Soil Use and Management

Soil Use and Management, September 2018, **34,** 427–436 doi: 10.1111/sum.12440

Crop rotation in no-till soils modifies the soil fatty acids signature

A. E. Ferrari[1], S. Ravnskov[2] & L. G. Wall[1]

[1]*Laboratorio de Bioquímica, Microbiología e Interacciones Biológicas en el Suelo, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, R. Sáenz Peña 352 B1876BXD Bernal, Buenos Aires, Argentina, and* [2]*Department of Agroecology, Faculty of Science and Technology, Flakkebjerg Research Centre, Aarhus University, Forsøgvej 1, Slagelse, DK-4200, Denmark*

Abstract

Analysis of whole-soil fatty acids (WSFA) was used to characterize no-till productive agricultural soils associated with different crop rotation managements on the Argentinean pampas, over two sampling seasons. Crop rotation (CR) treatment was compared with soybean monocropping (MC). Soils from nearby natural environments (NE) were used as reference treatments. The objective of this study was to characterize the soil lipid signature and seek putative markers of agricultural management. NE sites had greater concentration of total WSFA than agricultural sites, but no differences between CR and MC were identified. NE sites were characterized by straight chain and mono-unsaturated fatty acids, such as *16:1ω5c*, an established biomarker for arbuscular mycorrhiza. Comparing lipid profiles using multivariate methods allowed a comprehensive comparison among treatments. The CR and NE soil samples were more alike than those of MC, with several fatty acids in common. CR soils were associated with mixed, branched and hydroxylated fatty acids. MC profiles appeared to be enriched by *16:010Me* and *18:1ω7c* fatty acids, which could be potential treatment markers. Thus, use of the WSFA approach to study soil lipid signature appeared to be a sensitive method to characterize soil health and soil use and management. However, some of the fatty acids do not come from living cells but from soil organic matter, which sets a limitation on interpretation in terms of the microbial community but expands the biological origin of the soil lipid signature to any biological matter, alive or death, which is a constitutive part of the soil under study.

Keywords: Conservation agriculture, classification and regression trees analysis, cropping systems, soil biodiversity, soil lipids, soil organic matter

Introduction

Almost 90% of the agricultural lands in Argentina are managed by no-till agriculture, which improves physical soil properties and reduces erosion risks, especially when no-till is combined with crop rotation (Derpsch *et al.*, 2010). Argentinean soils have been mostly characterized by means of physical and chemical quality markers, while biological markers have not been extensively used, mostly because there is no consensus about what biological markers and reference values should be used (Wall, 2011). The analysis of soil lipids is one of the few available quantitative methods to study microbial communities that do not depend on microbe culturing (Frostegård *et al.*, 2011). In addition to the use of fatty acids from the phospholipids fraction (PLFA) as a method to look at microorganisms – as part of the organic pool in soil – the analysis of soil lipids as a whole provides a way for the global biochemical characterization of soils, including lipids in the soil organic matter pool and derived from either living or dead matter in the process of decomposition. Soil lipids can be studied by different methods. One of them is the direct saponification of soil, known as the whole cell fatty acid (WCFA) methodology, originally applied to microbial cultures (Miller, 1982), whereas other includes a fractionation method, which involves extraction of soil lipids followed by a separation into three fractions: phospholipids (PLFA), glycolipids and neutral lipids fatty acids (NLFA) (Zelles, 1999). The fractionation method (usually known as the PLFA/NLFA method) divides lipids from living organisms into membrane bound and storage lipids. However, the use

Correspondence: L. G. Wall. E-mail: wall.luisgabriel@gmail.com
Received November 2017; accepted after revision July 2018

of certain fatty acids as markers of taxonomic groups of micro-organisms has been criticized (Scott *et al*., 2010; Frostegård *et al*., 2011). This critique is more significant as huge amount of unculturable micro-organisms are being described by molecular methods and whose lipidic signatures are not known yet. The term 'WCFA' seems inappropriate, when the method is applied to soil samples instead of microbial cultures, the soil being much more than living cells. For this reason, we prefer to refer to this method, when it is applied to soil samples, as whole-soil fatty acid (WSFA) method. The WSFA method is faster, cheaper and appears to be more sensitive than the PLFA/NLFA method, though the results are more difficult to interpret in terms of the origin of the different fatty acids. We used three different approaches to analyse WSFA data: (i) the analysis of the individual fatty acids in the overall WSFA set, to find putative markers of soil use and management, (ii) the analysis of the pattern of chemical groups of fatty acids, as a hypothetical chemical adaptation response to changes in the microenvironments, modified by the soil management; and (iii) the analysis of specific fatty acids known as taxonomic markers of certain micro-organisms. These different kinds of approaches have been broadly used to compare soils under different tillage treatments (Drijber *et al*., 2000; Ritchie *et al*., 2000; Calderon *et al*., 2001; Ibekwe *et al*., 2002; Feng *et al*., 2003; Meriles *et al*., 2009; Van Groenigen *et al*., 2010; Sun *et al*., 2016), but there is little information about changes in WSFA profiles in no-till soil managed with different crop rotation intensities. The goal of this study was to look for lipid markers that characterize different agricultural soil management, considering that the lipid fraction of soil is more than microbial communities and including any biological material coming from living cells and also from nonliving organic matter.

