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REGULAR ARTICLE

The chemical composition of native organic matter influences the response of bacterial community to input of biochar and fresh plant material

Congying Wang · Craig Anderson · Manuel Suárez-Abelenda · Tao Wang · Marta Camps-Arbestain · Riaz Ahmad · H. M. S. K. Herath

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Abstract
Aim To investigate how the chemical composition of native organic matter of two contrasting soils varies with inputs of biochar and fresh material (including plant roots) and how these underlying changes influence microbial community structure.
Methods Corn stover (CS) and CS-derived biochars produced at 350 °C and 550 °C were applied at a dose of 7.2 t C ha^{-1} to two contrasting soils—an Alfisol and an Andisol. After 295 days of incubation, two undisturbed subsamples from each pot were taken: (i) in one, lucerne (*Medicago sativa* L.) was seeded (plant study, P) and (ii) in the other, the incubation was continued without the plants (respiration study, R); all subsamples were incubated for an additional 215 days. Soils without amendments were used as controls. At the end of the incubation (510 days), their bacterial community profiles were characterised using ARISA and the molecular composition of soil organic matter (SOM) was investigated by pyrolysis-GC/MS.
Results There were significant interactions between soil type, study type (P or R) and organic amendment. Organic amendments influenced overall SOM composition with microbial community response being mainly influenced by soil type but also strongly affected by the presence or absence of plants. For a specific soil type, ≥ 40 % of total variation in bacterial community ordination could be explained by the molecular composition of SOM.
Conclusions The molecular composition of SOM is proposed as an important factor influencing the microbial response to organic amendments, including biochar.

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C. Wang · T. Wang · M. Camps-Arbestain (✉) · R. Ahmad · H. M. S. K. Herath
New Zealand Biochar Research Centre, Institute of Agriculture and Environment, Private Bag 11222, Massey University, Palmerston North 4442, New Zealand
e-mail: M.Camps@massey.ac.nz

C. Wang
School of Environmental Science and Resources, Shanxi University, Taiyuan 030006, China

C. Anderson
The New Zealand Institute for Plant and Food Research, Private Bag 4704, Christchurch 8140, New Zealand

M. Suárez-Abelenda
Departamento de Edafoloxía e Química Agrícola, Facultade de Bioloxía, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

R. Ahmad
Department of Soil Science & Soil Water Conservation, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

H. M. S. K. Herath
Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla 9000, Sri Lanka

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Introduction

Organic amendments (OA) are commonly applied to agricultural soils to improve soil functions by enhancing soil carbon (C) storage, water holding capacity, aggregate stability and nutrient availability (Diacono and Montemurro 2010; Hargreaves et al. 2008) and thus help to increase crop yield. Simultaneously, OA can exert an effect on soil microbiological properties, through stimulating microbial growth (Schutter and Dick 2001; Elfstrand et al. 2007; Bastida et al. 2008) and/or influencing microbial community structures and their physiological functions (Gomez et al. 2006; Ng et al. 2014; Ros et al. 2006). These soil microbial factors are, in turn, considered as major players in manipulating soil organic matter (SOM) transformation and nutrient cycling (Costanza et al. 1997; Ng et al. 2014; Rutigliano et al. 2014). Knowledge of the interactions between soil microbial communities, OA and SOM dynamics is essential for predicting the fate of OA and the turnover of SOM as well as the responses of microbial community composition to OA additions (Fontaine et al. 2003; Goyal et al. 1999; Ng et al. 2014; Van Horn et al. 2013). However, due to the high complexity of both soils and OA along with environmental factors controlling microbial profiles, the extrapolation of patterns describing their interactions from one scale to another (e.g., local to regional scale) is always a challenge (Van Horn et al. 2013).

Many environmental variables, such as soil pH, ionic strength, moisture content, depth, SOM content and nutrient status (Fierer and Jackson 2006; Fierer et al. 2003; Van Horn et al. 2013; Zhou et al. 2002) have been proposed to explain the variation in soil microbial diversity and structure. However, ca. 50 % of this variation remains unexplained by the above-mentioned characteristics (Van Horn et al. 2013). This highlights the critical role that the quality of SOM (including OA and other inputs from roots) has in governing the structure and functions of the microbial community (Bending et al. 2002) and therefore requires an in-depth understanding of the OA and SOM chemistry at a molecular scale.

Organic amendments are often applied after certain "pre-processing" treatments, such as composting and pyrolysis, through which viable pathogens are eliminated (Ng et al. 2014). Conversion of organic wastes into biochars through pyrolysis has been promoted as a novel solution to improve soil fertility as well as to increase soil C sequestration (Lehmann et al. 2006; Lehmann et al. 2011). The greatest chemical difference between biochar and the feedstock (e.g. wood, litter, compost, manure and biosolids) from which biochar is produced, is the much larger proportion of condensed aromatic C in the former, for which a greater activation energy is needed to mineralise (Lehmann et al. 2015). Biochar application has been found to affect microbial diversity either positively (Gomez et al. 2014; Grossman et al. 2010; Kim et al. 2007), neutrally (Watzinger et al. 2014) or negatively (Khodadad et al. 2011). These paradoxical results could arise from the large variability in biochar properties and in those of soils to which biochar is applied, as well as their corresponding interactions. Variations in biochar and soil properties make individual microcosms and the microbial community response distinctive with respect to; i) nutrient status and C availability; ii) pH; iii) bacterial adhesion capacities; iv) capacity for protecting bacteria from predators; and v) effects of toxins and other environmental pressures, such as water stress (Lehmann et al. 2011). However, the effect of biochar on microbial community composition can be over-shadowed by the input of labile C sources from root exudates and the time elapsed since biochar application, both of which are key factors influencing SOM chemistry in laboratory incubations (Prayogo et al. 2014; Suárez-Abelenda et al. 2015). Therefore, it is hypothesised that the chemical nature of native SOM and fresh C input from plant roots are key determinants that influence the changes in soil bacterial community profiles following biochar addition. Ng et al. (2014) has pioneered the studies on the influence that biochar amendments have on the structure of soil microbial communities. Their results using a green waste biochar indicated that the structure of microbial communities was shaped by the chemical nature of soil C (which explained ≥50 % of the variation), as characterised by ^{13}C NMR spectroscopy. This technique enables acquisition of overall information regarding bulk SOM, but it is not adequate to monitor subtle changes in C forms of certain SOM fractions (more likely to be labile C) that are closely linked to microbial metabolism. Pyrolysis-GC/MS (Py-GC/MS)

can be used for such purposes as it characterises the molecular composition of volatiles derived from the pyrolysis of SOM. Volatile fractions are sometimes used as a proxy for labile C fractions (Wang et al. 2012; Zimmerman et al. 2011) and their composition is sensitive to slight alterations in SOM. Pyrolysis-GC/MS is often regarded as a complementary tool to NMR techniques (Kaal et al. 2007).

The objective of this study was to investigate how the chemical composition of native organic matter of two contrasting New Zealand pasture soils (an Alfisol and an Andisol) varies with inputs of biochar and fresh material (including plant roots) and how these underlying changes influence bacterial community profiles. To establish this, three OAs having an identical amount of total C but differing in the degree of C stability were added to soils and incubated for 510 days. The amendments were corn stover feedstock (CS) and two biochars produced from CS at 350 °C (CS-350) and 550 °C (CS-550). Incubations with (the plant study, P) and without plants (the respiration study, R) were compared to study the effect of fresh C input on the chemical composition of SOM and the microbial properties. Pyrolysis-GC/MS was used to characterise SOM at the end of the incubation (T510). Automated ribosomal intergenic spacer analysis (ARISA) was used to characterise the bacterial community profiles of the different soils at T510. This study was part of a larger project in which the stability of C in the OA was evaluated, along with their fate in physically- and chemically-defined organic C fractions and their effect on the decomposition of native SOM (Herath et al. 2014a, b). Changes in the chemical composition of SOM of the unamended soils over time in the presence and absence of roots have also been studied (Suárez-Abelenda et al. 2015).

