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

Blaud A.[a,+], Lerch T.Z.[b,c*], Chevallier T.[a], Nunan N.[b], Chenu C.[b], Brauman A.[a]

[a]*UMR Eco&Sols, (INRA-IRD-SupAgro), 2 place Viala, 34060 Montpellier, France*

[b]*UMR Bioemco, (CNRS- UPMC-IRD-ENS-AgroParisTech-UPEC), Campus AgroParisTech, 78850 Thiverval-Grignon, France*

[c]*UMR EGC, (INRA-AgroParisTech), Campus AgroParisTech, 78850 Thiverval-Grignon, France*

*Corresponding author: (T.Z. Lerch)

Address: Faculté des Sciences et Technologies, Université Paris-Est Créteil,

61 avenue du Général de Gaulle, 94010 Créteil Cedex, France.

Tel: +33 1 45 17 16 60.

Fax: +33 1 45 17 19 99.

E-mail adress: thomas.lerch@u-pec.fr

[+]Present adress :

Department of Animal and Plant Sciences, University of Sheffield, S10 2TN, U.K.

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}Clabelled 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.

Keywords: Soil Bacterial Communities Structure, ^{13}C-labelling, FAME-SIP, Soil Aggregation, Mineralisation, Microscale Biogeography

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 (Drazkiewicz, 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).

74 The process of aggregation occurs firstly by the formation of macroaggregates (> 200
75 µm), which are formed by mineral associations with particulate organic matter (POM) via
76 temporary binding agents (i.e. fungal hyphae and plant roots). Microaggregates (< 200 µm) are
77 formed within macroaggregates around POM (Oades, 1984; Six et al., 2000). The
78 decomposition of POM within macroaggregates by microorganisms produces
79 exopolysaccharides and other metabolites which act as persistent binding agents for the
80 formation of microaggregates (as reviewed by Degens, 1997). Thus, the dynamics of soil
81 aggregates create different ecological niches. There is a particularly strong niche differentiation
82 during the formation of macroaggregates around POM, as there are large amounts of readily
83 available substrate. Regions of the soil surrounding POM (extending no further than a few
84 millimeters) have been termed the 'detritusphere' and are described as microbial activity
85 hotspots (Gaillard et al., 1999; Poll et al., 2006). Macroaggregates contain more C, and more
86 labile young C, than microaggregates (Puget et al., 2000) and the C turnover is also higher in
87 macroaggregates (Buyanovsky et al., 1994; Monreal et al., 1997). In contrast, microaggregates
88 are more stable and persistent than macroaggregates (Jastrow, 1996; Bossuyt et al., 2002) and
89 are characterised by low predation rates, low nutrient availability and low gas diffusion rates as
90 reviewed by Ranjard and Richaume (2001).
91 The transient nature of soil aggregates makes it legitimate to ask how the dynamics of
92 microbial communities are related to the evolution of their habitat but also how this affects the
93 active communities involved in the degradation of POM. As microorganisms are active agents
94 of aggregate formation and stabilization (Tisdall and Oades, 1982), they impact on their own
95 habitat. The *de novo* formation of macroaggregates provides the best conditions for studying
96 this, as habitat differentiation is likely to be the most significant. Several studies (e.g. Denef et
97 al., 2001, 2002) have investigated the influence of nutrients addition on macroaggregates
98 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 ± 0.6 g kg^{-1}, the total nitrogen was 1.7 ± 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 ± 1.7 g kg^{-1} and 19.7 ± 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 ovendried 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 50 µm mesh sieve to obtain the 200-2000 µm and 50-200 µm soil fractions. The water and soil that passed through the 50 µm sieve was centrifuged (2000 rpm for 10 min, 4 °C) to obtain 0-50 µm fractions. Particulate organic matter (POM) fractions were discarded by flotation in water from the 200-2000 and 50-200 µm soil fractions in order to reduce the amount of plant residues and plant FAME. Each soil fraction was subsampled (from 0.5 g to 2 g) to measure the weight distribution of the soil fractions after oven drying at 40 °C. Subsequently, the subsamples were ground (< 200 µm) for C and ^{13}C

analyses, using an Elemental Analyser (EA, NA-2000, Carlo-Erba) and an Elemental Analyser coupled to an Isotopic Ratio monitoring Mass Spectrometer (EA-IRMS, NA-1500, Carlo-Erba), respectively. The remaining soil fraction material was stored at – 20 °C for FAME analysis.

2.5. FAME analyses

FAME profiles were produced following the protocol described by Schutter and Dick, (2000). Briefly, fatty acids were extracted and methylated from 3 g of soil (dry weight equivalent) with 15 ml of a mixture of NaOH (0.2 M) and methanol (1 M). The mixture was then vortexed 20 s and shaken for 1 h at 37 °C. After this incubation, 2-3 ml of acid acetic (1 M) was added to neutralise the pH. Hexane (15 ml) was added to each tube, vortexed for 1 min and centrifuged (1600 rpm) at 4 °C for 1 h to isolate the FAME in the organic phase. The organic phase (~5 ml) was transferred to a clean tube and evaporated under a stream of N_2. Dry FAME at bottom of the tubes were dissolved in 170 µl MTBE (Methyl Tertiary Butyl Ether)-hexane (1:1, v:v) and transferred to a vial with 30 µl of methyl stearate (internal standard, 0.01 M). FAME were quantified on a Gas Chromatograph (HP 6890) coupled to a Flame Ionisation Detector (GC-FID) and identified by GC (HP 6890) coupled to an Agilent 5973 electronic impact (70eV) quadripole mass spectrometer (GC-MS). The isotopic signature of each FAME was determined using a GC (HP 5890) coupled to an Isochrom III isotopic mass spectrometer (Micromass-GVI Optima) via a combustion interface (GC-c-IRMS). There were three analytical replicates for all of the FAME isotopic analyses. All GCs were equipped with the same SGE BPX-5 column (50m x 0.25mm x 0.32µm). The injector and detector were maintained at 280°C. Column temperature was programmed at 50 °C for 1 min and then ramped at 2 °C min^{-1} to 350 °C, followed by an isothermal period of 10 min. Samples were injected in splitless mode.

