

# Contribution of periphytic biofilm of paddy soils to carbon dioxide fixation and methane emissions

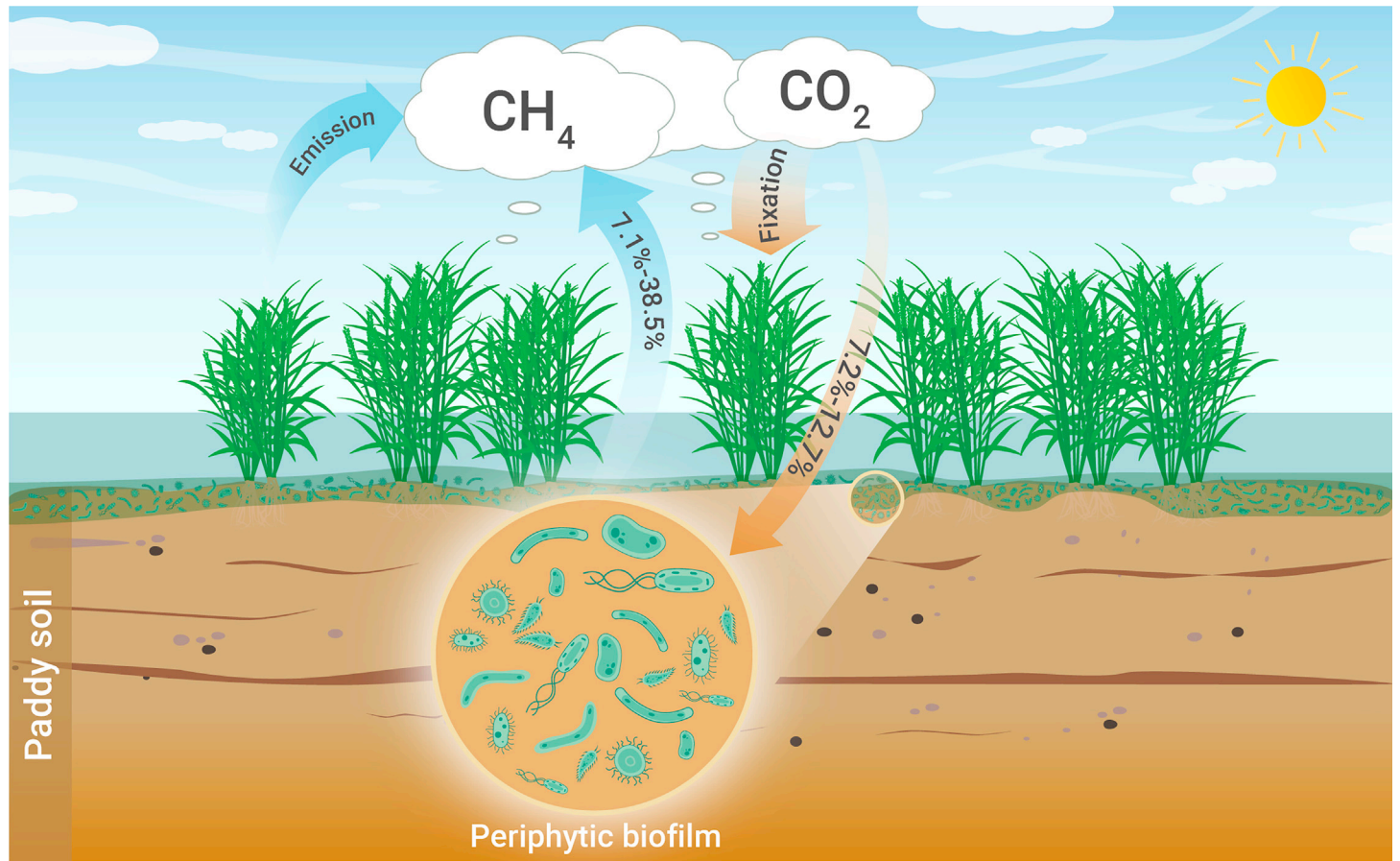
Sichu Wang,<sup>1,2,3</sup> Pengfei Sun,<sup>1,2</sup> Guangbin Zhang,<sup>1</sup> Neil Gray,<sup>4</sup> Jan Dolfing,<sup>5</sup> Sofia Esquivel-Elizondo,<sup>6</sup> Josep Peñuelas,<sup>7</sup> and Yonghong Wu<sup>1,2,\*</sup>

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## Graphical abstract



## Public summary

- Field experiments were conducted in tropical, subtropical, and temperate rice paddies
- Periphytic biofilm promoted CO<sub>2</sub> fixation and CH<sub>4</sub> emission
- Periphytic biofilm acted as a biotic converter of atmospheric CO<sub>2</sub> to CH<sub>4</sub>



# Contribution of periphytic biofilm of paddy soils to carbon dioxide fixation and methane emissions

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Rice paddies are major contributors to anthropogenic greenhouse gas emissions via methane (CH<sub>4</sub>) flux. The accurate quantification of CH<sub>4</sub> emissions from rice paddies remains problematic, in part due to uncertainties and omissions in the contribution of microbial aggregates on the soil surface to carbon fluxes. Herein, we comprehensively evaluated the contribution of one form of microbial aggregates, periphytic biofilm (PB), to carbon dioxide (CO<sub>2</sub>) and CH<sub>4</sub> emissions from paddies distributed across three climatic zones, and quantified the pathways that drive net CH<sub>4</sub> production as well as CO<sub>2</sub> fixation. We found that PB accounted for 7.1%–38.5% of CH<sub>4</sub> emissions and 7.2%–12.7% of CO<sub>2</sub> fixation in the rice paddies. During their growth phase, PB fixed CO<sub>2</sub> and increased the redox potential, which promoted aerobic CH<sub>4</sub> oxidation. During the decay phase, PB degradation reduced redox potential and increased soil organic carbon availability, which promoted methanogenic microbial community growth and metabolism and increased CH<sub>4</sub> emissions. Overall, PB acted as a biotic converter of atmospheric CO<sub>2</sub> to CH<sub>4</sub>, and aggravated carbon emissions by up to 2,318 kg CO<sub>2</sub> equiv ha<sup>-1</sup> season<sup>-1</sup>. Our results provide proof-of-concept evidence for the discrimination of the contributions of surface microbial aggregates (i.e., PB) from soil microbes, and a profound foundation for the estimation and simulation of carbon fluxes in a potential novel approach to the mitigation of CH<sub>4</sub> emissions by manipulating PB growth.