Materials and methods

Soil samples and biochars

A Typic Fragiaqualf (Alfisol, Tokomaru silt loam, TK) soil and a Typic Hapludand (Andisol, Egmont black loam, EG) soil were used for the study. Details on the soil collection and sample preparation have been provided by Herath et al. (2013). The TK soil was developed on loess with a layer of Aokutere ash (Rongotea, New Zealand) and is rich in silt and clay, which is dominated by 2:1 type clay minerals. The EG soil was developed on andesitic volcanic ashes of Oakura and Okato tephra (Wanganui-Hawera, New Zealand) and is dominated by allophane. Selected characteristics of these soils are shown in Table 1 (after Herath et al. 2014b). Briefly, soils had similar pH values (5.7 for TK and 5.8 for EG) whereas total C was 41.7 and 102.0 g kg^{-1} for TK and EG, respectively. Mineral N values were below 290 mg kg^{-1} and Olsen P ranged from 34 to 45 mg kg^{-1}. Pyrophosphate-extractable Al was 1.1 g kg^{-1} in the TK soil, and 7.3 g kg^{-1} in the EG soil. The effective cation exchange capacity (ECEC), determined according to Matsue and Wada (1985), was 17 and 29 cmol+kg^{-1} for TK and EG soils, respectively. Pyrolysis-GC/MS analysis of the TK and EG soils as described by Herath et al. (2014a) and Suárez-Abelenda et al. (2015) showed that the SOM in the TK soil had a relatively greater contribution of lignocellulose material than the EG soil; the latter had a more dominant contribution of degraded OM.

Corn stover (CS, *Zea mays* L.) collected from the Manawatu region (North Island, New Zealand) was cut into chips (<5 mm) and used as the feedstock for biochar production. Biochar was produced at final heating temperatures of 350 and 550 °C, with the resulting products referred to as CS-350 and CS-550, respectively. The selected physico-chemical properties of fresh corn stover (CS) and the two biochars have been described by Herath et al. (2013, 2014b) and are summarised in Table 2. Briefly, total C contents were 41.3, 63.5 and 71.7 % for CS, CS-350 and CS-550, respectively. Ash contents ranged from 10 to 12 % and fixed C (i.e. 100 %-ash%-volatiles%) ranged from 14 to 69 %. CS had the highest molar H/C ratio (1.98), whereas those of CS-350 and CS-550 were 0.64 and 0.45, respectively. Values of inorganic C were<1 % (dry weight basis) (data not shown). Liming equivalence was 110 and 127 g $CaCO_3$-eq kg^{-1}, for the CS-350 and CS-550 biochars, respectively.

Incubation experiment

The incubation experiment was conducted in PVC columns (100 mm in height and 150 mm in diameter) with the following treatments: i) control soils without amendment (TKc, EGc); ii) soils amended with fresh CS (TKCS, EGCS); iii) soils amended with biochar (TK350, TK550, EG350, EG550). All treatments were carried out in triplicate. Details of the experimental setup have been described by Herath et al. (2014b). Briefly,

Table 1 Main characteristics of soils used in this study (after Herath et al. 2014b)

Soil	pH	Total C g kg^{-1}	Mineral N mg kg^{-1}	Olsen P mg g^{-1}	Al_p[a] g kg^{-1}	ECEC[b] $cmol_+$ kg^{-1}	Main mineral types
TK	5.7	41.7	290	45	1.1	17	Micaceous clay minerals
EG	5.8	102.0	285	34	7.3	29	Allophane, kaolinite

TK denotes Tokomaru silt loam and EG Egmont black loam

[a] pyrophosphate extractable Al

[b] Effective cation exchange capacity

each organic amendment was added at a dose equivalent of 7.18 t C ha^{-1}. The water content was maintained at 70 % of water holding capacity (WHC) by adding water every two days. The incubation was conducted at room temperature of 20±6 °C.

After 295 days, two undisturbed subsamples (100 mm in height and 65 mm in diameter) were taken from each pot. One subsample was continuously incubated to investigate the influence of biochar on native SOM mineralisation (referred to as the respiration study, R), while the other one was planted with lucerne seed (*Medicago sativa* L.) to study the effect of interactions between newly plant-derived SOM and biochar on native SOM (henceforward referred to as the plant study, P) (for more details refer to Herath et al. 2014a). The water contents of the treatments with plants could not be maintained accurately at 70 % WHC when plant biomass increased drastically. Instead, the plant pots were watered to a similar weight to that of their corresponding non-plant treatments (according to Zhou et al. 2002, slight differences in water content does not influence microbial profiles significantly in C-rich soils such as those used in this study). The experiment was incubated for an additional 215 days, with the entire incubation duration being 510 days.

Pyrolysis-GC/MS analysis of soils

At the end of the incubation (510 days), and for both studies (R and P), soil samples were collected from each pot, then those of the same treatment- and study-type (e.g., R study, control treatment) were pooled together (n=3), thoroughly mixed, air-dried and ground prior to Py-GC/MS analysis. Justification for the pooling of samples is provided in the Supplementary Information (Fig. S1). In our previous studies (unpublished data), we found the variations in the relative abundances of identified pyrolysis products between replicates within the same treatment were relatively small (usually <10 % of the relative abundance of same compound) (Table S1). Therefore, we chose one mixed subsample for each treatment, which explains the absence of error bars in Fig. 1. Soil samples were treated with 2 % hydrofluoric acid (HF) overnight (for 4 times), thoroughly rinsed with deionised (DI) water and freeze-dried prior to analysis with Pt filament Py-GC/MS. Analysis was performed using a Pyroprobe 5000 (Chemical Data Systems, Oxford, USA) coupled to a 6890N GC and 5975B MSD GC-MS system (Agilent Technologies, Palo Alto, USA). Finely ground subsamples (ca. 1 mg) were embedded in fire-polished quartz tubes that

Table 2 Selected physicochemical properties of fresh corn stover (CS) and two biochars produced from CS (CS-350 and CS-550, respectively), all the data are presented on a dry weight basis (from Herath et al. 2013)

Sample	C %	N %	H %	O[a] %	Ash %	Fixed carbon %	Volatiles %	Molar H/C	Molar O/C
CS	41.3	0.83	6.08	40.66	11.4	14.6	74.1	1.98	0.74
CS-350	63.5	0.71	3.77	21.62	10.0	58.2	31.8	0.64	0.26
CS-550	71.7	0.76	2.92	13.55	11.8	69.2	19.0	0.45	0.14

[a] determined by difference as O%=[100 – (C+H+N+S+Ash)]%

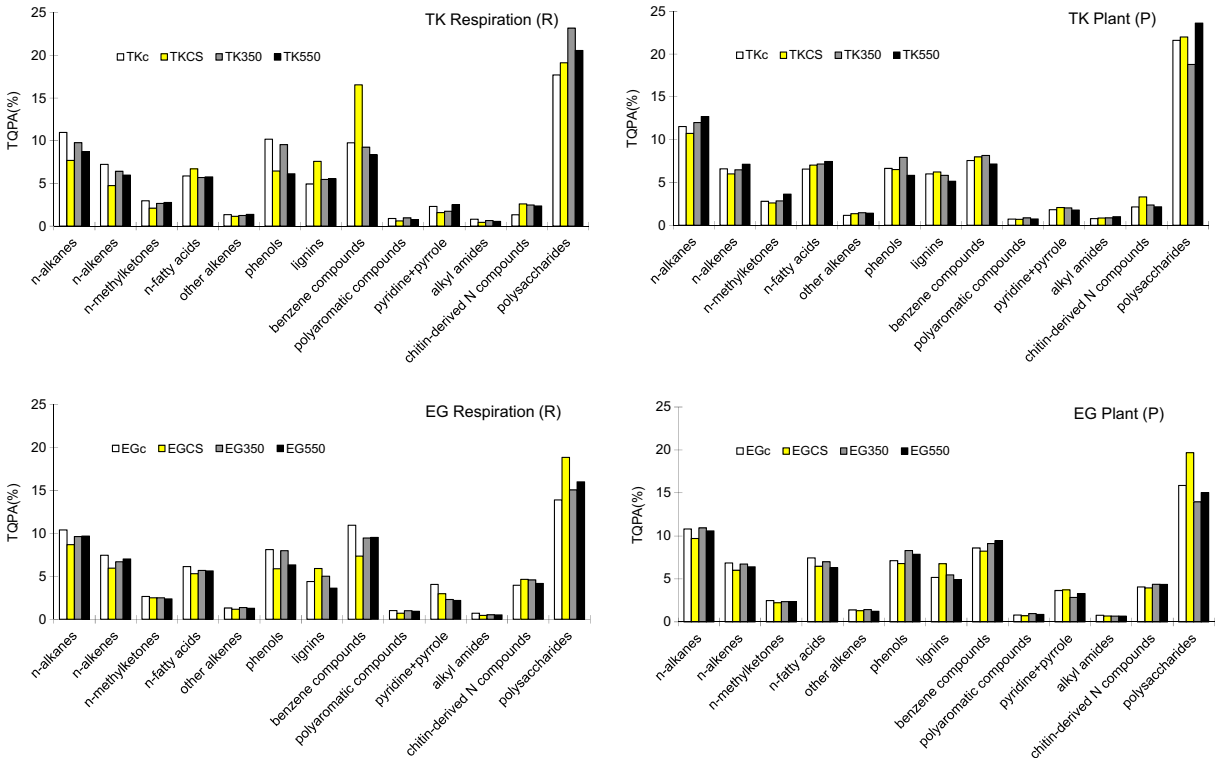

Fig. 1 Relative abundances of pyrolytic compounds grouped according to their possible origins. *TK* Tokomaru silt loam soil, *EG* Egmont black loam soil, *CS* corn stover, *CS-350* biochar produced from CS at a peak temperature of 350 °C, *CS-550* biochar produced from CS at a peak temperature of 550 °C. *Error bar* was not available as replicates of each treatment were pooled prior to Py-GC/MS measurements (justification provided in Fig. S1 and Table S1)

contained glass wool. The subsamples were pyrolysed at 550 °C for 10 s (10 °C ms^{-1}).