194 The fatty acid nomenclature used was that described by Frostegard et al. (1993). Mono-
195 unsaturated and cyclopropyl fatty acids were taken as gram-negative bacteria biomarkers
196 (Zelles, 1999), iso- and anteiso-fatty acids as gram-positive bacteria biomarkers (O'Leary and
197 Wilkinson, 1988; Zelles, 1999) and carboxylic acids with a methyl function on the carbon chain
198 as biomarkers for actinobacteria and in particular *Nocardia* (Zelles et al., 1994). A number of
199 FAME (C12:0, C14:0, C15:0, C16:0, C17:0, C16:4(3,7,11,15), C18:2(9,12), C18:1(9)cis,
200 C18:0, C19:0, C20:0 or C22:0) were detected in the lipid extract of the rice straw with an overall
201 $\delta^{13}C$ value of 8209±247‰ (data not shown). These were not used when analysing microbial
202 community structure as their origin (plant or microbial) was uncertain. Thus, 12 FAME that
203 were solely of bacterial origin, were selected: i-C15:0, a-C15:0, i-C16:0, brC17:0, i-C17:0,
204 aC17:0 for gram-positive bacteria; C16:1(9)cis, C16:1(11)cis, cycC17:0, cycC19:0 for gram-
205 negative bacteria; 10me-C16:0 and 10meC18:0 for actinobacteria.

206 *2.6. Estimating the amount of rice derived C within each soil fraction*

207 The standard notation for expressing isotopic contents as δ and the international internal
208 standard Vienna PeeDee Belemnite (R_{VPDB} = 0.0112372) were used (Coplen, 1995). The
209 methylation of isolated fatty acids introduced one additional carbon which was not present in
210 the parent compound and which altered the isotopic ratio of the original carboxylic acids. The
211 measured isotopic ratios of the FAME were corrected for the isotope ratio of the methyl
212 esterified moiety in order to obtain the isotope ratios of the fatty acids as described by Lerch et
213 al. (2007). In the amended soils (AS), equations (1), (2) and (3) were used to estimate the
214 quantity of C_{Straw} mineralised from bulk soil and the amount of C_{Straw} incorporated into each soil
215 fraction and into the FAME.

216 $$C_{AS} = C_{SOM} + C_{Straw} \quad (1)$$

$$\delta^{13}C_{AS} \times C_{AS} = \delta^{13}C_{SOM} \times C_{SOM} + \delta^{13}C_{Straw} \times C_{Straw} \quad (2)$$

where C_{AS} is the total amount of C in a given fraction of the amended soil, C_{SOM} is the amount of C derived from the soil organic matter (SOM) and C_{Straw} is the amount of C derived from the rice straw, $\delta^{13}C_{AS}$ is the isotopic signature of the amended soil, $\delta^{13}C_{Straw}$ is the isotopic signature of rice straw (6124‰) and $\delta^{13}C_{SOM}$ is the isotopic signature of the initial SOM. Here, the $\delta^{13}C$ of control samples was used to estimate $\delta^{13}C_{SOM}$. The amount of C_{Straw} incorporated into a given fraction (i.e. CO_2 evolved, bulk soil, physical fraction or FAME) was determined with equation (3), which is a combination of equations (1) and (2):

$$C_{Straw} = C_{AS} \times \left(\frac{\delta^{13}C_{AS} - \delta^{13}C_{CS}}{\delta^{13}C_{Straw} - \delta^{13}C_{CS}} \right) \quad (3)$$

2.7. Statistical analyses

Differences in bacterial community structure were analysed by principal components analysis. Significant differences among samples or among the scores of the PCA were tested by ANOVA (using the day of sampling and the physical fraction as factors). The normality and homoscedasticity of data were checked prior to statistical analysis. The evenness (*E*) of the labelled and total FAME profiles were compared to determine how the incorporation of labelled C was spread throughout the microbial communities. The evenness of FAME profiles was calculated as:

$$E = \left(-\sum_{i=1}^{q} p_i \log p_i \right) / \log q \quad (4)$$

where *p* is the relative abundance of each FAME (molar percentage) and *q* is the total number of FAME (Legendre and Legendre, 1998). All statistical analyses were performed using R version 2.9.0 (R Development Core Team. 2009. R: a language and environment for statistical computing).

3. Results

3.1. Dynamics of soil aggregation and carbon distribution among aggregates

During the incubation there was a shift towards macroaggregates, with a significant ($P < 0.001$) decrease in the quantity of fractions < 2000 μm and the *de novo* formation of macroaggregates (Fig. 1). The process occurred more rapidly in the amended soil but by day 21 the aggregate size distributions of control and amended soil were not significantly different ($P > 0.05$; Fig. 1). The carbon distribution profiles did not follow the same pattern (Fig. 2). First of all, there were no significant differences between control and amended samples, with the exception of a slight, but significant ($P < 0.05$), difference in the 200–2000 μm fraction, at sampling day 2. There was a significant ($P < 0.01$) decrease in the C concentration of the fractions < 200 μm during the incubation, with a concomitant increase in the C concentration of the fractions > 200 μm. However, at all sampling dates, the C concentration in the 0–50 μm fraction was higher than that of the other fractions. Initially, the C from the straw (C_{Straw}) was present mainly in the fractions < 200 μm (Fig. 3). The C_{Straw} concentration of the fractions > 200 μm increased dramatically by day 2. In contrast, the C_{Straw} concentration of the other fractions decreased. After 21 days of incubation, all the soil fractions showed a decrease in C_{Straw}, with the exception of the 0–50 μm fraction in which the C_{Straw} concentration remained steady. It should be noted that the amount of C_{Straw} incorporated into each soil fraction would be 24% lower when using the average $\delta^{13}C$ signature of plant fatty acids (8209‰) rather than the bulk (6125‰).

