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Changes in microbial community structure and function within particle size fractions of a paddy soil under different long-term fertilization treatments from the Tai Lake region, China

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Abstract

Greenhouse gas (GHG) production and emission from paddy soils impacts global climate change. Soil particle size fractions (PSFs) of different sizes act as soil microhabitats for different kinds of microbial biota with varying conditions of redox reactions and soil organic matter (SOC) substrates. It is crucial to understand the distribution of soil microbial community structure within PSFs and linkage to the GHG production from paddy soils of China. The change of bacterial and methangenic archaeal community and activity relating to CH_4 and CO_2 production with PSFs under different fertilizer applications was studied in this paper. The fertilization trial was initiated in a paddy soil from the Tai Lake region, Jiangsu, China with four treatments of non-fertilized (NF), fertilized with inorganic fertilizers only (CF), inorganic with pig manure (CFM) and inorganic with straw return (CFS), respectively since 1987, and the PSFs (<2 μm, 2–20 μm, 20–200 μm, and 200–2000 μm) were separated by a low energy sonication dispersion procedure from undisturbed samples. Analysis of bacterial community within different size particles was conducted by PCR-DGGE. The results indicated significant variation of bacterial community structure within different PSFs. The methane was predominantly produced in the coarser fractions, while more species and higher diversity of bacteria survived in the size of <2 μm fractions, in which the bacterial community structure was more significantly affected by fertilizer application practices than in the other coarser fractions. Higher bacterial species richness and more diversities in the smallest size fractions was due to the vicinity between microbes, access to carbon resource outside the microaggregates, and smaller pore size as protective agent suitable habitats for microbes rather than high SOC. Whereas, higher CO_2, CH_4 production and methanogenic archaeal community in coarser fractions may be contributed to storage of labile organic carbon in these fractions. It indicated that availability of SOC in PSFs is mainly factor affected survival of methanogenic archaeal community structure, whereas, bacterium community habitation more affected by physical protection of their location in PSFs. Their activity greatly depended on liability of SOC access to PSFs. Fertilizer application caused more change of bacteria community in clay fraction and greatly increased bacterium and methanogen activity in coarser fractions but only a slight effect on methanogenic archaeal community in the particle size fractions.
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Keywords: Paddy soils; Bacterial and methanogenic archaeal community; Bacteria and methanogenic activity; Fertilization practices; Particle size fractions

1. Introduction

Greenhouse gas (GHG) emissions from paddy soils and the impacts on global climate change have been increasingly concerned. However, the evolution of carbon dioxide and methane have been shown subject to fertilization practices [1–3], under which the SOC turnover and CH_4, CO_2 production potential could be affected by the changes in influencing community and activity of bacteria and methanogens [4]. Organic carbon content and C/N ratio under different long-term addition of fertilizer amendments caused the variation of soil bacterial and eukaryotic community structures [5]. According to Chen et al., acceleration of methane production related to increased amount of methanogenic bacteria under the application of Green manure [6].

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Soil particle size fractions (PSFs) of different sizes act as soil microhabitats for different kinds of microbial biota with varying conditions of redox reactions and SOC substrates [7], and thus, have been considered as the basic unit of soil entity in mediating mineral–organic interaction at microscale in many soil biochemical processes. Decomposition and transformation of SOC through blinding by various were already characterized for these fractions of varying sizes [8–10]. Recently, variation in microbial biomass, enzymes activity, and diversity, which may link to the different degree of carbon turnover in the different particle size fractions have been reported [11,12]. Whereas, influence of fertilization practices have been actively discussed on soil microbial community structures [5,13,14] and on the characteristic of microbiota's niche-aggregates [15] as well as the functioning of the soil microbial communities [16,17]. For microbes' acts in microhabitat level, how and to what extent these fertilization practices modify the microbial community structure and activity in PSFs are still in debt.