The temperature for both interface and the GC inlet (in splitless mode) was at 325 °C. The GC oven was heated from 50 to 325 °C (held 2 min) at 15 °C min^{-1}. The GC-MS transfer line was maintained at 325 °C, the ion source (electronic ionisation mode, 70 eV) at 230 °C and the quadrupole detector at 150 °C; scan range was *m/z* 50–500. The GC instrument was equipped with a (non-polar) HP-5MS 5 % phenyl, 95 % dimethylpolysiloxane column (30 m×0.25 mm i.d.; film thickness 0.25 μm). Helium was the carrier gas (flow rate: 1 ml min^{-1}).

A total of 215 pyrolysis products were identified according to their dominant mass ions (*m/z*) (Supplementary Information Table S2). The areas of peaks were calculated employing the Masslab software (Version 1.2.7; Fisons, Ipswich UK). The relative abundances of the pyrolysis products were expressed as the proportion (%) of total quantified peak area (TQPA; the sum of all identified peak areas). These were grouped based on their possible sources (Buurman et al. 2007; Suárez-Abelenda et al. 2014) as follows: (i) *n*-alkanes ($C_{9:0}$ – $C_{33:0}$); (ii) *n*-alkenes ($C_{9:1}$ – $C_{30:1}$);; (iii) *n*-methyl ketones ($C_{13:0}$ – $C_{35:0}$; K); (iv) *n*-fatty acids ($C_{5:0}$ – $C_{24:0}$; FA) and (v) other alkenes (Al); (vi) phenol and alkyl phenols (Ph); (vii) lignin-derived methoxyphenols (Lg); (viii) benzene-containing compounds (including Ar+B_3-B_{28}), (ix) polycyclic (PA + NA) aromatic hydrocarbons; (x) N-containing products, (xi) polysaccharide fragments (Ps), and (xii) sterol derived compounds (St). Due to the multiple possible origins of N-compounds, this group was split into three sub-groups: i) pyridines + pyrroles (associated to microbial-derived fractions when present along with tracers of degraded SOM); ii) chitin-derived N compounds (N5+N12, fungi- and arthropods-derived), and (iii) alkyl amides (AM) and indoles (N8, N9) − likely plant-derived, and nitriles (N6, N7) from charred material (Buurman et al. 2007; Suárez-Abelenda et al. 2014).

DNA extraction, amplification and ARISA fingerprinting

The application of ARISA enables a rapid assessment of changes in the bacterial community by exploiting the

size heterogeneity of the internal transcriber spacer (ITS) region between bacterial lineages (Havemann and Foster 2008; Khodadad et al. 2011). Two sets of soil samples were collected after 510 days from all treatments: one set represented soil from treatments that were planted with lucerne (P), the other set from the plant-absent respiration study (R). Only bulk soil samples without fresh roots from the plant study (P) were used for microbial and chemical tests. All samples were stored at −80 °C until DNA extraction.

Genomic DNA was extracted from 0.5 g (wet wt.) of each soil sample using a Powersoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Genomic DNA extractions were stored at −20 °C prior to amplification using polymerase chain reaction (PCR).

PCR was performed in 50-μl (final volume) mixtures containing 5 μl 10×reaction buffer (Invitrogen, Carlsbad, Calif.), 4 % (*v*/*v*) bovine serum albumin, 3 % (*v*/*v*) dimethyl sulfoxide, 1.25 mM $MgCl_2$, and each dNTP (Invitrogen) at a concentration of 0.1 mM. The bacterial primer set used (Integrated DNA Technologies, Coralville, IA, USA) was ITSF (5′ -GTCGTAACAAGGTAGCCGTA-3′) and ITSReub (5′-GCCAAGGCATCCACC-3′) (Cardinale et al. 2004). The forward primer (ITSF) was labelled with the fluorochrome 6-FAM (6-carboxylfluorescein) on the 5'end. The primer concentrations were 0.1 mM each, and 0.05 U of Taq DNA polymerase (Invitrogen) was added. Five μl of undiluted, 1/10 or 1/20 diluted genomic DNA were tested as template additions to assess for the presence of co-extracted PCR inhibitors, with 1/20 dilutions having the highest success rate and strongest amplification when visualised on 1 % agarose gels. The initial denaturation (95 °C for 1 min) was followed by 35 cycles consisting of a denaturation step at 94 °C for 1 min, primer annealing at 55 °C for 30 s, and extension at 72 °C for 1.5 min. There was a final extension step of 72 °C for 20 min. PCR products were stored at -20 °C until they were cleaned and analysed (usually less than 1 week).

PCR products were cleaned and desalted using ultrafiltration and the concentration (ng μl^{-1}) was then determined using a Nanodrop ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE). Appropriate volumes of cleaned PCR product (diluted with ultrapure H_2O if necessary) providing a final mass of DNA in the 20 to 50 ng range were then transferred to a 96-well sequencing plate and dried in a speedvac for 1 h at 60 °C. The dry sample was resuspended in 15 μl Hi-Di deionised formamide containing 0.25 μl of Genescan LIZ-1200 internal size standard. The sample was heated for 5 min at 95 °C to denature the DNA prior to ARISA analysis, which was carried out on an ABI 3130xl genetic analyser with POP7 chemistry and a 36 cm array (Applied Biosystems).

Data analysis

ARISA electrophoretograms were first checked for stable baselines, voltage and calibration in the range 100–1000 bp. If samples exhibited, abnormalities or maximum peak heights above a relative fluorescence intensity (RFI) of 6000 they were diluted and rerun to reach a target closer to the vicinity of 1000 RFI. Absolute peak heights and areas were initially established using PeakScanner software v 1.0 (Applied Biosystems) using a minimum of 10 fluorescence units. Data for peaks between 150 and 800 bp was exported and final minimum peak height threshold was determined with the T-REX online analysis tool (http://trex.biohpc.org/) (Culman et al. 2009) by removing peaks whose height was less than twice the standard deviation iteratively computed over all peaks. The data comprising the area of these 'acceptable' peaks was exported and peaks were binned using a custom R script 'interactive binner' (Ramette 2009) using a RFI cuttoff of 0.09 %, a window size of 2 and a shift size of 0.1. Further peaks were removed by this process and the window size was chosen to account for calibration errors and electrophoresis migration differences likely to occur within in the size range chosen (150–800 bp) and the 36 cm array used. The resulting standardised samples-by-binned-operational taxonomic unit (OTU) tables where then analysed using Primer 6 with PERMANOVA add-on (Primer-E Ltd, Plymouth, UK). A Bray-Curtis similarity matrix was calculated and the relationships among individual bacterial community profiles were then graphed using unconstrained multidimensional scaling (MDS) ordinations based on 250 restarts. Any observed relationships were then statistically tested using a 3-factor permutational ANOVA (PERMANOVA) via estimated components of variation. The Bray-Curtis similarity measures were then linked to the soil SOM data using a distance-based model (using the DISTLM routine to perform partitioning, test hypotheses and build models, with the additional dbRDA routine selected to produce an ordination of fitted values from the models) to

determine the SOM components that could best explain the variation in the bacterial ordination patterns. The 'Best' selection procedure was selected to build the final model because it examines the value of the selection criterion for all possible combinations of predictor variables to produce the model with the lowest residual SS. 'Adjusted R^2' was used as the selection criterion because it only accounts for the combination of predictor variables used to produce the model. The above-mentioned compound groups of pyrolysis products were used as predictor variables to simplify the distance-based model analysis. Sterol-derived compounds (St) were not included in the analysis because it was only found in trace amounts. The predictor variables for the DISTLM model are categorical (i.e. levels of factors) compared to usual regression models where the predictor variables are quantitative or continuous. The LINKTREE procedure was used for building multivariate regression trees (MRT) to provide further support for the DISTLM/dbRDA analysis. The LINKTREE procedure splits samples into logical groups based on their similarities and dissimilarities and then matches the 'best' variables from a complementary predictor data set to provide possible explanations as to why the samples have been split at each specific tree node. A minimum group size of 5 was selected with a minimum split size of 4. LINKTREE outputs a B% value that is a measure of absolute group differences at each tree node using ranks derived from the similarity matrix. LINKTREE also outputs ANOSIM R values (R=0 means 100 % similar, R=1 means completely dissimilar) to describe the relationships between samples split at each tree node along with threshold values for predictor variables that describe how the chemical data is related to the samples split at each node.