3.2. Carbon mineralisation

The total soil respiration was always significantly higher in amended soil than in control soil (data not shown). The difference in the total CO_2 between the non-amended soil and the amended soil was explained by the mineralisation of ^{13}C-labelled rice residues (79 ± 7 and 793 ± 19 μg ^{13}C–CO_2 g^{-1} soil, at days 2 and 21, respectively). In the amended soil, approximately half of the initial amount of rice straw was mineralised after 21 days of incubation (Table 1). The cumulative mineralisation of the soil organic matter in non-amended soil was always significantly ($P < 0.05$) higher than in amended soil. The rate of C-SOM mineralisation for non-amended and amended soil was 0.37 ± 0.01% and 0.33 ± 0.01% of C-SOM per day, respectively, after 2 days of incubation and then decreased to 0.24 ± 0.01% and 0.21 ± 0.01% of C-SOM per day, respectively. The mineralisation rate of C_{Straw} was more constant than rate of C-SOM through the incubation with 2.4 ± 0.22% and 2.3 ± 0.06% of C_{Straw}, after 2 and 21 days of incubation, respectively.

3.3. Dynamics of FAME profiles among soil aggregates

Fatty acid methyl ester profiles showed clear differences among soil fractions. These differences were evident principally along the first ordination axis of the PCA (Fig. 4). Lipid profiles of soil fractions 200–2000 and > 2000 μm were characterised by a high relative abundance of FAME characteristic of Gram-negative bacteria, such as C16:1(11)cis, C16:1(9)cis and cycC19:0, whereas the 50–200 μm and the 0–50 μm soil fractions were characterised by relatively more branched saturated FAME, characteristic of Gram-positive bacteria, such as aC15:0, iC15:0, aC17:0, iC16:0, i-C17:0 10me-C16:0, brC17:0 and 10me-C18:0. The second ordination axis revealed temporal changes in bacterial community structure (Fig. 4). In all soil fractions there was a trend towards a higher relative abundance of branched FAME on day 21 of the incubation, although ANOVA showed that this trend was only significant for the soil fractions > 50 μm. There was no significant effect of amendment except for the larger (> 200 μm) fractions after 2 days.

Molecular isotope analyses of selected FAME extracted at day 0 showed similar ^{13}C signature between control and straw amended soils, confirming that the groups of FAME analysed were not from plant origin. At days 2 and 21, all the selected FAME in the amended samples were significantly ^{13}C enriched (between 23.1‰ and 313.2‰) relative to the control soils (between −13.6‰ and −12.7‰), indicating an incorporation of C from labelled straw into the microbial biomass. The structure of bacterial communities that incorporated C_{Straw} into their biomass differed significantly ($P < 0.001$) among soil fractions (Fig. 5). Communities in macroaggregates differed from communities in microaggregates and there were further differences between communities associated with 0–50 and 50–200 µm fractions. Inspection of the PC loadings indicated that the lipids responsible for the differences among aggregate size fractions were the same for total and labelled lipids (Fig. 5B): Gram-negative bacteria in macroaggregates (> 200 µm) and Gram-positive bacteria in microaggregates (< 200 µm). Significant changes ($P < 0.05$) in community structure were observed between days 2 and 21 in all soil fractions except for 0–50 µm, although the changes were not consistent across aggregate-size fractions. Fig. 6 shows that the evenness of the control and amended sample lipid profiles were similar, with the exception of the 0–50 fraction and fractions > 200 µm at day 2. The FAME profiles were always more even for the total soil (0.98 ± 0.1) than for each soil fraction. While the total and the < 200 µm fractions did not vary with time, the largest soil fractions had a similar evolution, with a lower evenness value after 2 days. Fig. 7 shows that the evenness of ^{13}C-labelled FAME profiles were always lower than those of total FAME. In contrast to the total soil, changes in evenness occurred for all the soil fractions during the incubation. With exception of the 0–50 µm fraction, the labelled FAME profiles were more even after 21 days than after 2 days of incubation. The FAME-C_{Straw} to C_{Straw} ratio was used as an indicator of the amount of biomass relative to the available resource in the habitat. The ratio of FAME-C_{Straw} to C_{Straw} (Fig. S1) was always significantly lower in the finest fraction (0–50

312 μm) than in the other fractions, with the exception of the > 2000 μm fraction ratio, which
313 decreased after 21 days of incubation.

314 **4. Discussion**

315 *4.1. Carbon Mineralisation*

316 Nearly half of the added C_{Straw} was mineralised after only 21 days. This rate is higher
317 than those reported in other studies using similar ^{13}C-labelled plant residues. For instance,
318 Bernard et al. (2007) found that 33% of wheat straw residues were mineralised after 28 days
319 incubation. The difference is unlikely to be due to N availability as the C/N ratio was low
320 compared to other studies (Nicolardot et al., 2001), and no mineral N was added. The
321 experimental conditions might explain the high mineralisation rates as the mineralisation of
322 native C was also very high (5% in 21 days). The most likely reason is that both the soil and
323 the straw residues were air dried and crushed prior to incubation, which probably reduced the
324 physical protection of the organic matter (OM), resulting in enhanced mineralisation of both
325 native and added C (Chevallier et al., 2004; Salome et al., 2010). Furthermore, the soil was
326 rehydrated before incubation, which may have caused the desorption of organic compounds,
327 making them available for mineralisation (Van Gestel et al., 1991; Chevallier et al., 2004; Lerch
328 et al., 2011). Chevallier et al. (2004) found 2.4 times more C was mineralised after 35 days of
329 incubation for a soil that was air-dried and rewetted prior to incubation than for a fresh soil.
330 The high temperature of incubation used in this study (30 °C) may also explain the high rate of
331 degradation (Dalias et al., 2001).