Rice paddies in China have been recognized as an anthropogenic soil type formed due to long-time and hydroagric managements under seasonally submergence for rice growth, and as a major cropland soil with high C density and potential of C sequestration as well as high production capacity for food security of China [18–21]. These soils have been also concerned as playing a crucial role in the global carbon sequester and CH_4 budget [22–25]. For meeting the food demand for the increasing population, chemical fertilizers have been increasingly applied to the paddies both in China and Asia since 1980s [26]. Recently, we reported that different fertilization practices had profound impacts on microbial biomass C and gene diversity, and chemical fertilization reduced microbial biomass and diversity giving rise to a lower and more instable productivity in a paddy soil [14]. However, the changes of bacterial community structure as well as methanogenic archaeal community in particle size fractions as microhabitats in the paddy soil are still in debt. Thus, it is crucial to understand the distribution of soil microbial community structure in particle size fractions and any linkage to the GHG production from paddy soils of China for addressing determine better fertilizer application practices in GHG emission mitigation by rice agriculture.

The present study is to, taking an example of a rice paddy from the Tai Lake region, China, demonstrate the microbial community structure changes with the PSFs as well as with the different long-term fertilization practices by using 16S RNA-based DGGE technique, with special reference to the methanogenic archaea. The authors also aim at testing the relationship between the methanogens and bacterial community and their activity by methane, carbon oxide production potential under anaerobic incubation from different PSFs under the fertilization treatments.

2. Materials and methods

2.1. Site, fertilization treatments and sampling

The studied paddy (Ferric-Accumulic Stagnic Anthrosols), a typical rice paddy from the Tai Lake region with a rice production of 9 t/ha in late 1990s, is located in Wujiang County, Jiangsu Province, China (N: 31°05′900″; E: 120°46′924″). A subtropical monsoon climate governs the area with decadal mean annual precipitation of 1100 mm and mean annual temperature of 22 °C [14]. The fertilizer treatments initiated in 1987 have been consistently as follows: no fertilizer application (NF); inorganic fertilizer only (CF); inorganic fertilizer plus rice straw return (CFS) and inorganic fertilizer plus pig manure (CFM). The amount of inorganic fertilizer per year is nitrogen as urea 427.5 kg/ha, P_2O_5 as super phosphate 45 kg/ha, KCl 84 kg/ha and rice straw return in the amount of 4500 kg fresh weight/ha and manure in the amount of 16,800 kg fresh weight/ha, respectively. The size of each plot was 70 m^2 (10 m × 7 m). The layout of the experimental plots was in a completely randomized block design at three replications. All the plots have been cultivated under rice-rape rotation consistently since 1987. Undisturbed soil samples were taken after rice harvest in October 2004 from 0- to 15-cm depth intervals from each of the treatment plots, respectively using an Eijkelkamp Soil Sampler and then stored in stainless cans at 4 °C. The original basic soil physical and chemical properties are listed in Table 1.

2.2. Soil particle size fractions separation procedure

The fractionation procedure was based on the method developed by Stemmer et al. with minor modifications [17,27]. Fresh soil was dispersed in distilled water by use of a probe-type ultrasonic disaggregator (Shanghai Zhixin, JVD-650) with output energy of 0.2 kJ/g for 5 min. The fraction of 200–2000 μm was collected in sieves with a corresponding mesh size. The fraction of 20–200 μm was obtained by sedimentation after siphonage. The remainder was centrifuged to collect the fraction of 2–20 μm and the supernatant was centrifuged to collect the fraction of <2 μm. The fraction separates were freeze-dried prior to the following analysis.

Table 1
pH and basic nutrient pools of the studied soil

Treatment plot	pH (H_2O)[a]	SOC (g/kg)[a]	DOC (mg/kg)[a]	Total N (g/kg)[b]	Total P (g/kg)[b]	Available K (mg/kg)[b]
NF	6.13	16.43 c	64.3 b	1.72 b	0.24 c	82
CF	5.93	16.75 bc	45.5 c	1.80 b	0.37 b	105
CFM	5.74	17.22 b	79.9 a	2.05 a	0.72 a	98
CFS	5.88	17.93 a	81.4 a	2.06 a	0.37 b	88

Different low case letters in a single column indicate significant difference at $p < 0.05$.
[a] Data from Zhang et al. [14].
[b] Data from Qiu et al. [49].

