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SHORT COMMUNICATION

Legume and gramineous crop residues stimulate distinct soil bacterial populations during early decomposition stages

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Diouf, M., Baudoin, E., Dieng, L., Assigbetsé, K. and Brauman A. 2010. **Legume and gramineous crop residues stimulate distinct soil bacterial populations during early decomposition stages.** Can. J. Soil Sci. **90**: 289–293. This study characterized the genetic structure of the active soil bacterial populations involved in the decomposition of maize and soybean residues over 3 d. Significant compositional differences between the total bacterial community and its active component were observed that were residue specific, suggesting that residue management should be further evaluated as a driver of soil C cycle through selection of bacterial populations.

Key words: 16S rRNA DGGE, active bacterial community, organic matter, crop residue

Diouf, M., Baudoin, E., Dieng, L., Assigbetsé, K. et Brauman A. 2010. **Des résidus de culture de légumineuse et de graminée stimulent des populations bactériennes distinctes du sol au cours des stades précoces de décomposition.** Can. J. Soil Sci. **90**: 289–293. Cette étude a permis de caractériser la structure génétique des populations bactériennes actives du sol impliquées dans la décomposition de résidus de maïs et de soja sur trois jours. Des différences significatives dans la composition de la communauté bactérienne totale et de sa fraction active ont été observées et se sont avérées spécifiques du résidu appliqué, suggérant que la gestion des résidus devrait être évaluée en tant qu'effecteur du cycle du carbone dans le sol opérant par sélection de populations bactériennes.

Mots clés: 16S rRNA DGGE, communauté bactérienne active, matière organique, résidus de culture

The mineralization of organic matter is of great interest given its central role in soil energy fluxes, nutrient cycling and carbon sequestration (Trumbore 2006). Some studies have already noted changes in microbial community composition during plant material decomposition (Yang et al. 2003; Dilly et al. 2004). Yet, these studies did not specifically target the active microbial populations. Several molecular technologies enable a direct insight into the active components of the soil microbial community (Gray and Head 2001; McMahon et al. 2005). Among nucleic acid-based methodologies, the ribosomal RNA-based technique proved to be sensitive enough to investigate the impact of root physiology (Jossi et al. 2006) on soil microbial communities, but examples of its usefulness to characterize the plant-litter- degrading bacterial communities are still scarce (Aneja et al. 2004). In this context, our goal was to determine whether soil enrichment with crop residues of distinct biochemical qualities could trigger within days the activity of distinct subsets of the total bacterial community. Structural differences between the whole bacterial community and its active component were revealed by comparing the denaturing gradient gel electrophoresis (DGGE) profiles obtained from amplified 16S rDNA and reverse transcribed 16S rRNA fragments.

Arable soil (Lixisol, FAO classification) was collected in Senegal (Thyssé Kaymor). Its main characteristics were: sand 55.3%, silt 34.3%, clay 10.4%, pH (water) 5.8, CEC 5.4 meq g^{-1}, carbon 1.3%, nitrogen 0.1%. Soil was sieved (2 mm) and air-dried. Mature maize (*Zea mays* L.) and soybean (*Glycine maxima* L.) shoots were dried (65°C), ground and sieved (2 mm) but not autoclaved so as not to alter their biochemical attributes. It was assumed that the integrity of the bacterial RNA content of the residues was altered by their drying at 65°C (e.g., Ruwaida and Schlegel 1976). Crop residues displayed distinct biochemical attributes as evidenced by their C:N ratios (91 and 62, respectively) and near-infra red spectral signatures (data not shown).

Nine plastic flasks received 10 g dry soil and gravimetric soil water content was brought to 50%. Microcosms were incubated for 3 wk at 27°C and at 50% humidity in order to stabilize the microbial activity. Soil moisture was then increased to 70% in order to ease further residue moistening and diffusion of dissolved organic matter. Residues (200 mg, $n = 3$ each) were then incorporated into the soil and thoroughly mixed. Soil

contained in unamended microcosms was also mixed. Units were incubated for 3 d at 27°C in the dark and then frozen at −20°C.