332 It should be noted that the quantification of the C_{Straw} mineralisation may have been
333 over-estimated because of the ^{13}C-labelling method used. Indeed, it is difficult to ensure a
334 totally homogenous ^{13}C-labelling of the entire plant because of the isotopic fractionation that
335 naturally occurs during plant metabolism (Ghashghaie et al., 2003). This hypothesis was

confirmed when measuring the $\delta^{13}C$ values of fatty acids extracted from the rice straw (more than 8209‰) compared to the overall bulk value (around 6125‰). If the more ^{13}C enriched molecules were also the most labile, the amount of C_{Straw} would have been overestimated. This potential bias might also explain why an apparent negative priming effect (less native C mineralised in the amended soil compared to the control soil, Table 1) was observed during the incubation.

4.2. Formation of de novo soil aggregates

The incubation of crushed soil resulted in the rapid formation of *de novo* soil aggregates in both treatments after only 2 days. Soil macroaggregate formation may have been induced by a number of factors in both treatments. The wetting of dry soil, OM adsorption to clays, the physical entanglement of soil particles by fungi and the production of polysaccharide binding agents during microbial decomposition of OM are all known to cause aggregation (Tisdall and Oades, 1982; Bossuyt et al., 2001). Aggregate formation likely also occurred because of physical or electrostatic interactions between the clay minerals and oxides (Oades and Waters, 1991; Denef et al., 2004). Oxides are the main binding agent in Ferralsols (Oades and Waters, 1991). Despite the major role of oxides in the Ferralsol aggregation, the addition of plant residues increased the rate of aggregation with time (Fig. 1). The absence of significant difference between amended and non-amended soil aggregation after 21 days can be explained by the presence of oxides in both treatments and, to a lesser extent, the presence of native organic debris in the non-amended soil (only 48% were removed) which might also indirectly promote soil macroaggregation. In contrast, the amount of smaller soil fractions (< 200 µm) decreased with time, suggesting that the latter fractions were incorporated into macroaggregates as described in the aggregate hierarchy model (Oades and Waters, 1991; Bossuyt et al., 2001; Denef et al., 2001). Such *de novo* rapidly formed macroaggregates were likely microhabitats

with specific physico-chemical characteristics, such as organic resources, water and oxygen availability.

4.3. Dynamics of total bacterial communities

The analysis of microbial community structure using FAME profiling to distinguish microbial communities from different soils is now well established and gives results that are similar to DNA-based methods (Ritchie et al., 2000; Schutter and Dick, 2000). Although the extraction of total fatty acids can include non-microbial fatty acids, the method used in this study, the ester-linked (EL) procedure, reduces the risk of this happening (Schutter and Dick, 2000). Schutter and Dick (2002) showed that plant amendments did not elevate eukaryote fatty acids above background levels 3 days after amendment. This was also the case here. No difference in the fungal biomarker C18:2(9,12) was observed after the addition of rice straw and the significantly higher C18:2(9,12) content observed in fractions > 200 µm of the amended soil after 21 days of incubation (data not shown) can be attributed to an enhanced development of fungi in larger aggregates after the addition of straw. It is now firmly established that fungi participate in aggregate formation through hyphal growth (e.g. Oades and Waters, 1991; Degens, 1997; Bossuyt et al., 2001) and this result corroborates what has been found before. However, the isotopic signature of this fungal biomarker (> 800‰, data not shown) suggested that there was a contribution of rice straw lipids to the FAME profiles measured, despite the fact that this was not reflected in total lipid contents. Moreover, neutral and glycolipid fatty acids can end up in Eukaryote peaks when lipids are analysed by the EL-FAME method (personal communication R.P. Dick). If there was a high production rate of neutral and glycolipids during the decomposition of the rice straw, these lipids may have masked the fungal marker signal. The ambiguous origin of certain lipids such as C18:2(9,12) meant that ecological interpretation of the profiles was not straightforward. Therefore, the potential impact of non-microbial fatty acids being included in the analyses was eliminated by removing all fatty acids

385 detected in the lipid extract of the rice straw. Therefore, the differences among fractions and as
386 a function of time in both total FAME and ^{13}C-FAME profiles (Figs. 5 and 6) were likely caused
387 by changes in bacterial communities rather than by plant derived FAMEs.

388 The rapidity with which the bacterial community structure in the *de novo* formed
389 macroaggregates diverged from the community structure of the other fractions was totally
390 unexpected. Biological communities are known to be structured through interactions with their
391 habitat (O'Malley and Dupre, 2007). This is particularly true for microbial communities:
392 microbial biogeographic studies have shown that contemporary environmental effects (i.e.
393 habitat) play a significant role in structuring microbial communities (Martiny et al., 2006; King
394 et al., 2010). Here, there was a clear differentiation in bacterial community structure among soil
395 fractions, confirming the results of others (Fall et al., 2004; Mummey and Stahl, 2004) and
396 suggesting that there is a microscale biogeography in soil (Mummey et al., 2006; Ruamps et
397 al., 2011). The differences in bacterial community structure were primarily due to higher
398 relative abundances of actinobacteria and other Gram-positive bacteria in the fractions < 200
399 µm and greater proportions of Gram-negative bacteria in macroaggregates, again in agreement
400 with previous studies (e.g. Mummey and Stahl, 2004). The higher relative abundance of Gram-
401 negative bacteria in the present study might result from their role in aggregation, through the
402 production of extracellular polysaccharides: Amellal et al. (1998) showed that water-stable
403 aggregates of rhizosphere soil could be increased by inoculating the soil with Gram-negative
404 bacteria. The differentiation in bacterial community structure might also be due to differences
405 in local conditions in the soil fractions and, in particular, the uneven distribution of organic
406 matter between soil aggregates (Elliott, 1986; Puget et al., 1995). Gram-negative bacteria are
407 thought to be r-strategist/copiotrophic organisms with a high growth rate, using labile substrate;
408 actinobacteria and most Gram-positive bacteria on the other hand, are thought to be K-
409 strategist/oligotrophic organisms that are better decomposers of less labile soil organic matter

