A microcosm experiment to evaluate the influence of location and quality of plant residues on residue decomposition and genetic structure of soil microbial communities

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Abstract

The effects of location (soil surface vs. incorporated in soil) and nature of plant residues on degradation processes and indigenous microbial communities were studied by means of soil microcosms incubation in which the different soil zones influenced by decomposition i.e. residues, soil adjacent to residues (detritusphere) and distant soil unaffected by decomposition (bulk soil) were considered. Plant material decomposition, organic carbon assimilation by the soil microbial biomass and soil inorganic N dynamics were studied with\textsuperscript{13}C labelled wheat straw and young rye. The genetic structure of the community in each soil zone were compared between residue locations and type by applying B- and F-ARISA (for bacterial- and fungal-automated ribosomal intergenic spacer analysis) directly to DNA extracts from these different zones at 50% decomposition of each residue. Both location and biochemical quality affected residue decomposition in soil: 21% of incorporated \textsuperscript{13}C wheat straw and 23% left at the soil surface remained undecomposed at the end of incubation, the corresponding values for \textsuperscript{13}C rye being 1% and 8%. Residue decomposition induced a gradient of microbial activity with more labelled C incorporated into the microbial biomass of the detritusphere. The sphere of influence of the decomposing residues on the dynamics of soluble organic C and inorganic N in the different soil zones showed particular patterns which were influenced by both residue location and quality. Residue degradation stimulated particular genetic structure of microbial community with a gradient from residue to bulk soil, and more pronounced spatial heterogeneity for fungal than for bacterial communities. The initial residue quality strongly affected the resulting spatial heterogeneity of bacteria, with a significance between-zone discrimination for rye but weak discrimination between the detritusphere and bulk soil, for wheat straw. Comparison of the different detrituspheres and residue zones (corresponding to different residue type and location), indicated that the genetic structure of the bacterial and fungal communities were specific to a residue type for detritusphere and to its location for residue, leading to conclude that the detritusphere and residue corresponded to distinct trophic and functional niches for microorganisms.

Keywords: Biodegradation; Crop residues; Detritusphere; Microbial communities; Microcosms; Carbon 13; ARISA

1. Introduction

Soil tillage induces a lot of physical, chemical and biological changes in soil. Important modifications in soil processes are due to the location of crop residues in the soil profile with crop residues remaining at the soil surface in no till and incorporation of residues into the soil by ploughing in conventional tillage. In the long term, no-till systems generally lead to larger organic C and N stocks in the soil (Holland and Coleman, 1987; Balota et al., 2004) which mainly accumulate in the top few centimetres (Carter and Rennie, 1982; Salinas-Garcia et al., 2002). Less decomposition occurs in crop residues left at the soil surface than when soil incorporated (Holland and Coleman, 1987; Curtin et al., 1998; Coppens et al., 2006).
One main factor might be the climatic conditions in the mulch layer where alternate drying and moistening periods may restrict microbial activity compared to the permanently moist conditions existing around incorporated residues (Coppens et al., 2006).

Elsewhere, the number and amounts of microorganisms (i.e. soil microbial biomass) and their distribution within the soil profile are modified by tillage practices (Kandeler et al., 1999; Balota et al., 2004). Soil microbial diversity or composition may also be changed by no till compared to conventional tillage (Doran, 1980; Lupwayi et al. 2001a; Gomez et al., 2003). Several authors have suggested that the residue mulch is mainly decomposed by the fungal community since soil under no till contained larger amounts of fungi and larger proportions of fungi to bacteria than under conventional tillage (Holland and Coleman, 1987; Frey et al., 1999; Guggenberger et al., 1999; Six et al., 2002). These mulch-associated fungi carry out a simultaneous bidirectional translocation of soil-derived N and mulch-derived C. Most of this mulch-derived C can subsequently be located in the soil (Frey et al., 2003). Bacteria are generally considered to be the predominant decomposers of incorporated crop residues under conventional tillage (Holland and Coleman, 1987; Frey et al., 1999).

