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Article

Changes in Microbial Community of Agricultural Soils Subjected to Organic Farming System in Korean Paddy Fields with No-till Management

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Soil management for organic farming depends on the effects of soil microbial activities and aggregation. The seasonal changes were evaluated in the soil microbial community by fatty acid methyl ester (FAME) and total glomalin analysis in an organic farming system (OFS) with no-till management compared to those in a conventional farming system (CFS) with tillage and chemical amendments in a flooded paddy. The average concentrations of individual FAMEs and glomalin in the OFS were significantly higher than those in the CFS during rice-growing stages (p<0.001). OFS had significantly lower ratio of cy17:0/16:1ω7c and higher ratio of monounsaturated fatty acids to saturated fatty acids compared with those of CFS (p<0.001), indicating that microbial stress decreased due to organic soil inputs and the lack of chemical amendments, whereas communities of Gram-negative bacteria in OFS soils were significantly larger than those in CFS soils (p<0.001). Gram-negative bacteria should be considered as potential factor responsible for the clear microbial community differentiation observed between the OFS and the CFS in flooded paddy fields.

Key words: fatty acid methyl ester, glomalin, Gram-negative bacteria, microbial community, organic farming

Organic farming systems (OFSs), such as no-till rice production, are rapidly expanding worldwide. In comparison with conventional farming systems (CFSs), OFSs promote soil structure formation [Wright *et al*., 1999], enhance soil biodiversity [Mäder *et al*., 2002], and alleviate environmental stress [Altieri, 2002], because organic farming avoids synthetic chemical amendments and supplies organic fertilizer. Enhancing the soil microbial community in OFSs has promoted soil aggregation. Arbuscular mycorrhizal fungi (AMF) and glomalin have important roles in soil aggregation in OFSs. The AMF are present in most roots of plants and produce glomalin [Wright *et al*., 1996], which is a brown to reddish-brown glycoprotein that plays a major role in aggregate stabilization [Rilling *et al*., 2005]. Practices associated with OFSs have a positive effect on the soil microbial community and soil aggregation, but there is little evidence of a link between AMF and glomalin in flooded paddy [Stark *et al*., 2007]. Moreover, analyses of AMF and glomalin in flooded paddy soils are technically very difficult.

The fatty acid methyl ester (FAME) technique has been used to characterize root-associated microorganisms and AMF [Graham *et al*., 1995] and to describe microbial communities in agricultural soils [Buyer and Drinkwater, 1997; Macalady *et al*., 1998]. FAME composition provides quantitative insight into the microbial community, which can be sampled from the environment without the need for isolation [Pennanen, 2001]. In addition, FAME profiles of soils can be compared using multivariate statistical techniques to reveal differences in microbial

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communities [Frostegård and Bååth, 1996; Macalady *et al*., 1998]. The FAME method is also relatively simple and fast as well as effective for assessing microbial community structure [Schutter and Dick, 2000]. Previous reports have indicated that practices associated with organic farming have a positive effect on the soil microbial community and soil aggregation, but so far, there has been little evidence that the microbial communities in flooded paddy soil under OFS and CFS differ [Stark *et al*., 2007].

Therefore, the objectives of the present study were to measure seasonal changes in soil microbial communities by FAME, total glomalin concentration, and the ratio of glomalin to the AMF biomarker, as well as to identify potential factors responsible for the clear microbial community differentiation observed between OFS and CFS in flooded paddy soils.

Materials and Methods

Experimental site description. This study was conducted at a rice paddy in Hadong, Gyeongsangnam-do, Korea, from May in 2009 to October in 2010. The experimental fields were located at 35°02'47"N latitude and 128°51'27"E longitude. During the experimental period, the average temperature was 21.9°C and the annual precipitation was 1,414 mm in this area. According to the standard method of National Institute of Agricultural and Technology (NIAST) (2000), where the taxonomical classification of Korean soil is performed for analysis of soil and plant, the soil was of Jisan series (loamy soil consisting of 42.5% silt, 23.2% clay, and 34.3% sand) as revealed by pipette analysis. This soil was also classified by NIAST as fine loamy, mixed, mesic family of Fluvaquentic Endoaquepts. Components of the soil at the experimental sites were 39.8-42.8 g/kg organic matter, 181-195 mg/kg available phosphorus (P_2O_5), 0.87-0.96 $cmol_c$/kg exchangeable K^+, 9.0-9.7 $cmol_c$/kg exchangeable Ca^{2+}, 2.0-2.3 $cmol_c$/kg exchangeable Mg^{2+}, 0.32-0.37 $cmol_c$/kg exchangeable Na^+, 13-27 mg/kg NH_4^+-N, and 213~232 mg/kg available SiO_2, with pH ranging from 5.8-6.2. The soil samples were prepared at 1:5 of soil:distilled water and analyzed using standard methods of NIAST (2000).

