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Comparison of community structures of microbiota at main habitats in rice field ecosystems based on phospholipid fatty acid analysis

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ABSTRACT

The present study compared the community structures of microbiota at different habitats in Japanese rice fields by comparing their phospholipid fatty acids (PLFA) composition to understand the contribution of different habitats to microbiological diversity. The data were collected from four neighboring rice fields. Comparison was made for the PLFA compositions extracted from the floodwater, percolating water, rice soils under flooded and drained conditions, rice straw (RS) placed in flooded and drained rice soils, RS in composting process and RS composts placed in a flooded rice field. Average amounts of PLFAs were 33 μg L^{-1} in the floodwater, 17.1 μg L^{-1} in the percolating water from plow layers, 34.6 μg L^{-1} in the percolating water from subsoil layers, 108 μg g^{-1} dw (dry weight basis) in flooded rice soils, 382 μg g^{-1} dw in RS materials, 2510 μg g^{-1} dw in RS composts, 2850 μg g^{-1} dw in RS composts after application to a flooded rice soil, 222 μg g^{-1} ww (wet weight basis) in RS in drained rice soils, and 284 μg g^{-1} ww in RS in flooded rice soils. The total amount of PLFAs to the soil depth of 10 cm was estimated to be about 12 g m^{-2}. The PLFA compositions were different from each other depending on the habitats. Rice soils were characterized by the predominance of actinomycetes and Gram-positive bacteria in comparison with the other habitats. In contrast, the microbial communities in the floodwater and percolating water were characterized by the predominance of Gram-negative bacteria and eukaryotes (presumably

algae), and Gram-negative bacteria, respectively. The microbial community of RS materials was dominated by fungi. Gram-positive bacteria became predominant in RS after application to flooded rice soils, while RS placed in a drained rice field after harvesting rice was characterized by the predominance of Gram-negative bacteria and fungi. The community structures at respective habitats were stable and specific irrespective of the season of sampling and the duration of decomposition of RS.

Key words: community structure, diversity, PLFA, rice field ecosystem

INTRODUCTION

Rice field is a unique agro-ecosystem, where the field is maintained under flooded conditions during the most period of rice cultivation and left under drained conditions during the off-crop season. Flooding the field results in several specific habitats for microbiota such as floodwater and reduced soil layer (Kimura 2000). The reduced soil layer becomes aerobic after drainage for harvesting rice. Rice straw (RS) and stubble, rice rhizosphere, applied RS compost and percolating water are also specific habitats for microbiota in the rice field ecosystem. Thus, a rice field consists of diverse habitats for microorganisms in time and space.

The phospholipid fatty acids (PLFAs) analysis characterizes the community structure

of microbiota belonging to not only eubacteria but also eukaryotes (Zelles 1999). Phospholipid fatty acids composition has been used as bio-markers for identifying specific groups of microorganisms (Lechevalier and Lechevalier 1988): straight, mono-unsaturated PLFAs for Gram-negative bacteria (Wilkinson 1988), branched-chain PLFAs for Gram–positive bacteria (O'Leary and Wilkinson 1988), methylated PLFAs for actinomycetes (Kroppenstedt 1992), 20:3ω6 and 20:4ω6 PLFAs for protozoa (Vestal and White 1989; Herrmann and Shann 1997) and straight, poly-unsaturated PLFAs for eukaryotes including fungi (Lösel 1988; Zelles 1999). Our group has used this technique to elucidate microbial communities in various habitats in rice fields such as floodwater, percolating water, RS in soil, RS under composting process, RS compost in soil, and rice soils as shown in Table 1. These studies showed the predominant members in microbial communities, relative successions and effects of fertilizer application on the community structure at the respective habitats. However, direct comparison has not been conducted among microbiota in various habitats to understand the "whole community structures of microbiota in rice field ecosystems". As rice field ecosystems consist of distinct habitats for organisms, which would make the ecosystem diverse as a whole, such comparison is requisite to understand the diversity of microbial world in the rice field ecosystem. The following question still remains unanswered: "Is the microbial diversity in rice field ecosystems mainly caused by the assemblage of various

microbial habitats, by the variations of environmental conditions at the respective habitats, or by both?" The present study compared the community structures of microbiota at different habitats in rice fields by measuring their PLFAs compositions in order to answer this question. The data sources for comparison were from the studies listed in Table 1. Flooded soil samples with several electron acceptors were also included in the present study. This experiment was conducted to examine the effects of electron acceptors on the composition of microbial communities of rice field soil (Takai and Kamura 1966; Kimura 2000).

MATERIALS AND METHODS

Study sites. The following four rice fields, which were located at Aichi-ken Anjo Research and Extension Center, central Japan (latitude 34°48' N, longitude 137°30' E), were subjected to PLFA composition analysis in the present study: 1) the fields under long-term fertilizer trial (LTFT), 2) A2 field, 3) D2 field, and 4) E2 field. The details of treatments including fertilizer application and managements of these fields and the soil properties were described previously (Okabe et al. 2000a, 2000b; Nakamura et al. 2003; Shimizu et al. 2002a, 2002b; Tanahashi et al. 2004) (Table 1). The soil was an Anthraquic Yellow Soil (Oxyaquic Dystrudept).

