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Effect of long-term fertilization on C mineralization and production of CH_4 and CO_2 under anaerobic incubation from bulk samples and particle size fractions of a typical paddy soil

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Abstract

Impacts of nutrient management on C mineralization and greenhouse gas (GHGs) emission from soils have been of much concern in global change. Using laboratory incubation, the production of CH_4 and CO_2 were studied from both bulk samples and the particle size fractions (PSF) of topsoil from a paddy under a long-term different fertilization trial (including non (NF), chemical without (CF) and with manure (CFM) fertilization, respectively) in the Tai Lake Region, China. Four PSFs (2000–200, 200–20, 20–2, <2 μm) were separated from undisturbed samples collected after rice harvest by a low-energy ultrasonic dispersion procedure. Both the bulk samples and PSFs were incubated under submerged condition for 72 days. The concentration of CH_4 and CO_2 evolved during incubation were determined by gas chromatography. C mineralization rates ranged from 0.13 to 0.52 mg C g^{-1} C day^{-1}, with different fertilizations and size of the PSFs, and were not correlated with C/N ratio. While CO_2 production predominated over CH_4 from C mineralization from both bulk samples and the size fractions, CH_4 production played a predominant role in the total global warming potential (GWP) under all treatments. C mineralization of bulk soil was significantly higher under CF than under CFM and NF. CH_4 production, however, was 3 times as under CFM and 27 times as under NF, indicating a tremendous effect of chemical fertilization alone on the total GWP. CO_2 production from the PSFs differed from CH_4 under a single treatment, which was notably from the coarse PSFs larger than 200 μm. Higher C mineralization and CH_4 production with a higher metabolic quotient under CF implicated a vulnerability of soil functioning of GHGs mitigation in the paddy receiving chemical fertilizers only. Thus, rational organic amendments should be undertaken for mitigating the climate change.

Keywords: Anaerobic incubation; C mineralization; CH_4 and CO_2 production; Global warming potential; Long-term fertilization; Paddy soil; Particle size fractions

1. Introduction

C mineralization of agricultural soils has been actively dealt with in the research of C cycling of terrestrial ecosystem and global change. While both CH_4 and CO_2 production from soil result from C mineralization under submerged condition, production and emission of CH_4 has been widely documented with regard to the effects of soil factors such as temperature, moisture, pH and Eh as well as plant cultivars (Neue and Sass, 1994; Schimel, 1995; Xu et al., 1997; Ding et al., 2003; Kerdchoechuen, 2005). Rice paddy, a unique anthropogenic wetland ecosystem, is commonly believed to play a crucial role in the global CH_4 budget. Annual CH_4 emissions from world rice fields have been estimated in a range of 25–100 Tg (IPCC, 1992; Cao et al., 1996; Neue et al., 1990), occupying 10–15% of the total CH_4 emissions (Wang and Adachi, 2004). As an essential agricultural measure, the application of N fertilizers shows profound effect on the CH_4 production and flux from flooded soil (Dan et al., 2001; Rath et al.,

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2002). The effect of different N fertilization on methane emission from paddy soils has been widely studied (Lindau et al., 1991; Banik et al., 1996; Cai et al., 1997; Bodelier et al., 2000). However, there has been little information either on the relative dominance of CH_4 and CO_2 production during submerged C mineralization or on the effect of chemical fertilization alone on C mineralization and CH_4 and CO_2 production compared to that of combined application. While chemical fertilizers are increasingly applied in paddys of Asia (FAOSTAT, 2005), the effect of chemical fertilizers or combined applications of organic and chemical fertilizers is particularly crucial for predicting the future trend of greenhouse gas (GHGs) emission from Asian paddies and possible approaches to mitigate climatic change by agricultural practices.

To understand the biophysical processes performing at microscale in field soils, variation of C and N, microbial activities and production of GHGs from particle size fractions (PSFs) or (micro-) aggregates have been widely studied (Nacro et al., 1996; Gregorich et al., 1997; Schulten and Leinweber, 2000; Six et al., 2000; Li et al., 2007; Chin et al., 2005; Schumacher et al., 2005). Agricultural management practices have profound influences on the relative composition of the fractions and the contents of nutrients and activities of microbes in these different PSFs (Sessitsch et al., 2001; Li et al., 2007). Studies on the distribution, characteristics and sensitivity to environmental change of organic C in soil PSFs based on different methods have been widely reported (Angers and Carter, 1996; Puget et al., 2000; Eynard et al., 2005). Recently, studies on methane production from the different PSFs had been reported with respect to C lability and the response to warming (Van den Pol-van Dasselaar and Oenema, 1999; Drury et al., 2004). However, little has been done on production of GHGs from the PSFs from paddy soils and the possible control by C availability in, and by microbial access to organic C pools in the PSFs.