Most published work on the effect of location of the organic matter on its subsequent decomposition has involved large-scale field experiments and either the whole soil profile or different soil layers (e.g. Biederbeck et al., 1997; Jackson et al., 2003; Gomez et al., 2004; Zhang et al., 2005). Soil heterogeneity has rarely been taken into account except in Lupwayi et al. (2001b). Moreover, the effect of residue location could not be evaluated because of the different and uncontrolled climatic conditions in the various experimental tillage systems. Few studies have investigated the effect of residue location on degradation under controlled conditions (Coppens et al., 2006). One interesting approach was developed by Gaillard et al. (1999) under controlled conditions in which soil heterogeneity was considered in relation to distance from the point source of organic substrate.

Many of the works focussed on dynamics and structure of the soil microbial community in tillage systems only considered the bacterial and/or fungal biomass (e.g. Entry et al., 2003; Lupwayi et al., 1999; Ibeke et al., 2002; Feng et al., 2003; Spedding et al., 2004) and physiological diversity (e.g. Gomez et al., 2004; Lupwayi et al., 1998, 2001b; Zhang et al., 2005). As a result, most such studies tended to consider the entire microbial community as a functional “black box” whereas microbial communities are complex assemblages of species, with different metabolic characteristics and physiological requirements, and each one driving at least one of the multiple reactions of organic matter transformation. In this context, a major challenge in microbial ecology has been to precisely identify those microbial populations and activities involved in carbon degradation and the reciprocal structuration of soil communities according to carbon source. New molecular methods based on the analysis of nucleic acids directly extracted from the soil matrix have been developed over the past ten years. These circumvent the limitations resulting from the selectivity and unrepresentativity of culture-based methods and have revealed new aspects of soil microbial diversity (for review see Ranjard et al., 2000). However, despite these methodological developments, few studies have looked at the genetic structural dynamics of the soil microbial community during plant residue decomposition (Lundquist et al., 1999) and under the effect of mulching (Tiquia et al., 2002).

Thus, our main objectives were to study the effects of the location of various types of plant residues on the structure of the soil microbial community involved in their decomposition. This would consist of highlighting the significant differences in soil microbial community structure which were induced by the initial quality of the carbonaceous substrate and its initial location in the soil. Our study was performed under controlled conditions and considered the different soil zones (residues, detritusphere and bulk soil) that were variously influenced by the decomposition processes. $^{13}$C-labelled plant materials were used to study residue decomposition and the assimilation of organic carbon by the soil microbial biomass. Microbial biomass in each location was measured by fumigation extraction method. This has been widely used as a bioindicator to evaluate the effect of different agricultural practices on soil microorganisms (Marschner et al., 2003). The genetic structures of the bacterial and fungal communities indigenous to the different soil and residue zones under the different incubation conditions were assessed by the DNA “fingerprinting approach”, known as automated-ribosomal intergenic spacer analysis (ARISA).

2. Materials and methods

2.1. Soil type and preparation

The soil was an Orthic Luvisol collected at the Estre´es-Mons INRA experimental station (49.53°N, 3.01°E) and sampled from the 0–15 cm layer. Calibrated soil aggregates (2 mm diameter) were obtained by sieving the soil sample and were stored at 4°C until use. The main soil characteristics are presented in Table 1.

