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Diversity of aerobic anoxygenic phototrophic bacteria in paddy soil and their response to elevated atmospheric CO_2

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Summary

Aerobic anoxygenic phototrophic bacteria (AAnPB) are recognized as an important group driving the global carbon cycling. However, the diversity of AAnPB in terrestrial environment remains largely unknown as well as their responses to the elevated atmospheric CO_2. By using culture-independent techniques, the diversity of AAnPB in paddy soil and the changes in response to the rising atmospheric CO_2 were investigated within China FACE (Free-air CO_2 enrichment) platform. There was a phylogenetically diverse AAnPB community with large population size residing in paddy soil. The community structure of AAnPB in bulk and rhizospheric soils stayed almost identical, while the population size was higher in rhizospheric [$2.0–2.5 \times 10^8$ copy number of *pufM* genes g^{-1} dry weight soil (d.w.s.)] than that in bulk ($0.7–0.8 \times 10^8$ g^{-1} d.w.s.) soils. Elevated atmospheric CO_2 appeared to significantly stimulate AAnPB abundance (up to $1.4–1.5 \times 10^8$ g^{-1} d.w.s.) and result in a higher AAnPB percentage in total bacterial community (from 0.5% up to 1.5%) in bulk soil, whereas no significant effect was observed in rhizospheric soil. Our results would extend the functional ecotypes of AAnPB and indicate that environmental changes associated with the rising atmospheric CO_2 might affect AAnPB community in paddy soil.

Introduction

It was widely recognized that purple phototrophic bacteria (PPB) thrive only in anoxic environment, and oxygen inhibits the expression of light harvesting system, which hampers their growth (Pfennig, 1967; Bauer and Bird, 1996). However, this concept has been changed by the discovery of aerobic bacteriochlorophyll α-containing bacteria similar to known PPB. These obligately aerobic species of bacteria are termed as aerobic anoxygenic phototrophic bacteria (AAnPB) (Shimada, 1995; Yurkov and Beatty, 1998; Kolber *et al*., 2001). Sequencing analysis of 16S rRNA and light harvesting genes suggested that AAnPB have evolved from PPB (Xiong *et al*., 2000) and they share the conservative regions of *pufM* gene encoding the reaction centre of light harvesting system (Nishimura *et al*., 1996; Yurkov and Beatty, 1998). However, the growth of AAnPB does not strictly depend on light. The oxidative phosphorylation is the major pathway for AAnPB propagation. Light energy is utilized by AAnPB only for reducing energy requirements of growth (Beatty, 2002). Due to the highly heterogeneous constituents of AAnPB and no selective medium for culture-reliant approaches, the *pufM* gene is widely used as a biomarker for investigating the biogeographical distribution of AAnPB in nature (Waidner and Kirchman, 2008).

AAnPB are found in a wide variety of environments (Yurkov, 2006) and thought to be of ecological significance (Kolber *et al*., 2001; Jiao *et al*., 2007). Most researches have focused on the ecophysiological distribution of AAnPB in marine ecosystem, and little is known in terrestrial environment. Paddy field may represent the most important agricultural ecosystems in Asia since rice is the main source for food supply. Flooding management makes paddy field a unique aquatic system because of the co-occurrence of both anoxic and oxic niches. For instance, during rice growing period, soil is mainly characterized as an anaerobic system, while there are oxic zones such as rhizospheric and oxidized surface soils (Roger, 1996). In addition, soil is basically oxic during wheat growing and winter fallow seasons. Therefore, paddy soil could be an ideal environment for the growth of AAnPB. In the meantime, AAnPB require more organic carbon for growth due to the oxidative phosphorylation pathway when compared with the phototrophic lifestyle of other anoxygenic phototrophs. In fact, AAnPB outcompete other phototrophs, become the dominant anoxygenic

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phototrophs in oceans and are responsible for the ocean's carbon cycling (Kolber *et al.*, 2000; 2001). This new physiological group of bacteria might thus play an important ecological role in carbon cycling in paddy soil (Beatty, 2002).

Atmospheric CO_2 concentration has increased dramatically since pre-industrial times, and a further twofold increase (540–970 μmol mol^{-1}) is expected by the end of the 21st century (Cheng *et al.*, 2010). Elevated atmospheric CO_2 concentration can result in higher biomass of plants and root exudates (Ainsworth, 2008), which in turn influences soil microorganisms (Sadowsky and Schortemeyer, 1997). The China free-air CO_2 enrichment (FACE) project began on 2002 to explore the responses of crops and soil microorganisms in a paddy field to this climate change by artificially elevating atmospheric CO_2 concentration 200 ± 40 μmol mol^{-1} above current CO_2 concentration. We hypothesized that the community of heterotrophically growing AAnPB might be indirectly affected by the rising atmospheric CO_2 in paddy soil. Therefore, by using culture-independent methods on the basis of the *pufM* gene (Waidner and Kirchman, 2008), we investigated the community composition and the population size of AAnPB and their responses to elevated atmospheric CO_2 and N fertilization under *in situ* conditions in the China FACE platform.