2.3. Chemical analysis and CH_4, CO_2 production test

Soil organic carbon and total nitrogen (TN) of all freeze-dried fractions was measured using a CNS Macro Elemental Analyzer (Elementar Analysen System GmbH, Germany) after removing the carbonates with HCl (10%, v/v). For methane production potential test, 20 g sample of the size fraction separates was placed in a 120 ml serum bottle and 40 ml distilled H_2O was added enough to make the soil saturated and incubated under 25 centigrade for 21 days. To stimulate soil reduction, the bottles were flushed with 300 ml-N_2/min current for 10 min in order to replace the air in the headspace with N_2 before incubation. During incubation, a 0.25-ml sample of the gas evolved was collected by syringe pressure every 7 days and analyzed with Agillent 4890D gas chromatograph equipped with a porapak Q column (80/100 mesh) and a flame-ionization detector (Agillent 4890D, USA). The detection limit of CH_4 in gas evolved by GC was 1 ppmv. The temperature of the column was set at 35 °C and carrier gas flux was 30 ml/min [25]. Since a peak of methane production generally appeared on the 21st day after incubation started, CH_4 and CO_2 production rate of third week was used to compare the methanogenic activity among both the particle size fractions and the different treatments.

2.4. DNA extraction and PCR-DGGE analysis

DNA of particle size fractions was extracted using a fast DNA spin kit as described in manufacturer's instructions (Qbiogene, Inc.). The primers used for amplifying a region of 16S rDNA of bacteria and methanogenic archaea were PRBA338F-GC and PRUN518R, and 357F-GC and 0691F (TaKaRa), respectively [28,29]. PCR products were obtained using Ready-To-Go™ PCR Beads (Amersham Biosciences, 27-9557-01). Amplification was performed on a PTC-225 thermocycle (MJ Research Inc.), with the following program: 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 6 min. For methanogenic archaea, a minor modified procedure used for bacteria was conducted: initial denaturation at 95 °C for 5 min; 33 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min; and a single final extension at 72 °C for 6 min. The PCR products were run on 2% agarose gel to test their quality, and quantified by the spectrophotometer (Beckman DU7400) and quartz cuvette. PCR products resolved on 8% (w/v) polyacrylamide gels in 0.5 TAE buffer using denaturing gradient ranging from 30 to 60% (where 100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was carried out at 70 V (for bacteria) for 12 h and 100 V (for methanogenic archaea) for 14 h at 60 °C. Gels were stained with ethidiumbromide, visualized on a UV transilluminator and photographed (Bio Rad).

2.5. Data analysis

The profiles of DGGE were analyzed using the Quantity One software (Bio Rad) to calculate bacterial community similarity values (UPGMA, Dice coefficient of similarity). Microbial species richness and diversity indices were determined by band quantity and intensity [30,31]. All data of soil organic carbon, particle size distribution, CH_4 flux, and microbial community structures similarity coefficient were compared by a one-way ANOVA. Significance test was done using SPSS 11.0 for windows (LEAD Technologies, Inc, USA).

3. Results

3.1. SOC content and CH_4, CO_2 production rate within PSFs under different fertilizer treatments

Fertilizer treatments apparently influenced PSFs distribution (Table 2). The particle fractions proportion significantly increased in size of 200–2000 μm and decreased in size of 2–20 μm under CFM and CFS compared to under NF. The most profound variation of SOC was found in 200–2000 μm size fraction under different fertilizer treatments. CFM and CFS significantly increased SOC content of 200–2000 μm fraction compared to CF treatment.