Factor analysis was carried out on the semi-quantitative pyrolysis results using Statistica software (Statistica 8, Statsoft Inc., Tulsa, OK, USA) following Buurman et al. (2007) to assess the underlying structure and biochemical composition of SOM in the different treatments (2 soil types × 2 study types × 3 OA types). The data set was centred log ratio transformed prior to factor analysis. In total 215 variables (identified pyrolysis compounds) × 16 soil samples composed the data matrix. The eigenvalues, factor loadings and factor scores were calculated by extracting factors (Buurman et al. 2007). The variance-explaining value of the first factor was largest, sequentially followed by the second factor, the third factor and so on. In this study, only the first two factors (which explained >50 % of the total variance as described in the Results section) were selected. Components with loadings of less than 0.5 were not considered (Buurman et al. 2007).

Results

Molecular composition of SOM at day 510 (T510) and its factor analysis

General description In the R study, the sum of the abundances of aliphatic compounds (*n*-alkanes, *n*-alkenes, *n*-methyl ketones, *n*-fatty acids and other alkenes) in the TKc and EGc soils was larger than their OA-amended counterparts (>28 % vs. <25 %), and this was mostly attributed to the greater contribution of *n*-alkanes and *n*-alkenes in the former (Fig. 1). In the P study, this trend was also observed for the EG soil, although less accentuated, but not for the TK soil. The general relative abundances of these compounds (sorted in decreasing order) were *n*-alkanes > *n*-alkenes ≥ *n*-fatty acids > *n*-methyl ketones in all soils (Fig. 1).

In the R study, the abundance of phenols in the control and the CS-350-treated soils was larger than that of the other two treatments. This trend was also evident in the P study, although attenuated. The sum of all lignin moieties in the TKc soils (mean: 5.5 %) were found to be slightly larger than those in the EGc treatments (4.8 %) (Fig. 1). Irrespective of the study type, the application of fresh CS and poorly carbonised CS (CS-350) tended to increase—with few exceptions − the relative abundances of lignin biomarkers in both soils, compared to their corresponding controls, with the fresh CS amendment inducing a larger increment, as expected.

The relative abundances of the aromatic fraction (i.e., benzene compounds and polyaromatic compounds) did not follow any particular trend and were apparently not influenced by OA type, soil type or study type, probably because these compounds can have multiple origins and are thus non-selective pyrolysis products (Schulten et al. 1991). In terms of the total abundance of the N-compounds identified—pyrroles, pyridines, indoles, nitriles, and chitin-derived compounds (Table S2) −, the EG soils had higher values than the TK soils (data not shown). Both OA application and lucerne growth induced increases in the relative abundances of total N-compounds in the TK soils but not in the EG soils (data

not shown). In the R study, the sum of the total abundances of pyridines, pyrroles and indoles tended to decrease in the presence of OA, but this trend was not clear in the P study (data not shown). In general, the EG soils showed higher abundances of chitin-derived N compounds than the TK soils (Fig. 1). Neither OA nor plant growth had a clear influence on the relative abundances of these compounds in the EG soils but OA caused an increase in the TK soils in the absence of plants (Fig. 1).

In both study types, the abundances of polysaccharides were higher in the TK soil treatments (17–24 %) than their corresponding EG soil counterparts (13–20 %) (Fig. 1). Application of fresh CS increased the contribution of polysaccharides in both soils regardless of the study type. In the R study, biochar amendments tended to increase the abundances of polysaccharides, whereas the no clear trend was observed in the study with plants.

Factor analysis Factor analysis was carried out based on the relative abundances (% TQPA, Table S2) of pyrolysis compounds. Six factors resulted in a total explained variation of 78 %. Factor 1 (F1) and factor 2 (F2) explained ca. 50 % of the total variation. The distribution of scores and loadings from samples on F1 and F2 are shown in Fig. 2a and b.

In the plot of factor scores (Fig. 2a), samples were clearly separated in the F1F2 space according to soil type and study type. Soils from the R study plotted in positive values of F1 whereas soils from the P study were located in the negative projection. Moreover, F2 tended to separate the Andisol samples (EG) from those of the Alfisol (TK) (positive vs. negative values, respectively). Biochar-amended soils generally plotted to the right (i.e., to the largest positive values of F1) of their respective controls. For the F2 space the response of SOM composition to the amendments was influenced by the presence or absence of plants as follows. In the R study, OA-amended (both CS and biochar) soils plotted with the most negative loadings of F2 (especially the CS-amended samples), away from their corresponding controls. In contrast, OA-amended soils in the P study generally plotted towards positive values of F2. The distances between OA-amended soils and their controls were shorter in the P study than in the R study.

Figure 2b presents a graphical representation of the F1F2 loadings. The F1 negative loadings, which corresponded to samples from the P study, were dominated by long-chain *n*-alkanes ($C_{23:0} - C_{33:0}$), *n*-alkenes ($C_{28:1} - C_{30:1}$), long-chain *n*-fatty acids (including $C_{16:0}$ and $C_{24:0}$), a C_1 side-chain syringyl lignin moiety (4-methyl syringol, Lg7), syringol (Lg6), C_1 and C_2 side chain guaiacol (methyl and ethyl guaiacol, Lg2 and Lg4, respectively), an ethyl phenol (Ph4) along with large contents of N-containing compounds [including phenyl pyridine (N10) and methyl pyrrole (N3), methyl indole (N9), diketodipyrrole (N11), a nitrile compound (N7); and finally a regular series of alkyl amides (AM20 to AM34)].

The positive side of F1, which was occupied by samples from the R study in factor scores, was dominated by mid- to short-chain *n*-alkanes ($<C_{23}$) and *n*-alkenes ($<C_{28:1}$) along with benzene-containing compounds and PAHs, and polysaccharide-derived compounds including: furaldehydes (Ps3 and Ps4), levoglucosenone (Ps9), cyclopentenones (Ps5), furanone (Ps2), benzofuranes (Ps11 and Ps12) and a methylated furan (Ps18).

In the F2 factorial space, the negative direction (viz. TK samples) was dominated by lignin (4-vinylphenol, Lg3; 4-vinylguaiacol, Lg5; 4.methylsyringol, Lg7 and 4-(prop-1-enyl) syringol, Lg9) and pyrolysis products of polysaccharides such as levoglucosan (Ps17), dianhydro-D-glucopyranose (Ps16), dianhydrorhamnose (Ps10), 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one (Ps7), along with some long-chain *n*-alkane (C_{31} and C_{33}), and long-chain *n*-methyl ketones (MK18-MK35). The positive direction of F2 (viz. EG samples) was dominated by aliphatic moieties (ranging from medium- to short-chain length), a large series of alkyl benzenes, aromatic fractions (including mono- and poly-cyclic structures) and a few N-compounds (e.g. AM21, AM22 and N2).

Bacterial community profiles

The bacterial communities from all samples were analysed by ARISA and compared using Bray Curtis similarity and MDS ordination (Fig. S2). A three-factor test using PERMANOVA [soil type × OA type × study type (P versus R); where, soil type = So, OA = Oa, and study type = St)] and resulting estimated components of variation (Table 3) indicated a significant three-way interaction ($P=0.01$) along with significant 2-way interactions between So × St and So × Oa (each $P=0.001$).