The size of each experimental plot was 1,500 m^2 (50 m ×30 m), and all experiments were conducted in a randomized complete block design with three replications. The rice plant was transplanted on 10 June with 30 cm× 15 cm plant density and was harvested on 15 October every year. Every year on 25 May, before transplanting OFS experimental plot was prepared with no-tillage and treated with organic fertilizer (N-P_2O_5-K_2O=90-15-60 kg/ha), whereas the CFS plot was tilled and treated with a chemical fertilizer containing the same nutrients as the organic fertilizer. The CFS was applied in split application, one third as the basal dose was applied on 25 May (137 kg/ha urea, 75 kg/ha fused phosphate and 70 kg/ha potassium chloride), and the remaining fertilizer was applied on 25 July (59 kg/ha urea and 30 kg/ha potassium chloride). The experimental plots were submerged from 26 May through the middle of July and from the middle of August through the middle of September every year. The CFS plot was treated with herbicide on 20 June and pesticide on 7 August each year. The herbicide penoxsulam (0.08% active ingredient) in granule form was added into soil to make the final concentration of 2.0 mg/kg. The pesticide was pencycuron (25% active ingredient) in wettable powder and added into soil to make the final concentration of 0.15 mg/kg. Soil samples were collected from the topsoil (0-15 cm in depth) before transplanting stage, tillering stage, heading stage, and harvesting stage. Soil samplings were done in triplicate. The samples were freeze-dried and kept in a freezer at −20°C before analysis.

Analysis of total glomalin in soil samples. Total glomalin was extracted from the soil with 100 mM sodium pyrophosphate, pH 9.0 [Wright *et al*., 2006]. The pH was adjusted with HCl. Three replicates of 2.0 g of freeze-dried 1-2 mm soil aggregates were put into centrifuge tubes, and 8 mL of 100 mM sodium pyrophosphate (pH 9.0) was added. The soils in the tubes were stirred for 10 s to allow soil:solution contact and then autoclaved for 60 min at 121°C. Each sample was extracted until the supernatant showed the straw color typical of glomalin. After completion of the extraction cycles, the samples were centrifuged at 5,000×g for 10 min to remove the soil particles, and the protein content in the supernatant was subjected to the Bradford assay using a UV-1650PC spectrophotometer (Shimadzu, Kyoto, Japan) with bovine serum albumin as the standard. The concentration of glomalin was extrapolated to mg/g of aggregated soil particles by correcting for the dry weight of coarse fragments included in the weight of aggregates and for the volume of extractant.

Assay of soil microbial communities. Microbial community structure was characterized by the extraction and analysis of ester-linked fatty acid methyl esters from soil, as described by Schutter and Dick [2000]. In the first step, 15 mL of 0.2 M KOH in methanol was added to a 35-mL teflon-lined, screw-cap glass centrifuge tube containing 3 g of soil. The contents of the tubes were blended for 20 s, and incubated at 37°C for 1 h (with vortexing every 10 min), during which time ester-linked fatty acids were released and methylated. In the second step, 3 mL of 1.0 M acetic acid was added to neutralize

the pH of the tube contents. The FAMEs were partitioned into an organic phase by adding 10 mL of hexane and then vortexed for 60 s, followed by centrifugation at 2,000×g for 20 min. No washing step was needed in this procedure, although the Sherlock Microbial Identification System (MIDI) method requires that all acidic residues be removed from the organic phase to prevent damage to the gas chromatography (GC) column. After the hexane layer (5 mL) was transferred to a clean glass test tube, the hexane was evaporated under a stream of N_2 for 40 min. In the final step, the FAMEs and 30 μL of internal standard 19:0 were dissolved in 170 μL of 1:1 hexane: methyl-tert butyl ether, transferred into a 250-μL glass insert, and placed in a GC vial for analysis. Fatty acids were analyzed by Agilent 6890 GC with a flame ionization detector carried out by an MIDI Sherlocks microbial identification system (Microbial ID, Ins., Newark, DE).