2.2. Plant residues

Two different plant residues were used in this study: mature wheat straw (Triticum aestivum) and young rye leaves (Secale cereale L.). Both were labelled with $^{13}$C by growing the plants in a labelling chamber. The growth conditions are detailed in Aita et al. (1997) and Coppens (2005), respectively. The plants were dried at 80°C and cut into pieces 1 cm in length. The C and N contents and $^{13}$C isotopic abundance were determined with an NA 1500 elemental analyser (Fisons, Milan, Italy) coupled with an
Table 1
Main characteristics of soil used for incubation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay (&lt;2 μm)</td>
<td>g kg⁻¹</td>
<td>185</td>
</tr>
<tr>
<td>Silt (2–20 μm)</td>
<td>g kg⁻¹</td>
<td>296</td>
</tr>
<tr>
<td>Silt (20–50 μm)</td>
<td>g kg⁻¹</td>
<td>457</td>
</tr>
<tr>
<td>Sand (50–200 μm)</td>
<td>g kg⁻¹</td>
<td>57</td>
</tr>
<tr>
<td>Sand (200 μm–2 mm)</td>
<td>g kg⁻¹</td>
<td>5</td>
</tr>
<tr>
<td>Organic C</td>
<td>g kg⁻¹</td>
<td>8.31</td>
</tr>
<tr>
<td>Total N</td>
<td>g kg⁻¹</td>
<td>0.99</td>
</tr>
<tr>
<td>C:N ratio</td>
<td></td>
<td>8.4</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>g k g⁻¹</td>
<td>3.1</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Microbial biomass C</td>
<td>mg kg⁻¹</td>
<td>178</td>
</tr>
</tbody>
</table>

Table 2
Analytical characteristics of plant materials

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Unit</th>
<th>Rye plants</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>g 100 g⁻¹ d.m.</td>
<td>44.20</td>
<td>44.47</td>
</tr>
<tr>
<td>N</td>
<td>g 100 g⁻¹ d.m.</td>
<td>2.14</td>
<td>0.67</td>
</tr>
<tr>
<td>Isotopic excess</td>
<td>Atoms ¹³C 100 atoms⁻¹</td>
<td>2.88</td>
<td>1.99</td>
</tr>
<tr>
<td>Water-soluble C</td>
<td>g 100 g⁻¹ ¹³C</td>
<td>37.5</td>
<td>11.1</td>
</tr>
<tr>
<td>Van Soest fractions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble</td>
<td>g 100 g⁻¹ d.m.</td>
<td>52.7</td>
<td>23.3</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>g 100 g⁻¹ d.m.</td>
<td>24.7</td>
<td>28.7</td>
</tr>
<tr>
<td>Cellulose</td>
<td>g 100 g⁻¹ d.m.</td>
<td>21.2</td>
<td>42.8</td>
</tr>
<tr>
<td>Lignin</td>
<td>g 100 g⁻¹ d.m.</td>
<td>1.4</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Isochrom mass spectrometer (Fisons, Manchester, UK). Water-soluble C was obtained by extracting 50 mg of dried plant material in 25 mL water (20 °C, 30 min), and analysing the dissolved C with a 1010 TOC analyser (OI Analytical, College Station, TX). The soluble, hemicellulose, cellulose and lignin fractions were determined using the methods described by Linéres and Djakovitch (1993) and Van Soest (1963). The analytical characteristics of the plant materials are presented in Table 2.

2.3. Treatments, preparation of soil cores and incubation

Decomposition of the plant materials was studied in repacked soil cores (7 cm diameter, 4.8 cm height) obtained by uniaxial confined compression of a mass of calibrated wet aggregates in a cylindrical mould (Fazzolari et al., 1998) (5 replicates for each experimental treatment and sampling date). For residues left at the soil surface, the equivalent of 268.4 g dry calibrated soil aggregates was first introduced into the mould and compacted to obtain a final bulk density of 1.45. For the soil-incorporated residues, half the mass of calibrated soil aggregates (equivalent to 134.2 g dry soil) was first introduced in the mould and compacted to obtain a final bulk density of 1.45. The equivalent of 1.0 g dry plant residues was then introduced into the mould and distributed on the surface of this half core, after which the rest of the calibrated soil aggregates (equivalent to 134.2 g dry soil) was introduced into the mould to cover the plant residues. The soil aggregates and residues were then compacted together to obtain a final bulk density of 1.45. Soil cores with no addition of plant residues were also considered (control soil, 5 replicates for each experimental treatment and sampling date).

The soil moisture content was fixed and maintained at a matrix potential of −0.05 MPa by adding deionised water. No inorganic N was added to the soil before incubation. Each soil core was placed in a 3 L airtight glass jar with a CO₂ trap (30 mL 1 M NaOH). The soil cores were incubated at 15±0.3 °C for 168 days. The jars were opened periodically to renew the atmosphere and replace the CO₂ traps.