Results

DGGE fingerprinting of pufM *genes in paddy soil*

DGGE revealed that two dominant bands (2 and 5) consistently appeared in soil samples from all treatments, while the digitalized band intensity of band 5 increased by 3.1–37.5% under elevated atmospheric CO_2 (Fig. 1). Indeed, the intensities of 10 less dominant bands (1, 3, 4, 6, 7, 12, 13, 15, 16 and 17) also appeared to be enhanced by elevated atmospheric CO_2, while the intensities of bands 14 and 18 decreased by 9.5–100% and 7.1–100% respectively. Shannon's diversity indices were 2.56–2.93 for bulk soils and 2.63–2.98 for rhizospheric soils, which indicated a subtle difference of AAnPB composition between bulk and rhizospheric soils. The effect of N fertilization on *pufM* genes was also hardly differentiated in paddy soil.

Phylogenetic identification of pufM *genes in paddy soil*

Phylogenetic analysis indicated the presence of a phylogenetically diverse AAnPB community in paddy soil (Fig. 2). The *pufM* gene sequences could be phylogenetically placed within three groups. The *Alphaproteobacteria* phyla contained the sequences of bands 1, 10, 13, 16, 17 and 18, while DGGE bands 8 and 14 were affiliated within the phyla of *Gammaproteobacteria*. These *pufM* genes

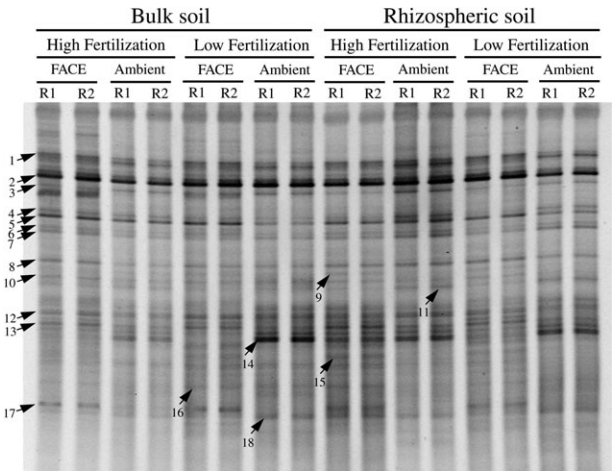

Fig. 1. DGGE fingerprinting pattern of *pufM* genes in paddy soils. R1 and R2 indicate the duplicate of DGGE fingerprints. The DGGE bands denoted by arrow from number 1 to 18 were excised for sequencing.

were closely related to the members of isolated AAnPB. The third group was formed by the dominant DGGE bands 2 and 5, which clustered with *Methylobacterium*- and *Bradyrhizobium*-like AAnPB respectively. However, the growths of the isolated *Methylobacterium* or *Bradyrhizobium* do not depend on aerobic anoxygenic photosynthetic metabolism, despite the fact that both of them are capable of producing bacteriochlorophyll α and carotenoids in cells (Urakami and Komaga, 1984; Evans *et al.*, 1990).

Real-time PCR quantification of pufM *and 16S rRNA genes in paddy soil*

Because primer sets *pufM* 557F/WAW and *pufM* 557F/750R were both used to investigate AAnPB diversity in the previous reports (Hu *et al.*, 2006; Waidner and Kirchman, 2008), they were chosen and validated each other in this study. The higher copy number of soil *pufM* gene was detected by *pufM* 557F/WAW than *pufM* 557F/750R assays (Fig. 3A), although both assays revealed the similar changing pattern of *pufM* genes in paddy soil. This indicated the former assay might be better suited for *pufM* gene quantification in paddy soils than the latter. The *pufM* gene abundance by *pufM* 557F/WAW assay varied from $0.7–1.5 \times 10^8$ g^{-1} d.w.s. in bulk soil and $2.0–2.5 \times 10^8$ g^{-1} d.w.s. in rhizospheric soil (Fig. 3A). It indicated that AAnPB abundance in rhizospheric soil was significantly larger than that in bulk soil ($P < 0.05$). Furthermore, the copy numbers of *pufM* genes in bulk soil were higher under FACE treatment than Ambient treatment ranging from $0.7–0.8 \times 10^8$ to $1.4–1.5 \times 10^8$ g^{-1} d.w.s., whereas no apparent difference was observed in rhizospheric soil. In addition, N fertilization treatment had little effect.