As expected, all pair-wise tests for the term (So x Oa x St) for pairs of levels for factor So, regardless of level, returned significant results ($P=0.002$ to $P=0.016$)

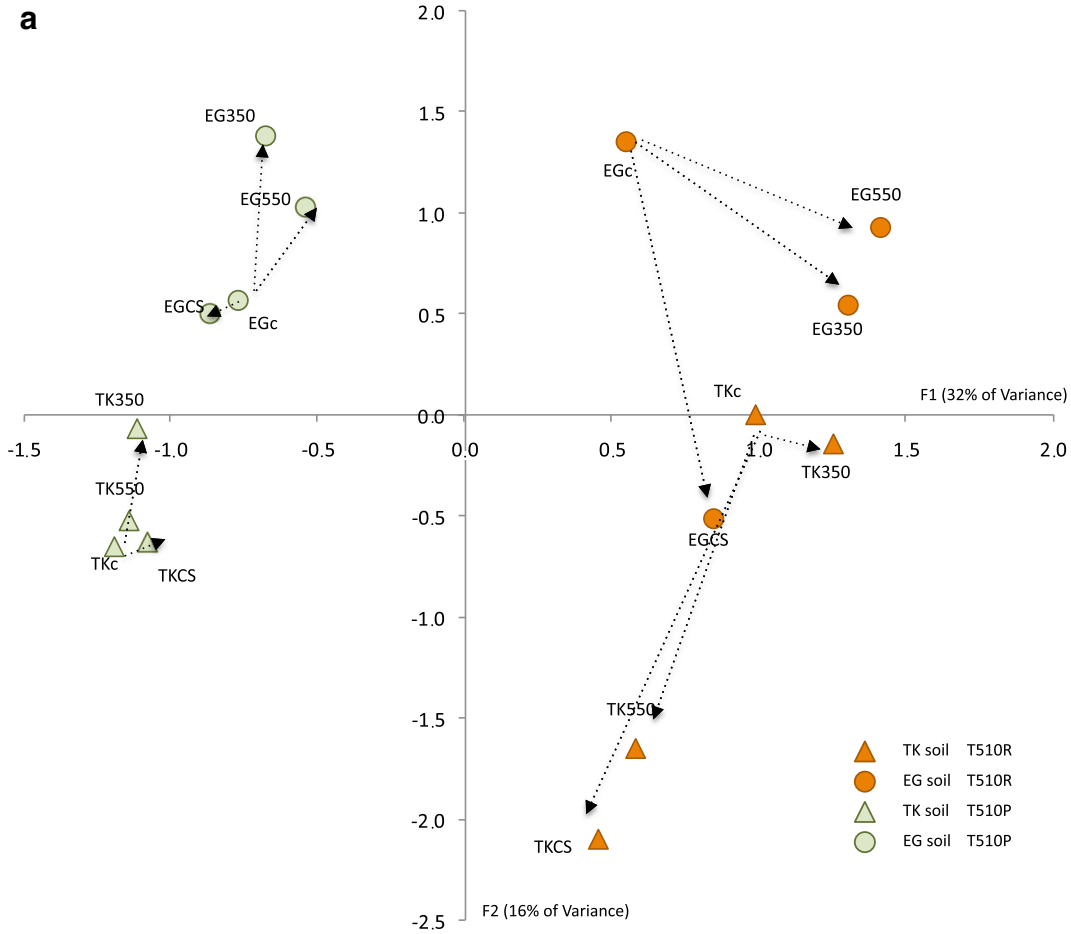

Fig. 2 Factor scores (**a**) and factor loadings (**b**) in the F1:F2 factorial space of the Alfisol (TK) and the Andisol (EG) samples incubated in the respiration study (R) and in the plant study (P) at time 510 day. *CS* corn stover, *CS-350* biochar produced from CS at a peak temperature of 350 °C, *CS-550* biochar produced from CS at a peak temperature of 550 °C. Factor analysis was carried out with centred logarithm data of the relative abundances (%TQPA) of identified pyrolysis compounds as described in the text. Loadings explaining less than 25 % of variance are not represented. Chemical compounds are grouped as (i) *n*-alkanes ($C_{9:0}$ – $C_{33:0}$); (ii) *n*-alkenes ($C_{9:1}$ – $C_{30:1}$); (iii) other aliphatic compounds (Al); (iv) *n*-methyl ketones ($C_{13:0}$ – $C_{35:0}$; K); (v) *n*-fatty acids ($C_{5:0}$ – $C_{24:0}$; FA); (vi) phenol and alkyl phenols (Ph); (vii) lignin-derived methoxyphenols (Lg); (viii) benzene containing compounds (including Ar and B_3-B_{28}); (ix) PAHs (polycyclic aromatic compounds, PA) including naphthalene compounds (NA); (x) N-containing products, (xi) polysaccharide fragments (Ps), and (xii) sterol derived compounds (St). Due to the multiple possible origins of N-compounds, this group was split into three sub-groups: i) pyridines + pyrroles (associated to microbial-derived fractions when present along with tracers of degraded SOM), indoles (N8, N9) – likely plant-derived, and nitriles (N6, N7) from charred material ii) chitin-derived N compounds (N5 + N12, fungi- and arthropods-derived), and (iii) alkyl amides (AM)

indicating that soil type has a strong influence in defining differences in bacterial community structure (this is also evident in Fig. S2, Fig. 3a and b where there are marked separations in data points related to soil type). Pair-wise tests for the term (So x Oa x St) for pairs of levels for factor St also returned significant results (P= 0.002 to P=0.041) for all comparisons except EG-CS (P=0.165). This implies that there are significant differences in the bacterial community depending on the presence or absence of plants but these differences are variable depending on the combination of soil type and OA.

Pair-wise tests for the term (So x Oa x St) for pairs of levels for factor Oa (Table 4) indicated that the bacterial community structure was not influenced by OA amendment when TK soil was planted (P study). However for TK soil in the absence of plants (R study) there were differences in the bacterial community structure for both

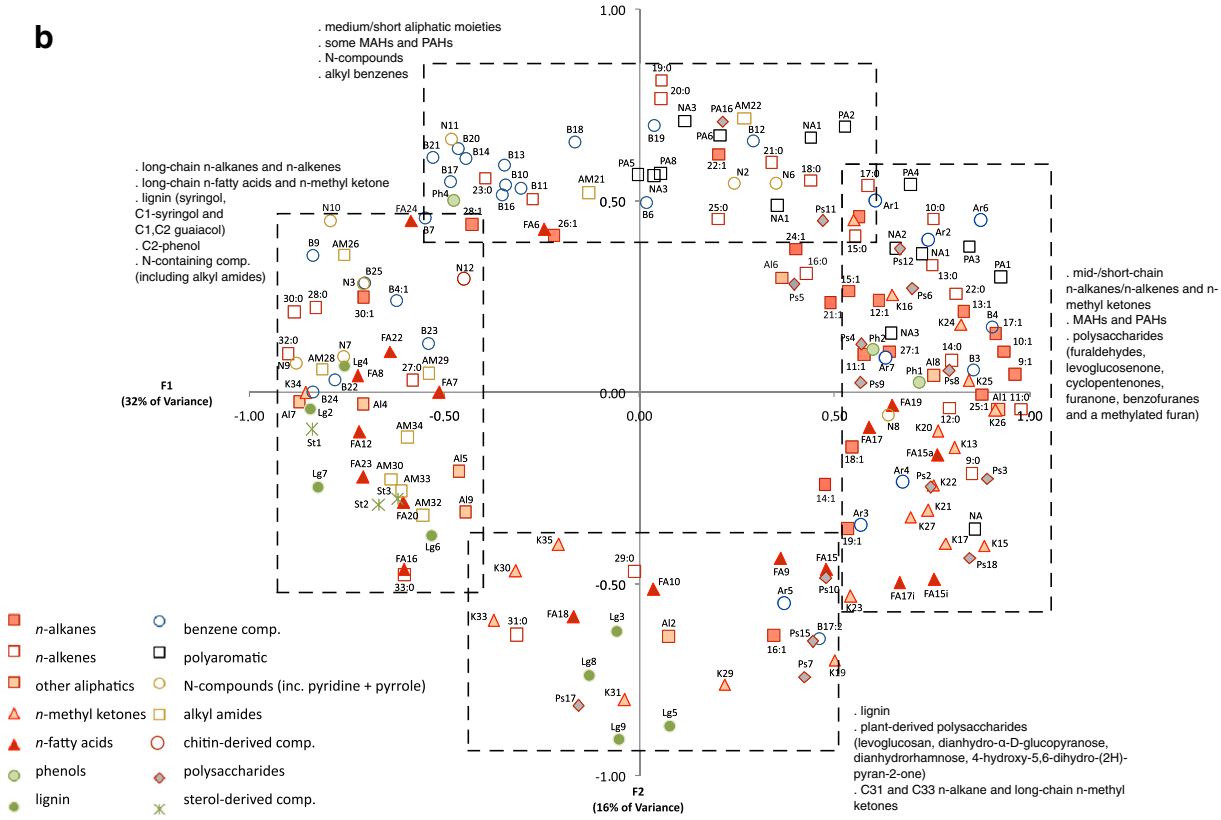

Fig. 2 (continued)

the CS and CS-350 amendments when compared to the un-amended TKc treatment, along with a marginal difference for the CS-550 amendment compared to the control (P=0.057). In this same treatment there were also no real differences in bacterial communities when comparing CS amendment to the CS-350 and CS-550 amendments, but these were detected when comparing the CS-350 and CS-550 amendments directly. These results suggest that OA does influence bacterial community structure in TK soil but the presence of plants actually obscures any effects that OA amendment might have. This is also apparent in the MDS ordinations where bacterial communities in the P study for TK soil do not form any obvious patterns (Fig. 3b) whereas