Paddy soils of China with an area of 130 Mha have a greater potential of C sequestration than their dryland counterparts (Pan et al., 2004). However, rice fields are usually flooded during most of rice growing seasons and the CH_4 emission from them amounts to about 7.7 Tg year^{-1} in Mainland China, greatly contributing to the China's and, indeed, global total methane budget (Yan et al., 2003). Nevertheless, knowledge on the effect of fertilization on CH_4 and CO_2 production under anaerobic conditions from size fractions of paddy soils and their relative contribution to the bulk total with regard to C source and microbial activities in soils is still insufficient.

The present study was conducted to demonstrate the variation of total C mineralization, CH_4 and CO_2 production and global warming potential of bulk soil with different fertilization regimes. It was also the subjective of this study to characterize the distribution pattern of CH_4 and CO_2 production among the different size fractions and the effect of fertilizer application on the potential of GHGs production from them.

2. Materials and methods

2.1. Soil and site description

The studied soil was from a paddy under a long-term fertilization trial initiated in 1987. The paddy was located at Jinjiaba Township, Wujiang Municipality, Suzhou City, Jiangsu Province, China (31°05′N and 120°46′E). The soil belongs to a Ferric-accumulic Stagnic Anthrosols (Gong, 1999). The mean annual precipitation and temperature was 1100 mm and 18.3 °C for the last 20 years. The paddy has been continuously cultivated with rice–rape rotation since1987 and the rice yield in most years reached to 9 t ha^{-1}, a typical yield for the region (Zhang et al., 2004).

2.2. Fertilization treatments

The long-term fertilization field trial was used to monitor the soil fertility and productivity changes under different fertilizer applications. Prior to the fertilization treatment, conventional fertilization was practiced with N as urea, P as super phosphate plus manure amendments. Three fertilization treatments were included in the trial, which had been continuously performed since 1987: no fertilization (NF), chemical fertilizers only (CF), chemical fertilizers plus manure (CFM). The applied chemical fertilizers were in same amount as urea for N, potassium chloride for K and super phosphate for P in the fertilized plots. The layout of the experimental plots was in a completely randomized block design with three replications. The size of each plot was 70 m^2 (10 m × 7 m). The rate of the fertilizer application per year was given in Table 1.The selected soil physical and chemical properties analyzed are given in Table 2.

2.3. Soil samples and treatments

Three soil samples were collected from 0 to 5 cm depth randomly in each plot after rice harvest in 2003. The samples were taken in two portions. One portion for PSF separation and CH_4 and CO_2 gas production test was directly stored in the dark in a refrigerator at 4 °C before incubation. Another portion was shipped to laboratory and subsequently air-dried, ground and sieved through a 0.25 mm sieve for the determination of SOC and total N.

Table 1
Fertilization treatments of studied paddy soil (kg ha^{-1})

Treatment	Manure[a]	N	P_2O_5	KCl	Chemical fertilizer ($N:P_2O_5:K_2O$)	Rice yield[b] (t ha^{-1} a^{-1})
CFM	16,800	427.5	45.0	54.0	1:0.11:0.20	8.24
CF	None	427.5	45.0	54.0	1:0.11:0.20	7.04
NF	None	None	None	None	None	4.17

[a] *Note*: In fresh weight.

[b] Mean value since 1988.