Materials and methods

Sites description

Detailed description of soils, sites and crop managements can be found in Figuerola *et al*., 2012; and Rosa *et al*., 2014;. Briefly, soil samples were collected from productive fields chosen by their different agricultural management according to criteria of the Argentinian No-Till Farmer Association (AAPRESID, in Spanish). Two treatments were defined as follows: Crop Rotation (CR) and Monocropping (MC). The CR treatment consisted of intense summer soybean–maize rotations with winter wheat and alternative use of cover crops, and with integrated management of fertilizer application and pest control. The MC treatment tended to soybean monocropping with occasional rotation, no winter cover crops and conventional pest management (intense fungicide and pesticide application). Natural environments (NE) close to the sampled agricultural fields (not more than 2–5 km distance), with no history of agricultural use, were chosen as reference. The three soil treatments were replicated in productive fields with more than 15 yr of no-till managements, at three different locations on the Argentinean Pampas, Bengolea (33°01′31″S; 63°37′53″W), Monte Buey (32°58′14″S; 62°27′06″W), Pergamino (33°56′36″S; 60°33′57″W) along a 340 km west–east transect. The soils of Bengolea were Entic Haplustolls with sandy loam texture in the surface horizons and loamy subsurface, being limited by climate and having a small water holding capacity due to coarse texture. Monte Buey soils were represented by Typic Argiudoll silt loam. Pergamino soils were Typic Argiudoll with silt loam in the surface horizons and silty clay loam in deeper layers. In Bengolea and Monte Buey, the climate was temperate subhumid with a mean annual temperature of 17 °C; Pergamino has a temperate humid climate with a mean annual temperature of 16 °C. Mean annual precipitation at Bengolea, Monte Buey and Pergamino, respectively, was 870, 910 and 1000 mm. The slope at all sites was <0.5%, and the average altitude was 223, 110 and 66 m.a.s.l. for the three areas, respectively (Bedano *et al*., 2016). Five subsamples were taken at each site over two seasons (February and early September, summer and winter, respectively, in the southern hemisphere). Each sample was a mix of 20–25 soil cores of 2.5 cm of diameter, from the 0–10 cm layer, carried to the laboratory at 5 °C and stored in freezer until analysis. Before analysis of lipids, soil samples were freeze-dried and milled under liquid nitrogen.

Lipid analysis

1 gram of soil from each freeze-dried and milled sample was saponified with a NaOH-methanol mixture, methylated with HCl-methanol, extracted with hexane/methyl tert-butyl ether (MTBE), amended with 33.75 μg of nanodecanoic (*19:0*) methyl ester as internal standard, extract washed with NaOH, evaporated under N_2 stream, resuspended in 100 μL of hexane and injected into an Agilent 6890 plus gas chromatograph. Oven temperature was increased from 170 to 260 °C with a 5 °C/min ramp, followed by another ramp (40 °C/min) until a final temperature of 310 °C. Hydrogen and nitrogen were used as carrier and make-up gases, respectively. A phenyl-siloxane (2.5%) column was used (25 m long, 200 μm ID, 0.33 μm film). A flame ionization detector was used, fed by a hydrogen–air mixture. Fatty acids were analysed through the MIDI microbial identification protocol (Sherlock version 4.5 MIDI, Microbial ID, Newark, DE, USA). More analytical details can be found in Ferrari *et al*. (2015). The concentration of each fatty acid identified by the MIDI software was assessed in relation to the *19:0* standard and expressed as nanomoles per gram of dry soil.

Fatty acid nomenclature

Suffix *c* indicates *cis* isomer. *Cy* means cyclopropyl ring. The hydroxy-substituted fatty acids are represented as *2OH* and *3OH* (hydroxyl group in position 2 and 3 from the carboxyl end, respectively) and *N alcohol* indicates unknown OH position. Fatty acids methylated in C10 are represented as *10Me*. Mono-unsaturated (*MUFA*) and poly-unsaturated fatty acids (*PUFA*) are designated by the omega (ω) nomenclature. Branched fatty acids in *iso* and *anteiso* position correspond to a lateral methyl group in the second and third C atom, respectively, from the methyl end. Fatty acids with the same retention time in the chromatogram are grouped as '*summed in feature*'. The fatty acids were classified into the following chemical groups: straight chain, branched, MUFA, PUFA, cyclic, methylated, hydroxyl and hydroxy branched and the mentioned 'sum in feature', plus a category of fatty acids from mixed chemical functions ('mixed or others') (Ferrari *et al*., 2015).

Statistics

One-factor analysis of variance (ANOVA) was used to assess the level of significance ($P < 0.05$) in the comparison of treatments (land use). The three locations were considered replicates as the location x land use interaction in the two-factor ANOVA was not significant. Each replicate was obtained as a mean value of five repetitions (subsamples). Means of fatty acid concentrations for each treatment were compared by the LSD Fisher test. Variance homogeneity was checked by means of the Bartlett test, and data were log-transformed when necessary.

Two multivariate methods of ordination were used; discriminant analysis was used to assess which parameters contribute mostly to the separation of treatments and was built with those fatty acids present in at least 20% of all samples, in order to eliminate very rare fatty acids from the data set that appeared in only one of five subsamples. Classification and Regression Trees analysis was used to determine which fatty acids allow a better discrimination among treatments. All the analyses were performed with the Statgraphics Plus 5.1 software package.

Results

Overall profile

Seventy-four different fatty acids were identified by the MIDI system, belonging to ten chemical classes (Table S1). The discriminant analysis based on the whole set of detected fatty acids showed a clear separation of the three treatments, either in summer or in winter, according to different relevant elements of the eigenvectors (Figure 1). NE sites were strongly correlated with the fatty acids *16:1ω5c* and *15:0* in both seasons. In summer, a strong discrimination between NE sites and agricultural sites was found according to the first discriminant function accounting for 95% of the total variance. In the discrimination between cultivated samples according to the second axis, CR summer samples had high loadings for *15:0iso*, *15:0anteiso*, *17:0iso*, *18:0* and *16:0N alcohol*. In winter, the first discriminant function was able to separate the three treatments, with CR sites samples between NE and MC ones (Figure 1b). CR winter samples were associated to the fatty acids *feature 3*, *14:0iso*, *18:0*, *15:1isoG* and *19:0cy*. The fatty acids *16:0*, *17:0anteiso*, *16:03OH* and *16:0Nalcohol* were related both to NE and to CR sites.

Selected fatty acids

In the one-factor ANOVA for individual WSFA, several fatty acids showed a decreasing trend NE > CR > MC, these differences being statistically significant or not. Those 12 fatty acids showing this trend were considered relevant for the purpose of searching for discrimination between CR and MC soils, and they are listed in Table 1.