but have a lower growth rate (Fierer et al., 2007; Elfstrand et al., 2008). More C derived from the rice straw was found in the macroaggregates, indicating that there was a more readily available C source for r-strategists such as the Gram-negative bacteria mainly found within macroaggregates. Finally, sample preparation may also have had an effect on the rapid change in bacterial community structure in the *de novo* formed macroaggregates. The soil was crushed (< 200 µm) to remove all the macroaggregates, possibly resulting in microbial cell death and the release of labile organic matter (Balesdent et al., 2000). If the labile organic matter thus released was primarily available to microorganisms in the *de novo* formed macroaggregates, this might explain the development of rapidly growing r-strategist type bacteria in these aggregates. Bacterial community succession was similar in all aggregate size fractions, with a higher relative abundance of Gram-positive and actinobacteria at the end of the incubation. This pattern of microbial succession, where rapidly growing r-strategist organisms are replaced by slower growing K-strategist organisms n the latter stages of organic matter decomposition, has been previously described (Fierer et al., 2007; Bastian et al., 2009; Baumann et al., 2009; Lerch et al., 2011). This study also produced the first evidence for greater variation in bacterial community structure over time in large aggregate size fractions (> 200 µm). These fractions were also the only ones in which the total C concentration (Fig. 2) and the straw-C concentration (Fig. 3) varied during the incubation, suggesting that the amplitude of change in bacterial community structure is closely related to changes in the microbial habitat

4.4. Dynamics of straw degrader communities

All the FAMEs studied were ^{13}C-labelled after only 2 days for the amended soil, indicating that the straw was rapidly incorporated into the bacterial biomass. This is consistent with the rapid mineralisation observed during the same period and has been observed by others previously (McMahon et al., 2005). The distribution of ^{13}C-labelling in FAME showed that both Gram-negative and Gram-positive bacteria were involved in the decomposition, which

435 again confirmed the results of McMahon et al. (2005). Using molecular methods for taxonomic
436 identification of bacteria (DNA- and RNASIP), Bernard et al. (2007) showed that the
437 assimilation of ^{13}C from wheat residues was composed exclusively of Gram-negative bacteria
438 after 14 days incubation. The difference in results may be due to the different methodological
439 approaches, soil and plant residue differences aside. DNA- or RNA-SIP required a very high
440 degree of ^{13}C-labelling compared to lipid-SIP in order to separate "heavy" from "light" nucleic
441 acids by density centrifugation and microorganisms that incorporated low levels of ^{13}C may
442 have remained undetected by DNA-SIP. The fact that Gram-negative bacterial lipids presented
443 between 2 and 19 times more ^{13}C-labelling than lipids characteristic of Gram-positive bacteria
444 in the > 50 µm soil fractions and unfractionated soil corroborates the assertion that DNA-SIP
445 may not be sensitive enough to detect low levels of labelling.

446 Principal component analysis showed that the degrader community structure followed
447 the same general trends as the whole unlabelled community: relatively more Gram-negative
448 bacteria in the largest fractions and an increase in the relative abundance of Gram-positive
449 (including actinobacteria) in all size fractions during the incubation (Fig. 5). Although all the
450 FAMEs analysed were enriched in ^{13}C, the evenness of the ^{13}C enriched lipid profiles relative
451 to the total lipid profiles (Figs. 6 and 7) suggested that the specificity of the bacterial degraders
452 was higher than the total bacterial community. These results are in agreement with that obtained
453 by Bernard et al. (2007). The lower evenness found in the largest size fractions (> 200 µm) also
454 suggested a higher specificity of degraders compared to the whole biomass. The evenness of
455 the labelling increased in the largest fractions between 2 and 21 days (Fig. 7), probably due to
456 the occurrence of cross-feeding (Gallagher et al., 2005) and also simply because nearly all soil
457 was present in macroaggregates by day 21. Furthermore, the FAME-C_{Straw}/C_{Straw} ratio (Fig. S1),
458 used as an indicator of the amount of biomass relative to the available resource in the habitat,
459 decreased for the largest soil fractions between 2 and 21 days of incubation. The fraction 200–

2000 µm showed the lowest evenness values after 21 days of incubation for the total and the degrader communities. The low evenness of the community in this fraction seems to be constant throughout the incubation, which might highlight a more stable habitat, in term of nutrient availability, than the > 2000 µm fraction.

5. Conclusions

This study provides a basis for understanding how the dynamics of microbial habitat formation through soil aggregation and fresh organic matter mineralisation drives microbial community structure. The study clearly shows that the variation in bacterial community structure was greater in the *de novo* formed habitats (large aggregate size fractions > 200 µm) than in the pre-existing ones (size fractions < 200 µm). Furthermore, the *de novo* formed habitats tended to have a greater proportion of fast growing organisms (i.e. r-strategists). These results are consistent with Odum's theory of ecosystem succession (Odum, 1969), which suggests that mature system tend to be more stable than the young ones and that young systems are characterised by high growth rates. Differentiation of bacterial community structure among different habitats was demonstrated to occur very rapidly (i.e. 2 days), indicating that bacterial community structure is closely linked to habitat. Using ^{13}C-labelling substrates, it was demonstrated that the active component of the bacterial communities, the straw degrader communities, were mainly present in, but not limited to, the macroaggregates.