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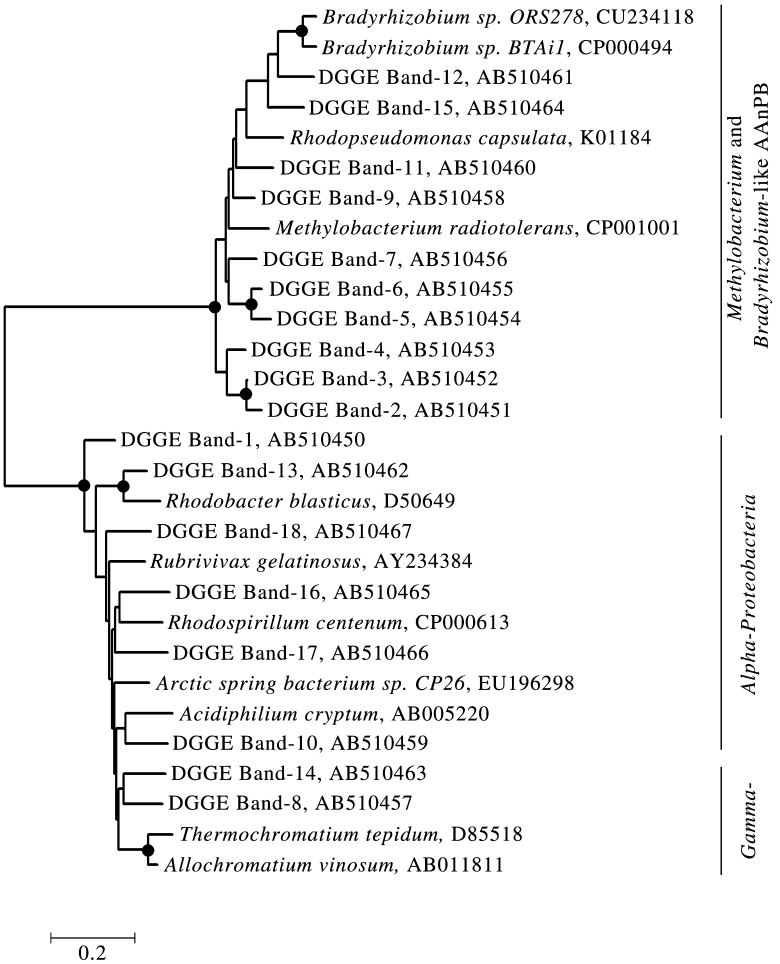

Fig. 2. Phylogenetic tree analysis showing the relationship of *pufM* genes (277 bp in length) in paddy soil to the closest relatives deposited in GenBank. The filled cycle indicated internal nodes with at least 50% bootstrap support. Scale bar indicates the number of nucleotide acid substitutions per site.

There were 0.9–1.6 $\times$ 10^{10} copy number of 16S rRNA gene g^{-1} d.w.s. in bulk soil and 2.7–3.7 $\times$ 10^{10} g^{-1} d.w.s. in rhizospheric soil respectively (Fig. 3B), which is consistent with the data from other soil (Pittl *et al.*, 2010). No significant difference of bacterial 16S rRNA gene copy numbers was observed between FACE and Ambient conditions for all treatments (Fig. 3B). However, elevated CO_2 appeared to result in a higher AAnPB percentage in total bacterial community (from 0.5% up to 1.5% for *pufM* 557F/WAW assay) in bulk soil, but not in rhizospheric soil.

Discussion

There was a diverse AAnPB community with large population size residing in paddy surface soil. Although the depth of photic paddy soil is usually between 0.2 and 2 cm (Roger, 1996), the unilluminated zone of surface paddy soil that is ploughed as a result of agricultural practices will become the illuminated zone following plowing the next year. Thus surface soil at a depth of 0–5 cm is presumably suitable for the investigation of phototrophs. In fact, the investigations of the diversities of algae (Fujita and Nakahara, 2006) and cyanobacteria (Song *et al.*, 2005) are also conducted at such depth in paddy surface soil. AAnPB are found to be widespread in almost all extant ecosystems (Yurkov, 2006). Culture-independent analysis in this study indicated some *pufM* genes of AAnPB could occur in geographically distinct ecosystems, while certain unique AAnPB could be particularly favoured in paddy soil. The *pufM* genes in paddy soil within the phyla of *Alpha-* and *Gammaproteobacteria* were similar to those of AAnPB isolated from ocean such as *Rubrivivax gelatinosus* and *Rhodobacter blasticus* and from lake water such as *Acidiphilium cryptum* (Hu *et al.*, 2006; Waidner and Kirchman, 2008). However, the deeply branching cluster of *pufM* genes related to *Methylobacterium* sp. and *Bradyrhizobium* sp. was detected only in paddy soil, which might extend the functional ecotypes of AAnPB. Although several non-AAnPB species appeared in the phylogenetic tree (Fig. 2), the genotypes related to these non-AAnPB were possibly from some unclassified AAnPB due to the low bootstrap values. The mix of alpha and gamma subdivisions of *Proteobacteria* in the phylogenetic tree built on the *pufM* gene might result from the