## INTRODUCTION

Rice paddies are globally important sinks for carbon dioxide (CO<sub>2</sub>) and sources of methane (CH<sub>4</sub>), with high levels of carbon exchange at the soil-atmosphere interface.<sup>1,2</sup> Annual emissions of CH<sub>4</sub> from rice paddies are estimated to be 20–100 Tg and represent about 5%–20% of annual global anthropogenic emissions.<sup>3,4</sup> However, uncertainties in estimates of CO<sub>2</sub> fixation and CH<sub>4</sub> emission from rice paddies still exist.<sup>5</sup> One reason is the underestimation of the contribution of soil-surface microbial aggregates to these carbon fluxes. For CH<sub>4</sub> emission estimations and simulations, it is generally assumed that methanogenic substrates are derived only from soil microbes, rice plants, and farmyard manure.<sup>6–8</sup> Organic carbon derived from microbial aggregates on the soil surface has been poorly considered. Furthermore, the biogeochemical function of microbial aggregates on the soil surface has been neglected due to the challenges of distinguishing their contribution from those of the soil microbes.<sup>9</sup>

Periphytic biofilm (PB) is a ubiquitous microbial aggregate that develops at the soil or sediment surface in aquatic ecosystems (Figure S1). These biofilms comprise a dominant biophase (bacteria, algae, fungi, protozoa, and metazoa), along with a minor abiotic phase composed of extracellular polymeric substances, minerals (iron, Fe; aluminum; and calcium), and nutrients (nitrogen, phosphorus).<sup>10,11</sup> In aquatic ecosystems, PB accounts for 7%–97% of the total primary productivity,<sup>12,13</sup> and its biomass in rice paddies may reach 50–620 kg ha<sup>-1</sup>.<sup>14</sup> Algal biomass in decaying PB is more readily fermented than plant biomass<sup>15</sup> and, as a result, PB-derived carbon influences the composition of soil organic carbon (SOC) and provides varied substrates for soil respiration and methanogenesis.

In addition to affecting CO<sub>2</sub> assimilation and substrate supply, PB inevitably also affects topsoil and floodwater properties directly associated with CH<sub>4</sub> production and oxidation. During the colonization and growth phase of the PB, auto-

trophs, such as microalgae, dominate the microbial aggregates.<sup>16</sup> Oxygen (O<sub>2</sub>) released during algal photosynthesis may diffuse into the soil,<sup>17</sup> leading to inhibition of CH<sub>4</sub> production<sup>18</sup> and facilitating oxidation of CH<sub>4</sub> at the interface of soil and water, preventing CH<sub>4</sub> fluxes into floodwater and from there to the atmosphere. During the decay phase of PB, heterotrophs replace autotrophs as the dominant community members.<sup>16,19</sup> Heterotrophic respiration and the release of labile carbon may lead to greater consumption of O<sub>2</sub><sup>20</sup> resulting in a reductive environment and higher levels of CH<sub>4</sub> production. In addition, unlike microalgae in floodwater, the multispecies aggregates (PB) release extracellular polymeric substances to form a barrier by clogging the pore space at soil-water interface,<sup>21</sup> limiting carbon transfer to the atmosphere and promoting its reaction with minerals and competing ions in the matrix. To the best of our knowledge, however, none of these hypotheses have been experimentally verified, and the net contributions of PB to carbon fluxes have not been properly evaluated.

While a limited number of studies have investigated the effects of periphyton or phytoplankton on carbon fluxes in aquatic ecosystems,<sup>15,22–24</sup> most have tended to focus on a single species of algae in rivers and lakes. Rice paddies are much shallower and have a lower flow velocity than rivers and lakes, with contrasting transport of gases from sediment to floodwater.<sup>23</sup> Compared with single microbial species, PB possesses a more complex microbial composition and structure.<sup>25</sup> This is important as successional processes and diversity of metabolisms among species dynamically influence soil and water properties, with associated impacts on carbon fluxes.