Table 2
Basic physical and chemical properties of the plots under different treatments sampled and measured in 2003

Treatments	pH (H_2O)	CEC (cmol kg^{-1})	SOC (g kg^{-1})	Total N (g kg^{-1})	C_{mic} (mg kg^{-1})	DCB-Fe (g kg^{-1})
CFM	5.74	24.18	17.22	1.50	310.5	15.98
CF	5.93	22.86	16.75	1.43	285.3	18.23
NF	6.13	21.12	16.43	1.37	268.1	15.91

2.4. Separation of particle size fractions

Separation of the PSFs of the paddy soil was performed following a procedure described by Stemmer et al. (1998) with minor modifications. Essentially, the soil–water suspension was dispersed by low-energy ultrasonication (output energy of 0.2 kJ g^{-1}) and subsequently fractionated by a combination of wet sieving (2000–200 μm), siphonage (200–20 μm) and centrifugation (20–2 μm). The remaining suspension was the size fraction of <2 μm particles. The PSF samples were freeze-dried prior to analysis.

2.5. Laboratory incubation and CH_4 and CO_2 production test

The incubation was performed in a LRH-250-S incubator (Medicine Machinery Co. Ltd., Guangdong, China) at 25 ± 1.0 °C for 72 days. Incubation of bulk samples and the separated PSF was conducted as follows: 20 g of samples (dry weight equivalent) were placed in 120 ml serum bottle and 40 ml distilled H_2O was added to keep the soil saturated. Each bottle was sealed with a rubber septum to allow gas sampling from the headspace. To stimulate soil reduction, the headspace of each bottle was flushed with N_2 gas (300 ml min^{-1}) for 10 min before incubation. During incubation, a 0.25 ml sample of the gas evolved was collected by syringe pressure every 5 days since the third day after incubation was initiated. The slurries in each bottle were stirred for 3 min using a magnetic bar before gas sampling, and then, the bottle were flushed with N_2 again (300 ml min^{-1}) for 10 min in order to keep an air of N_2 in the headspace (Wang et al., 1999). The Eh in the slurry was monitored and measured with a Mettler-Toledo pH-Eh meter connected to a platinum electrode. Eh was maintained below −150 mV during the whole period of incubation. The CH_4 and CO_2 concentrations of the sampled gas evolved during incubation were analyzed by Agilent 4890D Gas Chromatography equipped with a stainless steel column (Porapak Q) (80/100 mesh) and a flame-ionization detector (FID). Column, injector and detector temperature were 35, 130 and 250 °C, respectively. Nitrogen gas (carrier gas), FID hydrogen and FID airflow was set at the rate of 30, 45 and 400 ml min^{-1}, respectively. A blank of 40 ml distilled water was used as the control of the gas concentration in the bottle. The incubation was conducted in triplicate bottles.

2.6. Chemical analysis

Total organic carbon (C_{org}) and total nitrogen (TN) of dried samples was measured with HCl (10%, v/v) using a CNS Macro Elemental Analyzer (Elementar Analysen Systeme GmbH, Germany). Microbial biomass carbon was determined by fumigation as described by Vance et al. (1987). The moisture content of the samples was determined by oven-drying at 105 °C for 6 h. Content of free iron oxides was determined by dithionite–citrate–bicarbonate (DCB) extraction (Lu, 2000).

2.7. Calculation of global warming potential (GWP)

The effect of different fertilization treatments was standardized by determining the GWP from the production potential of CH_4 and CO_2 released during the incubation. GWP for each of these gases was calculated on the basis of CO_2 as the reference gas. According to IPCC (2001), 1 μmol-CO_2 is assumed as 1, and 1 μmol-CH_4 is 62 for 20-year duration. GWP is then calculated as described by Cai (1999):

$$\mathrm{GWP(CH_4)} = \frac{m(\mathrm{CH_4})}{16} \times 62 \qquad (1)$$

$$\mathrm{GWP(CO_2)} = \frac{m(\mathrm{CO_2})}{44} \times 1 \qquad (2)$$

$$\mathrm{GWP(total)} = \mathrm{GWP(CO_2)} + \mathrm{GWP(CH_4)} \qquad (3)$$

where m is the mass of the gas, which is hereby translated into the production rate during incubation. The GWP value is unit-less.

2.8. Statistics

Statistical differences were tested with analysis of variance procedure (ANOVA) using the SPSS11.0 statistical package. Statistical significant was determined at the 95% or 99% confidence level.