The only fatty acids showing a significant trend NE > CR > MC in both sampling seasons were *16:03OH* and *feature 7* (Table 1). The fatty acid *15:1isoG* showed the same trend NE > CR > MC but only in winter. Also in winter, *15:0iso3OH* were at significantly higher levels in CR than in MC. The MUFA *18:1ω7c* showed statistical differences between CR and MC in both seasons, always with significantly higher levels in MC (Table 1). The methylated fatty acids *16:010Me* and *18:010Me* only showed significantly larger values under MC than CR in summer. Over the summer, the fatty acid mixture *feature 2* showed a significant trend NE > CR > MC, although there were no differences between CR and MC in winter. The abundance of all relevant fatty acids, selected as explained above, was further studied by multivariate methods. The discriminant analysis using the reduced subset of 12 selected fatty acids still showed a good separation among the three treatments (Figure 2). The CR sites were strongly associated with *12:0 3OH*, *16:0 3OH*, *16:0anteiso*, *17:0 cy* and *feature 2* in summer (Figure 2a), and to *15:0iso3OH*, *19:0cy*, *12:0 3OH*, *15:1isoG*, *17:1ω8c* and *feature 5* in winter (Figure 2b). The Classification-Regression Tree showed a leading role of fatty acid mixture *feature 7* in the separation among treatments, especially in winter where larger values in NE sites, intermediate in CR and small in MC were observed (Figure 3). In summer, MC samples were also related to high levels of *12:03OH* and low levels of *18:1ω7c*.

Particular fatty acids extracted with the WSFA method

Most fatty acids obtained in this study had been found previously when the same set of samples was analysed by the PLFA/NLFA method (Ferrari *et al*., 2015). Nevertheless,

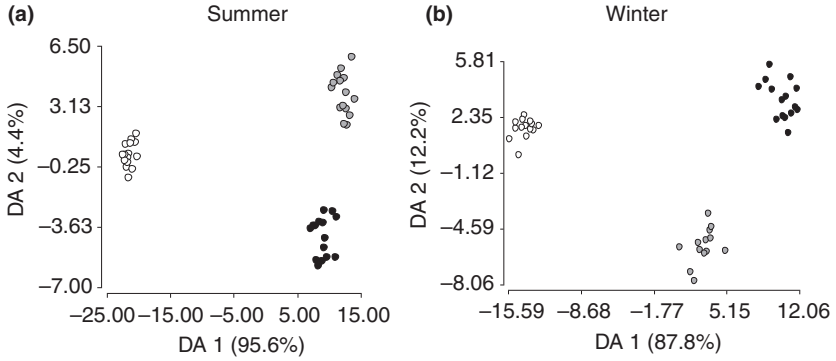

Figure 1 Discriminant analysis of individual fatty acids from the WSFA profile. Samples taken in summer (a) and in winter (b) (Symbols: white: natural environment; grey: crop rotation; black: monocropping).

Table 1 One-factor ANOVA for the comparison of mean concentrations (nanomoles/gram dry soil) of selected WCFA among all locations and subsamples, for every agricultural treatment

	Summer			Winter		
	NE	CR	MC	NE	CR	MC
15:0 iso 3OH	4.6 a (1.5)	3.4 a (0.93)	2.4 a (0.88)	4.4 b (1.7)	3.8 ab (0.91)	1.0 a * (0.41)
15:1 iso G	5.4 a (1.0)	4.8 a (1.3)	4.1 a (1.2)	12 c (2.2)	4.3 a (0.88)	1.6 a (0.54)
16:0 3OH	12 c (0.61)	8.4 b (0.79)	5.4 a (2.0)	14 b (1.4)	9.5 a (0.68)	7.0 a * (0.62)
16:0 10Me	69 b (5.5)	50 a (4.2)	55 a (14)	94 b (10)	56 a (4.3)	61 a (5.2)
16:0N alcohol	46 b (6.4)	16 a (3.0)	11 a (6.1)	43 b (6.9)	13 a (2.1)	11 a (2.0)
16:1w5c	289 b (47)	105 a (17)	107 a (41)	274 b (51)	82 a (14)	67 a (7.1)
18:2w6,9c	190 b (24)	95 a (8.5)	166 ab (36)	216 a (27)	257 a (114)	119 a (13)
18:1w7c	117 b (16)	61 a (6.8)	111 b (23)	154 b (21)	78 a (15)	143 b (21)
18:1w9c	378 b (45)	169 a (14)	204 a (61)	433 a (49)	398 a (169)	184 a (15)
19:1w6c	411 c (35)	154 a (11)	114 a (55)	390 c (23)	134 b (21)	78 a (3.6)
20:4w6	22 b (3.6)	13 a (2.5)	10 a (3.8)	24 a (4.7)	38 a (19)	20 a (3.0)
16:03OH/18:1w7c	0.13 b	0.16 b	0.056 a	0.12 b	0.12 b	0.069 a

Different letters indicate significant difference for $P = 0.05$ (NE: natural environment; CR: crop rotation; MC: monocropping). The asterisk indicates significant difference ($P < 0.05$) between CR and MC when NE data are excluded from the analysis (Standard error between brackets).

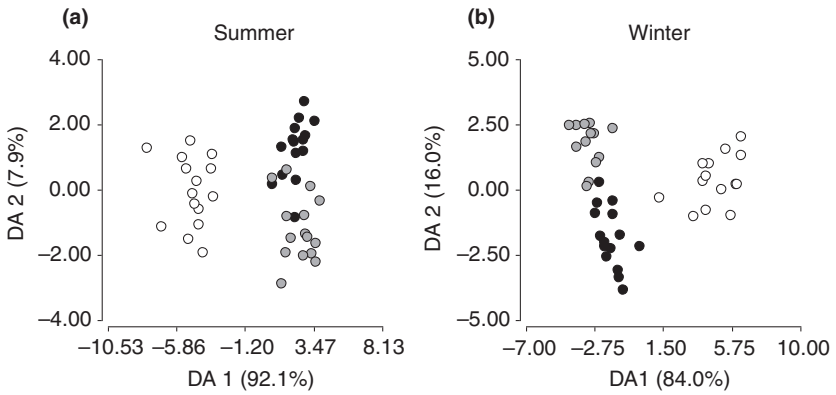

Figure 2 Discriminant analysis using a subset of selected relevant fatty acids from the WSFA data set, which showed a trend of differences among treatments according to one-factor ANOVA (see Table 1). Samples taken in summer (a) and winter (b) (For identification of symbols: see legend of Figure 1).

there were several fatty acids in the WSFA profile that were not found in the previous analysis with the PLFA/NLFA method. As mentioned earlier, lipids extracted by the WSFA method can come from living or dead organic matter. To determine whether particular lipids were preferentially extracted by the WSFA method, the concentrations of all fatty acids in the WSFA profile were compared with their concentrations in the PLFA/NLFA profile (adding the concentrations in the PLFA and the NLFA fractions). In this way, the WSFA/(PLFA+NLFA) ratio of concentrations was calculated for every fatty acid present in the WSFA profile (Table S2). In this way, it was evident that many fatty acids were preferentially extracted using the WSFA method (WSFA/(PLFA+NLFA) ratio greater than 1), but only some were exclusively extracted by the WSFA method, being absent in the PLFA+NLFA profile (denoted as 'WSFA exclusive' in Table S2).