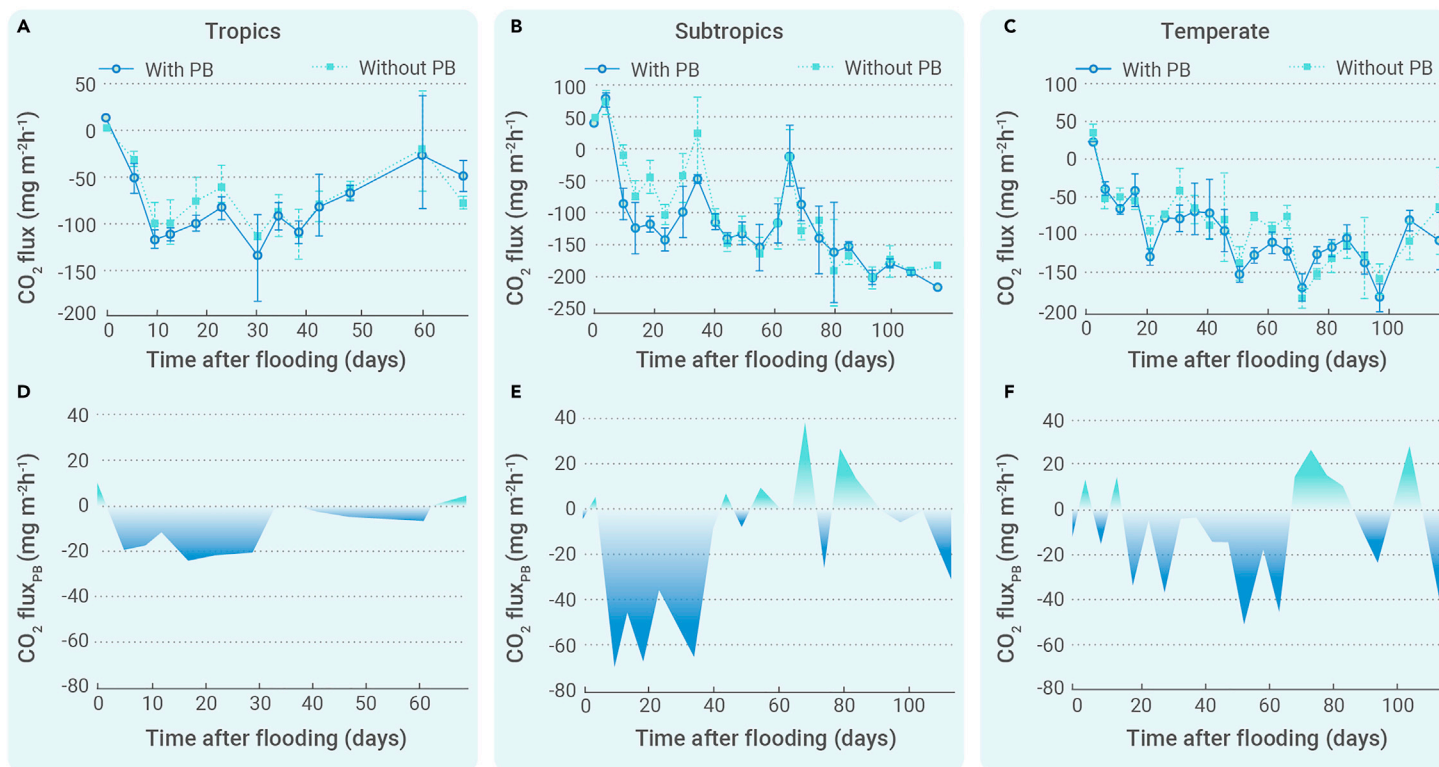
In this study, we quantified the contribution of PB to CO<sub>2</sub> fixation and CH<sub>4</sub> emission in rice paddies distributed across three climatic zones. We also used incubation and microcosm experiments to explore the underlying mechanisms of PB effects on CH<sub>4</sub> production and aerobic oxidation. The composition and metabolisms of soil methanogenic and methanotrophic communities in the presence of PB were measured using qPCR, high-throughput sequencing, and metabolomics analyses. Our results provide proof-of-concept evidence for the quantization of the contribution of surface microbial aggregates (i.e., PB) to CO<sub>2</sub> fixation and CH<sub>4</sub> emissions and a solid foundation for potential novel approaches to CH<sub>4</sub> emission mitigation by manipulating PB growth.

## RESULTS

### CO<sub>2</sub> and CH<sub>4</sub> fluxes in fields

CO<sub>2</sub> and CH<sub>4</sub> fluxes from rice paddies with and without PB were monitored *in situ* during the whole rice growing season. CO<sub>2</sub> fluxes decreased with time in the first 10 days and were usually negative from the fifth day after flooding onward (Figures 1A–1C). Totals of 626, 1,680, and 1,655 kg ha<sup>-1</sup> CO<sub>2</sub> were fixed in the tropical, subtropical, and temperate experimental paddy fields, respectively, in the presence of PB during the rice season daylight periods. The presence of PB promoted CO<sub>2</sub> uptake (Figures 1D–1F): fixation of CO<sub>2</sub> by the PB was at 79, 211, and 118 kg ha<sup>-1</sup> in the tropical, subtropical, and temperate paddies, representing 12.7%, 12.5%, and 7.2% of the total CO<sub>2</sub> fixation. PB tended to fix CO<sub>2</sub> during the early half of the rice season.

CH<sub>4</sub> fluxes increased after flooding and transiently declined to ~0 mg m<sup>-2</sup> h<sup>-1</sup> during the mid-season aeration period in the subtropical and temperate paddy fields and essentially stopped after the 80<sup>th</sup> day, when the soil was dried for rice harvest (Figures 2A–2C). The cumulative CH<sub>4</sub> emissions from the tropical,



**Figure 1. CO<sub>2</sub> flux in the experimental paddy fields** CO<sub>2</sub> flux in the (A) tropical, (B) subtropical, and (C) temperate experimental paddy fields during a rice growth season with and without periphytic biofilm (PB), and the fluxes induced by PB (D, tropical; E, subtropical; and F, temperate). In (A–C), the error bars indicate the standard deviations (SD) of triplicate results. In (D–F), positive values indicate that gas fluxes in the presence of PB were greater than those in the control (absence of PB), and negative values indicate the reverse.

subtropical, and temperate experimental paddy fields with PB were 68.2, 262.4, and 41.2 kg ha<sup>-1</sup>, respectively. The presence of PB increased CH<sub>4</sub> emission (Figures 2D–2F): the total CH<sub>4</sub> emission induced by PB amounted to 4.8, 101, and 4.3 kg ha<sup>-1</sup> over a single rice growing season across tropical, subtropical, and temperate rice paddies. These represented 7.1%, 38.5%, and 10.4% of the total CH<sub>4</sub> emission of the experimental paddies. During mainly the later half of the rice season, fluxes of CH<sub>4</sub> were lower in the presence of PB than in its absence.

#### CH<sub>4</sub> production and oxidation by PB

To test whether PB itself produces CH<sub>4</sub>, we incubated PB in serum bottles and measured CH<sub>4</sub> concentrations in the headspace over time (Figure 3A). We found that under anaerobic conditions CH<sub>4</sub> emission was not detected in the presence of PB, even with an additional supply of acetate, which is a methanogenic substrate (CH<sub>3</sub>COOH → CH<sub>4</sub> + CO<sub>2</sub>),<sup>26</sup> in contrast, CH<sub>4</sub> emission was observed from the anaerobic soil, and the presence of PB increased CH<sub>4</sub> flux in the initial 2 days.