Table 3 Estimated components of variation (and percentage of the estimated variation) from the 3-factor PERMANOVA contributing to differences in microbial community structure

Source	Variation (Standard Deviation)	% of estimated variation
Soil (So)	709.75 (26.64)	23.5
Organic Amendment (Oa)	75.65 (8.69)	2.5
Study type (St)	224.51 (14.98)	7.4
(So x Oa)	173.89 (13.19)	5.8
(So x St)	425.69 (20.63)	14.1
(Oa x St)*	37.31 (6.11)	1.2
(So x Oa x St)	155.30 (12.46)	5.1
Residuals	1215.00 (34.86)	40.3

* Not significant, P=0.083

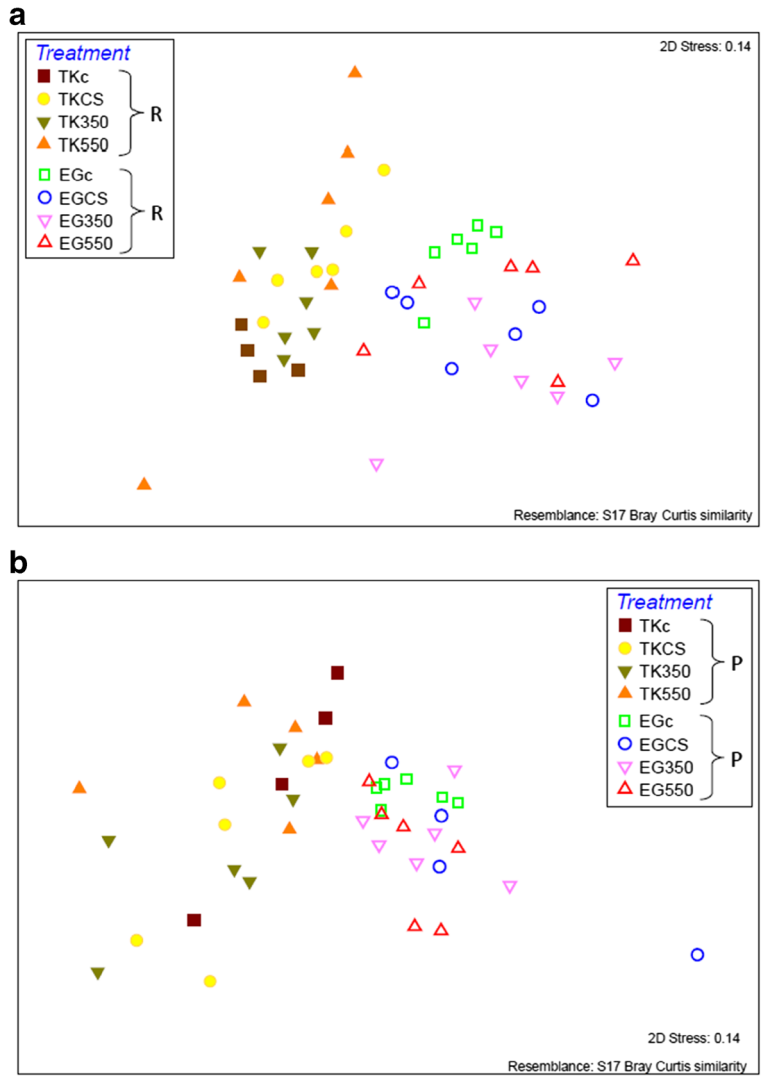

Fig. 3 MDS ordinations of ARISA data from the Respiration Study (**a**) and the Planted Study (**b**). *TK* Tokomaru silt loam soil, *EG* Egmont black loam soil, *R* respiration study, *P* planted study, *CS* corn stover, *CS-350* biochar produced from CS at a peak temperature of 350 °C, *CS-550* biochar produced from CS at a peak temperature of 550 °C. Bacterial community profiles were analysed using ARISA and profiles were then compared using Bray Curtis similarity. The MDS represents an unconstrained ordination of the Bray Curtis similarity measures from 250 restarts and allows examination of the broad relationships between microbial communities from each treatment. The 2D-stress values (both 0.14) indicate good ordinations with a moderate chance of a misleading interpretation, although not too much reliance should be placed on the detail of the plot without further analysis of the statistical relationships

those from the R study, especially the TKc and TK-350 communities, are more 'clustered' (Fig. 3a).

For EG soil, whether planted or not, there were significant bacterial community differences between un-amended control soil (EGc) and the CS, CS-350 and CS-550 amendments (Table 4). As for the TK soil, there were no significant differences when comparing CS with CS-350 or CS with CS-550 in either the P study or the R study (although the test was marginal for the CS with CS-550 in the P study; P=0.057). The only other evidence for bacterial community differences in the EG soil was in the R study when comparing the CS-350 amendment with the CS-550 amendment.

Overall, these results provide good evidence that the bacterial communities are soil type specific, while also

Table 4 P-values for pair-wise tests for term (So x Oa x St) for pairs of levels of factor organic amendment (Oa)

Soil (So)	TK		EG	
Study (St)	R	P	R	P
Control, CS	0.029	0.140	0.005	0.013
Control, CS-350	0.028	0.287	0.003	0.006
Control, CS-550	0.057	0.144	0.001	0.030
CS, CS-350	0.186	0.591	0.120	0.265
CS, CS-550	0.425	0.507	0.205	0.057
CS-350, CS-550	0.048	0.205	0.026	0.285

TK Tokomaru silt loam soil, *EG* Egmont black loam soil, *R* respiration study, *P* planted study, *CS* corn stover, *CS-350* biochar produced from CS at a peak temperature of 350 °C, *CS-550* biochar produced from CS at a peak temperature of 550 °C

suggesting that organic amendments can influence the bacterial community structure. Coupled to this there is evidence to suggest that the effect of these amendments on the bacterial community structure can be strongly affected, even overshadowed, by the presence or absence of plants depending on the soil type.

Linking microbial community differences to molecular composition of SOM

In order to explore the relationship between bacterial community profiles and molecular composition of SOM, distance based linear modelling (DISTLM and