The discriminant analysis of these exclusively WSFA-extracted fatty acids showed an association of CR samples with *10:0 3OH*, *16:0 3OH*, *11:0* and *feature 2* in summer; and with *18:0 3OH*, *15:1ω6c* and *10:0 3OH* in winter (Figure 4). The Classification-Regression Tree showed the importance of fatty acids *16:0 2OH* and *feature 7* in the separation of treatments in summer and winter, respectively, on the basis of the exclusively WSFA-extracted fatty acids (Figure 5).

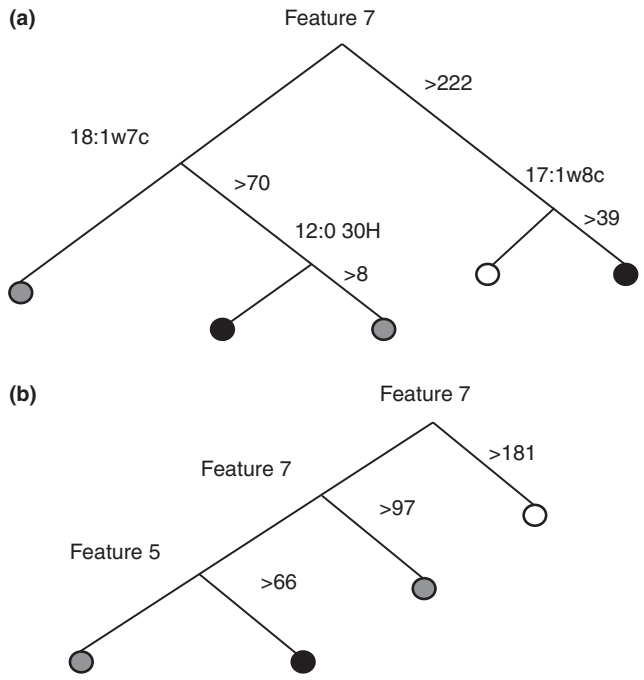

Figure 3 Classification-Regression Trees for selected relevant WSFA. Samples taken in summer (a) and in winter (b). (Symbols: see legend of Figure 1).

Fatty acids gathered in chemical groups

To identify whether the chemistry of the diversity of fatty acids could be considered as a characteristic of the use and management of soil, the data set of WSFA was reorganized as groups where fatty acids were gathered according to their chemical characteristics. It was possible to discriminate soil samples by treatments considering this kind of data organization (Figure 6), irrespective of the season of soil sampling, although the discrimination was not as good as with the individual fatty acids data set (Figure 1). In both sampling seasons, CR samples were associated with branched and hydroxylated fatty acids, and NE sites were correlated with MUFAs and MC sites with methylated and PUFAs (Figure 6). Classification-Regression Trees showed a clear separation among treatments showing a different pattern according to season. NE samples were mainly

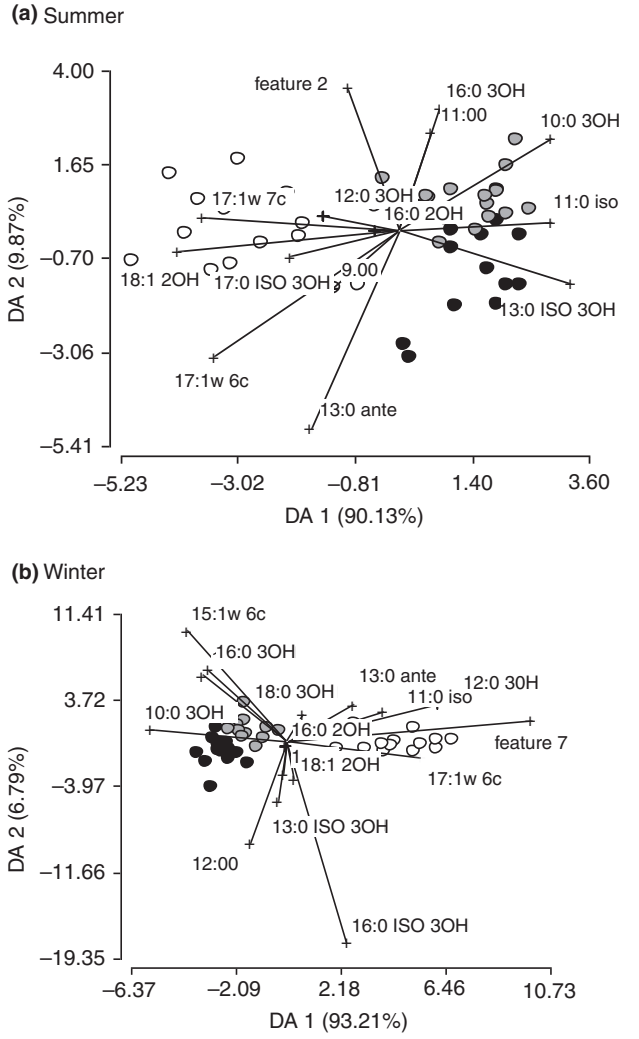

Figure 4 Discriminant analysis biplot for those fatty acids that were exclusively extracted by the WSFA method. Samples taken in summer (a) and winter (b) (Symbols: See Figure 1).