3. Results

3.1. Distribution of the PSFs and the variation of C and N contents

The composition of the PSFs and their contents of total C and total N from the studied paddy soil are summarized in

Table 3
Distribution (%) of soil particle size fractions and their C and N contents from the plots under different fertilizations

Treatment	2000–200 μm	200–20 μm	20–2 μm	<2 μm
Mass content (%)				
CFM	37.8 ± 2.5aA	30.1 ± 1.7aB	26.7 ± 1.6bC	5.3 ± 0.3aD
CF	33.7 ± 0.5bA	28.3 ± 0.3bB	28.9 ± 0.3bB	5.9 ± 0.5aC
NF	29.7 ± 0.7cB	29.1 ± 0.5bB	35.1 ± 0.5aA	6.1 ± 0.4aC
C_{org} (g kg^{-1})				
CFM	19.32 ± 0.37aA	16.68 ± 0.95aC	13.50 ± 0.19aD	18.64 ± 0.19aB
CF	17.81 ± 0.24bB	16.71 ± 0.57aC	13.32 ± 0.23aC	18.58 ± 0.41aA
NF	16.70 ± 0.48cB	16.43 ± 0.14aB	12.80 ± 0.14bC	18.23 ± 0.28aA
TN (g kg^{-1})				
CFM	1.50 ± 0.01bB	1.44 ± 0.06aB	1.28 ± 0.02aC	2.16 ± 0.05aA
CF	1.47 ± 0.05bB	1.48 ± 0.16aB	1.20 ± 0.01aC	2.04 ± 0.12abA
NF	1.76 ± 0.02aA	1.20 ± 0.06bB	1.20 ± 0.02aB	1.84 ± 0.06bA

Notes: "±" indicates standard deviation. Different lower case letters in a single column and different capital letters in a row indicate significant difference at $p < 0.05$ between the fertilization treatments and PSFs, respectively.

Table 3. While insignificant amounts (<10%) of soil particles were in the <2 μm category, significant difference in the mass content of size fractions were found in the PSF of 2000–200 μm between the different fertilization plots. The mass content of the PSF of 2000–200 μm and that of 20–2 μm under different treatments was in the order: CFM > CF > NF and NF > CF > CFM, respectively. This indicated an enhanced aggregation of smaller particles in size of 20–2 μm into large particles. Under a single treatment, high contents of OC and N was found in the PSF of <2 and 2000–200 μm and low contents in that of 20–2 μm in size category. There was observed significant difference in C and N of a single PSF between the fertilization treatments. The C/N ratio of the PSFs tended to decrease with the decreasing size while it was significantly higher in the PSF of 2000–200 μm under CFM than under CF and NF. In addition, soil N content was higher in fertilized plots than in non-fertilized ones (also see Table 2).

3.2. Total C mineralization of the bulk soil and the PSFs

The result of C mineralization of both the bulk samples and the PSFs is shown in Table 4. The mean accumulative C mineralized from bulk samples was at the level of 30, 35 and 15 mg OC under CFM, CF and NF, respectively, being significantly different between the fertilization treatments. However, the highest C mineralization rate of bulk sample on the basis of OC content was found under CF. Generally, C mineralization of the PSFs varied in a decreasing trend with their sizes and SOC content except for the clay sized fractions high in SOC. While significant difference in SOC was detected only in the PSF of 2000–200 μm between from the treated plots, C mineralization of a single PSF was significantly different between the treated plots. The data of mean contribution by a single PSF to the bulk total C mineralization were summarized in Table 4. Apparently, significantly higher contributions to the total C mineralization were in the of 2000–200 and 20–2 μm PSFs.

3.3. Production and global warming potential of CH_4 and CO_2 under different treatments

As products of C mineralization, considerable amounts of CH_4 and CO_2 were produced during the anaerobic incubation. As shown in Table 5, total CO_2 production after 72 days of incubation varied from 240.5 μg g^{-1}soil

Table 4
C mineralization rate of the individual size fractions and of the bulk soil

Treatments	Bulk soil	2000–200 μm	200–20 μm	20–2 μm	<2 μm	Recovery (%)
C mineralization rate ($(CH_4 + CO_2)$–C mg g^{-1} C day^{-1})						
CFM	0.42 ± 0.03B	0.47 ± 0.05A	0.43 ± 0.01A	0.47 ± 0.03A	0.33 ± 0.01A	108
CF	0.50 ± 0.02A	0.42 ± 0.02A	0.32 ± 0.01B	0.37 ± 0.02B	0.19 ± 0.02B	73
NF	0.21 ± 0.03C	0.28 ± 0.01B	0.13 ± 0.00C	0.21 ± 0.01C	0.17 ± 0.00B	98
Mean contribution of PSF to the bulk total C mineralization (%)						
CFM	–	42.3	31.3	29.8	4.2	108
CF	–	28.3	18.1	21.4	2.2	70
NF	–	39.7	18.0	35.1	5.0	98

Notes: "±" indicates standard deviation. Different capital letters in a single column indicates significant difference at $p < 0.01$ between the treatments. The mean contribution by a single PSF to the bulk total C mineralization was calculated as: contribution (%) = ((C mineralization rate of a given PSF × mass percentage of the PSF)/bulk total C mineralization rate) × 100%.