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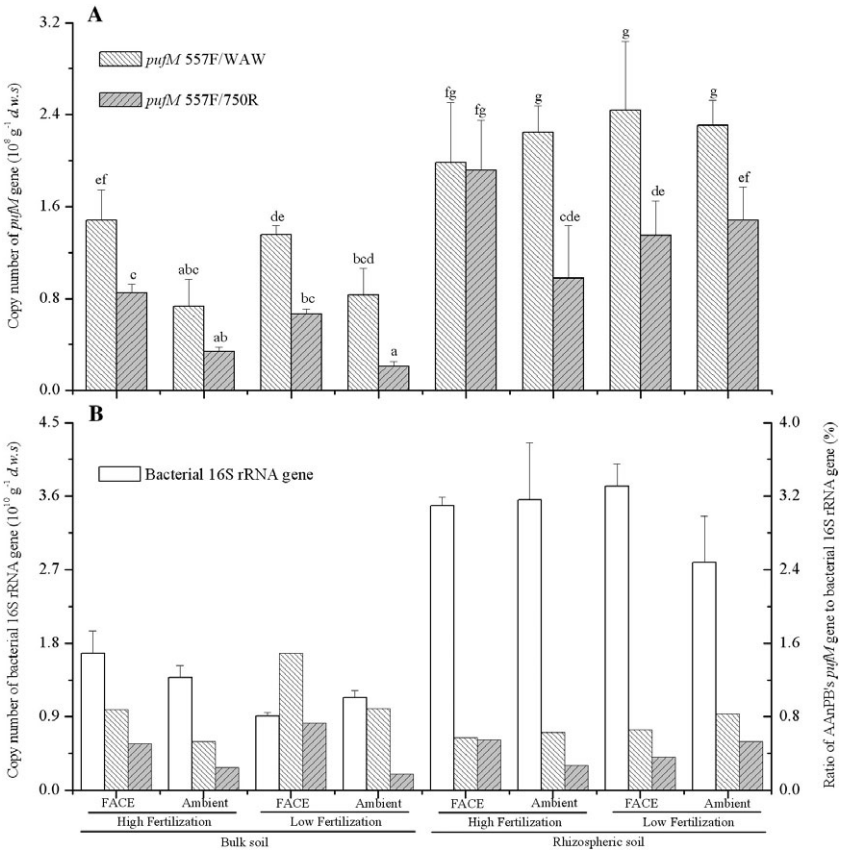

Fig. 3. The copy numbers of *pufM* gene in paddy soils with different treatments quantified by two different assays (A); The copy numbers of bacterial 16S rRNA gene and the ratios of *pufM* gene to bacterial 16S rRNA gene (B). The significant difference was indicated by the different letters above the standard error bar ($P < 0.05$).

horizontal transfer of photosynthetic gene (Nagashima *et al.*, 1997). The process of horizontal gene transfer is accelerated in particular in eutrophic environment (van Elsas and Bailey, 2002). Besides distinct genotypes, AAnPB community had large population size in paddy soil (Fig. 3A), which was generally 2 to 3 orders of magnitude higher than those detected in aquatic environments (Du *et al.*, 2006; Waidner and Kirchman, 2007). Compared with the oligotrophic condition of lake or marine ecosystem, paddy soil is much eutrophic and is presumably in support of the growth of strictly heterotrophic AAnPB (Yurkov, 2006), which is supported by the result from aquatic environments that higher AAnPB abundance is found in the region richer in nutrient (Jiao *et al.*, 2007; Waidner and Kirchman, 2008).

The abundance of AAnPB was higher in rhizospheric soil than that in bulk soil despite the subtle difference in their compositions. Flooded paddy soil can be considered a unique ecosystem characterized by oxidation–reduction status through the soil profiles, where microbial community structures might differ greatly between bulk and rhizospheric soils (Liesack *et al.*, 2000). It was thus surprising that DGGE fingerprinting analysis showed no dramatic change in the composition of *pufM* genes between bulk and rhizospheric soils (Fig. 1). The intensified agricultural practice such as plowing and tillage management could enhance the homogeneity of not only soil but also AAnPB distribution between bulk and rhizospheric soils. For instance, AAnPB from surface soil might be dispersed with depth during plowing, while AAnPB in deep soil might persist for a long time so that they could inoculate the bulk surface soil following plowing the next year. However, the copy numbers of *pufM* genes were significantly higher in rhizospheric soil than those in bulk soil, implying the stimulated growth of certain AAnPB (Fig. 3) in rhizospheric soil. The promoted growth of AAnPB in rhizospheric soil might be explained by the elevated concentrations of oxygen and labile organic carbon released from rice root. Given the highly versatile metabolic ability of AAnPB to adapt to a large variety of conditions, further studies at mRNA level probably will allow the better differentiation of active AAnPB composition in so complex environments.