The temperature program ramped from 170 to 270°C at 5°C per min. FAMEs were identified by comparison of retention time and equivalent chain length with known standards (Microb analyzer sample kit, Agilent Technologies) and confirmed by GC-mass spectrometry (MS). Individual fatty acids were used as biomarkers for various groups of microorganisms. The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, i17:0, a17:0, 17:0, cy17:0, 18:1ω7c, and cy19:0 were chosen to represent bacterial FAMEs [Macalady *et al*., 1998; Schutter and Dick, 2000]. The fatty acids 16:1ω7c, 18:1ω7c, cy17:0c, and cy19:0 were chosen to represent Gram-negative bacteria [Zelles, 1997]. The branched, saturated FAMEs i15:0, a15:0, i16:0, i17:0, and a17:0 were chosen to represent Gram-positive bacteria [Zelles, 1997]. The FAME 10Me18:0 was used to indicate soil *Actinomycetes* [Schutter and Dick, 2000]. The soil FAMEs 18:1ω9c and 18:2ω6c were used as indicators of saprophytic fungi [Bradleya *et al*., 2006] and the FAME 16:1ω5c was used as an indicator of AMF [Frostegård *et al*., 1993]. The ratios between the bacterial fatty acids cy17:0 and cy19:0 and their metabolic precursors, 16:1ω7c and 18:1ω7c, and the ratio of total mono-unsaturated fatty acids (MUFA) to total saturated fatty acids (SFA) have previously been used as indicators of environmental stress in bacterial communities [Guckert *et al*., 1986; Bossio and Scow, 1998]. For each sample, the abundance of individual FAMEs were reported in absolute amounts (nmol/g soil) and then converted to nmol percent based on total FAMEs.

Statistical analysis. All data were statistically analyzed using the SAS software version 9.3 for Windows (SAS Institute, Cary, NC). Comparisons of individual soil microbial communities, soil organic matter, and microbiological variables measured were performed using two-way analysis of variance (ANOVA). In cases where significant effects of the interactions between the independent variables were detected, the *F*-test was used to detect and separate the mean treatment differences at 0.1, 1.0, and 5.0% levels of significance ($p<0.001$, $p<0.01$, and $p<0.05$, respectively). In addition, significant effects of the microbial communities and their several ratios in soils between in 2009 and in 2010 were detected, and the Bonferroni t-test (Minimum Significant Difference) was used to detect and separate the mean treatment differences at 5.0% levels of significance ($p<0.05$). After normalizing the data as relative mol %, the soil microbial communities were analyzed by principal component analysis (PCA) to determine the overall effects of OFS and CFS on soil microbial communities during the rice growth stages.

Results and Discussion

The ratios of the microbial community and the biomass of total FAMEs in the soils at each sampling time are shown in Table 1. The average concentration of total FAMEs in the soils during rice-growing stages was higher in OFS (726 nmol/g) compared to CFS (389 nmol/g). Our observation of increased soil microbial biomass during rice growth stages in OFS has also been reported by other researchers in soil management systems from various geographical locations and agroecosystem types [Frey *et al*., 1999; Helgason *et al*., 2007]. In no-till soils, the reduction in physical disturbance to the system slows the rate of organic matter decomposition and contributes to the longevity of microorganisms [Beare *et al*., 1994].

Furthermore, the average concentration of FAME 16:1ω5c in soils during the rice growth period was higher in OFS (7.2 nmol/g) than CFS (5.0 nmol/g). The FAME profile of 16:1ω5c may be a biomarker for AMF [Frostegård *et al*., 1993; Balser *et al*., 2005]. In both treatments, the FAME 16:1ω5c content was generally higher before the submerging stages (20 May 2009 and 2010) and was then significantly decreased until the middle of July in OFS soils due to insufficient oxygen and until the middle of August in CFS soils due to insufficient oxygen and chemical amendments ($p<0.001$). The FAME 16:1ω5c concentrations were generally lower during the submerged period in both OFS and CFS ($p<0.001$). These results showed that concentration of FAME 16:1ω5c in paddy soils was more affected by flooding than by chemical amendments. Similarly, the applications of pesticide and fungicide in the field and laboratory resulted in a significant decrease in the soil AMF and glomalin [Rillig *et al*., 2003; Wang *et al*., 2008]. Therefore, the above results should be of great