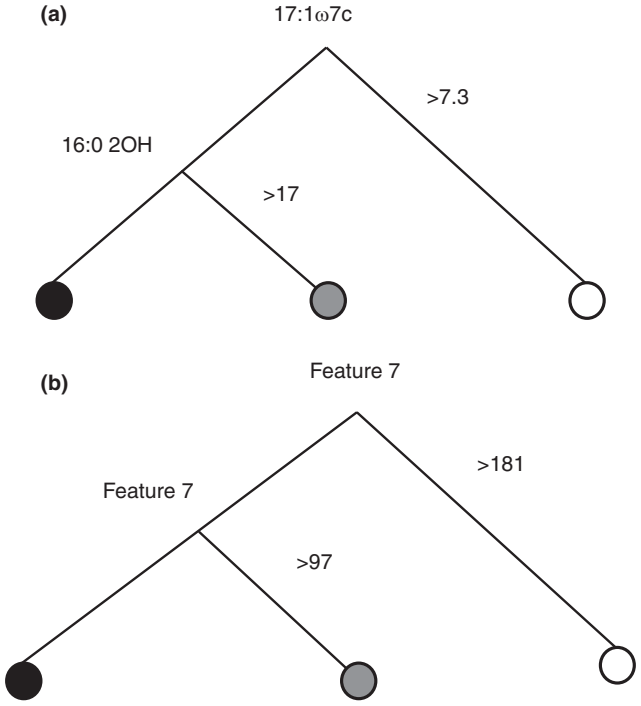

Figure 5 Classification-Regression Trees for exclusively extracted WSFA. Samples taken in summer (a) and in winter (b). (Symbols: see legend of Figure 1).

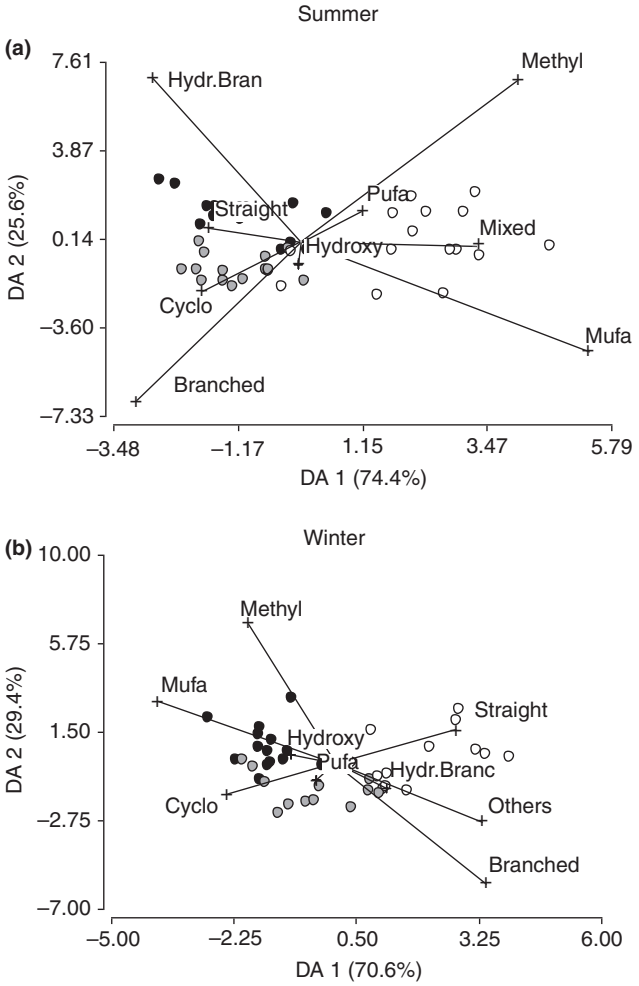

Figure 6 Discriminant analysis of WSFA gathered in chemical groups. Samples taken in summer (a) and winter (b). (Symbols: see legend of Figure 1).

associated with high levels of straight chain fatty acids in summer but with high levels of MUFAs in winter (Figure 7). CR samples were associated with high levels of branched and hydroxylated fatty acids in summer and to cyclic fatty acids in winter. MC samples were not positively discriminated by a particular chemical group, but by levels below a certain value for every chemical group of fatty acids (Figure 7).

The total amount of WSFA per gram of soil was significantly greater from NE sites than in agricultural sites, either in summer or in winter, as well as for most of the chemical groups (Table 2). The concentration of hydroxyl fatty acids showed a decreasing trend NE > CR > MC, though the difference between CR and MC was only significant in winter (Table 2) and only if NE data were excluded from the analysis. The 'mixed' category showed also a decreasing trend, but the differences were not significant in any of the seasons. If data from summer and winter were combined, only the hydroxylated group showed significant differences between CR and MC, with a significant trend NE > CR > MC (data not shown).

Discussion

Several fatty acids were exclusively extracted with the WSFA approach, not being found in the PLFA/NLFA method applied to the same soil sample set (Ferrari *et al*., 2015). Fernandez *et al*. (2013) made similar observations although they focused their analysis in soil microbial communities. In our case, those particular fatty acids showed the potential to discriminate between different uses and management of soil, especially the beta-hydroxy fatty acids (*10:0 3OH*, *16:0 3OH* and *18:0 3OH*), usually associated with Gram-negative bacteria (Ibekwe & Kennedy, 1999). The multivariate analysis was able to clearly discriminate between the three different soil treatments, especially in winter, suggesting a differential lipid signature in soils with different management, as suggested by Drijber *et al*. (2000) comparing native mixed prairie sod and agricultural soils.

NE sites showed the largest total amount of WSFA, whose value expressed in nanomoles per gram of soil should be indirectly proportional to the total microbial biomass (viable and dead). This observation is in agreement with results by other authors. Romaniuk *et al*. (2011) found

greater microbial biomass in natural environments than in conventional horticultural sites; Liebig *et al.* (2005) found higher total biomass in reduced till sites than in conventional tillage site; Sun *et al.* (2016), using the PLFA technique, found differences between organic and nonorganic agriculture, but not between conventional and minimum tillage.