Total soil DNA and RNA were simultaneously extracted from four-replicate 500-mg samples per microcosm (Griffiths et al. 2000). For each microcosm, the pooled nucleic acid extract was quantified in a 2% agarose gel electrophoresis (representative bands of total DNA and total 16S rRNA) along with calibrated dilutions of calf thymus DNA using TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK). The purified total RNA pool was immediately prepared from total nucleic extracts with RQI RNase-free DNase I (Promega, Charbonnières, France). The reaction was carried out in a total volume of 5 μL containing 0.5 U of the enzyme and 10 ng of 16S rRNA. The presence of residual DNA in DNase-treated extracts was routinely checked by PCR amplification using the universal eubacterial primers EUB338 and UNIV907R (see below for amplification conditions), and no contamination was evident in any sample. Purified RNA extracts were then reverse-transcribed (see below) and stored at −40°C before use.

cDNA was synthesised from purified RNA templates using the ThermoScript™ RT-PCR system kit (Invitrogen, Cergy Pontoise, France) and a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Courtaboeuf, France). RNA template (5 ng) was mixed with random hexamers primers (50 ng) and dNTP (10 mM) in a final volume adjusted to 12 μL with DEPC-treated water. The mixture was then incubated for 5 min at 65°C and immediately stored on ice. Subsequently, reverse transcription was initiated by adding 8 μL of Thermoscript™ RT reaction mix with primer annealing at 25°C for 5 min, followed by cDNA synthesis at 50°C for 50 min. The reaction was stopped by incubation at 85°C for 5 min. Residual RNA was eliminated by adding 1 μL of RNase H (Invitrogen) to the resulting cDNA mixture incubated at 37°C for 20 min.

16S rDNA genes were amplified from total DNA and cDNA with the universal eubacterial primers EUB338 and UNIV907-R (Amann et al. 1992) using PureTaq™ Ready-To-Go PCR Beads (GE Healthcare, Orsay, France) as previously described (Assigbetsé et al. 2005). A 40-pb GC-clamp was added on the forward primer EUB338 for DGGE analysis (Muyzer et al. 1993). 16S rDNA amplicons were resolved by DGGE using 6% acrylamide gels and a gradient of 45–70% denaturant accordingly to Ndour et al. (2008). Staining and scanning of gels are described elsewhere (Assigbetsé et al. 2005).

DGGE patterns were encoded into matrices related to band presence and relative intensity by using TL120 software. Similarity level between DGGE patterns was assessed by principal component analysis of matrices (covariance matrix) with ADE4 software (Thioulouse et al. 1997). Significant differences between mean ordination scores of representative points in the principal plan were analysed by Fisher's LSD tests (Statview, 4.55 version, Abacus Concept, CA). As the PCA principal plans absorbed at least 80% of the total inertia of the data set, such statistical differences between mean ordination scores were indicative of salient dissimilarities between genetic structures.

The hypothesis underlying this work is that organic matter pools of contrasted composition are likely to stimulate within days the activity of distinct populations of the soil bacterial community. Microcosm enrichment with crop residues induced a significant increase of soil DNA and 16S rRNA contents (2.5- and 6-fold, respectively) (Table 1). Soil RNA contents were not driven by compositional differences between maize and soybean residues, but DNA yields were significantly higher under soybean residues. While the larger soil DNA pool size can partly be attributed to the DNA content of residues, higher soil RNA contents should be strictly correlated to real-time enhanced bacterial metabolic activities and cell division as RNAs are highly labile and reactive molecules that were undoubtedly degraded in original plant materials owing to their physical treatment before incorporation into soil. Thus, this fast increase in RNA contents strongly suggests an immediate stimulation of microbial activity following residues application, and is in agreement with studies focusing on the evolution of various bacterial features following soil enrichment with plant material, such as clover (Zelenev et al. 2005), rye (Lundquist et al. 1999), ryegrass (McMahon et al. 2005) or pea (Ha et al. 2008), and including a first sampling at early stages similar to our timescale (3–5 d).