The stimulated growth of AAnPB and their higher percentage in total bacterial community resulting from elevated CO_2 implied AAnPB could be ecophysiologically important to carbon cycling in paddy soil. Elevated atmospheric CO_2 stimulates the photosynthesis of plants (Ainsworth, 2008). This in turn results in more carbon input into soil by stimulated root exudates and the decomposition of increased plant biomass (Rogers *et al.*, 1994; Daepp *et al.*, 2000; Jastrow *et al.*, 2000). The studies

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reveal that elevated atmospheric CO_2 leads to the increases in SOC by 14% (Feng *et al*., 2009) and dissolved organic C (DOC) by 42% (Cheng *et al*., 2010) in paddy soil. This process might enhance the bioavailability of organic matters and final stimulate the heterotrophic growth of AAnPB in paddy soil. The increase in AAnPB abundance and the pattern of AAnPB percentages under elevated atmospheric CO_2 in bulk soil indeed are in agreement with the conclusion from aquatic ecosystems that higher nutrient results in higher AAnPB abundance and percentage (Jiao *et al*., 2007; Waidner and Kirchman, 2008). In addition, the higher percentage indicated that AAnPB could be more sensitive to the changes in soil organic matters in paddy soil than other bacteria and implied that they might play an important ecological role in carbon cycling in paddy ecosystem.

Interestingly, it was noteworthy that no similar results were obtained in rhizospheric soil. The microbial processes are more complicated in rhizospheric soil than those in bulk soil due to the interference of plant root activities (Paterson *et al*., 1997). Many factors might result in the variation in the responses of AAnPB to elevated atmospheric CO_2 between bulk and rhizospheric soils. Soil sulfide concentration was suggested to be one of influencing factors. In general, sulfate-reducing bacteria (SRB) are attached to plant roots (Nielsen *et al*., 2001). The SRB population size 4–8 times larger and sulfide concentration 4–6 times higher are detected in paddy rhizospheric soil than those in paddy bulk soil, and elevated atmospheric CO_2 enhances the population size of SRB by 250% in rhizospheric soil (Feng *et al*., 2009). Thus, despite the fact that higher amount of root exudates released into rhizospheric soil under elevated CO_2 (Paterson *et al*., 1997) could stimulate AAnPB growth when compared with ambient condition, we hypothesize that the stimulative effect might be counteracted to a greater extent by the higher sulfide concentration as well as other unknown environmental factors. In fact it seemed that AAnPB abundance in paddy soil was affected by the presence of rice root to a greater extent than elevated atmospheric CO_2.

Experimental procedures

Site description

A FACE experiment was carried out with a rice–wheat rotation system at the Nianyu Experimental Station, Jiangsu Province, China (31°35′N, 120°30′E). The soil is classified as stagnic anthrosols. There has been no substantial change of agronomic practice in the field for more than 50 years and this region is the representative of the majority of agricultural lands in Jiangsu province, a key area for rice production in China. The soil had a 9.2% concentration of sand (1–0.05 mm), a 65.7% concentration of silt (0.05–0.001 mm), a 25.1% concentration of clay (< 0.001 mm), a 1.2 g cm^{-3} bulk density, a 15.0 g kg^{-1} soil organic C (SOC), a 1.59 g kg^{-1} total N, a 1.23 g kg^{-1} total P, a 10.4 mg kg^{-1} available P and a pH of 6.8. The station sits in the subtropical climatic zone with a mean annual precipitation of 900–1000 mm, mean annual temperature 16°C, the average daily integral radiation of 12.3 MJ m^{-2}, a total sunshine time of more than 2000 h and a frost-free period of more than 230 days per year.

FACE system

The China FACE project began on 2002 rice cultivation and has been carried out for 6 years. A detailed description in the FACE system has been given before (Okada *et al*., 2001; Liu *et al*., 2002). Briefly, it consists of three octagonal rings with a target CO_2 concentration of 200 $\pm$ 40 μmol mol^{-1} above the current concentration of 355 $\pm$ 15 μmol mol^{-1} (hereinafter referred to as the 'FACE ring'), and three comparison rings without CO_2 enrichment (hereinafter referred to as the 'Ambient ring'). FACE rings are at least 90 m apart from Ambient rings. Each ring has a diameter of 12.5 m. Facing the centre of FACE rings, pure CO_2 at high pressure was released about 50 cm above the crop canopy throughout the daylight hours from tubes surrounding crops. CO_2 release was strictly controlled by a computer program with an algorithm based on the wind-speed and direction to keep the target pCO_2 level within the FACE ring. Both FACE and Ambient rings were divided into two identical plots, to which low nitrogen (LN) (150 kg N ha^{-1}) and high nitrogen (HN) (250 kg N ha^{-1}) were applied. To prevent N transportation between HN and LN plots within each ring, a polyvinyl chloride (PVC) board was inserted 25 cm below the plow layer and was extended 30 cm above the water. A 50-cm-long strip next to either side of the PVC was set as a buffer zone from which no samples were taken.