Soil samples collected from the plow layer in the E2 field were used in a pot

experiment for determining microbial composition in the percolating water (Kimura et al. 2001a) and in laboratory experiments for examining the effects of N fertilizers and of electron acceptors for anaerobic respiration on the microbial communities of rice straw and flooded soil, respectively (Kimura et al. 2001b).

Sample collection for the determination of PLFA composition of microbiota. Methods for collection and preparation of samples have been previously described in the references listed in Table 1.

In addition, flooded soil samples treated with several electron acceptors were analyzed in the present study. Soil collected from the E2 rice field on 14 September, 1999 (drained conditions) was air-dried first, and then treated with glucose at the rate of 4 g kg^{-1} air dry soil. Then, 10, 10, 30 and 6 g of $Ca(NO_3)_2$, MnO_2, ferric oxide/hydroxide precipitate or $(NH_4)_2SO_4$ was amended as electron acceptors to 1 kg of the air-dry soil samples. Ferric oxide/hydroxide precipitate was obtained by alkalinizing $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ solution with NaOH. The precipitate was washed several times with distilled water, and air-dried. Distilled water (15 mL) was added to treated and untreated soil (20 g) in a test tube, and the soil was submerged carefully without remaining any bubbles. Then, the test tubes were closed with a W-shaped butyl rubber stopper, and the air inside the test tube was exchanged several times with N_2 gas. The soil samples thus prepared were incubated for 40 days at 24-28 °C. PLFA

analysis was conducted after 10 and 40 days for samples treated with ferric oxide/hydroxide precipitate, 10 and 30 days for samples treated with MnO_2, 15 and 40 days for samples treated with $Ca(NO_3)_2$, 10 and 30 days for samples treated with $(NH_4)_2SO_4$ and 10 and 40 days for untreated samples. The sampling periods during incubation were determined depending on reduction conditions of the respective treatment soils.

Phospholipid fatty acids (PLFAs) analysis. Analysis of PLFAs was performed as reported by Okabe et al. (2000b) that was a modification of the methods of Bligh and Dyer (1959) and White et al. (1979). The GC operation was conducted according to the method of Okabe et al. (2000b).

Fatty acids were designated based on the total number of C atom and the number of double bonds, followed by the position of the double bond from the methyl end (aliphatic end [ω]) of the molecule. *Cis* and *trans* configurations were indicated by 'c' and 't', respectively. The prefixes 'ai' and 'i' referred to *anteiso* and *iso* branchings, respectively, 10 Me referred to a methyl group on the 10th carbon atom from the carboxyl end of the molecule, and "cy" referred to cyclopropane fatty acids.

We used the proportion (mol %) of PLFAs and the Simpson's (1-*D*) and Shannon-Wiener's (H') diversity indexes for evaluating the diversity of PLFAs composition. The indexes were calculated according to Ito and Sato (2002).

Statistical analysis. The proportions of straight, saturated PLFAs, straight, mono-unsaturated PLFAs, straight, poly-unsaturated PLFAs and branched-chain PLFAs in the total PLFAs extracted from respective habitats and the degree of stress at respective habitats based on the *trans/cis* ratio of 16:1ω7 PLFA were compared by nonparametric analysis with the Stat macros program for Excel (Hirota 2001). To compare the microbial communities among samples, differences in the composition of PLFAs extracted from the samples were analyzed using cluster analysis and principal component analysis (PCA). The values were normalized for the analyses. Cluster analysis was performed using JMP IN (SAS Institute, Tokyo) by the Ward method. PCA was performed using EXCEL STATISTICS 97 for Windows (SRI, Tokyo). Correlation matrix was used in the analysis. Analysis of molecular variance (AMOVA) was conducted for the comparison of the PLFA patterns of microbiota. The calculation of Φ_{ST} and a significant test for the Φ_{ST} value based on the permutation procedure were performed according to Excoffier et al. (1992).

RESULTS AND DISCUSSION

Amounts of PLFAs at respective habitats

Average amounts of PLFAs in aquatic habitats were 17.1-34.6 μg L^{-1} (Table 2). Wide

variations in the amounts in the floodwater were attributed to the field managements including fertilizer treatments; the average amount of PLFAs in the floodwater in the A2 plots was 86.8±48.8 μg L^{-1} (n=10; wheat was the second crop and wheat straw was left on the field), while that in the rice fields under long-term fertilizer trial was 7.1±3.2 μg L^{-1} (n=20; the fields were left fallow during winter season). On the contrary, variations in the amount of PLFAs in flooded rice soils were smaller than those in aquatic habitats (108±33 μg g^{-1} dw [dry weight basis]).

Average amounts of PLFAs were 382-2850 μg g^{-1} dw for RS or RS composts and 222-284 μg g^{-1} ww (wet weight basis) for RS in rice soils. As wet RS samples were expected to contain about 90% of water in weight, amounts of PLFAs were considered not to be significantly different among RS composts, RS composts in soil and RS materials in soil. In addition, as the soil samples used in the present study contained about 13 g C kg^{-1}, similar amounts of PLFAs were estimated to be present in soil to those in RS composts and RS in soil per a unit weight of organic matter (C contents of RS were supposed to be about 35%).