Fertilizer application enhanced CO_2 production rate of all PSFs. Contrast to CF, CO_2 production rate significantly increased both in the size of 200–2000 μm and <2 μm fractions under CFM and CFS. CH_4 production rate decreased with particle size descending and was negligible from the size of <2 μm fractions under fertilizer treatments. Fertilizer application greatly increased CH_4 production rate from 200–2000 μm fractions. Whereas, higher CH_4 production rate from fractions of larger than 20 μm size was observed under CFM treatment.

3.2. Bacterial community structures within PSFs under different fertilizer treatments

Genetic fingerprinting by DGGE of bacterial16S rDNA amplified fragments showed a few strong dominating bands appearing across the different PSFs (Fig. 1). Together with those strong signals, a great number of fainter well resolved bands appeared in the profiles, which were all considered when analyzed by the Bio-Rad Quantity One software. Change of dominating bands in sizes fractions under fertilizer treatment showed the variety of bacteria due to fertilizer practices. In the size fractions of <2 μm, Band B became dominated under all treatments except for under NF, Band C appeared more significantly under CFM and CFS than under NF and CF, while Band A existed only under CF. Both bacterial species richness and diversities retrieved from DGGE profiles were higher under CFS than those under CF, while no significant difference among other fertilizer treatments (Fig. 2A and C). In addition, bacterial species richness and diversity increased with decreasing the size fractions except for the 200–2000 μm fractions. The size of <2 μm fractions survived higher bacterial species richness and diversity than other particle size fractions (Fig. 2B and D). The mean similarity coefficient of bacterial community was lowest in <2 μm fractions and highest in 200–2000 μm fractions under different fertilizer application (Table 3). Bacterial community structures under CFS and CFM were similar in <2 μm and 200–2000 μm fractions, respectively, while similar bacterial community structures were shown under NF and CF (Fig. 3).

Table 2
Particle size distribution, soil organic carbon (SOC), C/N ratio, and CH_4 production rate in the long-term experiment[a]

Particle size (μm)	Treatment[b]	Fraction content (g kg of soil^{-1})	SOC (g of C_{org} kg of bulk soil^{-1})	C/N ratio	CH_4 production rate[c] (μg CH_4 of g^{-1} soil day^{-1})	CO_2 production rate (μg CO_2 of g^{-1}soil day^{-1})
200–2000	CFM	378.3 ± 20 a	19.3 ± 0.6 ab	11.7	3.87 ± 0.39 a	58.6 ± 4.7 a
	CF	369.0 ± 2 ab	17.8 ± 0.3 c	11.3	2.43 ± 0.45 c	47.0 ± 2.8 b
	CFS	337.6 ± 3 c	19.1 ± 0.3 b	12.1	1.61 ± 0.02 d	36.2 ± 3.1 c
	NF	297.0 ± 4 d	16.7 ± 0.2 d	11.6	1.67 ± 0.14 d	31.3 ± 0.8 d
20–200	CFM	301.4 ± 17 d	16.7 ± 0.4 d	11.6	2.36 ± 0.17 c	34.3 ± 2.6 cd
	CF	283.4 ± 3 de	16.7 ± 0.3d	11.9	1.79 ± 0.14 d	29.7 ± 2.9 de
	CFS	301.4 ± 4 d	17.8 ± 0.4 c	11.3	0.03 ± 0.01 g	26.6 ± 1.7 ef
	NF	290.9 ± 5 de	15.1 ± 0.1 e	12.5	UT	13.9 ± 0.8 I
2–20	CFM	266.9 ± 16 e	13.5 ± 0.1 f	10.6	2.86 ± 0.16 b	44.9 ± 3.0 b
	CF	288.6 ± 3 de	13.3 ± 0.2 f	10.1	0.73 ± 0.14e	32.1 ± 2.8 cd
	CFS	302.3 ± 4d	13.8 ± 0.7 f	11.1	0.43 ± 0.03 ef	24.9 ± 2.7 fg
	NF	350.9 ± 3 bc	13.0 ± 0.2 f	10.7	0.16 ± 0.02fg	18.1 ± 1.4 hi
<2	CFM	53.4 ± 3 f	18.6 ± 0.5 bc	9.6	UT	33.2 ± 2.3 cd
	CF	59.0 ± 1 f	18.6 ± 0.4 bc	7.6	UT	17.2 ± 0.7 hi
	CFS	58.6 ± 1 f	20.2 ± 0.2 a	9.4	UT	31.6 ± 1.3 d
	NF	61.2 ± 3 f	16.5 ± 0.4 d	9.1	UT	21.4 ± 1.7 gh