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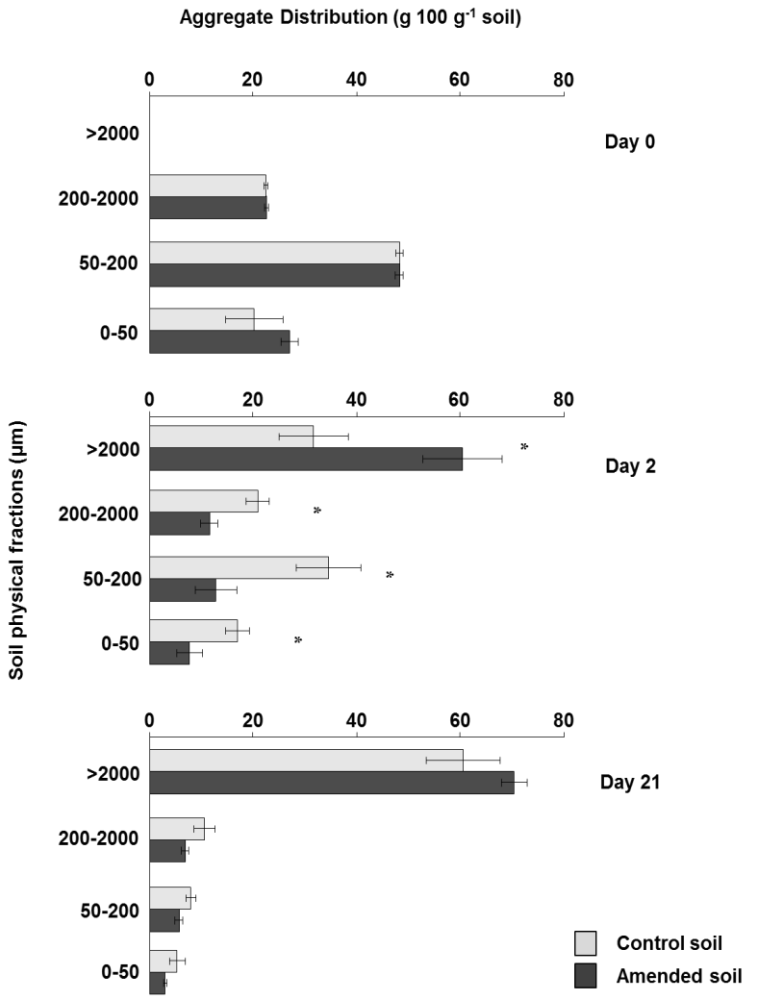
Figures and tables

Table 1: Cumulative amounts of C_{SOM} mineralised in control and amended soil expressed in percentage of initial C_{SOM}, and cumulative amount of C_{Straw} mineralised in amended soil expressed in percentage of initial C_{Straw}. Standard deviations were calculated for 3 experimental replicates.

Source of C mineralised	2 days	21days
%C_{SOM} in control soil	0.75±0.03	5.0±0.13
%C_{SOM} in amended soil	0.67±0.02	4.5±0.21
% C_{Straw} in amended soil	4.80±0.44	48.2±1.17



687

688 **Fig. 1.** Weight distribution of the soil fractions, in control soil (grey bars) and amended soil
689 (dark grey bars) through time. Error bars represent the standard deviation of 3 experimental
690 replicates. * show a significant difference between control and amended soil for a specific date
691 and soil fraction ($P < 0.05$).

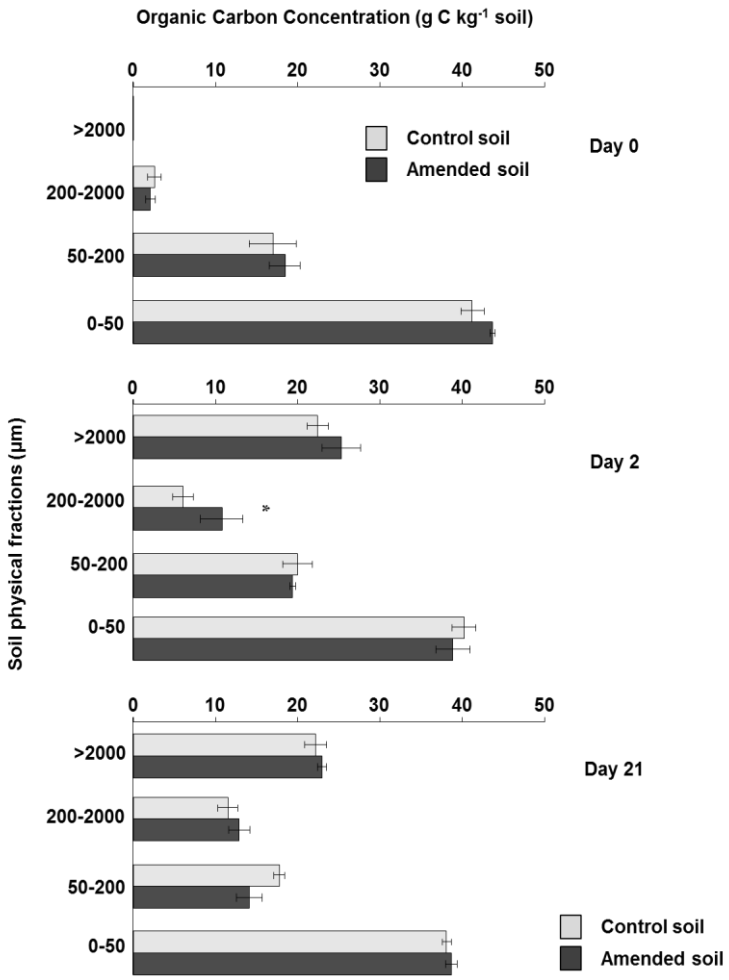


Fig. 2. Total organic carbon concentration of the soil fractions, in control soil (grey bars) and amended soil (dark grey bars) through time. Error bars represent the standard deviation of 3 experimental replicates. * show a significant difference between control amended soil for a specific date and soil fraction ($P < 0.05$).

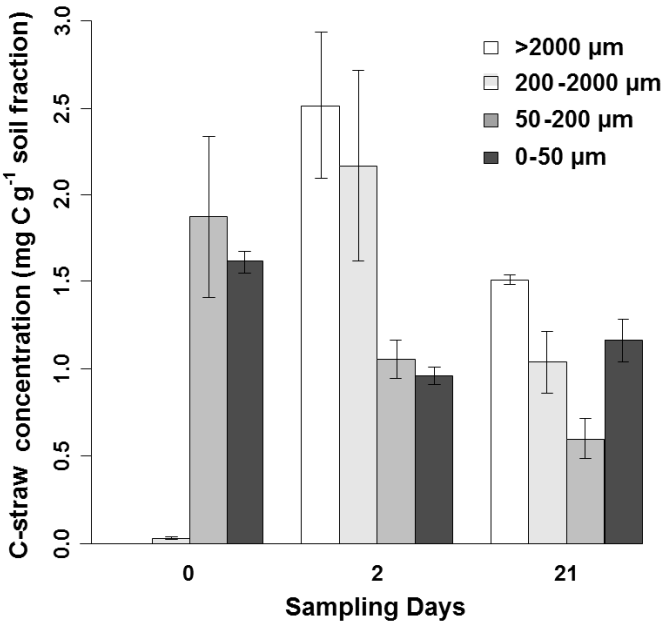


Fig. 3. Carbon concentration derived from the rice straw added (C_{straw}) in the soil fractions of the amended soil through time. Error bars represent the standard deviation of 3 experimental replicates.