These average values permit rough estimation of the total amount of PLFAs in the surface part of rice fields. Microbial biomass (g m^{-2}) as expressed by the amounts of PLFAs were 1.64 x 10^{-3} in floodwater, 0.86 x 10^{-3} in soil water, 11.9 in plow layer soil, 1.11 in RS, and 0.57 in RS compost. Here, the depths of floodwater and plow layer are supposed to be 5

cm and 10 cm, respectively. In addition, the pore space and the bulk density in the plow layer are postulated to be 50% and 1.1, respectively. The total amount of PLFAs in the 0-10 cm soil layer was about 12 g m^{-2} and it accounted for more than 99% of total microbial biomass resulting from the sum of microbial biomass values of the floodwater and the plow layer. Six tons dw ha^{-1} of RS roughly corresponds to a rice yield of 5 tons ha^{-1} under field conditions (the average yields of brown rice and RS in Japan are 5.2 and about 6 tons ha^{-1}, respectively); this amounts correspond to about 10 tons fresh weight of RS compost. The PLFAs in RS and RS compost amounted to about 10 and 4 %, respectively, of the average PLFA values of the plow layer soil. As the weight losses of RS and RS compost after their application to the plow layer were not considered, more than 90% of PLFAs were estimated to remain in the plow layer soil even after their application to soil.

PLFA composition of microbiota at respective habitats

RS materials contained largest proportion of straight, saturated PLFAs (median: 53%), which tended to decrease by microbial decomposition of RS in soil or in the composting process (Fig. 1). The proportions of straight, saturated PLFAs were significantly larger in the habitats with RS materials (RS in flooded rice soils, RS in drained/aerobic rice soil, RS in composting process, and RS compost in flooded rice soil) than in habitats not treated with RS (floodwater,

percolating water from the plow layer, percolating water collected from the culvert, soils under flooded conditions, and soils under drained/aerobic conditions) (P < 0.001, Mann-Whitney U test). Proportions of straight, mono-unsaturated PLFAs fluctuated widely among the habitats from 16 % of RS in flooded soils to 59% in the percolating water collected from culverts. However, aquatic environments such as the floodwater and percolating water contained significantly larger proportion of unsaturated PLFAs than other environments (P < 0.001, Mann-Whitney U test). As straight, mono-unsaturated PLFAs are generally the biomarker of Gram-negative bacteria (Wilkinson 1988), Gram-negative bacteria probably predominated in ~~those~~ aquatic environments of the rice field ecosystem. The proportions of branched-chain PLFAs showed the following order; both flooded and drained soils (43%), RS of flooded soil (39%) and RS under composting process (37%) > percolating water from plow layer (34%) and RS compost of flooded rice soil (30%) > percolating water from culvert (16%), floodwater (14%), RS in drained soil (9.4%) and RS materials (6.4%) (P < 0.01, Kruskal-Wallis test and Bonferroni procedure). This indicates that Gram-positive bacteria played important roles in soils and in the composting process of RS. The proportion of straight, poly-unsaturated PLFAs, the biomarker of eukaryotes, was significantly higher in RS materials (16%) and RS of drained rice field soil (17%) than in the other samples (2-9%) (P < 0.001, Mann-Whitney U test), and this behavior was attributed to the predominant

colonization of RS materials by fungi as mentioned below. However, the possibility that straight, poly-unsaturated PLFAs originated from rice plant remained undecomposed in RS materials cannot be discarded.

Figure 2 compares the average percentage of each PLFA in the samples among different habitats. An interesting aspect was lower percentages of 10Me-group PLFAs in floodwater and percolating water than in soil samples, which indicates that these aquatic environments do not represent good habitat for actinomycetes. On the contrary, relatively larger proportion of 20:4ω6c PLFA was found in the floodwater and the percolating water collected from the culverts than in the other habitats, indicating the presence of protozoa. All the standard deviations of respective PLFAs were small except for 16:0, 16:1ω7c and 18:2ω6c PLFAs, indicating that the community structure of microbiota in the rice field ecosystem is stable and site-specific, which were also revealed by cluster and principal component analyses (see below). These characteristics may have contributed to the biological diversity of the rice field ecosystem as a whole.

Statistical analyses

Cluster analysis and Principal component analysis were performed to all samples to estimate the development of specific communities of biota and the effect of field management on

microbial communities of respective habitats in the rice field ecosystem.

Cluster analysis firstly enabled to divide the PLFA patterns of microbial communities in the rice field ecosystem into two groups (Fig. 3). The PLFA patterns of microbial communities of clusters A and B were significantly different (P<0.001) upon AMOVA analysis. The first group consisted of floodwater, percolating water, RS compost of flooded soil, and RS samples in flooded and drained soils. The RS samples of flooded and drained soils belonging to this group were mainly at the early stage of decomposition. The second group included soil samples, RS samples in the composting process and degraded RS of soil. The first group (cluster A) was further separated into two sub-clusters (A1 and A2): with aquatic samples in the sub-cluster A1 and all other samples in the sub-cluster A2. The second group (cluster B) was also divided into two sub-clusters, namely the sub-cluster B1 with RS samples of soils and the sub-cluster B2 with soil samples and RS samples of the composting process. The PLFA patterns of microbial communities of the sub-clusters (A1 and A2, and B1 and B2) were significantly different (P<0.001) upon AMOVA analysis.