[a] Values are the means ($n = 3$) of all different treatments with ±standard error of the mean. Values in the same column followed by different letters are significantly different ($p < 0.05$).
[b] No fertilizer application (NF); chemical fertilizer only (CF); chemical fertilizer plus rice straw return (CFS) and chemical fertilizer plus pig manure (CFM).
[c] UT under GC test limitation

3.3. Methanogenic archaeal community within PSFs under different fertilizer treatments

There were 6–8 dominating bands in the DGGE profiles of methanogenic archaeal community in >2 μm size fractions, while 4–5 dominating bands in <2 μm size fractions (Fig. 4). However, more or less similar band patterns except for extra band A3 were detected from the larger particle fractions under NF. Four bands (in the fractions of <2 μm under CFM and CFS and of 200–2000 μm under CF and NF treatments) affected

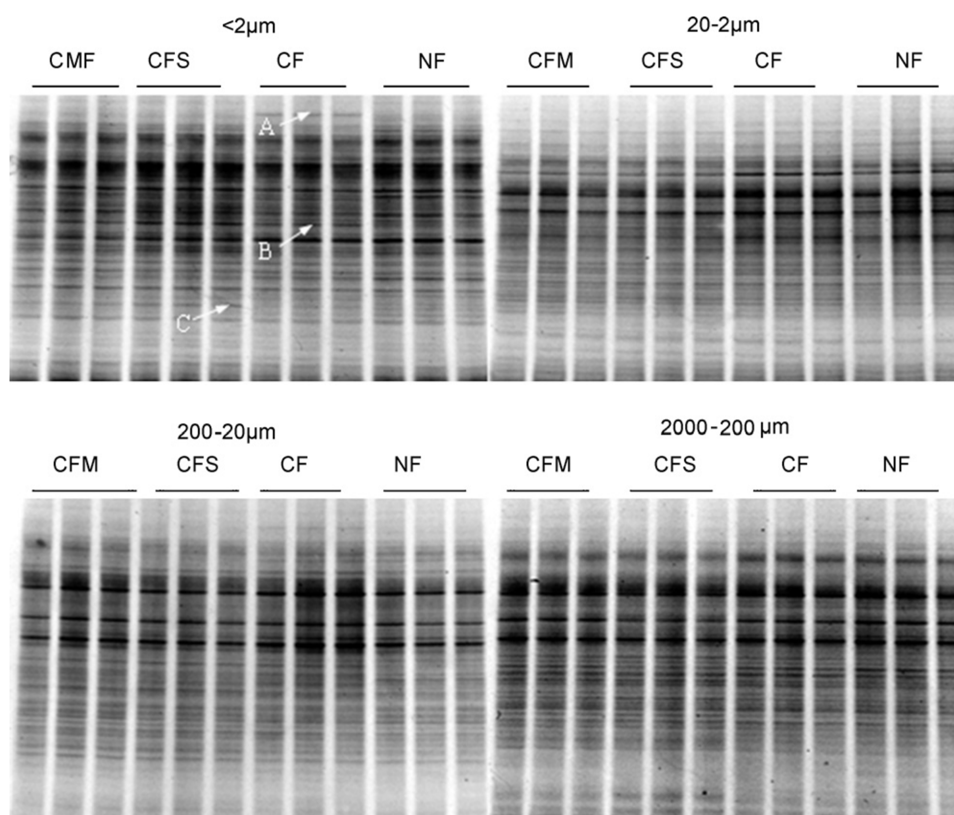

Fig. 1. DGGE profiles (negatively converted) of bacterial community structure within different size particles under fertilizer treatments.