Table 1. Changes in soil microbial biomarkers during rice plant growth stages

System	Year	Date	A[b]/B	G(+)/G(-)	F/B	cy17:0 /161ω7c	cy19:0: /18:1ω7c	MUFA /SFA	FAMEs nmol/g	16:1ω5c nmol/g	Glomalin mg/g
CFS[a]	2009	20 May	0.06±0.012[e]	0.96±0.01	0.52±0.02	0.28±0.03	0.63±0.04	0.76±0.03	501±76.3	7.7±1.0	2.46±0.049
		10 Jul.	0.05±0.001	1.20±0.01	0.38±0.02	0.27±0.04	0.56±0.03	0.65±0.03	362±7.8	4.1±0.1	1.85±0.033
		21 Aug.	0.06±0.007	0.97±0.04	0.42±0.01	0.45±0.06	0.57±0.06	0.69±0.01	316±12.1	4.0±0.4	1.85±0.069
		5 Oct.	0.04±0.004	0.96±0.14	0.36±0.03	0.25±0.11	0.57±0.04	0.68±0.04	323±16.0	2.8±0.2	1.18±0.152
	2010	20 May	0.05±0.004	1.02±0.07	0.49±0.03	0.29±0.01	0.49±0.06	0.77±0.03	392±50.7	6.6±0.3	1.82±0.030
		21 Jul.	0.04±0.005	1.13±0.03	0.43±0.01	0.30±0.03	0.46±0.01	0.70±0.02	412±12.3	4.9±0.5	1.51±0.040
		23 Aug.	0.05±0.002	1.07±0.02	0.43±0.01	0.28±0.01	0.55±0.12	0.68±0.02	342±16.5	4.1±0.3	1.38±0.097
		8 Oct.	0.04±0.003	1.05±0.09	0.48±0.03	0.24±0.04	0.46±0.05	0.76±0.05	461±47.7	5.7±0.7	1.30±0.074
	Average		0.05±0.009	1.04±0.10	0.44±0.05	0.29±0.08	0.54±0.08	0.71±0.05	389±71.1	5.0±1.6	1.67±0.040
OFS	2009	20 May	0.07±0.015	0.75±0.07	0.46±0.05	0.23±0.04	0.67±0.07	0.81±0.06	711±81.0	7.6±1.5	2.64±0.088
		10 Jul.	0.05±0.001	0.75±0.08	0.42±0.04	0.30±0.09	0.56±0.25	0.78±0.10	598±136.7	6.1±0.5	2.21±0.143
		21 Aug.	0.06±0.010	0.67±0.11	0.58±0.12	0.21±0.08	0.49±0.09	0.87±0.13	816±198.8	5.9±0.5	2.27±0.110
		5 Oct.	0.05±0.002	0.63±0.06	0.45±0.01	0.17±0.01	0.43±0.07	0.93±0.04	785±84.7	8.6±1.3	2.35±0.023
	2010	20 May	0.06±0.005	0.76±0.04	0.64±0.02	0.20±0.01	0.47±0.02	0.82±0.04	754±40.0	8.5±0.6	3.14±0.035
		21 Jul.	0.05±0.010	0.85±0.09	0.54±0.07	0.22±0.03	0.50±0.15	0.77±0.07	676±55.1	7.5±1.7	2.62±0.318
		23 Aug.	0.04±0.009	0.78±0.01	0.39±0.15	0.18±0.01	0.41±0.02	0.67±0.18	712±69.9	7.2±0.2	2.12±0.059
		8 Oct.	0.03±0.005	0.72±0.13	0.53±0.04	0.16±0.07	0.40±0.05	0.82±0.11	757±69.1	6.1±0.5	1.63±0.061
	Average		0.05±0.014	0.74±0.10	0.50±0.11	0.21±0.06	0.49±0.13	0.81±0.11	726±116.6	7.2±1.3	2.37±0.438
Significance[c]	System		**	***	***	***	NS	***	***	***	***
	Date		***	***	***	**	**	NS	NS	***	***
	System×Date		*	NS	*	*	NS	NS	NS	***	***
Year	2009		0.05±0.01	0.86±0.20	0.45±0.08	0.27±0.10	0.56±0.11	0.77±0.11	551±214	5.8±2.1	2.10±0.450
	2010		0.05±0.01	0.92±0.16	0.49±0.09	0.23±0.06	0.47±0.08	0.75±0.09	563±180	6.3±1.5	1.94±0.630
	MSD[d] (5%)		0.006	NS	NS	NS	0.057	NS	NS	NS	NS

[a]CFS, conventional farming system; OFS, organic farming system. [b]A, *Actinomycetes*; B, total bacteria; G(+), Gram-positive bacteria; G(-), Gram-negative bacteria; F, fungi; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; FAMEs, total fatty acid methyl esters. [c]Significant effects were obtained from two-way analysis of variance: NS, not significant at $p<0.05$; *, significant at $p<0.05$; **, significant at $p<0.01$ and ***, significant at $p<0.001$. [d]MSD, minimum significant difference. [e] Mean±standard deviation.