DGGE patterns from 16S rDNA and reverse transcribed 16S rRNA were obtained for all experimental units (Fig. 1). Total DGGE bands with different mobilities amounted to 33 across all patterns. Bands in DNA-based patterns were more numerous (30) than in RNA-based ones (19). Fourteen DNA bands were not detected in RNA lanes and three of them were identified in all DNA fingerprints. Three bands were specifically detected in RNA lanes and one of them was present in all RNA patterns. Overall, 17 DGGE bands held a discriminatory value, in terms of band presence/absence, and nine bands were shared by all profiles. The most conspicuous ones among the last are highlighted in Fig. 1 (coded A to H). Fifteen bands (out of 33) and 13 bands (out of 19) were observed in all DNA and RNA patterns,

Table 1. Soil nucleic acids extraction rates

Treatments	DNA yield	16S rRNA yield
Unamended	697 (186)*a*	265 (62)*a*
Maize	1683 (71)*b*	1571 (51)*b*
Soybean	1730 (352)*c*	1701 (148)*b*

Extraction rates expressed in ng g^{-1} dry weight soil. Means ($n = 3$) and associated standard errors are in parentheses.
a–c Different letters within a column indicate significant differences ($P < 0.05$).

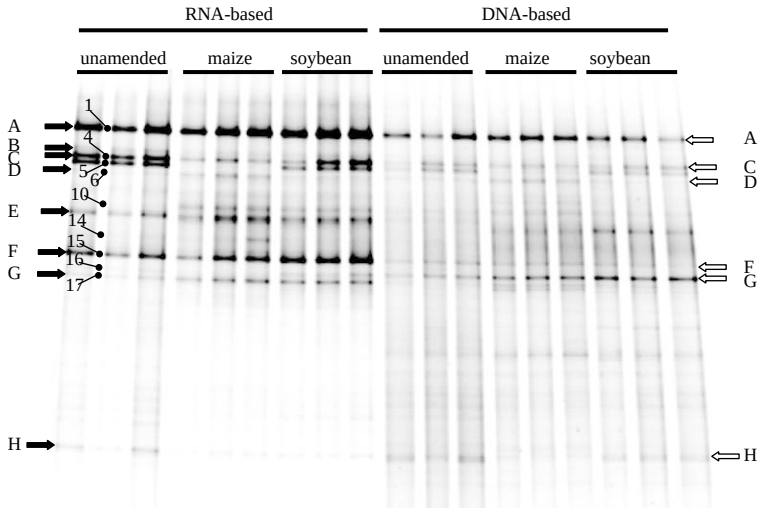

Fig. 1. 16S rDNA DGGE fingerprints based on soil rRNA for the first nine patterns, and soil rDNA for the last nine. Full arrows highlight conspicuous bands that are common to all RNA-based fingerprints (coded A to H), while open arrows indicate a subset of these bands that are also detected in all DNA-based fingerprints. Spots and associated numbers between the first two lanes indicate positions of the bands identified as discriminant in the PCA ordination of RNA-based profiles (see Fig. 2b).

respectively. This last result suggests that a majority of similar bacterial taxa were activated in response to plant materials of distinct moieties.

About 65% of the bacteria detected in unamended soil, as identified by band positions in DNA profiles, were active as deduced from the identification of RNA bands at identical positions. The presence of active bacterial populations within unamended microcosms could probably stem from mineralization of autochthonous soil organic matter that was less protected from microbial activity following the increase of soil moisture from 40 to 70% and partial disruption of aggregates during soil mixing. The proportions of metabolically active bacteria in maize and soybean soils were 57 and 52%, respectively. The influence of organic inputs on structural diversity was further evidenced by computing a PCA ordination of DGGE fingerprints (Fig. 2a). More than 80% of the total inertia was absorbed by the principal plan, conferring on it a high discriminatory value. As expected, total bacterial communities were unambiguously segregated from their corresponding active subsets along the first principal component whatever the treatment. Compositional shifts between total and metabolically active bacterial communities have already been reported within rhizosphere soils from chrysanthemum and grassland turfs (Duineveld et al. 2001; Griffiths et al. 2004). In this background, maize and soybean residues clearly sustained different total and active bacterial communities as indicated by plot discrimination along the first and second axes, respectively. Such an effect of residue quality on the composition of the total bacterial community was already observed with beech and spruce leaves (Aneja et al. 2004) or pea shoots of distinct growth stages (Ha et al. 2008). Treatment ordination was confirmed when RNA-based fingerprints were analysed separately (Fig. 2b). As suggested above on the basis of the high number of bands common to all RNA lanes, structural shifts between active populations evidenced by PCA were mainly driven by changes in the relative abundance of active members (i.e., relative band intensity) in response to distinct organic inputs. This observation could translate the existence of a particular level of functional redundancy in this soil, which is frequently observed in broad bacterial functions such as plant material decomposition.