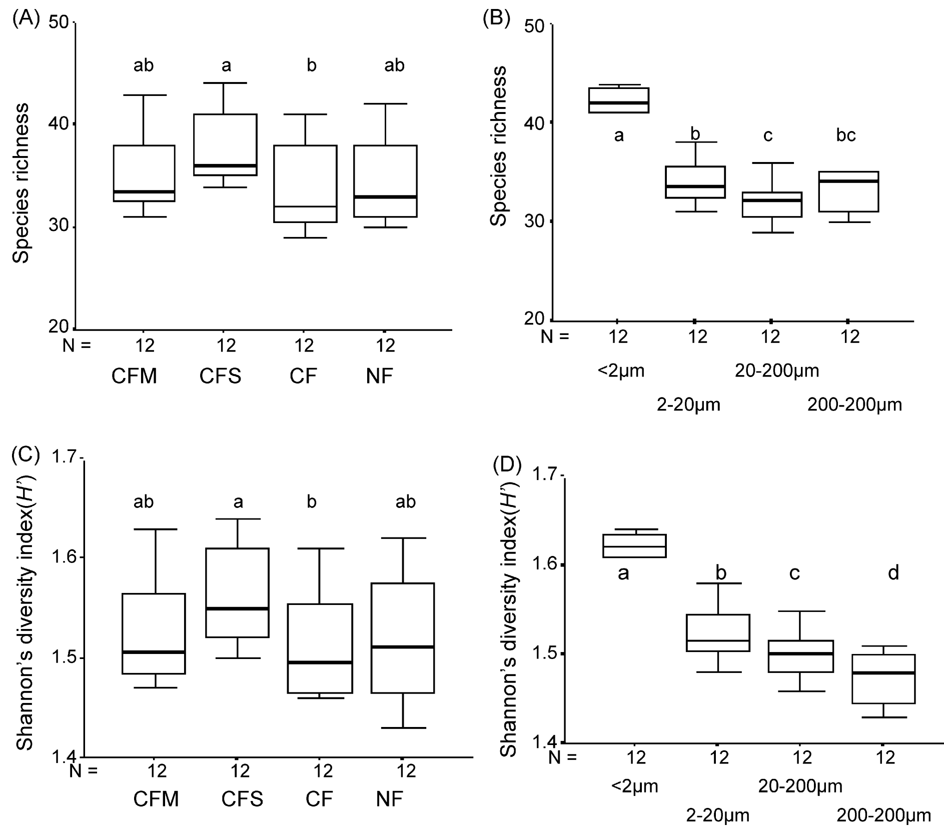

Fig. 2. Box-plot representation of bacterial species richness for different treatments (A) and for different size particles (B), Shannon's diversity index (H') for different treatments (C) and for different size particles (D). Boundaries of the boxes closet to, and further form X axes indicate the 25th and 75th percentiles, respectively. The bold line within box marks the medium. Bars above and below the boxes indicate the 90th and 10th percentiles, respectively. Average values with the same letter in each figure indicate no significant differences between different treatments and different size particles ($p < 0.05$, Fisher LSD).

by marginal utility were not included when analysis were conducted.