To test whether PB is capable of CH<sub>4</sub> oxidation, we incubated different amounts of PB in Woods Hole medium with a high initial concentration of CH<sub>4</sub> (~10,000 ppm/6,530 μg L<sup>-1</sup>). The CH<sub>4</sub> was consumed over time in all bottles (Figure 3B), including in the control without PB. Nevertheless, by day 4, the depletion of CH<sub>4</sub> in the incubations that had received the largest amounts of PB amendment was significantly greater ( $p < 0.05$ ) than in the absence of PB. Consistent with this evidence of biologically mediated CH<sub>4</sub> removal, in separate experiments we detected higher concentrations of dissolved O<sub>2</sub> in floodwater in the presence of PB (Figure S2).

#### Soil organic carbon

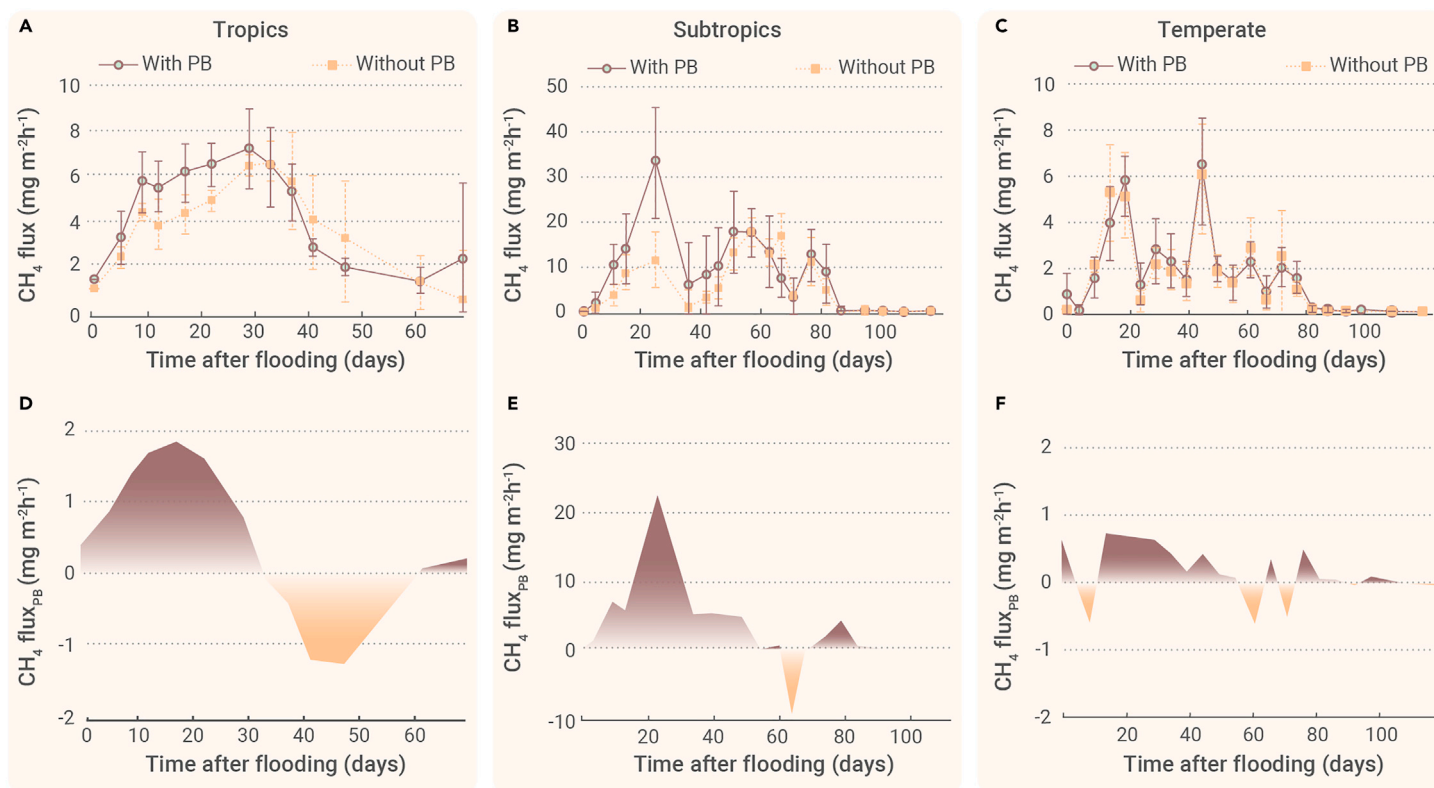
Flooded soil was the main CH<sub>4</sub> source in rice paddy. To evaluate the effect of PB on soil properties, soil columns were incubated for 40 days in microcosms under flooding (Figure S3A). The PB grew rapidly in the first 10 days (growth phase), reached a maximum biomass, and then began autogenic sloughing between day 10 and day 20, and then autogenic sloughing became the dominant process (decay phase) from day 20 onward,<sup>10</sup> as indicated by the F<sub>0</sub> in Figure S4A and observed in Figure S5.

The presence of PB increased the concentration and changed the composition of the SOC. Effects were most pronounced in the 0–1 cm soil layer, with a significant increase in total organic carbon (TOC) concentrations, from 21.5 ± 1.8 to 24.6 ± 0.9 g kg<sup>-1</sup> (Figure 4A). Dissolved organic carbon (DOC) concentrations in the soil pore water in the presence of PB gradually increased after flooding, from 66.2 ± 8.1 to 81.9 ± 5.6 mg L<sup>-1</sup>, while DOC concentrations in soil without PB were significantly lower, fluctuating between 48.6 ± 5.9 and 58.8 ± 4.6 mg L<sup>-1</sup> (Figure 4B). In the presence of PB, concentrations of 491 different soil organic molecules increased (Figure 4C), with lipids and organic acids accounting for 45% and 13% of those increased organic molecules, respectively (Figure S6A), while the concentrations of 214 soil organic molecules, mostly heterocyclic compounds (Figure S6B), reduced.