Outline of cropping systems

The rice–wheat rotation system in this study is a popular cropping system in the regions under a comparable subtropical climate in China. Wheat is sown in early November and harvested in late May or early June of the following year. Rice seeds are sown on a nursery bed in mid May and the seedlings transplanted in mid June and harvested in mid to late October. Urea and compound fertilizer are applied as N fertilizer. For rice cultivation, N fertilizer is applied three times: 36% of nitrogen is basally applied in mid June, and the remainder nitrogen is top-dressed in mid June (24%) and mid July (40%). For wheat cultivation, N fertilizer is applied also three times: 50% of nitrogen is basally applied in early November, and the remainder nitrogen is top-dressed in mid February (10%) and mid April (40%). Detailed information on rice and wheat cultivation has been given before (Xu *et al*., 2006; Zhu *et al*., 2009).

Samples collection and determination

On 23 September 2008, one of the three ambient rings and one of the three FACE rings were randomly chosen for soil sampling at rice anthesis. Soil samples from each plot treated with HN or LN were collected in triplicate. The rhizospheric

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soil was defined as soils adhering to rice root up to 2-mm-thick layer and recovered by gently washing away most soil particles around rice root. The bulk soil was collected from 0–5 cm depth surface layer, which was undisturbed by rice growth with 20–30 cm away from rice plant.

DNA extraction, PCR-DGGE, sequencing and phylogenetic analysis

For each of the triplicate soil samples 0.5 g of soil was used for DNA extraction using a FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The extracted soil DNA was dissolved in 50 μl TE buffer, quantified by spectrophotometer and stored at −20°C before use. The *pufM* gene fragment of 277 bp in length was amplified with the forward primer *pufM* 557F: CGCACCTGGACTGGAC, and reverse primer *pufM*_WAW: AYNGCRAACCACCANGCCCA as previously described in detail (Waidner and Kirchman, 2008).

A DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA) was used for DGGE analysis. Approximately 150–250 ng PCR amplicons of *pufM* genes from each soil sample were electrophoresed on a 10% acrylamide–bisacrylamide gel containing a denaturant gradient from 45% to 75% at 130 V for 8 h in 1× TAE running buffer at 60°C (Feng *et al*., 2009). The gels were visualized by using a Gel Doc EQ imager combined with Quantity one 4.4.0 (Bio-Rad) and the band intensity was digitalized. The genetic diversity of soil microbial communities was analysed by Shannon indices (*H*) method according to the equation of Zak and colleagues (1994).

Dominant DGGE bands were excised and eluted overnight in sterilized distilled water at 4°C. The eluted DNA was used as a template to re-amplify the *pufM* gene as described above for the mobility check, then cloned into a pMD18-T vector (TaKaRa) and transformed into *Escherichia coli* DH5α. Three random clones containing the correct gene size for each DGGE band were sequenced by the Invitrogen Sequencing Department in Shanghai. DNASTAR software package was used to manually check and compare the clone sequences. One representative clone sequence with high quality after sequence comparison from each band was used for phylogenetic analysis.

Together with the top BLAST hit, the second and third highest BLAST hits of homologous gene sequences, the *pufM* gene sequences were used to build a basic phylogenetic tree by the neighbour-joining method using the software package of MEGA 4.0 version (Molecular Evolutionary Genetics Analysis) (Tamura *et al*., 2007). The tree topology was further evaluated by different methods including minimum evolution and maximum parsimony. The phylogenetic relationship of *pufM* gene sequences to the closest homologue in the GenBank was then inferred. The sequences generated in this study have been deposited in the DDBJ database under accession numbers AB510450 to AB510467.