While the strongest structural shift at the scale of total bacterial communities was induced by soybean residues as compared with control treatment, the active bacterial populations were more responsive to maize inputs (Fig. 2a). It could have been anticipated that shoot nitrogen content would trigger the strongest shift within the active populations since (i) organic matter mineralization is often limited by nitrogen availability (Trinsoutrot et al. 2000) and (ii) N availability is known to be implied in the formation of recalcitrant compounds during plant materials decay (Fog 2008). However, it should not be ruled out that original biochemical attributes of plant material such as polyphenolic compounds could also have impacted bacterial activities and community composition. Importantly, such effects could mostly have been mediated through the organic soluble fraction released from decaying residues, as illustrated with differential ^{13}C incorporation in microbial biomass from leached/unleached ryegrass residue (McMahon et al. 2005).

This study indicates that the structural diversity of active bacterial assemblages is responsive to distinct

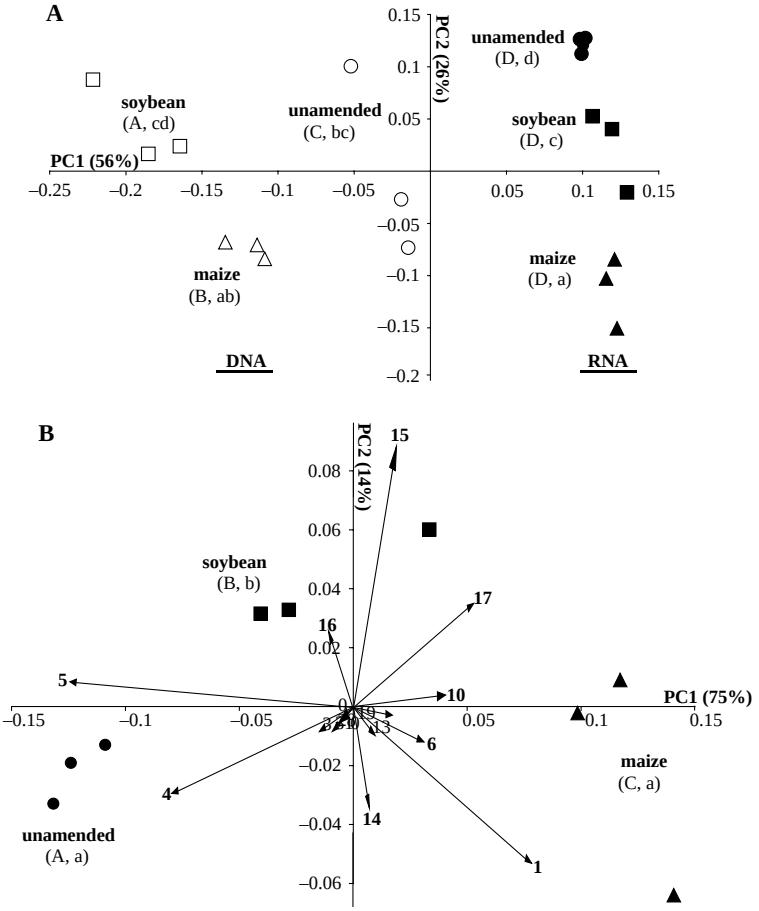

Fig. 2. (A) PCA ordination of DNA-based (white symbols) and RNA-based (black symbols) 16S rDNA DGGE fingerprints. Circle, unamended; triangle, maize; square, soybean. Different capital and lowercase letters in parentheses indicate significant differences ($P < 0.05$) between mean abscisses and ordinates, respectively. (B) PCA ordination of 16S rRNA-based DGGE fingerprints. Symbols and associated letters have the same meaning as in Fig. 2 (A). Annotated arrows represent the most discriminating DGGE bands and their numbers refer to the identification ranks of all detected bands between 1 and 33 along the DGGE gel (see spots in Fig. 1).

biochemical attributes of decaying residues. Future research should determine to which extent this early and specific elicitation of active bacteria affects in turn the C sequestration versus mineralization ratio from decaying crop residues and how managing the biochemical quality of crop residues could favour C sequestration in arable soils.

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