To test whether the increased SOC derives from the CO<sub>2</sub> fixed by PB, we labeled CO<sub>2</sub> in the incubation chamber containing soil columns with and without PB (Figure S3B). The <sup>13</sup>C<sub>2</sub> (enrichment >98 atom%) was injected into the sealed chamber, then the <sup>13</sup>C enrichment of PB and surface soil was determined after incubation. The atomic proportion of <sup>13</sup>C/<sup>12</sup>C in the PB reached 2.41% ± 0.002% (Figure 4D), while the soil TOC was more <sup>13</sup>C enriched in the presence of PB (1.10% ± 0.01%) than when PB was absent (1.08% ± 0.004%). These results suggest that PB fixed CO<sub>2</sub> from the atmosphere and released the carbon to the surface soil.

#### Redox potentials

Redox potentials (E<sub>h</sub>s) of surface soil and floodwater were profiled during the growth phase (day 10 after flooding) and the decay phase (day 40 after flooding) of PB. At day 10, E<sub>h</sub>s of the floodwater and at the 1.5-cm soil depth were similar, but the E<sub>h</sub> decreased sharply between the depths of 1.5 and 3.5 cm (Figure 5A). The presence of PB significantly ( $p < 0.01$ ) increased the E<sub>h</sub> of floodwater and surface soil (–1.5 to 1.5 cm depth) from 237 to 257 mV. At depths >3.5 cm, the E<sub>h</sub> in soil declined to –30 mV, both with and without PB. At day 40 after flooding there was no change in E<sub>h</sub> of the floodwater and surface soil (0–1.5 cm layer) where PB was absent, and in soil >3.5 cm deep, the E<sub>h</sub> decreased to –158 mV (Figure 5B). In the presence of PB, there was a steeper decrease in E<sub>h</sub> of the surface soil, from 248 mV at the water surface to –200 mV in soil >3.5 cm deep. Regardless of the presence of low-E<sub>h</sub> microsites likely present throughout the



**Figure 2. CH<sub>4</sub> flux in the experimental paddy fields** CH<sub>4</sub> flux in the (A) tropical, (B) subtropical, and (C) temperate experimental paddy fields during a rice growth season with and without PB, and the fluxes induced by PB (D, tropical; E, subtropical; and F, temperate). In (A–C), the error bars indicate the SD of triplicate results. In (D–F), positive values indicate that gas fluxes in the presence of PB were greater than in the control (absence of PB), and negative values indicate the reverse.

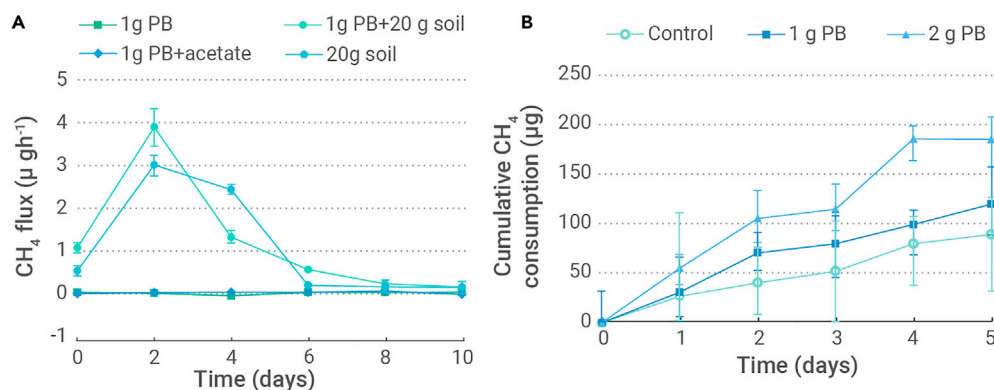
soil column, this low Eh in bulk soil below 3.5 cm is consistent with the potential onset of methanogenesis.<sup>27</sup>