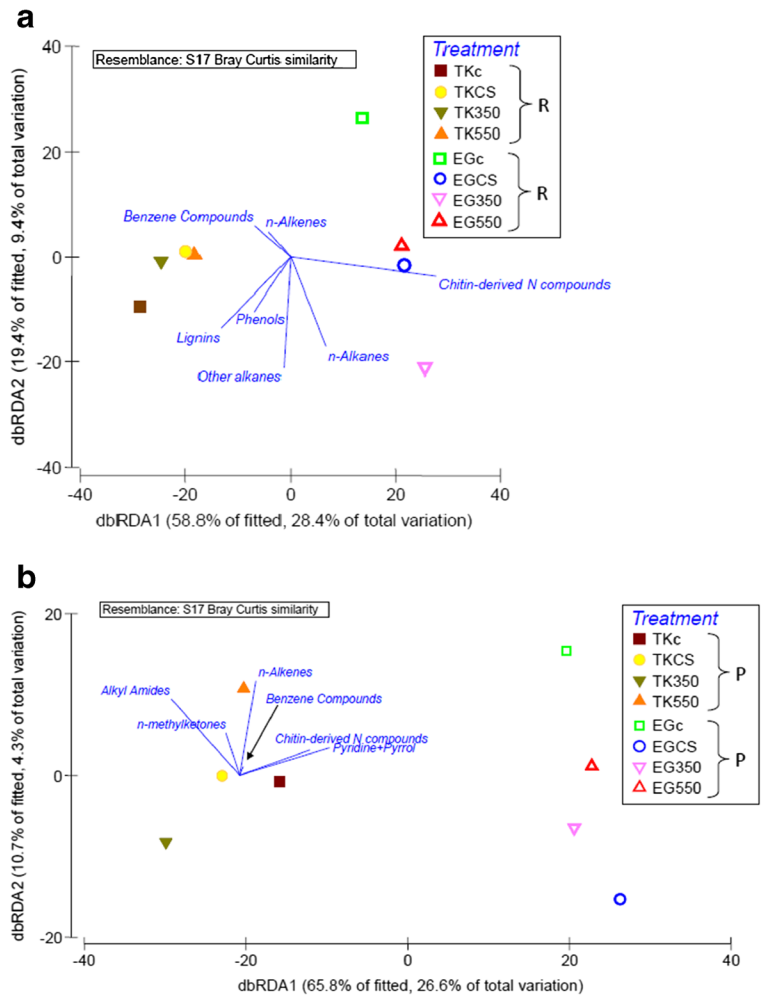

Fig. 4 dbRDA plots from the DISTLM modelling of the fitted relationships between the multivariate data cloud of ARISA profiles and SOM predictor variables from the Py-GC/MS analysis. Panel **a** represents samples from the Respiration Study while panel **b** represents samples from the Planted Study. *TK* Tokomaru silt loam soil, *EG* Egmont black loam soil, *R* respiration study, *P* planted study, *CS* corn stover, *CS-350* biochar produced from CS at a peak temperature of 350 °C, *CS-550* biochar produced from CS at a peak temperature of 550 °C. The variance partitioning data for these plots appears in Tables S3 and S4. Thirteen predictor variables were used that represented combinations of chemically similar compounds detected in the Py-GC/MS analysis. These models were used to investigate specific soil SOM drivers that could influence microbial community structure in samples from the respiration study versus those from the planted study. The models were run using the 'Best' procedure which examines the value of the selection criterion for all possible combinations of predictor variables to produce the model with the lowest residual SS. 'Adjusted R^2' was used as the selection criterion because it only accounts for the combination of predictor variables used rather than all the variables. The models estimate that up to 48 and 40 % of the total variation in the microbial communities can be potentially explained by differences in soil SOM characteristics in the respiration and planted studies respectively. DISTLM modelling of samples grouped with respect to soil type as opposed to study type can be found in Figs. S3 and S4

dbRDA, Figs. 4a, b, S3, S4) and multivariate regression trees (MRT, Supporting information Figs. S5 and S6) were used.

Results from the DISTLM 'Best' procedure (soil type and OA type were considered) indicated that up to~ 48 % and~40 % of total variation in bacterial community ordination from the R study (Table S3) and from the P study (Table S4), respectively, could be explained by the molecular characteristics of SOM. For the fitted models, the first two axes accounted for 78.2 and 76.6 % of the explained variations respectively (Fig. 4, Tables S3 and S4). The strongest predictor variable in the R study was chitin-derived N compounds while in the P study both chitin-derived N and pyridine+pyrrole became important − more so for the EG soil in both R and P studies (Fig. 4 and Fig. S3). These vectors lay in the X-axis direction that separates the two soils and described ~26 to 28 % of the total variation (Fig. 4a and b). In the presence of plants, the bacterial communities in both soils responded to alkyl amides – with these becoming the strongest predictor variable (Fig. 4b) − defining a separation between samples from the P study versus the R study in the TK soil (Fig. S4). Other SOM drivers in this soil included alkyl compounds (including *n*-alkanes, *n*-alkenes and *n*-methyl ketones) and phenols that separated between control treatments and amended treatments of a specific study type, and polysaccharide-derived compounds, which separated between the P versus R study types (Fig. S4). In addition to microbial-derived N compounds (pyridine + pyrrole), lignin was an important predictor variable for EG soil defining strong separation between bacterial communities from EGc and EG350 especially in samples from the respiration experiment (Fig. S3).

The MRT analysis (Figs. S5 and S6) produced similar indications about which SOM variables influenced microbial community structure to those obtained from the DISTLM / dbRDA analysis. More specifically, irrespective of the presence or absence of plants, microbial community profiles could be separated into two groups according to soil type related to the difference in the abundance of chitin-derived N compounds and alkyl-compounds. The degree of similarity in microbial community compositions for specific soil types (a lower ANOSIM R value and a lower B% suggest a higher similarity; Figs. S5 and S6) varied with the type of OAs and was influenced by plant growth. The degree of similarity mainly corresponded to differing abundances of microbially-derived N compounds (pyridine+ pyrrole), polysaccharide-derived compounds, lignin, phenols and alkyl compounds (Figs. S5 and S6).

Discussion

Molecular characterisation of SOM at day 510 (T510)

According to factor analysis (Fig. 2a and b), different soil types were separated along the F2 axis. The EG soil treatments (including both control and OA-amended samples) were associated with higher abundances of i) short/mid-chain aliphatic moieties (mostly *n*-alkanes ranging between C_{17} and C_{24},likely produced by microbial chain length shortening of longer chain aliphatic structures; Buurman et al. 2007); ii) mono- and polycyclic aromatic compounds (accumulated upon decay due to their intrinsic recalcitrance; Buurman et al. 2005); iii) polysaccharide-derived compounds including methylbenzofuran (Ps11), dibenzofuran (Ps16), and to a lower extent furfuraldehyde (Ps6) and cyclopentenone (Ps8) (all proposed as indicators of degraded SOM formed by bacterial and fungal activities; Buurman et al. 2007; Suárez-Abelenda et al. 2014, 2015); and iv) N-compounds including pyridine (N2), C_1-pyrrole (N3), phenylpyridine (N10), the chitin marker diketopiperazine compound (N12), and diketodipyrrole (N11). Pyridines, pyrroles and indoles may originate from microbial activities (Buurman et al. 2007; Schulten et al. 1997; Suárez-Abelenda et al. 2014). Acetamides and diketopiperazine are pyrolysis products of chitin and chitin-entangled protein, respectively (Stankiewicz et al. 1997). Large contributions of acetamides and diketopiperazine suggest the existence of active SOM decomposition by fungi and arthropods (Nierop et al. 2005).

In contrast, soils from the TK soil treatments featured larger abundances of preserved litter-derived SOM such as i) lignin moieties (Lg3, Lg5, Lg7 and Lg9); ii) plant polysaccharide fragments such as levoglucosan (Ps17; marker of cellulose; Stuczynski et al. 1997; Poirier et al. 2005), 1,4:3,6-Dianhydro-alpha-d-glucopyranose (Ps16), dianhydrorhamnose (Ps10), 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one (Ps7); iii) long-chain *n*-alkanes (C_{31} and C_{33}) (which are typical pyrolysis products from cuticular waxes in fresh plant materials; Eglinton and Hamilton 1967), and long chain *n*-methyl ketones (>C_{18}). Furthermore, the relative proportion of C_3 side-chain lignin moieties (C_3-syringol; Lg9) to the

relative abundance of syringol (Lg6) (C_3S:S) – which is commonly used as index of lignin degradation (Schellekens et al. 2009) − was lower in the EG soils than in the TK soils (e.g. 1.3 and 0.7 for TKc and EGc in the R study respectively, Table S5 in Supplementary information) indicating a better preservation of lignin in the TK soils. Hence, it was concluded that *preserved* SOM (i.e. plant-derived Ps) was more dominant in the TK soil type (Alfisol) treatments; whereas *degraded* SOM (i.e., microbially-processed) was more abundant in the EG soil type (Andisol) treatments, as demonstrated in the control soils at T0 (Herath et al. 2014a), despite the considerable decrease in preserved SOM experienced by the TK soil during the 510-day incubation in the absence of plants (Suárez-Abelenda et al. 2015).

Soils from the P study were clearly separated from those of the R study along the F1 axis. In the P study, pyrolysis products were dominated by long-chain *n*-alkanes ($C_{23:0}$ − $C_{33:0}$), *n*-alkenes ($C_{28:1}$ − $C_{30:1}$), long-chain *n*-fatty acids (including $C_{16:0}$ and $C_{24:0}$), and long-chain *n*-methyl ketones (MK31-MK35) (Fig. 2b). All these compounds were ascribed to pyrolysis of aliphatic biopolymers and cuticular waxes of higher plants (Eglinton and Hamilton 1967; Kögel-Knabner 2002; Nierop 1998; Tegelaar et al. 1989), whilst long-chain *n*-methyl ketones probably originated from the microbial oxidation of long chain *n*-alkanes (Raven et al. 1997; Jansen et al. 2006; Suárez-Abelenda et al. 2015). Soils from the P study were also rich in alkyl benzenes, which are possibly produced by cyclization of root-derived aliphatic biopolymers; and alkyl amides series (AM20 to AM34), which are associated with fresh litter (Buurman et al. 2007). In addition, lignin tracers (Lg2, Lg4, Lg6 and Lg7), and plant-derived sterol compounds substantiated the importance of fresh input from plant roots to SOM pools. Soils from the R study were characterised by abundant mid- to short- chain *n*-alkanes ($<C_{23}$) and *n*-alkenes ($<C_{28}$); mid- to short-chain *n*-methyl ketones (MK13-MK28), and degraded polysaccharide-derived compounds such as furaldehydes (Ps3 and Ps4), cyclopentenones (Ps5) and furanone (Ps2). All these indicated distinct SOM decay resulting from microbial activity during the 510-day incubation and the lack of fresh litter input.