Real-time quantitative PCR of pufM *and 16S rRNA genes in paddy soil*

The copy numbers of soil *pufM* gene from AAnPB and bacterial 16S rRNA gene in paddy soil were quantified by real-time quantitative PCR analysis with an Opticon 2 continuous fluorescence detection system (MJ Research) by the assays of the *pufM* 557F/WAW and bacterial 519F/907R (Biddle *et al*., 2008). The reliability of soil *pufM* genes quantification was further assessed by the *pufM* 557F/750R as described previously (Waidner and Kirchman, 2008). The detailed protocol for qPCR was shown below. To generate real-time PCR standard curve, a single clone containing the correct insert was grown in Luria–Bertani medium and then plasmid DNA was extracted, purified and quantified. A 10-fold dilution series of the plasmid DNA was made to generate a standard curve covering seven orders of magnitude from 10^2 to 10^8 copies of template per assay. Assays were set up using the SYBR *Premix Ex Taq* Kit (TaKaRa). The 25 μl reaction mixture contained 12.5 μl of SYBR *Premix Ex Taq*, 0.5 μM of each primer, 200 ng BSA μl^{-1}, 1.0 μl template containing approximately 2–9 ng DNA. A negative control was always run with water as template instead of soil DNA extract. Specific amplification were confirmed by agarose gel electrophoresis of real-time PCR amplicons showing an expected band of PCR amplicons and melting curve analysis always resulting in a single peak. Real-time PCR was performed in triplicate and amplification efficiencies of 97.4–104% were obtained with R^2 values of 0.966–0.977. Based on the standard curve plotted by using the known gene copy number against the cycle threshold (C_T), the copy numbers of *pufM* and bacterial 16S rRNA genes were calculated by extrapolating its C_T value at which fluorescence emission crosses a threshold within the logarithmic increase of target genes. The threshold was defined as 10 times the standard deviation around the average intensity of background fluorescence. The differences in copy number of *pufM* and bacterial 16S rRNA genes between treatments were statistically evaluated using one-way analysis of variance under significant differences $P < 0.05$ using SPSS 13.0 statistical package.

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References

Ainsworth, E.A. (2008) Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. *Glob Change Biol* **14:** 1642–1650.

Bauer, C.E., and Bird, T.H. (1996) Regulatory circuits controlling photosynthesis gene expression. *Cell* **85:** 5–8.

Beatty, J.T. (2002) On the natural selection and evolution of the aerobic phototrophic bacteria. *Photosynth Res* **73:** 109–114.

Biddle, J.F., Fitz-Gibbon, S., Schuster, S.C., Brenchley, J.E., and House, C.H. (2008) Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proc Natl Acad Sci USA* **105:** 10583–10588.

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Cheng, L., Zhu, J., Chen, G., Zheng, X., Oh, N.H., Rufty, T.W., *et al*. (2010) Atmospheric CO_2 enrichment facilitates cation release from soil. *Ecol Lett* **13:** 284–291.

Daepp, M., Suter, D., Almeida, J.P.F., Isopp, H., Hartwig, U.A., Frehner, M., *et al*. (2000) Yield responses of *Lolium perenne* swards to free-air CO_2 enrichment increased over six years in a high N input system on fertile soil. *Glob Change Biol* **6:** 805–6816.

Du, H., Jiao, N., Hu, Y., and Zeng, Y. (2006) Real-time PCR for quantification of aerobic anoxygenic phototrophic bacteria based on *pufM* gene in marine environment. *J Exp Mar Biol Ecol* **329:** 113–121.

van Elsas, J.D., and Bailey, M.J. (2002) The ecology of transfer of mobile genetic elements. *FEMS Microbiol Ecol* **42:** 187–197.

Evans, W.R., Fleischman, D.E., Calvert, H.E., Pyati, P.V., Alter, G.M., and Rao, N.S.S. (1990) Bacteriochlorophyll and photosynthetic reaction centers in *Rhizobium* strain BTAi 1. *Appl Environ Microbiol* **56:** 3445–3449.

Feng, Y., Lin, X., Wang, Y., Zhang, J., Mao, T., Yin, R., and Zhu, J. (2009) Free-air CO_2 enrichment (FACE) enhances the biodiversity of purple phototrophic bacteria in flooded paddy soil. *Plant Soil* **324:** 317–328.

Fujita, Y., and Nakahara, H. (2006) Variations in the microalgal structure in paddy soil in Osaka, Japan: comparison between surface and subsurface soils. *Limnology* **7:** 83–91.

Hu, Y., Du, H., Jiao, N., and Zeng, Y. (2006) Abundant presence of the γ-like proteobacterial *pufM* gene in oxic seawater. *FEMS Microbiol Lett* **263:** 200–206.

Jastrow, J.D., Miller, R.M., and Owensby, C.E. (2000) Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformations to soil organic matter in grass land. *Plant Soil* **224:** 85–97.

Jiao, N., Zhang, Y., Zeng, Y., Hong, N., Liu, R., Chen, F., and Wang, P. (2007) Distinct distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic bacteria in the global ocean. *Environ Microbiol* **9:** 3091–3099.

Kolber, Z.S., Van Dover, C.L., Niederman, R.A., and Falkowski, P.G. (2000) Bacterial photosynthesis in surface waters of the open ocean. *Nature* **407:** 177–179.

Kolber, Z.S., Plumley, F.G., Lang, A.S., Beatty, J.T., Blankenship, R.E., Vandover, C.L., *et al*. (2001) Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. *Science* **292:** 2492–2495.

Liesack, W., Schnell, S., and Revsbech, N.P. (2000) Microbiology of flooded rice paddies. *FEMS Microbiol Rev* **24:** 625–645.