### Microbial composition and metabolism

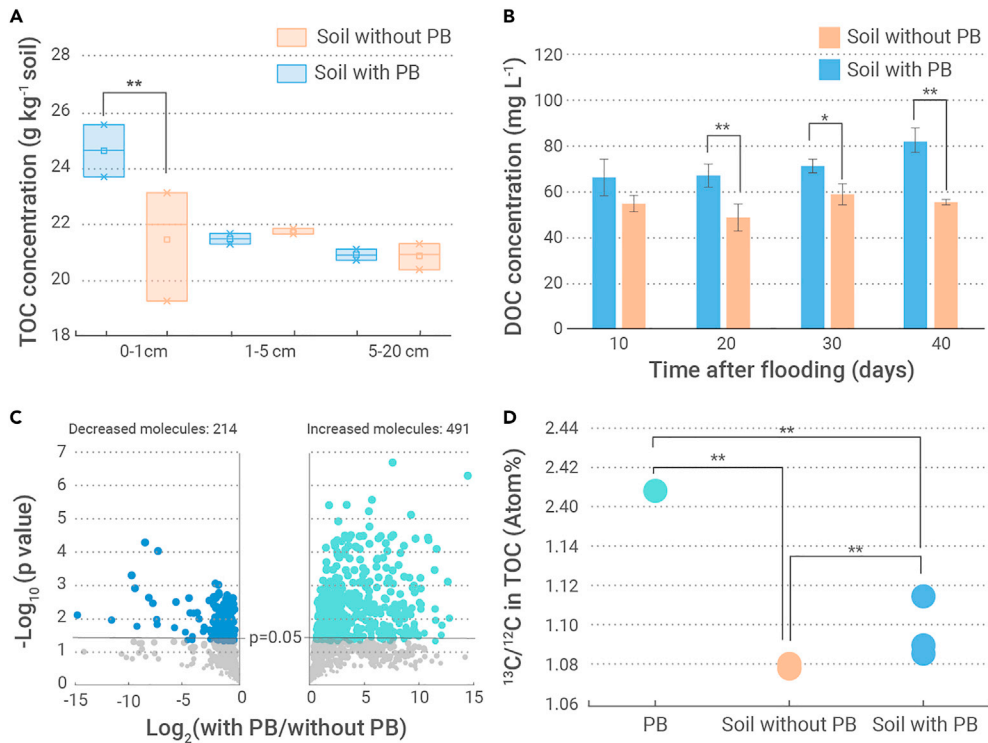
The size and composition of the communities of methanogens and aerobic methanotrophs in the soil microcosms were influenced by the presence of PB. Regardless of the presence or absence of PB, the *mcrA* and *pmoA* gene abundances (indicators of methanogen and methanotroph, respectively) both appeared to be higher in the surface (0–1 cm) soil layer than the abundances at deep soil layers (5–20 cm) (Figures 6A and 6B). This pattern implies that CH<sub>4</sub> production and its aerobic oxidation were generally more prevalent in the surface soil. However, the presence of PB enhanced the size of the methanogen community in the surface soil ( $p < 0.05$ ), which increased from  $(6.25 \pm 0.68) \times 10^8$  to  $(8.97 \pm 0.78) \times 10^8$  gene sequences  $g^{-1}$  soil (Figure 6A). In contrast, *pmoA* gene abundances were higher ( $p < 0.05$ ) in soil without PB (Figure 6B), indicating that the proliferation of aerobic methanotrophs was inhibited in the presence of PB. The community size of methanogens and aerobic meth-

anotrophs showed no significant differences ( $p > 0.05$ ) between microcosms with or without PB.

Based on *mcrA* and *pmoA* gene sequence analyses, there were similar methanogen and methanotroph genera found in the soils regardless of PB presence or absence, but the relative abundances in the relevant sequence libraries significantly differed ( $p < 0.05$ ). More specifically, the presence of PB tended to increase the relative abundance of *Methanosarcina* in both the surface and the deep soil, and led to decreases in *Methanobacterium* in surface soil (0–1 cm layer) and *Methanocorpusculum* in the deep soil (5–20 cm layer) (Figure 6C). For the methanotrophs, the presence of PB increased the relative abundance of *Methylocaldum* from 13.7% to 77.7% in the surface soil. In the deep soil, PB increased the relative abundance of *Methylocystis*, but decreased that of *Methylomonas* (Figure 6D). The methanogen and methanotroph genera found in PB were consistent with those in soil. *Methanobacterium* and *Methylocystis* were the most abundant methanogen and methanotroph genera, respectively, in PB, but were in proportions of only 3.8%–12.2% and 3.8%–23.8% in soil.



**Figure 3. Methanogenesis and CH<sub>4</sub> oxidation capacity of PB** (A) Methane emissions by PB and soil under anaerobic incubation. (B) Consumption of CH<sub>4</sub> in the headspace of the bottles with different amounts of PB. Error bars are SD of three replicates.



**Figure 4. Effects of PB on SOC in microcosms** (A) Concentration of TOC in different soil layers after incubation. (B) Temporal changes in concentration of DOC in soil pore water after flooding. (C) Changes in soil concentrations of organic molecules in the presence of PB. The horizontal axis represents the logarithm of the fold changes in the concentration of organic molecules calculated as average concentration in the PB treatment/average concentration in control. Light and dark blue circles indicate increases and decreases in concentrations of organic molecules, respectively, and gray circles indicate organic molecules with no change in concentration ( $p > 0.05$ ). (D) Proportion of  $^{13}\text{C}/^{12}\text{C}$  within PB biomass (light blue circles), soil when PB was absent (orange circles), and soil in the presence of PB (dark blue circles) after pulse labeling by  $^{13}\text{CO}_2$ . \*,  $p < 0.05$  and \*\*,  $p < 0.01$ .

Fixation of  $\text{CO}_2$  was accompanied by release of  $\text{O}_2$ , and might promote  $\text{CH}_4$  oxidation. Unexpectedly, PB promoted  $\text{CH}_4$  emission from soil (Figures 2 and 3A), possibly due to the effects of PB on the soil properties and soil microbial composition directly associated with  $\text{CH}_4$  production, e.g., availability of methanogenic substrates, soil Eh, and activity of methanogens.<sup>22,27,33-35</sup>

The main methanogenic pathways in paddy soil are acetoclastic methanogenesis ( $\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$ ) and hydrogenotrophic methanogenesis ( $4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ ).<sup>28</sup> We analyzed key metabolites involved in methanogenesis via metabolomics (Figure S7 and Table S1) and found that concentrations of coenzyme  $\text{F}_{420}$ , which is a key electron transporter involved in two steps of hydrogenotrophic methanogenesis from methenyltetrahydromethanopterin to methyltetrahydromethanopterin;<sup>29</sup>  $\text{F}_{420}\text{H}_2$ ; and their precursor, the coenzyme  $\text{F}_{420}\text{-1}$ , were upregulated in the presence of PB, leading to an acceleration of methanogenesis via the hydrogenotrophic pathway. In addition, the upregulation of the metabolites acetyl-coenzyme A, malonyl-coenzyme A, and 3-hydroxypropionyl-coenzyme A in the 3-hydroxypropionate (3HP) bicycle metabolic pathway<sup>30</sup> also suggested a more active carbon fixation in the PB group (consistent with Figure 3).