4. Discussion

4.1. Change of bacteria community and CO_2 production rate in PSFs

The results showed that bacterial community structure were different among PSFs. Both bacterial species richness and bacterial diversity were significantly affected by size of particle fractions. The size of <2 μm fractions survived higher bacterial species richness and diversity. Some researches demonstrated that the magnitude of change in the bacterial community response increased with the decreasing aggregate size [13] and higher bacteria community habitation in clay fractions [11,17]. Differences in soil organic matter composition and substrates availabilities were likely to influence the microbial community structure [32]. In the present study, higher SOC content and lower C/N ratio (from 7.6 to 9.6) of SOC of the size <2 μm fractions were suitable to bacterial survival. On the other hand, smaller size particles provided a protective habitat for microorganisms through pore size exclusion of predators, in which bacterium bonded of humified SOM and microbial metabolites

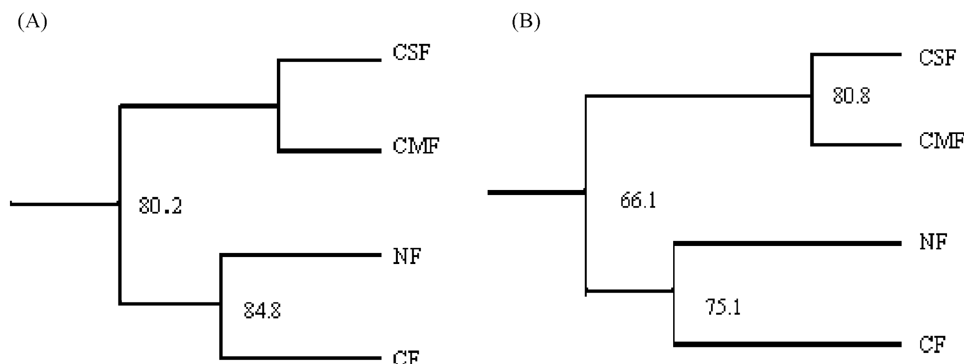

Fig. 3. Similarity tree retrieved from DGGE profile of bacterial community within 200–2000 μm fractions (A) and <2 μm fractions (B).

Table 3
Similarity retrieved from DGGE profiles (Fig. 1) after Bio-Rad Quantity One software analysis

Particle size fractions (μm)	Similarity (%)	CV (%)
<2	70.05 ± 6.6 c	9.47
2–20	75.16 ± 5.9 b	7.84
20–200	72.08 ± 4.5 bc	6.19
200–2000	82.09 ± 4.1 a	4.79

Note: Different low case letters in a single column indicate significant difference at $p < 0.05$.

to clay minerals and pedogenic oxides resist predators [33,34]. Therefore, <2 μm particle fractions could be considered as favorable habitats for bacteria due to the vicinity between microbes, access to carbon resource outside of the microaggregates, and smaller pore size as protective agent rather than to providing carbon resources stored inside. However, bacterial associated with <2 μm fractions appeared lower activity. Many studies showed that C compounds associated with clay fractions were generally stable, refractory and rich in recalcitrant materials, such as polyethylene, lipids [35–37,42–44]. Our previous researches found that content of humus and humic acid with lowest E4/E6 ratio was highest in the size of <2 μm fraction from the same paddy soil [38]. Furthermore, SOC availability was reduced by physical protection of clay absorption.

The size of 200–2000 μm fractions were lower bacterial species richness and diversities, but higher SOC and bacterial activity compared to <2 μm fractions. This could be attributed to the preferential colonization by fungi because these SOC had higher C/N ratios and abundant in decomposable SOC favorable for fungi and micro-fauna in these fractions [11,39]. Our previous studies showed that the coarse fractions contained more particulate organic matter for the same soil [40]. Some researches found that SOC quality affected the bacteria/fungi ratio and the gram-positive/gram-negative ratio in soil [14]. In addition, some species of bacteria could probably be out-competed by eukaryotic organism in coarse fractions; for example, those coarsely sized fractions favored some predators (mainly protozoa) to prey on bacteria [33].