2.4. Soil sampling and determinations

Three zones were considered for each sampling date: plant residues, detritusphere (6 mm thick soil layer adjacent to the plant residues, as suggested in Gaillard et al., 2003) and bulk soil (soil below the 6 mm detritusphere). First, the remaining plant residues were carefully separated from the soil with tweezers after opening the core along the straw layer in the case of incorporated plant residues. Then, the detritusphere and bulk soil were separated by sectioning the soil cores and half cores (incorporated residue treatments) with a cutting device (Millon, 2004). In the incorporated residues treatments, both detritusphere layers were put together as well as both bulk soil layers. Each soil zone (bulk soil and detritusphere) were then homogenized and sampled for further determinations.

The dry mass of the remaining plant residues (2 replicates/date) was determined after drying for 24 h at 80 °C. The C content and ¹³C isotopic abundances of the remaining plant residues, detritusphere and bulk soil (2 replicates/date) were determined using an NA 1500 elemental analyser coupled with an Isochrom mass spectrometer. Water-soluble C was extracted from the detritusphere and bulk soil (dry soil:water ratio = 1/5; agitation 30 min at 20 °C, 2 replicates/date) and determined using the 1010 TOC analyser. The ¹³C isotopic abundances of the freeze-dried extracts were then determined using the elemental analyser coupled with the mass spectrometer. Microbial biomass C and ¹³C in the detritusphere and bulk soil were determined after measuring soluble organic C in 0.025 M K₂SO₄ extracts of CHCl₃ fumigated and non-fumigated soil (dry soil:extractant ratio = 1/5; agitation 30 min at 20 °C, 3 replicates/date) (Trinsoutrot et al., 2000a). Inorganic N was extracted from the detritusphere and bulk soil with 1 M KCl (dry soil:KCl ratio = 1/5–3/10; 30 min at 20 °C, 2 replicates/date), and NH₄⁺–N and NO₃⁻–N were determined by continuous flow colorimetry with an auto-analyser (TRAACS 2000, Bran & Luebbe, Norderstedt, Germany) using adaptations of the methods proposed by
Kamphake et al. (1967) and Krom (1980). The CO₂ and ¹³CO₂ collected in NaOH traps (5 replicates) were determined using the techniques described in Trinsoutrot et al. (2000a). Finally the plant residues, detritusphere and bulk soil samples (3 replicates/date) used for DNA extraction were stored at −40°C prior to analysis.

2.5. Extraction and purification of total DNA from soil zones and plant residues

Microbial DNA was extracted from independent triplicates of the plant residue, detritusphere and bulk soil at the sampling time corresponding to degradation of half the residues (50% dry mass loss during the 168-day incubation = about 15 days for rye leaves and 1 month for wheat straw) according to the method described by Ranjard et al. (2003). Briefly, 1 g from 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 grammes 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

Fig. 1. Amounts of ¹³C labelled plant residues remaining in soil for the different experimental treatments. Bars indicate standard deviation values.

Fig. 2. Cumulative evolution of ¹³C labelled CO₂ for the different experimental treatments. Bars indicate standard deviation values.
The samples were incubated for 20 min at 70°C, then centrifuged at 14 000g 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 000g for 5 min. After precipitation with one volume of ice-cold isopropanol, the nucleic acids were washed with 70% ethanol. DNA was separated from the residual impurities, particularly humic substances, by centrifuging through two types of minicolumns. Aliquots (100 μL) of crude DNA extract were loaded onto PVPP (polyvinyl polypyrolidone) minicolumns (BIORAD, Marne-la-Coquette, France) and centrifuged at 1000g for 2 min at 10°C. The collected eluate was then purified with the Gene clean turbo kit (Q-Biogene, Illkirch, France).

