^{-1}$, respectively. The bulk isotopic signature of the rice straw C was 6124 ± 158‰. Before incorporation into the soil samples, the straw residues were oven-dried at 60 °C and finely ground to <500 μm.

2.2. Experimental design

Amended samples (20 g dry weight equivalent) were mixed with 0.08 g of rice straw residues, corresponding to a C addition of 7.3% of the total soil organic C. Control samples did not receive any amendment. Prior to commencing the incubation, the water potential of all samples was adjusted to −0.01 MPa by addition of Milli-Q water. The samples were placed in 1.2 l jars along with a vial of Milli-Q water (20 ml), to avoid drying of soil, and a vial of NaOH (20 ml, 0.5 M) for trapping the CO_2 released during soil respiration (see Section 2.3). Microcosms were incubated in the dark for 21 days at 30 °C. It was decided to incubate for 21 days based on the previous studies showing that microbial communities were mainly affected by the addition of plant residues during the first 15 days (McMahon et al., 2005) or 21 days (Schutter and Dick, 2001) of incubation which lasted 80 days. Six replicates of control and amended samples were destructively sampled before the incubation started (day 0), after 2 (day 2) and 21 days (day 21) of incubation. Three replicates of control and amended samples were immediately fractionated and three other replicates (unfractionated soil samples) were stored at −20 °C for further analyses.

2.3. Soil respiration

The CO_2 produced by soil respiration was determined by back titration at equivalent point pH 8.6 using HCl (0.2 M), of the NaOH placed in the microcosms with an excess of $BaCl_2$ (1.5 M). After each titration, the carbonate precipitate ($BaCO_3$) was filtered (fiber glass filter, Wathman GF/A ø 5.5 cm, retention 0.26 mm), washed, and dried at 40 °C. The ^{13}C content of the $BaCO_3$ was determined using an EA-IRMS (NA-1500, Carlo-Erba). The amount of ^{13}C mineralised was obtained as explained below.

2.4. Soil fractionation and analyses

The soil fractionation method used was adapted from Yoder (1936). The samples were immersed in Milli-Q water (4 °C) above a sieve (mesh size: 2000 μm) for 5 min. Large macroaggregates (>2000 μm) were then separated by automatically moving the sieve up and down 3 cm at 30 cycles per minute for 10 min. Aggregates retained on the 2000 μm sieve were considered to be large macroaggregates. The water and soil that passed through the 2000 μm sieve were then sieved using a 200 μm followed by a