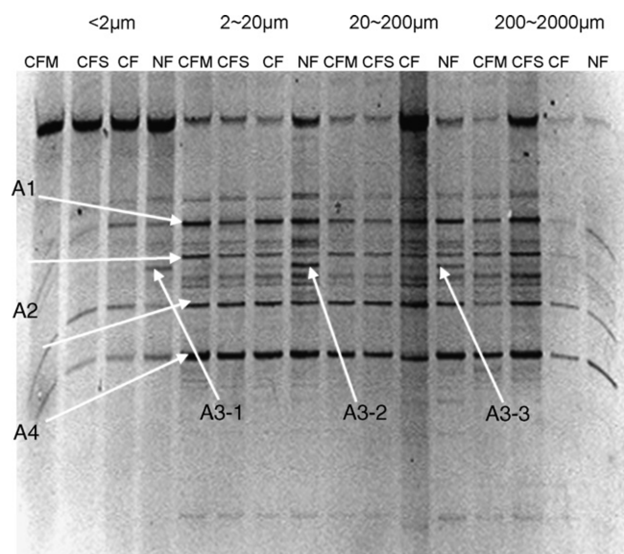

Fig. 4. DGGE profile (negatively converted) of methanogenic archaeal community structure within different size particles under fertilizer treatments.

4.2. Change of CH_4 production rate and methanogenic archaeal within PSFs

On the contrary to the bacterial community, methanogenic archaeal species was less in the clay-sized fractions than in the other PSFs, while CH_4 production capacity decreased with decreasing size of fractions and was negligible in the size of <2 μm fractions. Some studies had similar reports that the rates of CH_4 production were higher within larger size fractions [41,42]. The source of methanogenic substrates was fresh plant materials [43]. It was demonstrated that organic matter associated with macroaggregates was more labile than with micro-aggregates [9,20,44]. Preferential allocation of increased labile C in the coarse fractions has been already reported for paddy soils from south China [12,35,45,46]. Therefore, the higher methanogenic archaeal species and activity in larger size fraction could be attributed to more availability of carbon substrate to the methanogens in those fractions.

CH_4 production detected within the <2 μm fraction was negligible because of less availability of organic carbon in them for methanogen. Thus, the survival of species and activity of methanogens in the clay-sized fraction of the studied paddy soil was restricted by the relatively resistant refractory SOC source.

4.3. Effect of fertilizer treatment on bacteria community, methanogenic archaeal and their activity within PSFs

Fertilizer application caused more change of bacterial community within clay fractions than that within coarser fractions. Therefore, fertilizer treatments in the present study, which different organic matter input change the composition of organic matter associated with clay fractions, caused variation of bacterial community in these fractions. Furthermore, significant different of bacterial community similarity between <2 μm and 200–2000 μm fractions appeared that bacterial community in PSFs greatly depend on particle size rather than fertilizer application (Table 3).

Fertilizer application influenced, to a less extent, methanogenic archaeal community within SPSFs but caused significantly change of CH_4 production within the size of 200–2000 μm fractions. Methanogenic archaeal community in paddy soil was relatively stable, but its activity was very sensitive to the change of management and environmental condition [47,41]. CH_4 production was stimulated by the increase of rice root in soil [48]. Our previous research showed a significantly positive linear correlation between SOC of bulk soil with SOC of coarse size fractions under different fertilizer application at the same paddy soil [45]. It reflected that younger SOC of fresh root and plant residue of rice and rape increase by fertilizer application mainly accumulated in coarse size fractions. It was suggested that fertilizer treatments increase methanogenic archaeal activity within the coarse fractions

because of more the storage of fresh organic matter in these fractions rather than change of their community structure.

5. Conclusion

Bacterial, methanogenic archaeal community and activity appeared difference within different PSFs. Methane was predominantly produced in the size of 200–2000 μm fractions, more species and higher diversity of bacteria survived in the size of <2 μm fractions. Availability of SOC in PSFs was mainly factor affected survival of methanogenic archaeal community structure, whereas, bacterium community habitation more affected by physical protection of their location in PSFs. Otherwise, their activity greatly depended on lability of SOC associated with PSFs. Fertilizer application caused more change of bacteria community in clay fractions and significantly increased bacterium and methanogen activity in 200–2000 μm fractions but only a slight effect on methanogenic archaeal community in the PSFs.

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