Table 5
Production potential of CH_4 and CO_2 and the GWP from the bulk soil under the different treatments

Treatments	GHGs production ($\mu g\ g^{-1}$ soil)		GWP		Total GWP	Contribution by CH_4 (%)
	CH_4	CO_2–C	CH_4	CO_2		
CFM	46.3 ± 3.5B	470.4 ± 15.8A	179.4B	39.2A	218.6B	82.1B
CF	136.4 ± 15.3A	465.3 ± 44.8A	528.5A	38.8A	567.3A	93.2A
NF	5.2 ± 0.6C	240.5 ± 23.3B	20.1C	20.1B	50.1C	50.1C

Notes: "±" indicates standard deviation. Different capital letters indicates significant difference at $p < 0.01$ between the treatments.

under NF to 470.4 $\mu g\ g^{-1}$ under CFM. Total CH_4 production, however, varied in much wider range from 5.2 $\mu g\ g^{-1}$ soil under NF to 136.4 $\mu g\ g^{-1}$soil under CF. Apparently, CO_2 production predominated over CH_4 even under the anaerobic condition. Fertilization affects CH_4 production differently compared to CO_2, although both were significantly influenced by the different fertilizer treatments. There was no significant difference in the CO_2 production from the soils between the two fertilized plots (CF and CFM), which was as twice as much as the production from NF plot. Whereas, very significant difference in production of CH_4 of bulk soil existed between the different treatments as the CH_4 production under CF was 3 and 27 times as much as produced that under CFM and NF, respectively. According to IPCC (2001), the calculated total (CH_4 + CO_2) GWP potential under CF was as 2.4 and 14 times as much as that under CFM and NF, respectively (Table 5). Among the three different fertilization treatments, the bulk soil sample under CF exerted the highest greenhouse gases (here CH_4 and CO_2) production capacity ($p < 0.01$), especially for CH_4. While the CH_4 emission from both CFM and CF treatments dominated total GWP, the contribution of CH_4 to total GWP was increased from 82% under CFM to 93% under CF. Therefore, different fertilizer application practices affected not only the intensity of CH_4 production, but also the relative dominance of CH_4 in the total GWP of the paddy.

3.4. Production of CH_4 and CO_2 from PSFs

A significant difference occurred in CH_4 and CO_2 production from different sized PSFs under different fertilizer treatments (Fig. 1). For CH_4, the production capacity basically decreased with the size of the particles under all the studied treatments. A considerable amount of CH_4 production occurred from the particle sizes that were larger than 2 μm under CFM and CF. By contrast, CH_4 production was only detected from 2000 to 200 μm PSF under NF. Production of CH_4 was insignificant from clay-sized fraction under all the treatments. However, production of CO_2 was observed in significant amount from all the differently sized PSFs under all treatments with highest in the fractions with largest size. Unlike the variation of CH_4 production, there was no significant difference in CO_2 production between the PSFs smaller than 200 μm.

4. Discussion

4.1. C mineralization in anaerobic condition and the fertilization effect

C mineralization of bulk soil is of key importance in C cycling of paddy ecosystems. Zhou et al. (2003) showed mean C mineralization rate of three typical types of paddy soils ranging from 0.11 to 0.13 mg C g^{-1} OC day^{-1} using aerobic incubation at 25 °C for 112 days. Recently, Zhang et al. (2006) reported aerobic SOC mineralization of a paddy soil derived from Paludults from Jiangxi China in a range of 0.13–0.17 mg CO_2–C g^{-1} OC day^{-1}. Ranging from 0.2 to 0.5 mg CO_2–C g^{-1} OC day^{-1}, C mineralization of the bulk samples in the present study seemed much higher. The calculated mean values of C mineralized to C_{mic} ratio in this

