Soil Biology & Biochemistry 41 (2009) 262–275

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Impact of wheat straw decomposition on successional patterns of soil microbial community structure

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ARTICLE INFO

Article history:
Received 24 June 2008
Received in revised form
17 October 2008
Accepted 27 October 2008
Available online 24 November 2008

Keywords:
Plant residue
Community dynamics
Bacterial diversity
Fungal diversity
Detritusphere
Soil microcosms

ABSTRACT

The dynamics of indigenous bacterial and fungal soil communities were followed throughout the decomposition of wheat straw residue. More precisely, such dynamics were investigated in the different soil zones under the influence of decomposing wheat straw residue (i.e. residues, soil adjacent to residue = detritusphere, and bulk soil). The genetic structures of bacterial and fungal communities were compared throughout the decomposition process long by applying B- and F-ARISA (for bacterial and fungal-automated ribosomal intergenic spacer analysis) to DNA extracts from these different zones. Residue decomposition induced significant changes in bacterial and fungal community dynamics with a magnitude of changes between the different soil zones ordered as followed: residue > detritusphere > bulk soil, confirming the spatial structuration of the sphere of residue influence to the 4–6 mm soil zone in contact with residue. Furthermore, significant differences in the structure of bacterial and fungal communities were apparent between the early (14 and 28 days) and late (from 56 to 168 days) stages of decomposition. These could be related to ecological attributes such as the succession of r- (copiotrophs) and K- (oligotrophs) strategists. Microbial diversity at the early (28 days) and late (168 days) stages of degradation was further analysed by a molecular inventory of 16S and 18S rDNA in DNA extracts from the residue zone. This confirmed the succession of different populations during residue decomposition. Fluorescent *Pseudomonas* spp. and *Neurospora* sp. were dominant in the early stage with subsequent stimulation of Actinobacteria and Deltaproteobacteria taxa, as well as Basidiomycota fungal taxa and Madurella spp. According to the ecological attributes of these populations, microbial succession on fresh organic residue incorporated in soil would be dominated by copiotrophs and r-strategists in the early stages, with oligotrophs (K-strategists) increasing in relative abundance as substrate quantity and/or quality declines over time.

1. Introduction

The loss of soil organic carbon under arable farming has detrimental effects on the physical, chemical and biological properties of soils in agrosystems and is one of the most serious factors leading to their degradation (Gregorich et al., 1994; Rivero et al., 2004). As a result, soil organic matter (SOM) content and quality are considered as key factors when evaluating the sustainability of management practices (Gregorich et al., 1994, 1997, see Haynes, 2005, for review). Consequently, a major objective in any sustainable agricultural system is to maintain the stock of SOM by directly adding crop residues, various types of manure and composts, sewage sludge or municipal wastes. The influence of such organic inputs on soil physical, chemical and biological properties and of processes resulting in an increased stock of plant nutrients, enhancement of soil structure and therefore an improvement in soil ecosystem stability, have been well documented (Bossuyt et al., 2001; Crecchio et al., 2007; Dhillion, 1997).

Most of the biological changes induced by OM amendments in soil result from changes in the availability of carbon sources which lead to alterations in the dynamics and structure of the soil communities (Holland and Coleman, 1987). Most studies at the microbial community level have focused on functional capacity and the characterization of phenotypic traits (for review, see Bunemann

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doi:10.1016/j.soilbio.2008.10.024

et al., 2006). Microbial communities indigenous to soils in which the organic status has been improved by adding degradable organic matter generally exhibit an increase in catabolic diversity (Degens et al., 2000). Furthermore, most phenotypic characterizations of such communities support the hypothesis that organic fertilization significantly modifies the population structure (Bossio et al., 1998; Vepsalainen et al., 2004). The tendency in such studies has been to consider the overall microbial community as a taxonomic and functional "black box". However, these communities consist of a complex assemblage of species, with different metabolic characteristics, physiological requirements and ecological attributes, each species driving at least one of the multiple reactions of OM biotransformation. In this context, a major challenge in microbial ecology is to precisely identify those microbial populations and activities involved in organic carbon degradation and the reciprocal structuring of soil communities according to carbon source. A better knowledge of these different points may serve as a conceptual framework to help in the design of the link between the structure of the soil bacterial communities and their function in the agro-ecosystem (Drenovsky et al., 2008).

A critical advance in soil ecology has been achieved by utilizing culture-independent methods such as analyses of DNA and/or RNA extracted directly from the soil (Ranjard et al., 2000). As these techniques consider both the cultured (approximately 1% of total) and non-cultured members of soil communities, they provide a more comprehensive measure of their diversity and composition. Even if measures of the abundance of individual species are not necessarily indicative of microbial growth or activity, they should reveal any bacteria or fungi that are present in latent or dormant forms. Such approaches have already been used to elucidate the influence of soil organic status on composition and structure of the soil microbial community (Lejon et al., 2005, 2007; Crecchio et al., 2007).