Soil samples amended with several electron acceptors in the laboratory experiments were also included ~~with field soil samples~~ in the sub-cluster B2 because effects of the electron acceptors on the structure of microbial communities of rice field soil were small. Lueders and Friedrich (2000) also showed that sequential reduction process had little effects on archaeal

communities of rice soil in microcosm experiments. Floodwater and percolating water samples could be distinguished in the sub-cluster A1 (Fig. 3). Only 5 samples among 172 samples could not be included in the above mentioned sub-clusters. This analysis suggests that community structures of microbiota in the rice field ecosystem are determined predominantly by the characteristics of respective habitats and that the temporal fluctuations in the community structure at respective habitats are significantly small in comparison with the variations among the habitats.

The results of principal component analysis are shown in Figs. 4 and 5. The total percentages by the first, second and third principal components were 19.1, 12.6 and 10.7%, respectively. Principal component analysis elucidated microbial populations that were responsible for groupings in cluster analysis. In Fig. 4, samples in cluster A were mainly located in the left hand side and those in cluster B in the right hand side, and samples belonging to sub-clusters B1 and B2 were distributed in the first and fourth quadrants in the same figure, respectively. On the contrary, samples distributed in sub-clusters A1 and A2 were mainly located in the second and third quadrants, respectively (Fig. 5). The high positive and negative loads in the first principal component included biomarkers of Gram-positive bacteria (branched-chain PLFAs) and those of Gram-negative bacteria and eukaryotes (mono- and poly-unsaturated PLFAs), respectively, and the high negative loads in the second principal

component included biomarkers of actinomycetes and Gram-positive bacteria (10Me-group and branched-chain PLFAs). In addition, the high negative loads in the third principal component included two biomarkers of fungi (18:2ω6c and 18:1ω9c).

RS materials used in the present study distributed at the lower region in the third quadrant of Fig. 5, and the relative community structure was characterized by the predominance of fungi (18:2ω6c), whose proliferation was probably stimulated by dry moisture conditions. Nearly all RS samples placed in flooded rice soils distributed in the first quadrant in Fig. 4, which differed from the other samples. The scores of the first, second, and third principal components of RS samples in flooded rice soil correlated positively ($P < 0.05$), negatively ($P < 0.01$), and positively ($P < 0.01$) with the duration of placement, respectively. By considering the PLFAs with high positive loads in the first and second principal components of Fig. 4, the community structure of microbial population in RS of flooded rice soils was characterized by the predominance of Gram-positive bacteria. In contrast, RS placed in drained rice field after rice harvest distributed in the third quadrant of Fig. 5, and was characterized by the predominance of Gram-negative bacteria and fungi. These findings indicated that different microbial populations were responsible for RS decomposition in Japanese rice fields during the flooding and the drained-aerobic periods.

Floodwater and percolating water samples distributed mainly in the second quadrant

of Fig. 5, and differed from the other samples due to the predominance of Gram-negative bacteria and eukaryotes (presumably algae), and Gram-negative bacteria, respectively.

Soil samples distributed in the fourth quadrant of Fig. 4 were characterized by the predominance of actinomycetes and Gram-positive bacteria (10Me-group and branched-chain PLFAs) irrespective of the type of samples (from the rice field or from microcosm experiments), the treatment (the addition of electron acceptors) and field conditions (flooded or drained). RS from the composting process was also located in the fourth quadrant of Fig. 4, and shifted upper-leftward with the duration of composting. In contrast, RS compost samples that were placed in the flooded rice field were distributed at the center of Fig. 4, together with the decomposed RS samples of the composting process. The score plots of RS compost samples in soil did not show marked changes with the duration of placement compared to those of RS samples in flooded rice soil.

Both Figs. 4 and 5 confirm the characteristics (stable and site-specific) of the structure of microbial communities in the rice field ecosystem, as shown by cluster analysis (see above). Another interesting aspect is the predominance of Gram-positive bacteria (branched-chain PLFAs) in the solid habitats (soils, RS and RS compost) and Gram-negative bacteria (straight, mono-unsaturated PLFAs) in the aquatic habitats (floodwater and percolating water).

Simpson's (1-*D*) and Shannon-Wiener's (H') diversity indexes of PLFAs composition

were highest in samples of drained soil, flooded soil and RS compost (0.916 to 0.931 and 2.87 to 2.96 for 1-*D* and H', respectively). Samples of percolating water from plow layer, RS in flooded soil and RS compost in flooded soil showed intermediate diversity indexes (0.887 to 0.909 and 2.75 to 2.76 for 1-*D* and H', respectively), whereas those of percolating water from culvert, floodwater, RS in drained soil and RS materials were the lowest (0.780 to 0.873 and 2.08 to 2.54 for 1-*D* and H', respectively; $P < 0.01$, Kruskal-Wallis test and Bonferroni procedure). This suggests that microbial diversity might have been different among the three habitats as described above. It is important to underline that PLFA analysis does not allow assessing the diversity at genus or species level (Nannipieri et al. 2003).