Table 2. Soil microbial communities of different farming systems expressed as % total FAME at rice plant growth stages

System	Year	Date	B[b]	G(+)	G(-)	A	F	AMF	PC 1	PC2
CFS[a]	2009	20 May	31.1±0.8[e]	13.3±0.7	13.9±0.6	1.70±0.4	16.1±0.2	1.55±0.06	-0.07±0.25	1.15±0.68
		10 Jul.	32.1±1.5	15.2±0.7	12.7±0.7	1.44±0.1	12.2±0.9	1.14±0.02	-1.26±0.20	-0.07±0.19
		21 Aug.	32.1±1.3	13.7±0.1	14.2±0.6	1.83±0.3	13.6±0.6	1.27±0.10	-0.82±0.11	0.99±0.51
		5 Oct.	32.8±1.5	14.3±1.3	15.0±0.9	1.27±0.1	11.7±0.5	0.85±0.06	-0.96±0.67	-1.01±0.22
	2010	20 May	32.4±1.0	14.6±0.9	14.3±0.1	1.71±0.1	15.7±0.5	1.69±0.16	-0.49±0.45	0.66±0.11
		21 Jul.	32.3±0.1	15.1±0.2	13.4±0.2	1.34±0.2	13.8±0.3	1.18±0.15	-0.99±0.08	-0.53±0.35
		23 Aug.	32.0±1.7	14.8±0.8	13.8±0.6	1.58±0.1	13.8±0.7	1.20±0.14	-0.90±0.23	0.03±0.33
		8 Oct.	33.7±1.2	15.4±1.2	14.7±0.4	1.28±0.1	16.0±0.6	1.23±0.07	-0.65±0.51	-0.83±0.29
	Average		32.3±1.2	14.5±1.0	14.0±0.8	1.52±0.3	14.1±1.7	1.26±0.26	-0.77±0.46	0.05±0.84
OFS	2009	20 May	33.0±2.2	12.5±1.3	16.7±1.0	2.31±0.4	15.1±1.1	1.06±0.14	0.54±0.56	1.69±1.09
		10 Jul.	32.4±1.1	12.4±1.3	16.5±0.5	1.53±0.1	13.6±1.0	1.06±0.29	0.10±0.81	-0.02±0.72
		21 Aug.	29.2±1.9	10.3±1.1	15.5±1.7	1.81±0.3	16.7±2.7	0.75±0.14	1.46±1.02	0.35±0.74
		5 Oct.	32.4±0.6	11.5±0.7	18.4±0.8	1.55±0.1	14.5±0.1	1.09±0.07	1.07±0.29	-0.53±0.27
	2010	20 May	29.3±0.7	11.3±0.3	14.8±0.6	1.77±0.1	18.8±0.9	1.13±0.06	1.47±0.13	0.88±0.23
		21 Jul.	30.5±2.3	12.4±1.5	14.5±0.8	1.42±0.2	16.4±1.6	1.11±0.01	0.55±0.85	-0.00±0.50
		23 Aug.	30.0±1.1	11.8±0.3	15.1±0.4	1.25±0.2	11.8±4.9	1.02±0.07	0.11±0.63	-0.99±0.32
		8 Oct.	30.9±0.4	11.6±1.2	16.4±1.3	1.01±0.2	16.4±1.2	0.81±0.04	0.84±0.78	-1.78±0.55
	Average		30.9±1.9	11.7±1.1	16.0±1.5	1.58±0.4	15.4±2.7	1.00±0.17	0.77±0.77	0.84±1.15
Significance[c]	System		**	***	***	NS	**	***	***	NS
	Date		NS	NS	***	***	***	***	*	***
	System×Date		*	NS	**	*	*	***	NS	*
Year	2009		31.9±1.7	12.9±1.7	15.3±1.9	1.68±0.4	14.2±2.0	1.10±0.26	0.01±1.05	0.32±1.01
	2010		31.4±1.7	13.4±1.8	14.6±1.0	1.42±0.3	15.3±2.6	1.17±0.25	-0.01±0.97	-0.32±0.90
	MSD[d] (5%)		NS	NS	NS	0.19	NS	NS	NS	0.56

[a]CFS, conventional farming system; OFS, organic farming system. [b]B, total bacteria; G(+), Gram-positive bacteria; G(-), Gram-negative bacteria; A, *Actinomycetes*; F, fungi; AMF, arbuscular mycorrhizal fungi, PC, principal component. [c]Significant effects were obtained from two-way analysis of variance: NS, not significant at $p<0.05$; *, significant at $p<0.05$; **, significant at $p<0.01$ and ***, significant at $p<0.001$. [d]MSD, minimum significant difference. [e]Mean±standard deviation.

importance for OFSs [Rillig *et al*., 2001].