## DISCUSSION

### Indigenous capacity of periphytic biofilm to produce and oxidize $\text{CH}_4$

The incubation experiments indicated that PB has little indigenous capacity to produce  $\text{CH}_4$ , but may promote aerobic  $\text{CH}_4$  oxidation. However, based on the sequence analyses, both *mcrA* and *pmoA* genes were present in PB (Figure S8), indicating that methanogens and methanotrophs coexist in PB. As methanogens are strict anaerobes,<sup>18</sup> methanogenesis was suppressed by photosynthetic microalgae due to  $\text{O}_2$  release in the PB environment. Therefore, PB worked as a  $\text{CH}_4$  sink through both the production of  $\text{O}_2$  and the provision of a habitat for aerobic methanotrophs.

### The underlying reasons that PB promoted $\text{CH}_4$ emission and $\text{CO}_2$ fixation

The promotion of  $\text{CO}_2$  fixation by PB may be attributed to autotrophs present in PB. Following fertilizer application and flooding, PB colonizes rapidly on the soil surface due to favorable light and nutrient conditions.<sup>19</sup> Microalgae and other autotrophs in PB fix  $\text{CO}_2$  from the atmosphere during photosynthesis,<sup>16,17</sup> the temporal fluctuation in  $\text{CO}_2$  flux was consistent with variations in daily weather patterns (temperature and illumination).<sup>31</sup> After the early stages, PB decayed as the canopy cover of the developing rice increased, as a result of reduced light and nutrient availability.<sup>19</sup> The light dependence indicated that microbial  $\text{CO}_2$  fixation in rice paddies is phototrophic rather than chemotrophic.<sup>32</sup> Microbial  $\text{CO}_2$  fixation plays a significant role in the terrestrial carbon sink. The rate of microbial phototrophic  $\text{CO}_2$  fixation was significantly higher in rice paddies than in upland soils,<sup>32</sup> which might be attributed to the  $\text{CO}_2$  fixation of algae in PB (7.2%–12.7% of the total  $\text{CO}_2$  sink in this study).

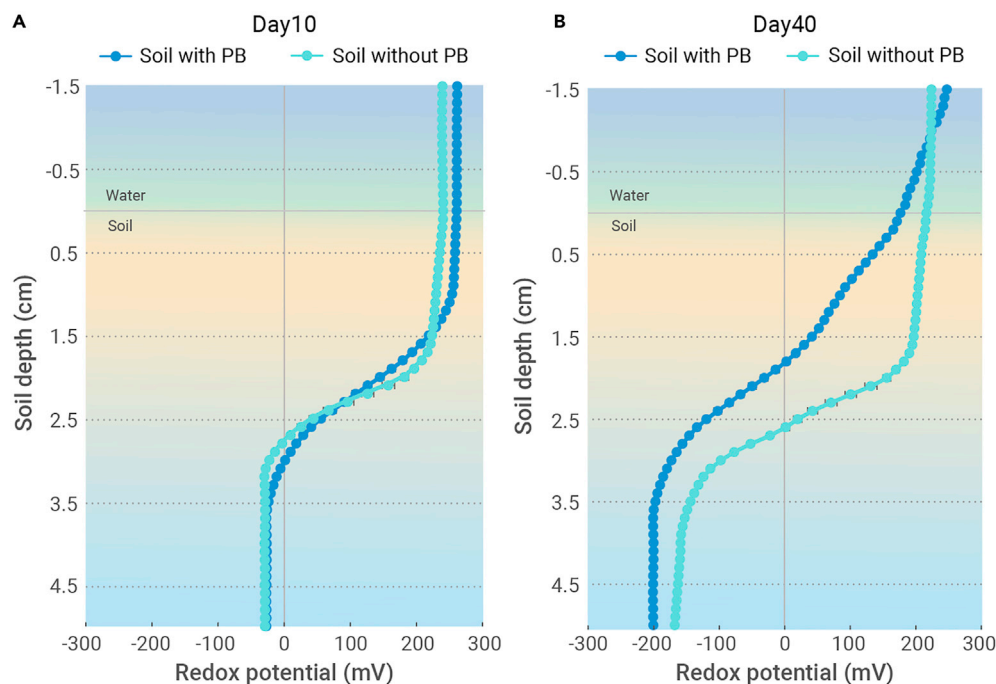
Fixed carbon was released to the soil as methanogenic substrates accompanied by the growth and decay of PB: over the growth and decay phases of PB, the  $\text{CO}_2$  that was fixed in its biomass was gradually released into the surface soil and pore water as exudations and debris, as indicated by the higher levels of TOC and DOC (Figures 4A and 4B). For instance, microbiomes in PB release organic compounds, such as complex organic acids and monosaccharides, into the extracellular environment;<sup>36-39</sup> biopolymers within the PB debris, such as cellulose, were likely depolymerized to form sugar monomers and lipids, which were converted to glycerol and long-chain fatty acids,<sup>26,40</sup> as indicated by the greater concentrations of lipids and organic acids (Figure S6) and diversity of other organic molecules in soils with PB (Figure 4C).

Variations in Eh reflected the dynamics of the release and depletion of electrons and terminal electron acceptors such as  $\text{O}_2$  and  $\text{Fe}^{3+}$ .<sup>20,41</sup> Photosynthetic microalgae were the dominant community during the growth phase of PB,<sup>16</sup> and  $\text{O}_2$  released by microalgae diffused into floodwater and surface soil and increased the Eh (Figure 5A). As PB began autogenic sloughing, the exudations and minimal debris were released to the soil and degraded by soil microbes, leading to a depletion of soil  $\text{O}_2$  and of other electron acceptors.<sup>20</sup> When  $\text{O}_2$  released by PB was in excess of that consumed during degradation of PB debris, the Eh decreased in the near-surface soil during the decay phase of PB (Figure 5B).

This methanogenic potential is also consistent with the observed reduction of the electron acceptor  $\text{Fe}^{3+}$  at this depth in the PB group soil at day 40 (Figure S9), which similarly implies a more reducing environment in the presence of PB. Following the typical model of a thermodynamically controlled redox succession in saturated soil,<sup>20</sup> the reduction of  $\text{Fe}^{3+}$  would be predicted to proceed prior to the onset of methanogenesis as a function of depth. Accordingly, it appears that the bulk-phase solid Fe had been reduced.