Changes in PLFAs in RS materials

RS materials contained large proportions of 16:0 and 18:2ω6c PLFAs (Fig. 2). Thus, fungi grew preferentially in RS materials. The proportions of 16:0 and 18:2ω6c PLFAs decreased during the decomposition of RS under flooded conditions, while branched-chain PLFAs such as i15:0, i16:1 and ai15:0 PLFAs increased, which indicated that Gram-positive bacteria contributed to the decomposition of RS in the flooded rice field. Probably a part of 16:0 PLFA was originated from RS materials and may have remained in RS samples in flooded rice soils for a long time. Although lower proportions of straight, mono-unsaturated

PLFAs also characterized RS of flooded rice soils, the PLFA patterns of microbial population in decomposing RS under flooded conditions became similar to those of soil samples after long decomposition. In contrast, the proportions of straight, mono-unsaturated PLFAs increased and 18:2ω6c PLFA remained high when RS was decomposed in the drained rice field after rice harvest. Thus, different microbial communities characterized RS decomposition in soil during the flooded rice cultivation and after rice harvest, being Gram-positive bacteria including actinomycetes predominant during the flooding and Gram-negative bacteria and fungi predominant after rice harvest.

The proportions of 16:0 and 18:2ω6c PLFAs also decreased during the composting process as RS decomposition in soil as described above, while branched-chain PLFAs such as i15:0, i16:0, i17:0, ai15:0, ai17:0 and 10Me-group PLFAs increased (Fig. 2). Thus, Gram-positive bacteria including actinomycetes predominated in the composting process of RS. It was interesting that the PLFA patterns of microbiota in composting RS were similar to those of soil samples, probably because that *Bacillus* (branched-chain PLFAs) and actinomycetes (10Me-group PLFAs) were active in the thermophilic stage of the composting process of RS (Cahyani et al. 2003).

Stressed conditions for microorganisms at respective habitats as estimated from the

trans/cis ratios of 16:1ω7 PLFA

The *trans/cis* ratios of 16:1ω7 PLFA for the microbiota at respective habitats are reported in Table 3. The average values of the ratio ranged from 0.06 for the microbiota in the floodwater to 0.29 for microbiota of RS in flooded soils. The *trans/cis* ratios of 16:1ω7 PLFA were significantly different among the microbiota of the investigated habitats (RS in flooded rice soils, RS compost in flooded rice soil, flooded soils, floodwater and percolating water from plow layer soil and culvert, RS in composting process, drained soil, RS materials, and RS in drained rice soil) ($P < 0.01$, Kruskal-Wallis test and Bonferroni procedure). The microbiota under flooded conditions showed the highest values and those in aquatic habitats the lowest ones, and this indicated that microbial stress was high under flooded conditions, and low in aquatic habitats. Reduced conditions and surplus of organic materials (substrates for microorganisms) might have been possible stresses for micorbiota. These conditions might have influenced the composition of microbial communities in the rice field ecosystem.

Conclusions

Rice soils were characterized by the predominance of actinomycetes and Gram-positive bacteria in comparison with other habitats. In contrast, the microbial communities of the floodwater and percolating water were characterized by the predominance

of Gram-negative bacteria and eukaryotes (presumably algae), and Gram-negative bacteria, respectively. The community structure of RS materials was characterized by the predominance of fungi. Gram-positive bacteria became predominant in RS after application to flooded rice soils, while RS placed in a drained rice field after rice harvest was characterized by the predominance of Gram-negative bacteria and fungi. PLFA patterns of composting RS were similar to those of soil samples. The community structures of biota at respective habitats were stable and specific irrespective of the season of sampling and the duration of decomposition, which was estimated to contribute to the biological diversity of the rice field ecosystem as a whole.

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Figure legends

Fig. 1. Proportions of straight, saturated PLFAs, straight, mono-unsaturated PLFAs, straight, poly-unsaturated PLFAs, and branched-chain PLFAs of microbiota inhabiting at respective habitats. Box shows 90 % range (5-95 %) of data. Solid and broken lines inside box indicate median and lower and upper quartiles (25 and 75 %) of the data, respectively.

Fig. 2. Comparison of the PLFA patterns among the floodwater, percolating water and soil samples that were shown by the average values with the standard deviation.

Fig. 3. Cluster analysis of the PLFA patterns of microbial communities in the rice field ecosystem. Arrows indicate the samples that were out of their respective sub-clusters. △, Floodwater; △△, Percolating water, ×, Rice straw materials; □, Rice straw in flooded soil; □□, Rice straw in drained soil; ⇐, Rice straw compost; ⇐⇐, Rice straw compost in flooded soil; ○, Flooded soil; ○○, Drained soil.