The average concentration of total glomalin in the soils was higher in OFS (2.37 mg/g) compared to CFS (1.67 mg/g) during the rice growth stages (p<0.001). This result is attributed to the tillage and chemical amendments in the CFS. Wright *et al*. [1999] revealed that the content of glomalin, an exudation product of AMF hyphae having a role in soil aggregation, was higher in no-till than tilled soils. The total glomalin content was highest before the submerged stage for both the OFS and the CFS; it rapidly and significantly decreased after submersion. According to Rillig *et al*. [2003], 50% of the total glomalin (4.91 mg/cm^3) was still detectable after over 400 days of incubation in the A horizon. Wright and Upadhyaya [1998] have established a strong curvilinear relationship between the AMF hyphal product "glomalin" and soil aggregate water stability across several soil types. Therefore, AMF and total glomalin will be excellent indicators for OFS [Johnson *et al*., 2001]. Further studies on total glomalin are necessary to reveal a suitable management strategy and a foundation for novel biotechnological application in OFS.

The ratios of cy17:0/16:1ω7c, cy19:0/18:1ω7c and MUFA/SFA have previously been used as indicators of environmental stress in bacterial communities [Guckert *et al*., 1986; Bossio and Scow, 1998]. OFS soils showed a lower average ratio of cy17:0/16:1ω7c (0.21) and a higher average ratio of MUFA/SFA (0.81) compared to CFS soils (0.29 and 0.71, respectively), indicating that microbial stress decreased in the OFS [Mechri *et al*., 2010] (Table 1). A lower cyclopropyl precursor ratio has been associated with an increase in bacterial growth rates and a decrease in carbon limitation [Kieft *et al*., 1997; Bossio and Scow, 1998]. Bossio *et al*. [1998] showed that different farming regimes, such as different organic inputs, influence fatty acid profiles and cause the MUFA to increase with organic input to the soil. It must be noted that in the present study, there was a strong effect of different cultivation systems on the abundances of MUFA in the soil [Bossio *et al*., 1998; Lundquist *et al*., 1999]. In addition, an increase in the production of cyclopropyl fatty acids has been shown for pesticide application and tillage [Macalady *et al*., 1998]. The fungi/bacteria ratio after submerging the paddy decreased in both CFS and OFS soils and was higher in the OFS (0.49) than in the CFS (0.42) during the rice growth stages (p<0.001). Fungi are more sensitive to disturbance than bacteria, and they tend to respond more quickly to changes in soil organic matter and submersion [Mamilov and Dilly, 2002; Hamman *et al*., 2007].

In addition, the microbial communities in the CFS and OFS soils expressed as % total FAMEs were determined

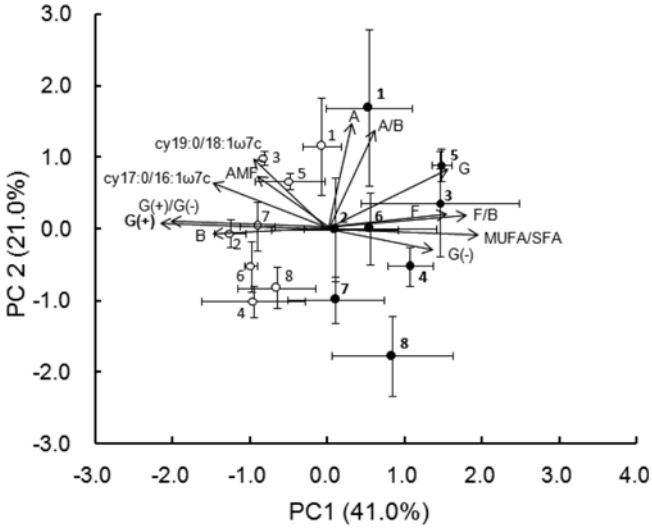

Fig. 1. Principal component analyses of soil microbial communities from an organic farming system (●) and a conventional farming system (○). The variance explained by each principal component (PC) axis is shown in parentheses. PC analysis shows loading values for the individual microbial biomarkers. The bars represent one standard deviation of the mean. 1) 20 May, 2009; 2) 10 Jul. 2009; 3) 21 Aug., 2009; 4) 5 Oct., 2009; 5) 20 May, 2010; 6) 21 Jul., 2010; 7) 23 Aug., 2010, and 8) 8 Oct., 2010. A, *Atinomycetes*; AMF, Arbuscular mycorrhizal fungi; B, total bacteria; F, fungi; G, total glomalin; G(+), Gram-positive bacteria; G(-), Gram-negative bacteria; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. Significant effects of PC1 were obtained from two-way analysis of variance. The system was significant at p<0.001, the date was significant at p<0.05, and system date interaction was not significant.