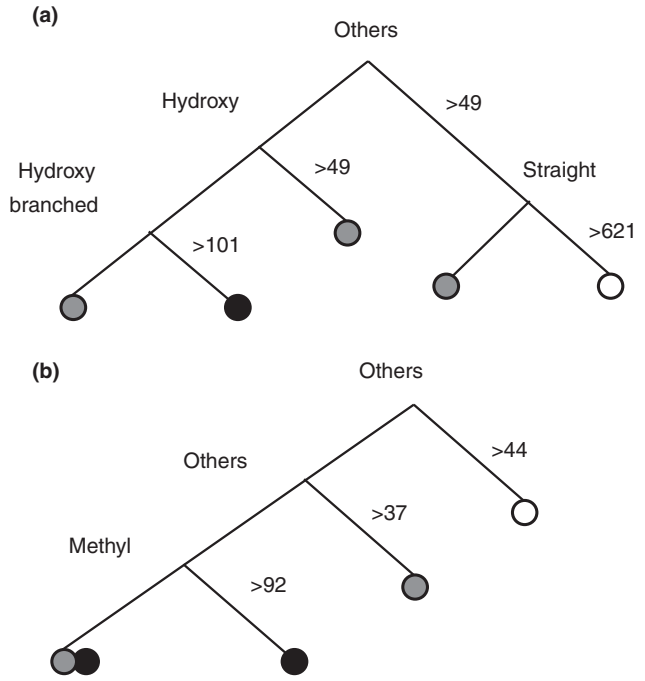

Figure 7 Classification-Regression Trees for WSFA gathered in chemical groups. Samples taken in summer (a) and in winter (b). (Symbols: see legend of Figure 1).

In this study, both agricultural sites were under no-till, and no differences were found between them, though CR samples showed slightly larger WSFA values than MC samples in winter. Similar results were found when the same soil samples were analysed in terms of PLFA/NLFA profiles (Ferrari *et al.*, 2015).

NE sites showed a strong association with *16:1ω5c*, a known marker of arbuscular mycorrhiza (Olsson, 1999; Grigera *et al.*, 2007), in both sampling seasons, suggesting that agricultural use decreases hyphae development but no differences between agricultural management practices were found. This result is in agreement with previous reports of our team (Ferrari *et al.*, 2015; Cofre *et al.*, 2017) using the PLFA/NLFA method. Thus, the WSFA profile followed the same trend as the NLFA profile (the larger lipid fraction) but not the PLFA trend, which showed larger values under the CR treatment (Ferrari *et al.*, 2015). The *hydroxylated* fatty acids, and especially *16:0 3OH*, showed a significant trend NE > CR > MC in both seasons. Thus, this fatty acid shows a high potential as a marker of agricultural management in the soils under study. The same significant trend was found for the sum of the hydroxylated and cyclic groups, and these two chemical groups are usually considered biomarkers of Gram-negative bacteria (Albertsen *et al.*, 2006; Ravnskov *et al.*, 2006; Scott *et al.*, 2010).

Table 2 One-factor ANOVA for the comparison of mean concentrations (nanomoles/gram dry soil) of selected WCFA groups among all locations and subsamples, for every agricultural treatment

	Summer			Winter		
	NE	CR	MC	NE	CR	MC
Total WCFA	3457 b (248)	1834 a (142)	1967 a (130)	3746 b (248)	2339 a (142)	1775 a (130)
Chemical groups						
MUFA	865 b (101)	362 a (33)	455 a (27)	942 b (101)	590 a (33)	425 a (27)
PUFA	49 b (3.6)	34 a (3.4)	32 ab (2.5)	49 a (3.6)	58 a (3.4)	30 a (2.5)
Hydroxyl	59 c (4.1)	44 b (4.1)	33 a (5.0)	63 b (4.1)	33 a (4.1)	24 a (5.0)
Methyl	114 b (13)	70 a (7.0)	84 ab (5.3)	170 b (13)	92 a (6.9)	100 a (5.3)
Cyclic	26 a (9)	31 a (6.6)	38 a (6.9)	84 b (9.5)	59 ab (6.6)	52 a (6.9)
Straight	993 b (113)	522 a (41)	555 a (31)	982 b (133)	589 a (41)	519 a (31)
Branched	426 b (29)	319 a (25)	301 a (18)	488 b (29)	316 a (25)	273 a (18)
Others	244 b (23)	112 a (12)	158 a (11)	123 b (23)	65 a (12)	40 a (11)
Sums and ratios						
HYD + CYC	85 a	75 a	71 a	147 b	92 a	76 a
Branch/PUFA	10 a	10 a	9.7 a	16 b	9 a	11 ab
MUFA/PUFA	18 b	11 a	15 ab	23 a	16 a	16 a

Different letters indicate significant difference for $P = 0.05$ (NE: natural environment; CR: crop rotation; MC: monocropping). (Standard errors between brackets).

The hydroxylated fatty acids in beta position are dominant in Gram-negative bacteria but can be also present in some species of Gram-positive bacteria, as a background, and even in fungi and higher plants. Nevertheless, WSFA would be detecting not only microbial lipids but also lipids from other sources, considering the soil as a whole living system. Another fatty acid with a significant trend NE>CR>MC in both seasons was *feature 7*, an unresolved mixture of known (e.g. *19:1ω6c*) and unknown fatty acids. The former is not a common fatty acid and does not have a defined taxonomic association; possibly, it could be linked to unculturable micro-organisms, but its origin is intriguing. In discriminant analysis, CR sites appeared to be more similar to NE sites than to MC sites, having some fatty acids in common over winter (*16:0*, *16:03OH* and *16:0N alcohol*). Straight chain fatty acids, such as *18:0*, are broadly distributed and have no defined taxonomic association. The fatty acid *16:0N alcohol*, an alcohol and not a fatty acid, is relatively rare and was associated with beta-proteobacteria from the *Moraxella* genus (Ritchie *et al*., 2000). Other 'rare' fatty acids, such as *15:1isoG* and *15:0iso3OH*, had greater values under CR than MC, but only for the winter sampling. The latter acid has been associated with gamma-proteobacteria from the *Xantomonas*/*Pseudomonas* group and to bacteroidetes from the *Cytophaga*/*Flexibacter*/*Bacteroides* group (Scott *et al*., 2010), the latter group mostly being considered as unculturable. The rare fatty acid *15:1isoG* was also strongly associated with CR sites either in PLFA or in NLFA profiles (Ferrari *et al*., 2015). CR sites were strongly correlated with branched and hydroxylated fatty acids, classical taxonomic biomarkers of Gram-positive and Gram-negative bacteria, respectively. The straight chain fatty acids (especially *15:0* and *18:0*) were also strongly associated with NE and CR but not with MC. It is worth noting that the NLFA profile had shown the fatty acid *20:0* as a strong marker of NE and CR treatments that did not appear as such in the WSFA approach, while MC sites appeared associated to methylated fatty acids, especially *16:010Me* (Ferrari *et al*., 2015). The fatty acids methylated at C10 are specific taxonomic markers of Actinobacteria. The PLFA and NLFA profiles have also shown larger values of this group under MC than CR, though the difference was significant only in winter (Ferrari *et al*., 2015). In our study, MC sites had higher levels of the methylated group under MC than CR over both seasons, suggesting that this group might be also considered as a putative marker for monocropping practices in this agricultural system.