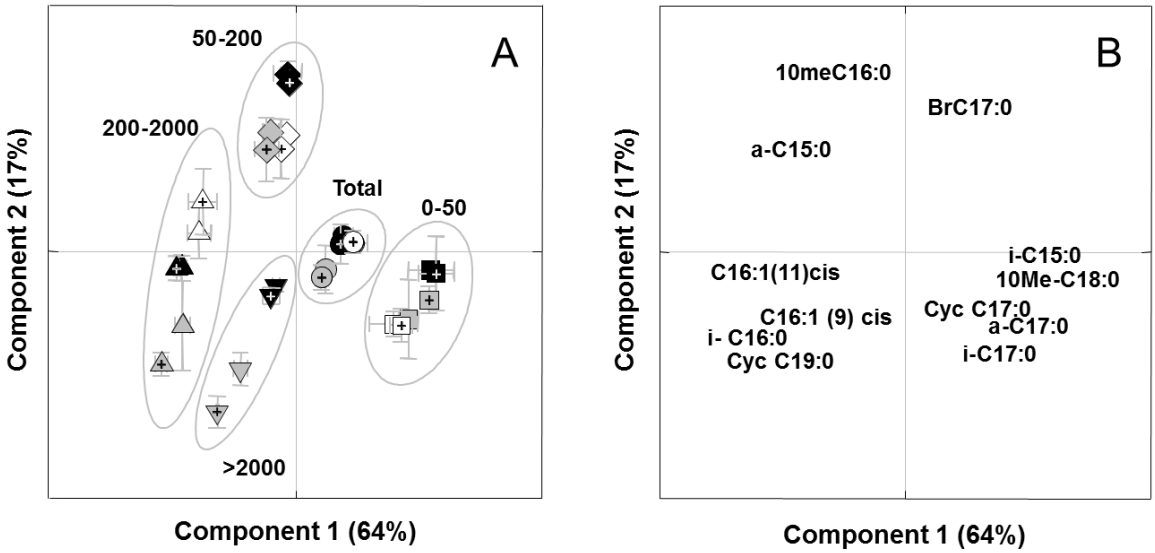
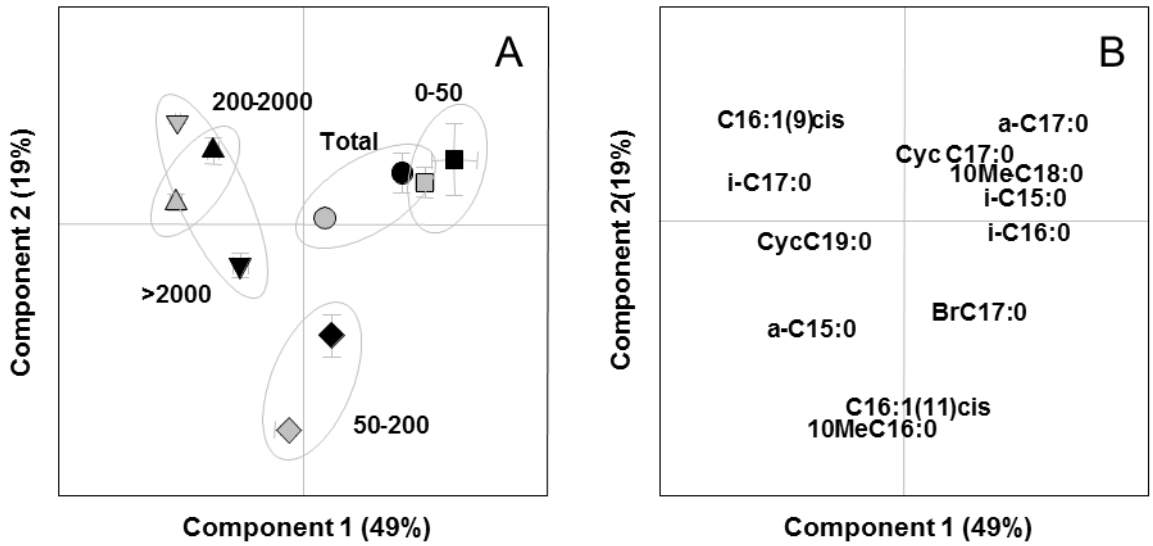
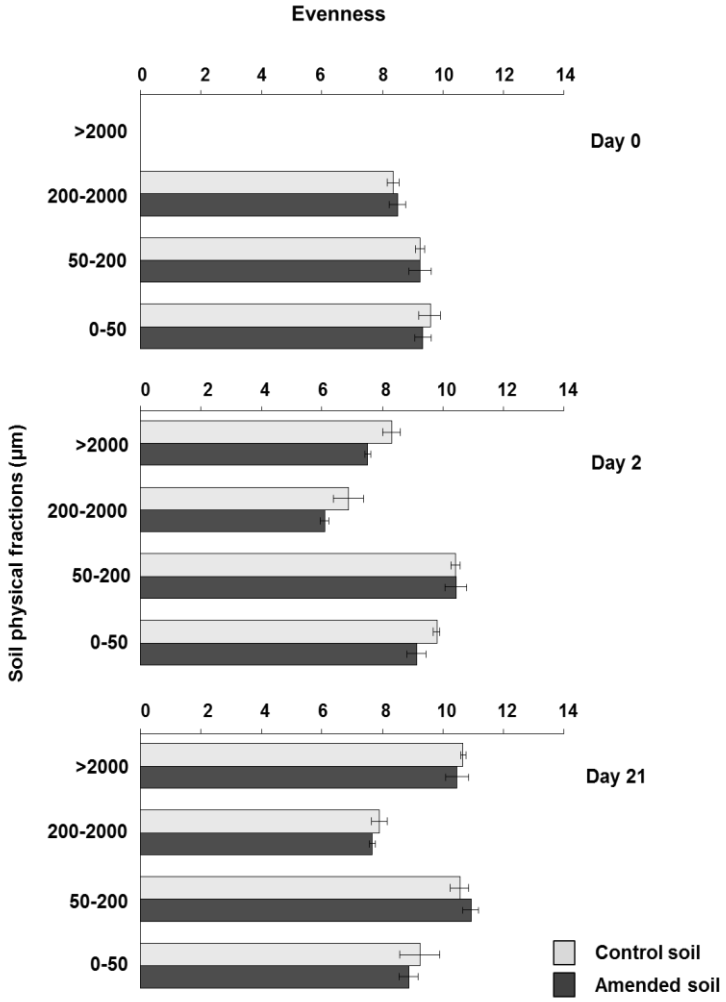