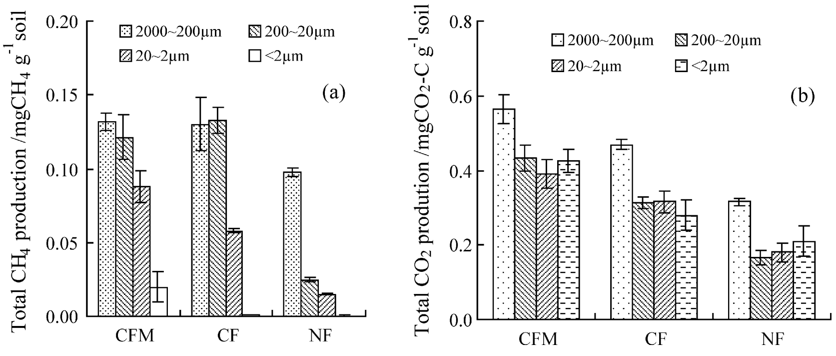

Fig. 1. Total CH_4 and CO_2 production from the size fractions: (a) CH_4 and (b) CO_2. CFM: chemical fertilizer plus pig manure; CF: chemical fertilizer only; NF: no fertilizer.

study was 0.9, 1.6 and 1.9 mg C mg^{-1} C_{mic} under NF, CFM and CF, respectively, being also higher than around 0.5 mg C mg^{-1} C_{mic} for the case of Zhang et al. (2006). Seemingly, C mineralization of paddy soils under submerged conditions was stronger than under non-submerged conditions, which could be due to the higher metabolic ultilization of SOC under anaerobic condition as observed also by Huang et al. (1998). C mineralization of the bulk samples was disproportional to their OC contents as similarly described by Zhou et al. (2003) and Zhang et al. (2006). This supported our findings of significant C sequestration in the paddy under CFM due to physical protection C in the form of particulate OC (Zhou et al., 2006a,b). However, C mineralization under chemical fertilization alone was enhanced with the highest metabolic quotient despite its relatively low OC content. This was coincident with the highest CO_2 flux from the CF plot than from the other treated ones in our field study (Zheng et al., 2006).

The dependence of C mineralization under aerobic conditions on soil N supply status has been well described for forest soils (Macdonald et al., 1999; Lerós et al., 1999) and for paddy soils (Zhang et al., 2006). The results of these studies showed that generally higher C mineralization could be expected in soils that have a low C/N ratio. Here, the C mineralization rate of both bulk soil and PSFs was plotted against the ratio of C/N in Fig. 2. Apparently, the variation of C mineralization under anaerobic condition either from a single treated plot with the size of tested fractions or of a single size fraction with the fertilization treatments could not be accounted for by the variation of C/N ratios of the tested samples. More recently, Zhou et al. (2006) have shown, based on the data of crop yield and SOC storage under the different treatments, that N effect both on C assimilation by rice and soil C sequestration varied significantly with the fertilizer application treatments. Failure of correlation of C mineralization with C/N ratio and high GHGs (CH_4 and CO_2 in this case) production under chemical fertilization could implicate a vulnerability of microbial community responsible for C mineralization rather than a change in microbial activity in response to N status. Indeed, Zhang et al. (2004) described a significantly reduced microbial diversity and crop productivity of the soil system in the CF plot by using molecular footprint technology. Coincidently, Cookson et al. (2005) reported also that soil microbial community structure could be altered as the result of addition of fertilizers with different C/N ratios.

4.2. Production of CH_4 and CO_2 and GWP under different fertilizations

It is notably important that fertilization significantly ($p < 0.01$) increased not only the total GWP of the paddy soil but also the contribution of CH_4 to the total compared to non-fertilized plot. This may be, in part, due to the increased organic carbon input that resulted from the higher biomass generated (Zhang et al., 2004; Qiu et al., 2005) as methanogens largely consumes fresh organic material from the rhizosphere. However, application of chemical fertilizer alone, in particular, caused much higher CH_4 production and total GHGs emission in contrast to lower crop yield than under combined fertilization of CFM. This is similar to our finding of higher CO_2 emission rate under CF evidenced by field monitoring when no rice was growing (Zheng et al., 2006). Production and emission of both CH_4 and CO_2 resulted from C mineralization, by which these gases are evolved as the end products with methane being the primary product under submerging conditions (Kimura et al., 2004). While fertilization enhanced SOC mineralization with the increased microbial biomass and enzyme activity, the predominant pathway of CH_4 and CO_2 formation was generally considered unchanged under different fertilizer treatments (Chidthaisong et al., 1999; Zhu'ge et al., 2005). The capacity of CH_4 and CO_2 production were likely to depend on available C resources for microbes. It has been well addressed that application of different fertilizers especially with manure could enhance the bioavailable