### 2.6. Automated-RISA fingerprinting

The genetic structure of the microbial communities was determined by ARISA, which exploits the variability in length of the intergenic spacer (IGS) between small (16S for bacteria and 18S for fungi) and large (23S for bacteria and 28S for fungi) subunit rRNA genes in the rrn operon. The bacterial and fungal ribosomal IGS were amplified with the primers S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18 and ITS1F/3126T, respectively, with PCR conditions as described by Ranjard et al. (2003). Fifty nanograms of DNA were used as template in PCR. A fluorescent-labelled primer was used for the LiCor® DNA sequencer (Scientec, Les Ulis, France) in B-ARISA and F-ARISA, namely the IRD 800 dye fluorochrome (MWG SA Biotech, Ebersberg, Deutschland). PCRs were performed using the S-D-Bact-1522-b-S-20 and 3126T primers labelled at their 5’ end with the IRD800 fluorochrome. The concentration of labelled PCR products was estimated, and between 0.5 and 1 μL of the product was added to deionised formamide and denatured at 90°C for 2 min. ARISA fragments were resolved on 3.7% polyacrylamide gels and run under denaturing conditions for 15 h at 3000 V/60 W on a LiCor® DNA sequencer (ScienceTec). The data were analysed using the 1D-Scan software (ScienceTec). This software converted fluorescence data into electrophoregrams in which the peaks represented the PCR fragments. The heights of the peaks were calculated together with the median filter option and the Gaussian integration in 1D-Scan, and represented the relative proportion of fragments in the total products. Lengths (in base pairs) were calculated by using a standard size with bands ranging from 200 to 1206 bp.

![Fig. 3. Distribution of 13C labelled water soluble C in the different soil zones for the different experimental treatments. Values are calculated for 1 kg of whole soil taking into account the proportion of soil zones (detritusphere represents, respectively, 12.5% and 25% of soil weight for residues at surface and incorporated residues). Bars indicate standard deviation values.](image-url)
2.7. Statistical analysis

The statistical analyses of the C and N data were performed using the ANOVA procedure of SAS software (SAS Institute, 2001), with a means comparison by Student–Newman–Keuls test ($p \leq 0.05$). Significant differences ($p \leq 0.05$) in DNA yields between the different sample sizes in each soil were determined using Statview-SE software and Student’s $t$-test.

Data obtained from the 1D-Scan software were converted into a table summarizing the band presence (i.e. peak) and intensity (i.e. Gaussian area of peak) using the PrepRISA programme (Ranjard et al., 2003). A robust analysis was ensured by integrating 100 bands for B- and F-ARISA profiles with a 2 bp resolution (Ranjard et al., 2003). PCA was carried out using a B-ARISA and F-ARISA covariance matrix on the data matrix (communities as rows and bands as columns). This method enabled the bacterial or fungal communities to be ordinated by plotting the scores for the first two principal components in two dimensions. PCA were performed using the ADE-4 software (Thioulouse et al., 1997).

Fig. 4. Distribution of inorganic N in the different soil zones for the treatments with input of residues and evolution of inorganic N in whole soil for control soil. Values are calculated for 1 kg of whole soil taking into account the proportion of soil zones (detritusphere represents, respectively, 12.5% and 25% of soil weight for residues at surface and incorporated residues). Bars indicate standard deviation values.
3. Results and discussion