Vaccenic acid (*18:1ω7c*) was in greater concentration under MC than CR over both seasons, showing a big potential as a marker of monocropping. Mono-unsaturated fatty acids belonging to the *ω7* series are typical of Gram-negative bacteria (Zelles, 1999), being found in all strictly anaerobic bacteria (Zelles *et al*., 1992). Large concentrations of *18:1ω7c* have been reported for many groups of micro-organisms, including mycorrhizal fungi (Olsson, 1999; Grigera *et al*., 2007; Karlinski *et al*., 2007), Gram-negative bacteria (MacNaughton *et al*., 1999; Ibekwe *et al*., 2002; Scott *et al*., 2010; Börjesson *et al*., 2012; Müller-Stover *et al*., 2012), proteobacteria associated with the *Enterobacter*/*Vibrio* and *Bacillus-Clostridium* groups (Hedrick *et al*., 2010; Scott *et al*., 2010), and specifically the *Rubellimicrobium* genus (Dastager *et al*., 2008; Weon *et al*., 2009). It is worth mentioning that in a DNA analysis of the same samples (Figuerola *et al*., 2012), the *Rubellimicrobium* group appeared to be specifically associated with MC soils when microbial diversity was studied by pyrosequencing and confirmed by qPCR. Despite important differences being found for fatty acids with a recognized taxonomic assignment, there were also differences for rare fatty acids, such as *16:0N alcohol* and *15:1 iso G*. These fatty acids may be associated with soil organisms that cannot be cultivated in the laboratory.

Conclusions

In this study, the whole soil fatty acid profile (WSFA) method has identified more fatty acids with the potential to be used as indicators of soil management than our previous study, which used only the PLFA/NLFA method. From our data, we concluded that, under no-till, crop rotation was differentially associated with *mixed*, *branched* and *hydroxylated* fatty acids, whereas monocropping appeared to have a potential marker based on the levels of methylated fatty acids, especially *16:010Me*, and *18:1ω7c*.

The fact that, when using WSFA, some fatty acids come not from living cells but from soil organic matter may be disadvantageous in terms of analysis of the microbial community, it does expand the biological origin of the soil lipid signature to any biological matter, alive or death, which is a constitutive part of the soil under study.

Acknowledgements

This work was supported by grants PAE 36976-PID 53 and PME 2006-1730 (Agencia Nacional de Promoción Científica y Tecnológica, Argentina), PIP2012-11220110100031 (CONICET, Argentina), PUNQ 0395/07 and PUNQ EXPTE 1411/15 (Universidad Nacional de Quilmes, Argentina). LGW is member of CONICET (Argentina).

References

Albertsen, A., Ravnskov, S., Green, H., Jensen, D.F. & Larsen, J. 2006. Interactions between the external mycelium of the mycorrhizal fungus Glomus intraradices and other microorganisms as affected by organic matter. *Soil Biology and Biochemstry*, **38**, 1008–1014.

Bedano, J.C., Domínguez, A., Arolfo, R. & Wall, L.G. 2016. Effect of Good Agricultural Practices under no-till on litter and soil invertebrates in areas with different soil types. *Soil and Tillage Research*, **158**, 100–109.

Börjesson, G., Menichetti, L., Kirchmann, H. & Kätterer, T. 2012. Soil microbial community structure affected by 53 years of nitrogen fertilization and different organic amendments. *Biology and Fertility of Soils*, **48**, 245–257.

Calderon, F.J., Jackson, L.E., Scow, K.M. & Rolston, D.E. 2001. Short-term dynamics of nitrogen, microbial activity and phospholipid fatty acids after tillage. *Soil Science Society of America Journal*, **65**, 118–126.

Cofre, M.N., Ferrari, A.E., Becerra, A., Dominguez, L., Wall, L.G. & Urcelay, C. 2017. Effects of cropping systems under no-till agriculture on arbuscular mycorrhizal fungi in Argentinean pampas. *Soil Use and Management*, **33**, 364–378.

Dastager, S.G., Lee, J.C., Ju, Y.J., Park, D.J. & Kim, C.J. 2008. *Rubellimicrobium mesophilum* sp. nov., a mesophilic, pigmented bacterium isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, **58**, 1797–1800.

Derpsch, R., Friedrich, T., Kassam, A. & Li, H. 2010. Current status of adoption of no-till farming in the world and some of its main benefits. *International Journal of Agricultural and Biological Engineering*, **3**, 1–25.

Drijber, R.A., Doran, J.W., Parkhurst, A.M. & Lyon, D.T. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. *Soil Biology and Biochemistry*, **32**, 1419–1430.

Feng, Y., Motta, A.C., Reeves, D.W., Burmester, C.H., van Santon, E. & Osborne, J.A. 2003. Soil microbial communities under conventional till and no-till continuous cotton systems. *Soil Biology and Biochemistry*, **35**, 1693–1703.

Fernandez, M.F., Saxona, J. & Dick, R. 2013. Comparison of whole cell fatty acids (MIDI) or phospholipid fatty acids (PLFA) extractants as biomarkers to profile soil microbial communities. *Microbial Ecology*, **66**, 145–157.

Ferrari, A.E., Ravnskov, S., Larsen, J., Tønnersen, T., Maronna, R. & Wall, L.G. 2015. Crop rotation and seasonal effects on fatty acid profiles of neutral and phospholipids extracted from no-till agricultural soils. *Soil Use and Management*, **31**, 165–175.