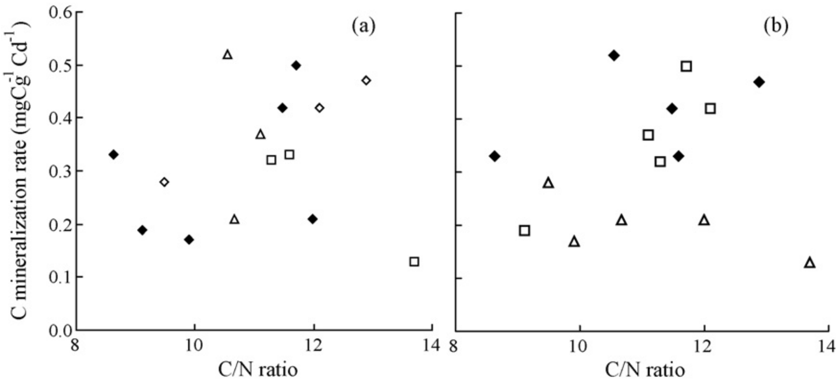

Fig. 2. Variation of C mineralization rate against the C/N ratio with size fractions including bulk samples (a) ((■) bulk sample; (◇) 2000–200 μm; (□) 200–20 μm; (△) 20–2 μm; (◆) <2 μm) and with different treatments (b) ((◆) CFM, chemical fertilizer plus pig manure; (□) CF, chemical fertilizer only; (△) NF, no fertilization).

pool of organic carbon (Zhang, 1995; Galantini and Rosell, 2006), and, in turn, the CH_4 production from readily bioavailable organic carbon utilization by methanogenic microbes was promoted. However, compared to CF in this study, higher production of CH_4 and CO_2 was not observed under CFM with comparable amount of chemical fertilizers and higher biomass production (Zhang et al., 2004). Furthermore, there was no significant difference in CO_2 evolved between under CFM and CF during the anaerobic incubation. Therefore, it could be inferred that there was no significant difference in the microbial community responsible for CO_2 production but rather in the community structure responsible for CH_4 production in anaerobic incubation between under the CF and CFM treatments. It is widely accepted the CH_4 emission from soils depended on the balance of CH_4 production and consumption due to oxidation (Cai and Mosier, 2000), which is controlled by the relative intensity of activity of methanogens for CH_4 production and methanotrophs for CH_4 consumption (Gilbert and Frenzel, 1995; Bosse and Frenzel, 1997). Ammonium-based fertilizers are generally considered to inhibit methanotrophs, as was demonstrated in soils and sediments (Conrad and Rothfuss, 1991; Bosse et al., 1993). The decreased CH_4 oxidation by long-term application of chemical N fertilizers had been well evidenced in a study by Mosier et al. (1996), who found that a sandy loam soil in shortgrass steppe fertilized with chemical N fertilizers for 5–13 years consumed 30–40% less CH_4 than the unfertilized counterpart. In this study, however, activity of methanotrophs for CH_4 oxidation was not possible under the anaerobic incubation. Furthermore, no remarkable difference in genetic diversity of methanogens was found between the fertilization treatments when analyzing 16S rDNA of methanogens using DGGE-PCR method (Zhang, 2006). Seemingly, the higher CH_4 production under CF plot could be in association with the relatively enhanced metabolic activity of methanogens under CF. The effect of the long-term different fertilizer application on activity of methanogens still needs further study.