3.1. Influence of residue quality and location on $^{13}$C fluxes in soil

As expected, rye leaves decomposed more quickly and to a greater extent than wheat straw (Figs. 1 and 2). Differences in decomposition were related to residue quality (Table 2) as reported by several authors (Trinsoutrot et al.; 2000b; Gaillard et al., 2003; Abiven et al., 2005) who showed that decomposition under controlled conditions was related to the initial water soluble or soluble compounds present in the plant materials. Wheat straw decomposition was poorly affected by location, differences in decomposition being statistically significant for CO$_2$ emission only during the first 14 days of incubation. In contrast, location did influence the decomposition of rye leaves and a little more CO$_2$ was emitted when the residues were superficial (Fig. 2), but more undecomposed rye leaves remained ($p<0.01$) when the residues were left on the soil surface (Fig. 1). This apparent discrepancy between CO$_2$ emission and amounts of remaining residues is explained by the fact that CO$_2$ emission corresponds to apparent C mineralization and does take into account the incorporation in microbial biomass (see below). Most authors (Douglas et al., 1980; Holland and Coleman, 1987; Curtin et al., 1998) have shown that decomposition under field conditions is enhanced when the residues are incorporated. Similar results were found by Coppens et al. (2006) for laboratory experiments with controlled rain events. In contrast, when studies involved soil incubation under controlled conditions, differences between the decomposition of plant materials at the soil surface or soil incorporated were shown to be weak or null (Cogle et al., 1989; Aulakh et al., 1991). In fact, differences in decomposition between residues incorporated in soil or left at the soil surface may relate to moisture conditions in the mulch layer (Aulakh et al., 1991; Coppens et al., 2006), while moisture conditions in our study were optimal for residue decomposition in all experimental treatments. Another factor of influence is the inorganic N availability which will be discussed later.

During biodegradation, water soluble C was transferred from the decomposing residues to the detritusphere and bulk soil as observed by Frey et al. (2003). Indeed, some water soluble $^{13}$C was continuously present in both soil zones during the incubation period (Fig. 3), but was always
less than 0.1% of the added $^{13}$C. The effect of residue location on the amounts present in the soil fractions was statistically non-significant, although a little more labelled soluble carbon was found in the detritusphere when the residues were incorporated. Nevertheless, the amounts of labelled soluble C were significantly higher in the detritusphere than in bulk soil and higher for rye leaves. In addition, the total residue-derived $^{13}$C present in the detritusphere and bulk soil represented 5.7–19.1% and 1.2–2.9% of the initial $^{13}$C input at the end of incubation (results not shown). This confirms the spatial limitation of the sphere of influence of residues as reported by Gaillard et al. (1999) who showed that decomposing plant residues produced a steep gradient in microbial activity and residue-derived $^{13}$C in the soil mainly in the 4-mm zone in contact with the residue.

3.2. Influence of residue quality and location on soil inorganic N dynamics in soil

Very low amounts of inorganic N were observed in the soil zones for wheat straw at both locations (Fig. 4). Wheat straw incorporation in fact induced net N immobilization in both zones, although small amounts of N were...
mineralized at the end of the incubation period. Surface-applied wheat straw induced net N immobilization in the detritusphere whereas N mineralization was observed for the bulk soil during the first 14 days followed by progressive N immobilization from day 14 to day 84. These data confirm the findings of several authors (Aulakh et al., 1991; Coppens et al., 2006) who reported greater N immobilization when residues were incorporated. The inorganic N for rye leaves and both locations, increased in both soil zones during the incubation period which indicated net N mineralization, with more N mineralized than in the control soil. These dynamics shows that the organic N in rye leaves is mineralized which is in accordance with the findings of many other authors (e.g. Trinsoutrot et al., 2000a; Mendham et al., 2004; Abiven et al., 2005) who showed that plant materials with a low C:N ratio induced net N mineralization during their decomposition in soil. Our results suggest that wheat straw decomposition was probably limited by the availability of soil inorganic N, as shown by Recous et al. (1995), which could explain the weak effect of location on decomposition. Finally, comparative N dynamics in the detritusphere and bulk soil suggest a transfer of inorganic N from bulk soil to detritusphere and vice versa. This may occur by physical processes (e.g. diffusion) and biological transfer.

3.3. Influence of residue quality and location on $^{13}$C incorporation in the microbial biomass

Significantly more labelled C was present in the microbial biomass of the detritusphere for both residues than in bulk soil (Fig. 5), confirming the conclusion of Gaillard et al. (1999) that microbial assimilation occurs essentially in the detritusphere. In fact, the soluble C transferred into the soil from decomposing residues is rapidly assimilated by soil microorganisms. Thus, the further the soil is from the residues, the less C is available to the soil microorganisms, thereby leading to considerable soil heterogeneity.