Fig. 4. Distribution of the score plots of the PLFA composition in the floodwater, percolating water, RS compost and soil samples in the coordinate system of the first and second principal components. ◇, Floodwater; ◆, Percolating water from plow layer; ◆, Percolating water

from subsoil; □, Rice straw materials; ■, Rice straw in drained soil; ■, Rice straw in flooded soil; ▲, Rice straw compost; △, Rice straw compost in flooded soil; ●, Flooded soil; ○, Drained soil.

Fig. 5. Distribution of the score plots of the PLFA composition in the floodwater, percolating water, RS compost and soil samples in the coordinate system of the first and third principal components. Symbols are same as those in Fig. 4.

Table 1. Sources of data used in this work and characteristics of the respective samples

Sample	Field	No. of samples	Year	Remarks	Reference
Floodwater	LTFT	21	1996	Three different fertilizer treatments	Okabe et al. 2000a
	A2 field	7	2000		Shimizu et al. 2002a
	A2 field	3	2001		Shimizu et al. 2002b
	E2 field	6	1997		Okabe et al. 2000b
Percolating water	A2 field	7	2000	From culverts (40 cm depth)	Shimizu et al. 2002a
	A2 field	6	2001	From L-shaped pipes and culverts	Shimizu et al. 2002b
	Laboratory	16			Kimura et al. 2001a
RS in flooded soil	E2 field	13	1998	Sheath and blade samples	Nakamura et al. 2003
	E2 field	10	1999	Sheath and blade samples	Nakamura et al. 2003
	Laboratory	28		Incubation in the E2 soil with N sources	Kimura et al. 2001b
RS in drained soil	E2 field	10	1998,1999	Sheath and blade samples	Nakamura et al. 2003
Composting RS		13			Cahyani et al. 2002
RS material		4			Cahyani et al. 2002
					Nakamura et al. 2003
RS compost in soil	D2 field	7	2002		Tanahashi et al. 2004
Flooded soil	A2 field	3	2000		Shimizu et al. 2002a
	E2 field	6	1997		Okabe et al. 2000b
	Laboratory	10		Incubation of E2 soil with amendments	Unpublished data
Drained soil	E2 field	2	1997		Okabe et al. 2000b

LTFT, Rice field under a long-term fertilizer trial; RS, rice straw.

Table 2. Average amounts of PLFAs at respective habitats

Habitat	No. of samples	Average±S.E.
RS in flooded soil	23	284±94 (μg g^{-1} wet weight)
RS compost in flooded soil	7	2850±420 (μg g^{-1} dry weight)
Flooded soil	13	108±33 (μg g^{-1} dry weight)
RS compost	13	2510±760 (μg g^{-1} dry weight)
RS material	3	382±7 (μg g^{-1} dry weight)
RS in drained soil	10	222±75 (μg g^{-1} wet weight)
Percolating water		
from plow layer	19	17.1±9.5 (μg L^{-1})
from culvert	10	34.6±9.9 (μg L^{-1})
Floodwater	30	32.8±46.4 (μg L^{-1})

Table 3. Average *trans/cis* ratios of 16:1ω7 PLFA at respective habitats

Habitat	No. of samples	Median (0.25-0.75 percentile)
RS in flooded soil	51	0.29 (0.20-0.38)
RS compost in flooded soil	7	0.29 (0.24-0.34)
Flooded soil	19	0.27 (0.19-0.31)
RS compost	13	0.24 (0.21-0.26)
Drained soil	2	0.17 (0.12-0.22)
RS material	4	0.18 (0.16-0.18)
RS in drained soil	10	0.13 (0.071-0.18)
Percolating water		
from plow layer	19	0.090 (0.034-0.14)
from culvert	10	0.053 (0.030-0.077)
Floodwater	37	0.052 (0.044-0.066)