4.3. C mineralization and greenhouse gases production from soil PSFs

Biophysical processes at the microscale in soils have been extensively studied by means of soil size fractionation as they differ in microbial community, enzyme activity and C forms (Stemmer et al., 1998; Kiem et al., 2002; Sessitsch et al., 2001). As seen in Fig. 1, production of CH_4 sharply and of CO_2 gently decreased with the decreasing size of the fractions under a single treatment. While the organic compounds available to methanogens are mainly CO_2 type substrates, methyl substrates and acetate (Madigan et al., 2000; Lange and Ahring, 2001), carbon availability in general was thought to be the important factor affecting CH_4 production from the size fractions. A study by Van den Pol-van Dasselaar and Oenema (1999) showed that similar total C mineralization from the different sized PSFs from a peat soil while significant CH_4 production occurred only in the fractions of large PSF with mainly younger OC material available as substrate for methanogens. In our study, the C mineralization and CO_2 production from the PSF of 2000–200 μm was significantly higher than that from the PSFs in smaller sizes and no remarkable difference was observed between the PSFs from a single plot. However, high CH_4 production rates occurred in the PSFs larger than 2 μm from fertilized plots and only in that of 2000–200 μm from the NF plot (Fig. 1). No significant correlation was found between SOC content and CH_4 production of the PSFs larger than 2 μm although both of SOC and CH_4 production decreased with particle size under different fertilizer treatments in the present study. The fact that negligible CH_4 production was found in the PSFs of <2 μm despite their high SOC content under all the treatments could suggest that the OC pool associated with fine size fractions was possibly unavailable for methanogens. The results here also support the findings by Kiem et al. (2002), John et al. (2005) and Quenea et al. (2004) who showed that SOC associated with fine PSFs were basically refractory and slow to turnover. Our recent work on humus fractionation in these different size fractions also showed increasing humification with the decreasing size of the PSFs (Ding et al., 2006). Accordingly, little substrate was available to methanogens due to the highly humified SOC from the smallest size of fractions. Ramakrishnan et al. (2000) also showed that CH_4 production in different sized PSFs was limited by their substrate and low CH_4 production from the clay-sized fraction was restricted by the relatively resistant C source which, in turn, could be unaffected by the different fertilization practice.

While the CO_2 production rate of a PSF of a given size under different treatments tended to vary with the OC content, fertilization treatment seemed to slightly influence the proportion of CO_2 production from a single fraction under a given treatment despite of an increased proportion of the PSFs smaller than 20 μm under CFM compared to under CF. Therefore, the effect of fertilization on CO_2 from the PSFs seemed to be less profound than on the CH_4 production.

Organic carbon associated with large PSFs was predominantly labile C and the content could be considerably altered by management practices in the short term. This was well addressed by Zhou et al. (2006a,b) on particulate organic carbon from the same soil under the different treatments, by Li et al. (2007) on variation of total OC of the studied soil and by Kandeler et al. (1999) on a fine-sandy loamy Haplic Chernozem by tillage system. Mertz et al. (2005) found the composition of organic matter associated with coarse PSFs changed with fertilization years while investigating the stabilization of organic matter among different PSFs. As the diversity of methanogens was not significantly different among the PSFs from a single treated plot (Zhang et al., 2006), the higher CH_4 production from large size fractions under all the treatments could be accounted for by the higher labile C supply and these large sized fractions could be considered prone to mineralization and release to the atmosphere. However, the effect of relative distribution of methanogens and methanotrophs and their activity changes with the different PSF on the CH_4 and CO_2 production is still unknown for this paddy soil.

5. Conclusion

C mineralization of both the bulk soils and size fractions of the paddy under anaerobic incubation was at significantly high rates and independent of soil total N. Total CH_4 and CO_2 production, and GWP under chemical fertilization alone were significantly higher than under combined application of chemical and organic fertilizers. Variation of CH_4 production with the size of particle fractions differed from that of CO_2 production, showing varying availability of C pools in different particle size fractions to methanogens and CO_2-producing microbes. While CO_2 production predominated over that of CH_4, chemical fertilization enhanced greatly the activity of methanogens and, in turn, the total GWP from the paddy. CH_4 production both from bulk samples and the particle size fraction of 2000–200 μm was promptly affected by the different fertilizer application practice. This raises a problem of great vulnerability of the microbial community under chemical fertilization alone in mediating GHGs emission from the paddy soil. Thus, rational fertilization with organic amendments should be considered as measure in agriculture for mitigating climate change. How the enhanced activity of methanogens and the vulnerability of the microbial community under chemical fertilization alone need further studies.

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