More $^{13}$C was present in the microbial biomass during the decomposition of rye leaves than of wheat straw and more $^{13}$C was incorporated into the microbial biomass when rye leaves were incorporated (Fig. 5). These results indicate a higher rate of decomposition when this residue is incorporated (Fig. 1) and coincide with a higher availability of the residue-derived water-soluble C (Fig. 3). An effect of location of the wheat straw residue was only observed in the detritusphere. These results confirm that rye leaves provide a much more decomposable substrate for soil microorganisms than wheat straw, as observed by Trinsoutrot et al. (2000a) who found that more C was assimilated by the soil microbial biomass from residues

![Fig. 7. Principal component (PC1 x PC2) plots generated from B-ARISA profiles obtained from the residue (R), the detritusphere (Dsph) and bulk soil (BS) in microcosms incubated with wheat straw or rye leaves at soil surface or incorporated. Ellipses represent 90% confidence limits.](image-url)
with high N and soluble C contents. The lower incorporation into the microbial biomass of labelled C from wheat straw is coherent with its lower decomposition (Figs. 1 and 2) and lower availability of released watersoluble C (Fig. 3). The poor effect of wheat residue location on microbial assimilation confirmed the limited biodegradation in both locations, possibly due to soil inorganic N availability (Fig. 4).

3.4. Influence of residue quality and location on spatial distribution of the microbial community structure

In this study, the genetic structures of the bacterial (B-ARISA) and fungal (F-ARISA) communities in plant residues, detritusphere and bulk soil, were compared at a single stage in degradation during the 168-day incubation, corresponding to a loss of 50% dry mass (about 15 days for rye leaves and 1 month for wheat straw). ARISA fingerprinting of the community, by direct determination of DNA extracts from the different soil zones, provided complex profiles with peaks ranging from 250 bp (i.e. 100 bp-IGS) to more than 1000 bp (1050-bp IGS) for bacteria and from 350 to 1000 bp for fungi (Fig. 6). Visual comparison of the B- and F-ARISA profiles showed that each soil zone was characterized by a specific pattern, suggesting a particular genetic structure of the community.

The PCA of B-ARISA confirmed and expressed these differences as a gradient from residue to bulk soil (Fig. 7) suggesting that residue degradation stimulated certain minor populations in the bulk soil and detritusphere (as indicated by arrows in Fig. 6). This spatial gradient in variability confirmed that a decomposing residue results in soil heterogeneity but that its sphere of influence is spatially limited (Gaillard et al., 1999). In addition, this spatial heterogeneity exhibits similarities with that observed in the rhizosphere between root tissue and bulk soil (Mougel et al., 2006). Altogether, these results confirm that both the rhizosphere of living plants and residues of dead plants represent “hot spots” of a readily available energy source, C and nutrients for soil microorganisms, and strongly modify the genetic structure of the community by stimulating particular populations, especially as the soil system is often substrate limited as regards microbial growth (Mougel et al., 2006; Lejon et al., 2007).

In the case of wheat residue decomposition, the weak discrimination between indigenous bacterial communities in the detritusphere and bulk soil, whatever the residue location (Fig. 7), did not coincide with the above-described spatialisation of the microbial biomass (Figs. 3–5), or with the previously reported assimilation of decomposing substrates (Gaillard et al., 1999; Ronn et al., 1996). These authors recorded spatial heterogeneity in microbial activity.
around decomposing wheat straw over a millimetre scale, with the area of influence limited to 4 mm from the residue. Altogether, these results suggest a weak relationship between the rate of residue decomposition and the composition of the community (Lejon et al., 2007). In contrast, a significant discrimination between the bacterial communities in each zone was apparent for rye leaves, and even more so when the residue was incorporated (Fig. 7). The observed differences between rye leaves and wheat straw suggested a strong interaction between the initial quality of the residue and resulting spatial heterogeneity, and confirmed the hypothesis of Gaillard et al. (2003). These authors concluded that the decomposition of young rye leaves led to a higher spatial heterogeneity of the decomposing microorganisms due to a greater release of soluble organic compounds.