Liu, G., Han, Y., Zhu, J.G., Okada, M., Nakamura, H., and Yoshimoto, M. (2002) Rice-wheat rotational FACE platform I. System structure and control. *Chin J Appl Ecol* **13:** 1253–1258.

Nagashima, K.V.P., Hiraishi, A., Shimada, K., and Matsuura, K. (1997) Horizontal transfer of genes coding for the photosynthetic reaction centers of purple bacteria. *J Mol Evol* **45:** 131–136.

Nielsen, L.B., Finster, K., Welsh, D.T., Donelly, A., Herbert, R.A., de Wit, R., and Lomstein, B.A. (2001) Sulphate reduction and nitrogen fixation rates associated with roots, rhizomes and sediments from *Zostera noltii* and *Spartina maritima* meadows. *Environ Microbiol* **3:** 63–71.

Nishimura, K., Shimada, H., Ohta, H., Masuda, T., Shioi, Y., and Takamiya, K.-I. (1996) Expression of the puf operon in an aerobic photosynthetic bacterium, *Roseobacter denitrificans*. *Plant Cell Physiol* **37:** 153–159.

Okada, M., Lieffering, M., Nakamura, H., Yoshimoto, M., Kim, H.Y., and Kobayashi, K. (2001) Free-air CO_2 enrichment (FACE) using pure CO_2 injection: system description. *New Phytol* **150:** 251–260.

Paterson, E., Hall, J.M., Rattray, E.A.S., Griffiths, B.S., Ritz, K., and Killham, K. (1997) Effect of elevated CO_2 on rhizosphere carbon flow and soil microbial processes. *Glob Change Biol* **3:** 363–377.

Pfennig, N. (1967) Photosynthetic bacteria. *Annu Rev Microbiol* **21:** 285–324.

Pittl, E., Innerebner, G., Wanek, W., and Insam, H. (2010) Microbial communities of arboreal and ground soils in the Esquinas rainforest, Costa Rica. *Plant Soil* **329:** 65–74.

Roger, P.A. (1996) *Biology and Management of the Floodwater Ecosystem in Ricefields*. Los Baños, The Philippines: International Rice Research Institute.

Rogers, H., Runion, G., and Krupa, S. (1994) Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environ Pollut* **83:** 155–189.

Sadowsky, M.J., and Schortemeyer, M. (1997) Soil microbial responses to increased concentrations of atmospheric CO_2. *Glob Change Biol* **3:** 217–224.

Shimada, K. (1995) *Aerobic Anoxygenic Phototrophs*. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Song, T.Y., Martensson, L., Eriksson, T., Zheng, W.W., and Rasmussen, U. (2005) Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. *FEMS Microbiol Ecol* **54:** 131–140.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24:** 1596–1599.

Urakami, T., and Komaga, K. (1984) *Protornonas*, a new genus of facultatively methylotrophic bacteria. *Int J Syst Bacteriol* **34:** 188–201.

Waidner, L.A., and Kirchman, D.L. (2007) Aerobic anoxygenic phototrophic bacteria attached to particles in turbid waters of the Delaware and Chesapeake estuaries. *Appl Environ Microbiol* **73:** 3936–3944.

Waidner, L.A., and Kirchman, D.L. (2008) Diversity and distribution of ecotypes of the aerobic anoxygenic phototrophy gene *pufM* in the delaware estuary. *Appl Environ Microbiol* **74:** 4012–4021.

Xiong, J., Fischer, W.M., Inoue, K., Nakahara, M., and Bauer, C.E. (2000) Molecular evidence for the early evolution of photosynthesis. *Science* **289:** 1724–1730.

Xu, Z.J., Zheng, X.H., Wang, Y.S., Wang, Y.L., Huang, Y., and Zhu, J. (2006) Effect of free-air atmospheric CO_2 enrichment on dark respiration of rice plants (*Oryza sativa L.*). *Agric Ecosyst Environ* **115:** 105–112.

Yurkov, V.V. (2006) Aerobic phototrophic proteobacteria. In *The Prokaryotes*, Vol. 5. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). New York, NY, USA: Springer, pp. 562–584.

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Yurkov, V.V., and Beatty, J.T. (1998) Aerobic anoxygenic phototrophic bacteria. *Microbiol Mol Biol Rev* **62:** 695–724.
Zak, J.C., Willig, M.R., Moorhead, D.L., and Wildman, H.G. (1994) Functional diversity of microbial communities: a quantitative approach. *Soil Biol Biochem* **26:** 1101–1108.
Zhu, C.W., Zhu, J.G., Zeng, Q., Liu, G., Xie, Z.B., Tang, H.Y., *et al*. (2009) Elevated CO2 accelerates flag leaf senescence in wheat due to ear photosynthesis which causes greater ear nitrogen sink capacity and ear carbon sink limitation. *Funct Plant Biol* **36:** 291–299.

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