(Table 2). The total bacteria community was generally larger in the CFS soils (average 32.3%) compared to the OFS soils (average 30.9%) during rice growth stages (p<0.01). The average community of Gram-positive bacteria in the CFS soils (14.5%) was higher than that in the OFS soils (11.7%), whereas the community of Gram-negative bacteria was lower in the CFS soils (14.0%) than in the OFS soils (16.0%) during rice growth period (p<0.001). Thus, the average ratio of Gram-positive bacteria/Gram-negative bacteria was higher in the CFS (1.04) compared to the OFS (0.74) during rice growth stages (Table 1). The Gram-negative bacteria were faster growing and more active than the Gram-positive bacteria and competed better for the available substrates released from organic matter [Mechri *et al*., 2010]. The average community of *Actinomycetes* did not differ significantly between CFS (1.52) and OFS (1.58) during rice growth period (Table 2). Interestingly, the patterns of the soil microbial biomass (Table 1) and the soil microbial communities (Table 2) between OFS and CFS were very similar for both 2009 and 2010. These findings were in

agreement with prior results obtained for tillage practice and cropping sequence [Rotenberg *et al*., 2007].

To explain the effect of OFS on soil microbial community structure, after normalizing the data as relative mol %, PCA was performed for all individual microbial communities in the soil samples derived from each cultivation system and each sampling time (Fig. 1). The coordinates of soil samples were plotted on the two first principal components. The first variable accounted for 41.0% of the variation, whereas the second variable accounted for 21.0% of the variation. PC1 showed significant difference between the OFS (average 0.77) and the CFS (average -0.77) during rice growth period ($p<0.001$). This was in agreement with the previous results obtained for different soil management practices [Meriles *et al*., 2009]. Practical considerations suggest that OFS showed higher enhancement of the soil microbial community profiles compared to CFS. These findings suggest that OFS had the potential to change microbial community structure. Drenovsky *et al*. [2004] found differences in the microbial community with changes in soil organic matter and soil water content in the California agricultural soils. To obtain detailed information on the factors responsible for the separation of different cultivation systems, correlations between the variables and the factors were calculated (Fig. 1). The FAMEs with positive eigenvector coefficients for PC1 were MUFA/SFA, fungi/bacteria, glomalin, fungi, and Gram-negative bacteria, whereas negative eigenvector coefficients were found for Gram-positive bacteria, Gram-positive bacteria/Gram-negative bacteria, total bacteria, and cy17:0/16:1ω7c. In particular, the community of Gram-negative bacteria had a positive correlation with OFS (Table 2 and Fig. 1). These findings suggest that the shifting community of Gram-negative bacteria should be considered as a potential factor responsible for the clear soil microbial community differentiation observed between OFS and CFS.

In conclusion, OFS was responsible for a strong effect on the microbial composition of paddy soils. Further work is needed to determine whether the new microbial activity affected the OFS separately from soil texture and submersion by FAME analysis.

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References

Altieri MA (2002) Agroecology: The sceience of natural resource managemen for poor farmers in marginal environments. *Agric Ecosyst Environ* **93**, 1-24.

Balser T, Treseder KK, and Ekenler M (2005) Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. *Soil Biol Biochem* **37**, 601-604.

Beare MH, Hendrix PF, and Coleman DC (1994) Water-stable aggregates and organic matter fractions in conventional-and no-tillage soils. *Soil Sci Soc Am J* **58**, 777-786.

Bossio DA and Scow KM (1998) Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microb Ecol* **35**, 265-278.

Bossio DA, Scow KM, Gunapala N, and Graham KJ (1998) Determinants of soil microbial communities: Effects of management, season and soil type on phospholipid fatty acid profiles. *Microb Ecol* **36**, 1-12.

Bradleya K, Rhae A, Drijberb RA, and Knopsc J (2006) Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *Soil Biol Biochem* **38**, 1583-1595.

Buyer JS and Drinkwater LE (1997) Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. *J Microbiol Meth* **30**, 3-11.

Drenovsky RE, Vo D, Graham KJ, and Scow KM (2004) Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microb Ecol* **48**, 424-430.

Frey SD, Elliott ET, and Paustian K (1999) Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biol Biochem* **31**, 573-585.

Frostegård Å and Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol Fertil Soils* **22**, 59-65.

Frostegård Å, Tunlid A, and Bååth E (1993) Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl Environ Microbiol* **59**, 3605-3617.