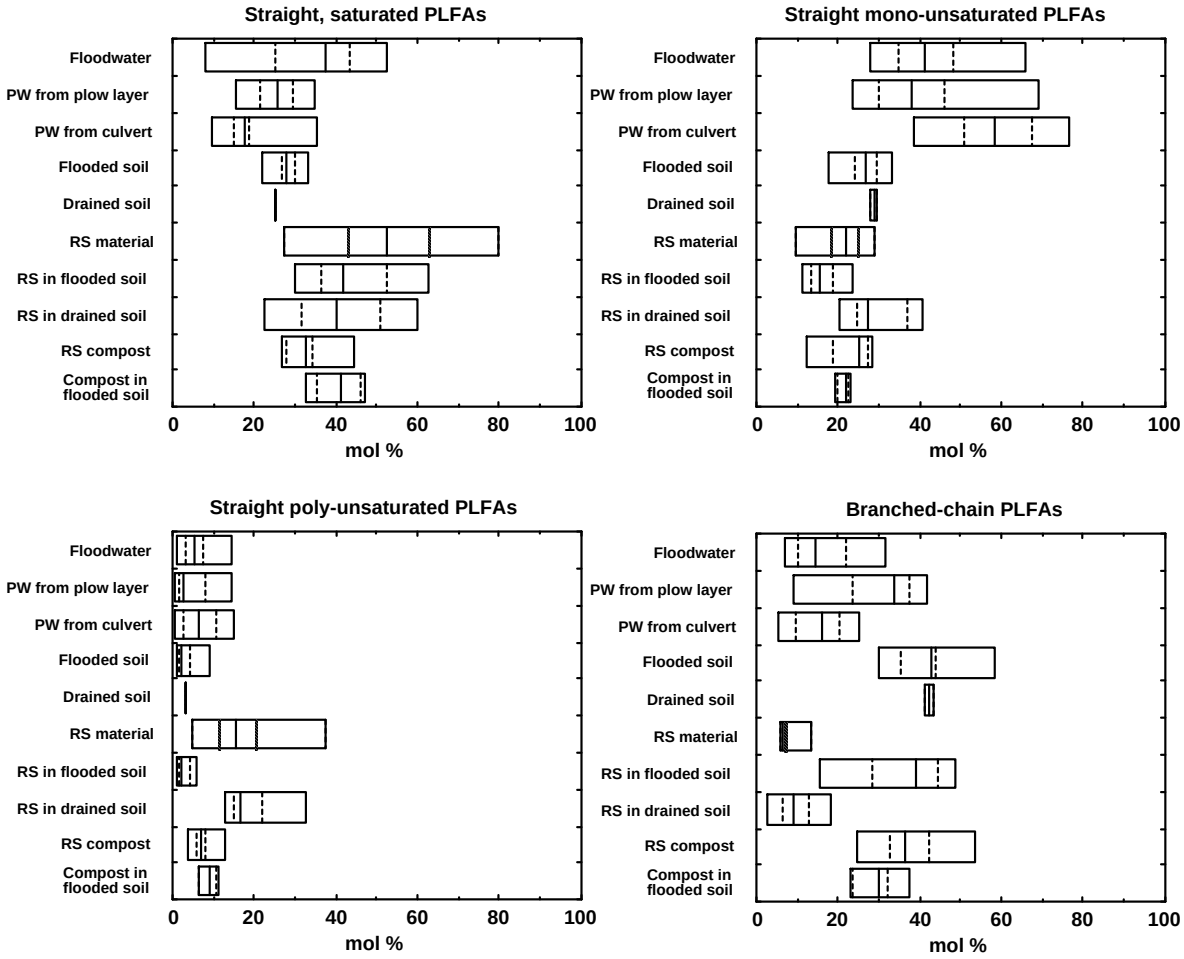

Fig. 1. Kimura and Asakawa

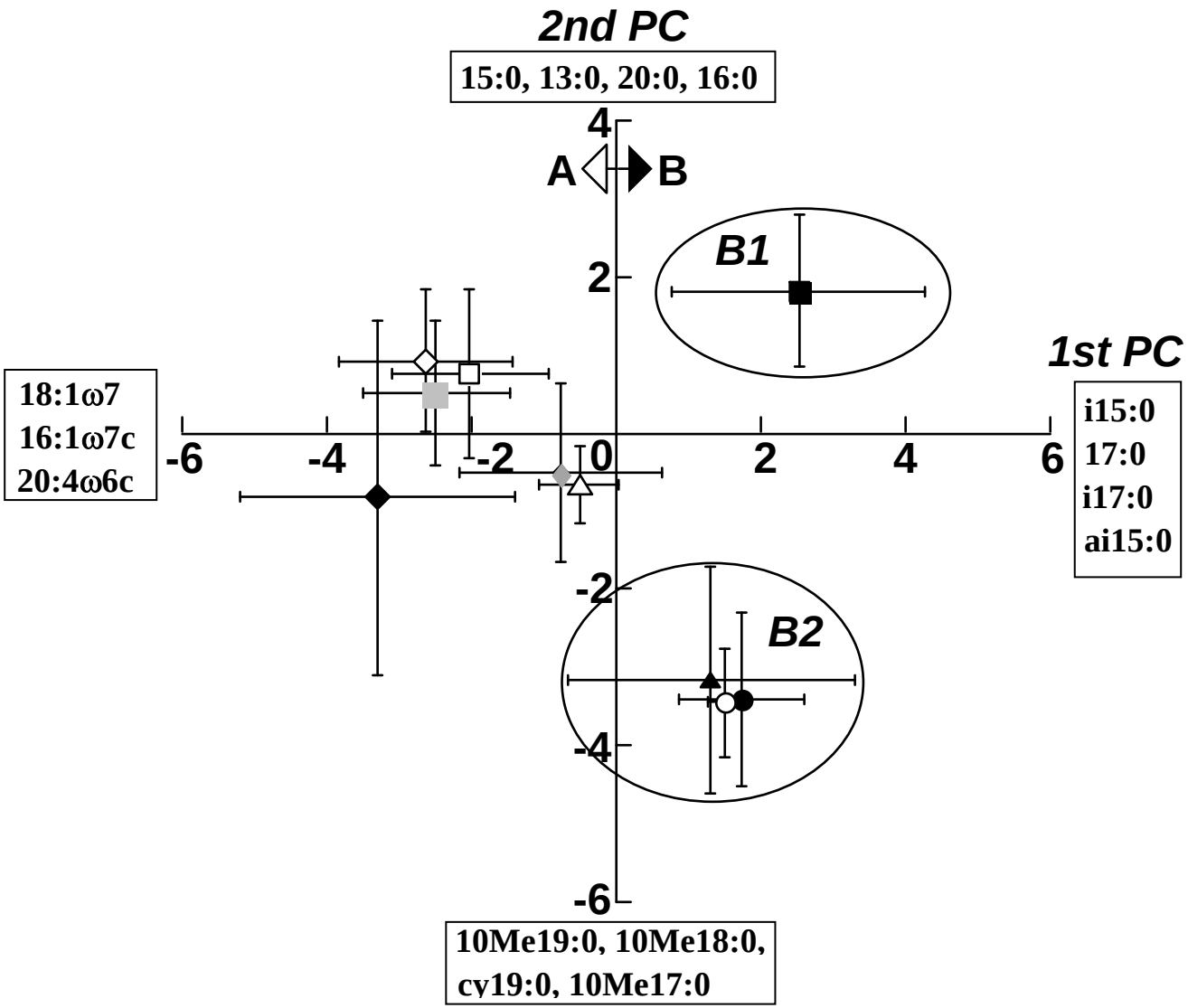