Fig. 4. Scores (A) and loadings (B) of the 2 main components of the PCA, representing 81% of the variability. Variables are the relative abundance of 12 FAME detected between Day 0 (white), Day 2 (grey) and Day 21 (black) of the incubation for each soil fractions and the unfractionated soil (Total) from non-amended and amended soil (cross within symbols). Square: 0-50 µm; Diamond: 50-200 µm; Triangle: 200-2000 µm; Inverted triangle > 2000 µm. Standard deviations of each soil fraction correspond to 3 replicates of sampling dates.



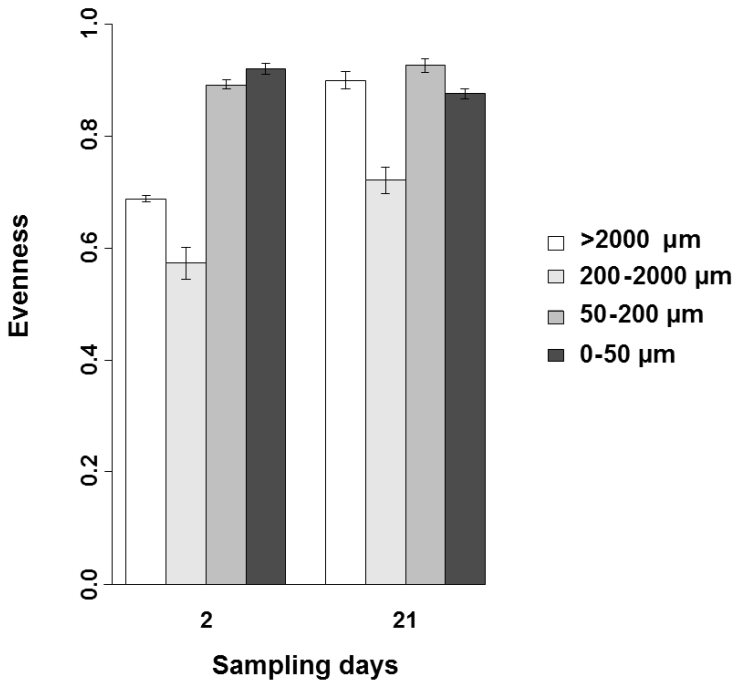
732

733 **Fig. 5.** Scores (A) and loadings (B) of the 2 main components of the PCA, representing 68% of
734 the variability. Variables are relative abundance of C_{Straw} in the 12 FAME detected between
735 Day 0 (white), Day 2 (grey) and Day 21 (black) of the incubation in each soil fractions and
736 unfractionated soil (Total) from amended soil. Square: 0-50 µm; Diamond: 50-200 µm;
737 Triangle: 200-2000 µm; Inverted triangle > 2000 µm. Standard deviations of each soil fraction
738 correspond to 3 replicates of sampling dates.



739

740 **Fig. 6.** Evenness index of total FAME from each soil fraction, in control soil (grey bars) and
741 amended soil (dark grey bars) through time. Error bars represent the standard deviation of 3
742 experimental replicates.



743

744 **Fig. 7.** Evenness index of ^{13}C-labelled FAME from each soil fraction of the amended soil

745 through time. Error bars represent the standard deviation of 3 experimental replicates.

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Supplementary information

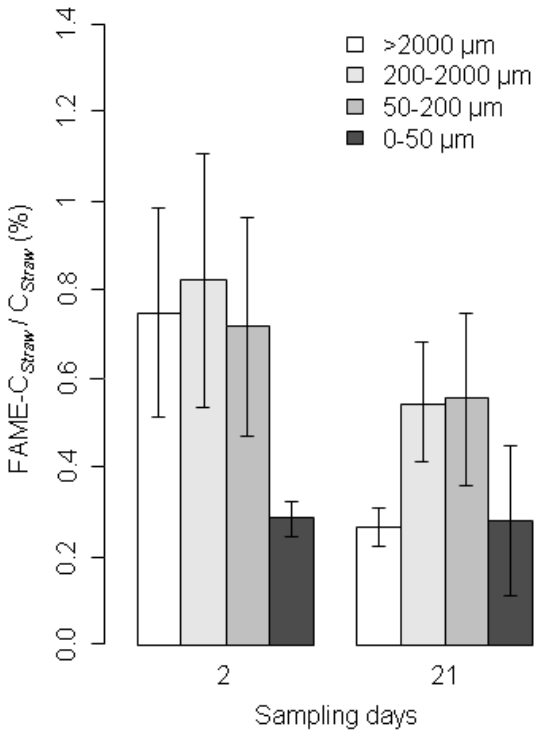

Fig. S1. Proportion of FAME derived from ^{13}C-labelled straw relative to the residual ^{13}C-labelled C among soil fractions, after 2 and 21 days of incubation. Standard deviations of each soil fraction correspond to 3 experimental replicates.