Graham JH, Hodge NC, and Morton JB (1995) Fatty acid methyl ester profiles for characterization of glomalean fungi and their endomycorrhizae. *Appl Environ Microbiol* **61**, 58-64.

Guckert JB, Hood MA, and White DC (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of Vibrio cholerae: Increases in cis/trans ratio and proportions of cyclopropyl fatty acid. *Appl Environ Microbial* **52**, 794-801.

Hamman ST, Burke IC, and Strombeerger ME (2007) Relationships between microbial community structure and soil environmental conditions in a recently burned system. *Soil Biol Biochem* **39**, 1703-1711.

Helgason BL, Walley FL, and Germida JJ (2007) Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. *Soil Sci Soc Am J* **73**, 120-127.

Johnson CK, Doran JW, Duke HR, Wienhold BJ, Eskridge KM, and Shanahan JF (2001) Field-scale electrical conductivity mapping for delineating soil condition. *Soil Sci Soc Am J* **65**, 1829-1837.

Kieft TL, Wilch E, O'connor K, Ringelberg DB, and White DC (1997) Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. *Appl Environ Microbiol* **63**, 1531-1542.

Lundquist EJ, Scow KM, Jackson LE, Uesugi SL, and Johnson CR (1999) Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. *Soil Biol Biochem* **31**, 1661-1675.

Macalady JL, Fuller ME, and Scow KM (1998) Effects of metam sodium fumigation on soil microbial activity and community structure. *J Environ Qual* **27**, 54-63.

Mäder P, Flieâbach A, Dubois D, Gunst L, Fried P, and Niggli U (2002) Soil fertility and biodiversity in organic farming. *Science* **296**, 1694-1697.

Mamilov AS and Dilly OM (2002) Soil microbial eco-physiology as affected by short-term variations in environmental conditions. *Soil Biol Biochem* **34**, 1283-1290.

Mechri B, Chehab H, Attia F, Mariem FB, Braham M, and Hammami M (2010) Olive mill wastewater effects on the microbial communities as studied in the field of olive trees by analysis of fatty acid signatures. *Eur J Soil Biol* **46**, 312-318.

Meriles JM, Vargas Gil S, Conforto C, Figoni G, Lovera E, March GJ, and Guzmán CA (2009) Soil microbial communities under different soybean cropping systems: Characterization of microbial population dynamics, soil microbial activity, microbial biomass, and fatty acid profiles. *Soil Till Res* **103**, 271-281.

Pennanen T (2001) Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH-a summary of the use of phospholipids fatty acids, Biolog® and 3H-Thymidine incorporation methods in field studies. *Geoderma* **100**, 91-126.

Rilling MC, Lutgen ER, Ramsey PW, Klironomos JN, and Gannon JE (2005) Microbiota accompanying different arbuscular mycorrhizal fungal isolates influence soil aggregation. *Pedobiologis* **49**, 251-259.

Rillig MC, Ramsey PW, Morris S, and Paul EA (2003) Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. *Plant Soil* **253**, 293-299.

Rillig MC, Wright SF, Nichols KA, Schmidt WF, and Torn MS (2001) Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* **233**, 167-177.

Rotenberg D, Joshi R, Benítez M, Chapin LG, Camp A, Zumpetta C, Osborne A, Dick WA, and McSpadden Gardener BB (2007) Farm management effects on rhizosphere colonization by native populations of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. and their contributions to crop health. *Phytopathology* **97**, 756-766.

Schutter ME and Dick RP (2000) Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. *Soil Sci Soc Am J* **64**, 1659-1668.

Stark C, Condron LM, Stewart A, Di HJ, and O'Callaghan M (2007) Influence of organic and mineral amendments on microbial soil properties and processes. *Appl Soil Ecol* **35**, 79-93.

Wang MC, Liu YH, Wang Q, Gong M, Hua XM, Pang YJ, Hu S, and Yang YH (2008) Impact of methamidophos on the biochemical, catabolic, and genetic characteristics of soil microbial communities. *Soil Biol Biochem* **40**, 778-788.

Wright SF and Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* **198**, 97-107.

Wright SF, Franke-Snyder M, Morton JB, and Upadhyaya A (1996) Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant Soil* **181**, 193-203.

Wright SF, Nichols KA, and Schmidt WF (2006) Comparison of efficacy of three extractants to solubilize glomalin on hyphae and in soil. *Chemosphere* **64**, 1219-1224.

Wright SF, Starr JL, and Paltineanu IC (1999) Changes in aggregate stability and concentration of glomalin during tillage management transition. *Soil Sci Soc Am J* **63**, 1825-1829.

Zelles L (1997) Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* **35**, 275-294.