Fig. 4. Kimura and Asakawa

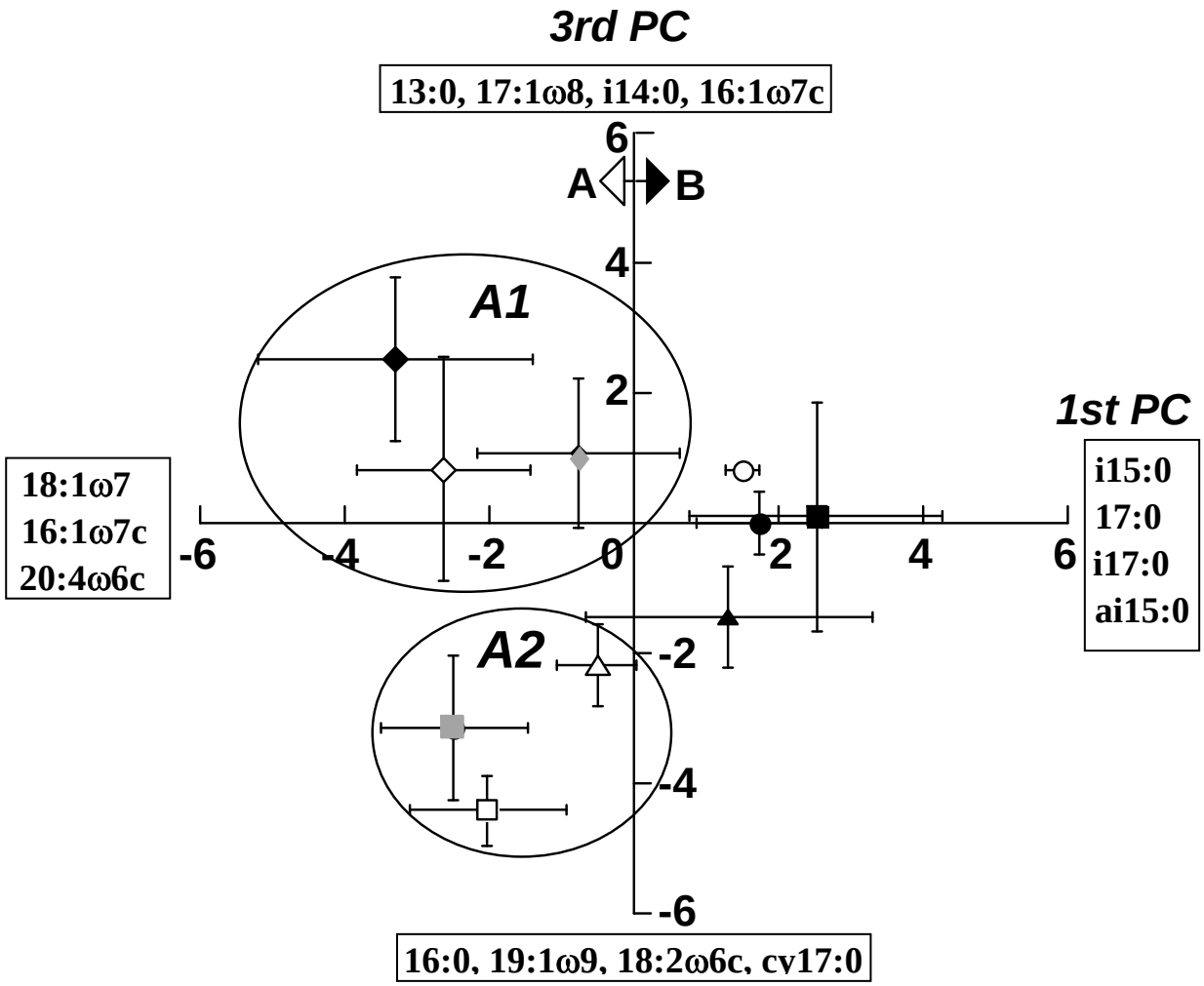

Fig. 5. Kimura and Asakawa