A more significant discrimination between bulk soil and detritusphere was recorded for the fungal community than for bacteria (Fig. 8), suggesting that plant residue degradation, whatever the type of residue and location, led to a more significant spatial heterogeneity of fungal genetic structure and thus a more significant impact of the residue in the detritusphere soil. This might be explained by the shape of fungal bodies and the growth patterns produced by the filamentous ramified mycelia that enable residue-colonizing fungi to radiate through the soil over several millimeters and thus modify the population structure in the detritusphere (de Boer et al., 2005). This hypothesis was supported by visual observation of the profiles which revealed bands common to residue and detritusphere but not detected in bulk soil (indicated by arrows in Fig. 6). However, several bands were also specific to the detritusphere suggesting that particular populations were stimulated in this zone possibly by the amount and/or specific quality of soluble carbon (Lejon et al., 2005, 2007). It should be noted that, in our case, residue location had no significant influence on spatial heterogeneity of the fungal community and did not confirm the possibly less important involvement of fungi in the decomposition process when residues are incorporated (Holland and Coleman, 1987; Frey et al., 1999).

![Fig. 9. B- and F-ARISA profiles obtained from DNA extracted from independent replicates of residues and detritusphere in microcosms incubated with wheat straw at soil surface (WS) or incorporated (WI) and rye at soil surface (RS) or incorporated (RI).](image)
3.5. Influence of plant residue quality and location on the microbial genetic structure in each soil zone

The data obtained from the ARISA profiles were computed to compare the genetic structures of the communities in each zone according to residue type (rye leaves vs. wheat straw) and location (incorporated vs. surface) (Fig. 9). Whatever the location, PCA revealed a significant discrimination between the detritusphere of rye leaves and wheat straw, suggesting that the structures of the indigenous bacterial and fungal communities in the detritusphere were more specific to the type of residue than to its location (Fig. 10). In soil, microorganisms are mainly heterotrophic and carbon limited and such discrimination could result from the contrasting availability and quality of the carbon source supplied by wheat straw and rye leaves decomposition as previously discussed (Fig. 3 and Table 2). A similar hypothesis was tested in forest and agricultural field studies where the distinct composition of microbial communities was believed to be due to differences in the soil organic management (Myers et al., 2001; Lejon et al., 2005; Yang et al., 2003) leading to different types of dissolved organic C in the soil (Marschner et al., 2003). From a functional point of view, discrimination between the community structures can be related to differences in the rate of decomposition (Figs. 1 and 2), these being more significant for residue type than for their location.

In the residue zone, PCA revealed a more significant discrimination of the bacterial and fungal communities between residue location than between residue type (Fig. 10). Significant discrimination was only observed between wheat straw and rye leaves for bacterial communities when the residues were on the surface. These unexpected results suggest that similar bacterial and fungal populations colonized both residue types and that the physical location of the residue had a greater influence than its quality on the microbial composition of colonizers. These data could be partly explained by the stress conditions, in terms of moisture content, generally observed in the mulch layer and by the colonization surface offered to microorganisms which is reduced when the residue is incorporated (Holland and Coleman, 1987; Aulakh et al., 1991; Coppens et al., 2006).

4. Conclusions

Altogether, our results emphasized that the presence of decomposing residues in soil induced a gradient from bulk soil to residue site of microbial density, processes and also in the composition of the community. In addition, this

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**Fig. 10.** Principal component (PC1 × PC2) plots generated from B- and F-ARISA profiles obtained from the residues and the detritusphere in microcosms incubated with wheat straw at soil surface (WS) or incorporated (WI) and rye at soil surface (RS) or incorporated (RI). Ellipses represent 90% confidence limits.
sphere of influence may be modified by type of residue as well as its location in the soil. This study represents an initial step in the ecological study of crop residue decomposition in soil. Nevertheless, it remained difficult to extrapolate our results in the field conditions considering agricultural practices such as organic management and tillage. Future research will need to confirm, at the field level, the influence of residue location on microbial community by taking into account agronomic resultant such as soil fertility, carbon turnover as well as ecological parameters such as microbial diversity and stability.

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References