The effect of OA additions on SOM molecular composition was largely overshadowed by the soil type and study type. However, there is evidence of an increase in the relative abundances of lignin and plant-derived polysaccharides in the R study as a result of OA (Fig. 1). The plant-derived polysaccharide fragments − including levoglucosan, pyranones and dyanhydro-α-glucopyranose; and lignin moieties, especially 4-vinylphenol and 4-vinylguaiacol − were found in large amounts among the pyrolysis products of CS and in smaller amounts in the incompletely pyrolysed (CS-350) biochar (Fig. S7). Moreover, biochar-amended soils showed an increase in the contributions of typical pyrolysis products of charred material such as PAHs (mostly naphthalene compounds) (Fig. 1) and polysaccharide-derived compounds resulting from thermal alteration (benzofuranes, Ps11 and Ps12, and methylated furan, Ps18; Kaal et al. 2008, 2009). The OA applications in the P study produced fewer changes in SOM molecular composition after 510-d incubation than in the R study as indicated by the smaller distances between the control soils and their corresponding OA-amended counterparts (Fig. 2a). It was thus inferred that fresh input from roots tended to dilute the influence of OA additions on SOM molecular composition.

To sum up, all factors considered−soil type (EG vs TK), study type (R vs P), and OA type (nil, CS, CS-350 and CS-550)—were found to have a strong influence on the chemical composition of SOM with the impact following the order of: study type > soil type > OA type. The growth of plant, *Medicago sativa* L., introduced large amounts of alkyl benzenes and alkyl amides along with labile compounds such as lignin moieties, structural polysaccharides, plant-derived sterols and long-chained aliphatic structures from cuticular waxes that stimulated microbial growth (as inferred from the relative abundances of chitin markers, e.g., diketopiperazine compound (N12) in the plant study; Fig. 2b). Compared to the TK soil treatments, EG soil treatments were dominated by a more microbial re-assimilated and decomposed (*degraded*) SOM fraction composed of mid- to short-chain *n*-alkenes/alkanes, aromatic and poly-aromatic compounds and high contents of degraded polysaccharide fragments. The EG soil also showed the largest signal of chitin-derived products, which is ascribed to fungal and/or arthropod activity (Stankiewicz et al. 1997). The source of exogenous C, especially fresh CS and CS-350 biochar, was shown to modify the molecular composition of SOM mainly through the contribution of lignin and plant-derived polysaccharides. Signals indicative of charred material in biochar-amended soils [mostly attributed to benzene containing compounds (excluding the long-chained alkylated benzenes), PAHs and furans] were less

recognisable in the P study due to the masking effect of plant-derived markers. The lesser influence of the CS-550 biochar on SOM fingerprints could to some extent be explained by the low signal intensity of its pyrogram, as the analytical pyrolysis temperature of Py-GC/MS used was the same to the one at which this biochar was produced (550 °C). Nonetheless, the lesser overall effect of OA type on SOM chemical composition compared to those of the study type and soil type was attributed to the relatively small contribution of the C from OA to total soil C and the relatively long incubation period that allowed considerable decomposition of exogenous labile C.

Relating microbial community to molecular properties of SOM

The structure and functioning of soil microbial communities reflect interactions between a host of biotic and abiotic factors. The biochemical composition of organic substrates available to microorganisms decomposing SOM is among the most important (Wardle and Giller 1996). It is necessary to indicate that although a thorough understanding of the changes in SOM characteristics was addressed at all stages of incubation (see Suárez-Abelenda et al. 2015); assessment of microbial responses was conducted exclusively at T510. It should be noted that to assess the long-term effect of OAs on microbial community structure, the most important comparison required is between unamended control vs. amended soils rather than addressing community changes in each treatment over time. The PERMANOVA results from the 3-factor analysis indicated significant interactions between soil type, study type and OA, but pairwise comparisons between all levels and combinations of these terms and the estimated components of variation suggested that soil type had the greatest overall influence on bacterial community variations (Fig. S2 and Table 3). The DISTLM analysis indicated that, for a specific soil type, ≥ 40 % of total variation in bacterial community ordination could be explained by the molecular characteristics of SOM (Tables S3 and S4).

The dominant role of soil type in controlling variations bacterial community structure (~24 % of variation explained as a main effect), compared to the other factors studied − study type (~7 %) and OA type (~3 %) − is in accord with results from Khodadad et al. (2011) and is attributed to the subtle differences in soil physicochemical properties such as pH and water availability (this is especially true for OC-poor soils, Zhou et al. 2003) or the obvious differences in soil mineralogy, nutrient availability (Carson et al. 2009; Khodadad et al. 2011), as well as soil C forms (Ng et al. 2014). In this specific study, it is possible that differences between soils are further accentuated by the peculiarities of allophanic soils (the Andisol, EG) which are rich in short-range order compounds (Parfitt 2009) compared to non-allophanic soils (the Alfisol, TK). In the presence of these compounds the fraction of available C as a proportion of the total C decreases (Nishiyama et al. 2001) as short-range ordered aluminosilicates are known to strongly interact with SOM and protect it against decomposition (Parfitt 2009; Chevalier et al. 2010). However, here emphasis will be placed on the C forms shaping the soil microbial community composition.

According to both DISTLM and MRT analyses, bacterial communities in TK soils were more likely influenced by *preserved* SOM, such lignin moieties and polysaccharides; while those in EG soils tended to be governed by *degraded* SOM, such as chitin-N compounds. This is consistent with previous results (Buurman et al. 2007; Suárez-Abelenda et al. 2011) in that Andisols (e.g., EG soil) are generally more dominant in *degraded* SOM than non-Andisols (e.g., TK soil) and Chevalier et al. (2010) has suggested that only small molecular weight molecules (e.g., microbial-derived) could become physically entrapped within the mesopore structure of allophane aggregates.

If application of OAs induced the alteration of the molecular composition of SOM, it could cause the shift in microbial community composition. This was evidenced in the EG soil in the R study. Due to the lack of exogenous C input to the EGc treatment, over time this soil became enriched with short- and mid-chained *n*-alkanes, *n*-alkenes, *n*-methyl ketones and microbial-derived polysaccharide fragments (e.g. cyclopentenone, levoglucosenone and furans) (Fig. 2) after the exhaustion of more labile forms of native soil C (e.g. plant-derived polysaccharides and lignin; Nierop 1998; Nierop and Jansen 2009). This probably made the microbial community composition of this treatment the most dissimilar to the EG treatments that received OA in the respiration (R) study (Fig. S5). Moreover, given that CS-550 biochar provided less labile C than its fresh and less carbonised counterparts (CS and CS-350), the EG soil amended with CS-550 was more dissimilar in microbial community properties than EG treatments

amended with CS and CS-350, which showed a high similarity (B%<40 % in Fig. S5). It is possible that the greater abundance of plant-derived polysaccharide fragments in the TK soil made the response towards the addition of OA less accentuated, especially with regard to those amendments (CS and CS-350) in which these compounds were present (Fig. 2 and Fig. S5).

Compared to the R study, the involvement of plants (P study) in the system generally decreased the similarity of bacterial communities between TK treatments but increased that between EG treatments (Fig. S6). One possible explanation could be that plant roots stimulated microbial activity thereby introducing considerable amounts of *degraded* SOM into the system. As EG soils already had a considerable contribution of *degraded* SOM, this additional microbial-derived C input would dilute the effect of the OA. For the same reason, additional microbial-derived C input in the TK treatments would offset the effect of plant-derived native SOM dominant in this soil and caused a shift in potential C sources from *preserved* SOM to *degraded* SOM. In addition, one should not disregard the effect of other soil physicochemical conditions affecting the response of microbial community composition to OA additions. However, the present study is not comprehensive enough to determine the relative importance of factors such as nutrient availability and aggregate formation in influencing soil bacterial community properties, which needs further investigation.

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