However, to fully exploit the potentials of microbiological plant residue degradation, a better understanding of the structural and functional diversity of microbial populations and their succession during plant degradation in healthy soils is required. Understanding the roles of the community in decomposing vascular plant residue will require: (i) the knowledge of the taxonomic composition of bacteria and fungi, (ii) monitoring the patterns of occurrence of individual taxa and (iii) to link structure and function, either directly by measuring residue incorporation into community members, or indirectly through monitoring decomposition alongside community change. Previous studies have shown that fungi dominate the decomposition pathways in semi-natural unfertilized soils but not in fertilized sites (Bardgett et al., 1993), but it is not clear whether the diversity of bacterial or fungal species varies. These authors demonstrated that the fungal communities were affected by agricultural practices but the evolution of biodiversity during the organic matter decomposition remained unclear.

In a previous study (Nicolardot et al., 2007), the effect of location (soil surface vs incorporated in soil) and the nature of plant residues (young rye vs wheat straw) on the degradation process and on indigenous microbial communities was evaluated. Such a study was performed by incubating soil microcosms in which were considered the different soil zones influenced by decomposition i.e. decomposing plant residues, soil adjacent to residues (detritusphere) and distant soil unaffected by decomposition (bulk soil). From a spatial point of view, residue degradation increased soil heterogeneity by stimulating specific genetic structure of microbial communities with a gradient from residue to bulk soil. It was concluded that the detritusphere and residue corresponded to distinct trophic and functional niches for microorganisms.

In this present study, our objective was to characterize the diversity and dynamics of bacterial and fungal communities during the degradation of soil-incorporated wheat residues. First, microbial community dynamics were directly assessed by Bacterial and Fungal-Automated Ribosomal Intergenic Spacer Analysis (B-ARISA or F-ARISA) on DNA extracted from the different soil zones (residue, detritusphere, bulk soil). Then, a molecular inventory of bacterial and fungi diversity, based on 16S and 18S rRNA clone libraries, was carried out at those incubation times revealing the most important modifications in community structure, to precisely identify the succession of populations occurring during residue degradation.

2. Materials and methods

2.1. Soil, microcosm set up and sampling strategy

The soil, used for microcosm incubation, was an Orthic Luvisol collected at the Estrées-Mons INRA experimental station ($X = 649{,}756$, $Y = 241{,}211$) sampled from the 0–15 cm layer. Calibrated soil aggregates (2 mm dia.) were then obtained by sieving the soil sample, and were then stored at 4 °C until use. Soil microcosms were constructed as described in a previous study (Nicolardot et al., 2007). Briefly, plant residues from mature wheat (*Triticum aestivum*) straw were dried at 80 °C and cut into 1 cm long pieces. The plant material was repacked in soil cores (7 cm dia., 4.8 cm height) obtained by uniaxial confined compression in a cylindrical mould of a mass of calibrated wet aggregates (Fazzolari et al., 1998). First, half the mass of calibrated soil aggregates (equivalent to 134.2 g dry soil) was placed in the mould and compacted to obtain a final bulk density of 1.45 g cm^{-3}. Then the equivalent of 1.0 g dry wheat straw was placed in the mould, distributed at the surface of this half core. Finally, the remaining calibrated soil aggregates (equivalent to 134.2 g dry soil) were added to cover the plant residues. The soil aggregates and residues were then compacted together to obtain a final bulk density of 1.45 g cm^{-3}. Soil cores without residue incorporation were also considered (control microcosms). The soil moisture content was fixed and maintained at a matrix potential of −0.05 MPa by adding deionised water. The soil cores were incubated at 15 °C for 168 days. For each sampling date (0, 14, 28, 56, 90 and 168 days) three soil fractions were considered: plant residues, detritusphere (6 mm thick soil layer adjacent to plant residues) and bulk soil (beyond the 6 mm detritusphere). Remaining plant residues were carefully separated from soil with tweezers after opening core along the straw layer as was previously described (Nicolardot et al., 2007). Then half soil cores were sectioned to separate detritusphere and bulk soil by using a cutting device (Millon, 2004). Plant residues, detritusphere and bulk soil samples (3 replicates per date) used for DNA extraction were stored at −40 °C prior analysis.

2.2. Extraction and purification of total DNA from soil, detritusphere and plant residues

Microbial DNA was extracted from the three replicates of plant residue, detritusphere and bulk soil obtained at each sampling date, according to the method described by Ranjard et al. (2003). Briefly, 1 g of each soil and residue sample was mixed with 4 mL of a solution containing 100 mM Tris (pH 8.0), 100 mM EDTA (pH 8.0) 100 mM NaCl, and 2% (wt/vol) sodium dodecyl sulfate. Two grams of 106 μm-diameter glass beads and 8 glass beads of 2-mm-diameter were added to the mixture in a bead-beater-tube. The samples were then homogenized for 30 s at 1600 rpm in a mini bead-beater cell disruptor (Mikro-dismembrator S. B. Braun Biotech International). The samples were incubated for 20 min at 70 °C and then centrifuged at 14,000 × g for 1 min at 4 °C. The collected supernatants were incubated for 10 min on ice with 1/10 volume of 3 M potassium acetate (pH 5.5) and centrifuged at 14,000 × g for 5 min. After precipitation with one volume of ice-cold isopropanol, the