Figuerola, E.L.M., Guerrero, L.D., Rosa, S.M., Simonetti, L., Duval, M.E., Galantini, J.A., Bedano, J.C., Wall, L.G. & Erijman, L. 2012. Bacterial indicator of agricultural management for soil under no-till crop production. *PLoS ONE*, **7**, e51075.

Frostegård, A., Tunlid, A. & Bååth, E. 2011. Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry*, **43**, 1621–1625.

Grigera, M.S., Drijber, R.A., Shores-Morrow, R.H. & Wienhold, B.J. 2007. Distribution of the arbuscular mycorrhizal biomarker C16:1*cis*11 among neutral, glycol and phospholipids extracted from soil during the reproductive growth of corn. *Soil Biology and Biochemistry*, **39**, 1589–1596.

Hedrick, D.B., Peacock, A. & White, D.C. 2010. Interpretation of fatty acid profiles of soil microorganisms. In: *Manual of soil analysis–Monitoring and assessing soil bioremediation* (eds R. Margesin & F. Schinner), pp. 251–259. Springer-Verlag, Berlin, Heidelberg.

Ibekwe, A.M. & Kennedy, A.C. 1999. Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. *Plant and Soil*, **206**, 151–161.

Ibekwe, A.M., Kennedy, A.C., Frohne, P.S., Papiernik, S.K., Yang, C.H. & Crowley, D.E. 2002. Microbial diversity along a transect of agronomic zones. *FEMS Microbiology Ecology*, **39**, 183–191.

Karlinski, L., Ravnskov, S., Kieliszewska-Rokicka, B. & Larsen, J. 2007. Fatty acid composition of various ectomycorrhizal fungi and ectomycorrhizas of Norway spruce. *Soil Biology and Biochemistry*, **39**, 854–866.

Liebig, M., Carpenter-Boggs, L., Johnson, J.M.F., Wright, S. & Barbour, N. 2005. Cropping system effects on soil biological characteristics in the Great Plains. *Renewable Agriculture and Food Systems*, **21**, 36–48.

MacNaughton, S.J., Stephen, J.R., Venosa, A.D., Davis, G.A., Chang, Y.J. & White, D.C. 1999. Microbial population changes during bioremediation of an experimental oil spill. *Applied Environmental Microbiology*, **65**, 3566–3574.

Meriles, J.M., Vargas Gil, S., Conforto, C., Figoni, G., Lovera, E., March, G.J. & Guzman, C.A. 2009. Soil microbial communities under different soybean cropping systems: characterization of microbial population dynamics, soil microbial activity, microbial biomass and fatty acid profiles. *Soil and Tillage Research*, **103**, 271–281.

Miller, L.T. 1982. Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *Journal of Clinical Microbiology*, **16**, 584–586.

Müller-Stover, D., Hauggaard-Nielsen, H., Eriksen, J., Ambus, P. & Johansen, A. 2012. Microbial biomass, microbial diversity, soil carbon storage and stability after incubation of soil from grass-clover pastures of different age. *Biology and Fertility of Soils*, **48**, 371–383.

Olsson, P.A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology*, **29**, 303–310.

Ravnskov, S., Jensen, B., Knudsen, I.M.B., Bødker, L., Jensen, D.F., Karlinski, L. & Larsen, J. 2006. Soil inoculation with the biocontrol agent *Clonostachys rosea* and the mycorrhizal fungus *Glomus intraradices* results in mutual inhibition, plant growth promotion and alteration of soil microbial communities. *Soil Biology and Biochemistry*, **38**, 3453–3462.

Ritchie, N.J., Schutter, M.E., Dick, R.P. & Myrold, D.D. 2000. Use of length heterogeneity PCR and fatty acid methyl ester profile to characterize microbial communities in soil. *Applied Environmental Microbiology*, **66**, 1668–1675.

Romaniuk, R., Giuffré, L., Constantini, A. & Nannipieri, P. 2011. Assessment of soil microbial diversity measurements as indicators of soil functioning in organic and conventional horticulture systems. *Ecological Indicators*, **11**, 1345–1353.

Rosa, S.M., Kraemer, F.B., Soria, M.A., Guerrero, L.D., Morrás, H.J.M., Figuerola, E.L.M. & Erijman, L. 2014. The influence of soil properties on denitrifying bacterial communities and denitrification potential in no-till production farms under contrasting management in the Argentinean Pampas. *Applied Soil Ecology*, **75**, 172–180.

Scott, J.J., Budsberg, K.J., Suen, G., Wixon, D.L., Balser, T.C. & Currie, C.R. 2010. Microbial community structure of leaf-cutter ant fungus gardens and refuse dumps. *PLoS ONE*, **5**, e9922.

Sun, H., Koal, P., Liu, D., Gerl, G., Schroll, R., Gattinger, A., Joergensen, R.G. & Munch, J.C. 2016. Soil microbial community and microbial residues respond positevely to minimum tillage under organic farming in southern Germany. *Applied Soil Ecology*, **108**, 16–24.

Van Groenigen, K.J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D. & Jones, M.B. 2010. Abundance production and stabilization of microbial biomass under conventional and reduced tillage. *Soil Biology and Biochemistry*, **42**, 48–55.

Wall, L.G. 2011. The BIOSPAS consortium: soil biology and agricultural production. In: *Handbook of molecular microbial ecology I: metagenomics and complementary approaches* (ed. F. de Bruijn), pp. 299–306. John Wiley & Sons Inc, Hoboken, NJ.

Weon, H.Y., Son, J.A., Yoo, S.H., Hong, S.B., Jeon, Y.A., Kwon, S.W. & Koo, B.S. 2009. *Rubellimicrobium aerolatum* sp. nov., isolated from an air sample in Korea. *International Journal of Systematic and Evolutionary Microbiology*, **59**, 406–410.

Zelles, L. 1999. Fatty acid pattern of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. *Biology and Fertility of Soils*, **29**, 111–129.

Zelles, L., Bai, Q.Y., Beck, T. & Beese, F. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. *Soil Biology and Biochemistry*, **24**, 317–323.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of 75 fatty acids identified by the MIDI system in the WSFA profile, classified in eight chemical groups.

Table S2. List of fatty acids preferably extracted by the WSFA methods in summer and winter, together with the WSFA/PLFA+NLFA ratio in every sampling season.