Changes in size and composition of microbial communities are closely related to their environments, and Eh and substrate composition and availability are major drivers of this change.<sup>42,43</sup> For instance, since methanogens are strict anaerobes,<sup>18</sup> a higher SOC availability, as well as lower Eh, promoted an increase in the methanogen community size in the surface soil with PB (Figure 6A). The majority of methanogens, e.g., genera *Methanocorpusculum*, *Methanosarcina*, and *Methanocella*, produce  $\text{CH}_4$  through the hydrogenotrophic pathway,<sup>44</sup> but *Methanosarcina* has a broader substrate spectrum,<sup>40</sup> which could also utilize acetate to produce  $\text{CH}_4$ .<sup>43,45</sup> Evidence for the shift toward *Methanosarcina* in the soil of the PB group (Figure 6C) suggested a promotion of acetoclastic methanogenesis.

Aerobic methanotrophs obligately require an aerobic environment,<sup>46</sup> and accordingly, we found that their abundance was lower (relative to the control) in the presence of PB, due to the lower Eh in the surface soil. In the surface



**Figure 5. Effect of PB on redox potential (Eh, mV) of floodwater and surface soil Eh profiles in the microcosms with and without PB during (A) the growth phase (day 10 after flooding) and (B) the decay phase (day 40 after flooding) of PB.** Error bars representing SD ( $n = 3$ ) are mostly smaller than the data symbols and hence not observable.

soil, limitation of  $O_2$  imposed by the PB selected for an increased relative abundance of the genus *Methylocaldum* (Figure 6D) against a backdrop of declining community size of methanotrophs (Figure 6B), which is perhaps because *Methylocaldum* is adapted to surviving periods of stress.<sup>47</sup> *Methylocaldum* is a potential thermophile, but additionally shows a capability for adapting to drastic temperature gradients often found in transiently warm environments such as paddies.<sup>47,48</sup> This property probably gives it an advantage over the genus *Methylocystis*, as it possesses survival strategies in response to the stress of limited  $O_2$  availability. Furthermore, as a type I methanotroph, *Methylocaldum* is thought to grow quickly under favorable conditions.<sup>45,46</sup>

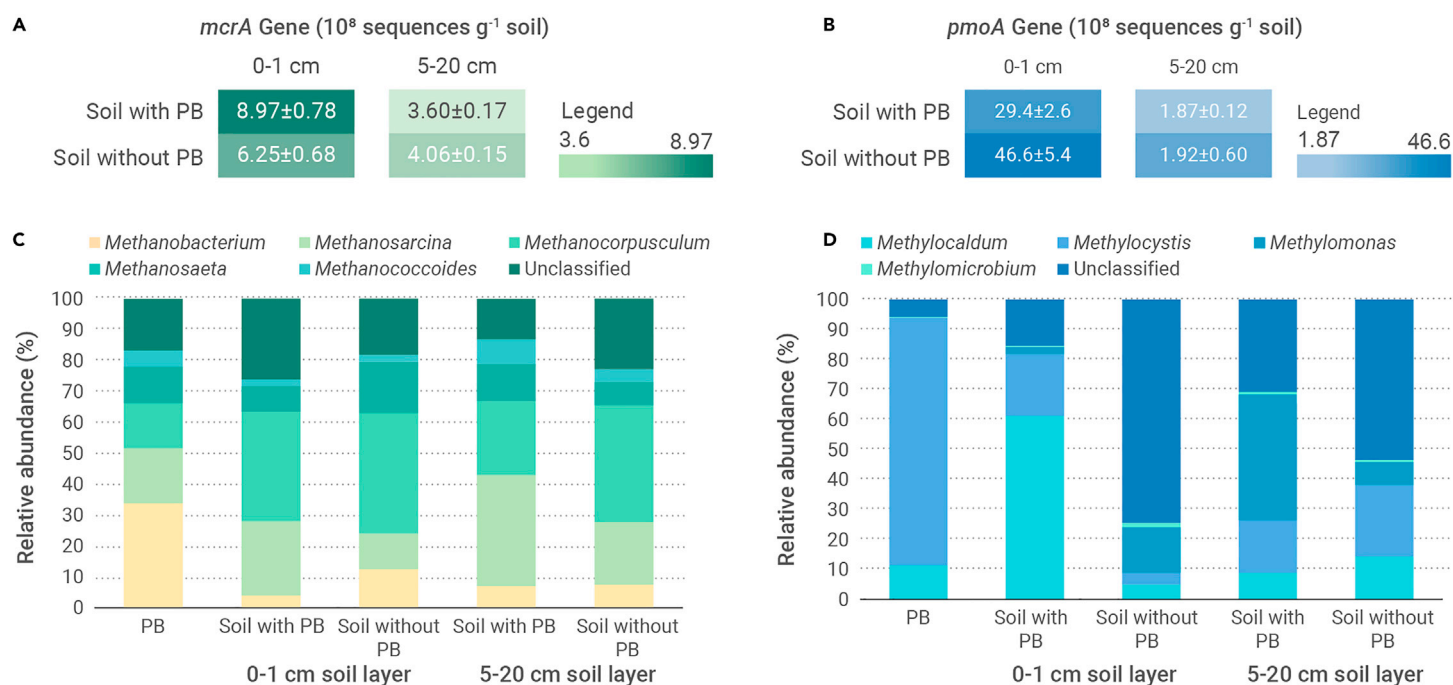
Taken together, our results show that the presence of PB affected  $CH_4$  emission through the regulation of microbial composition and metabolism, availability of methanogenic substrates, and soil Eh. During its growth phase, PB grew

more prominent role. This is the reason that PB promoted  $CH_4$  emission in the field.

Previous studies in mangrove reported a decrease in  $CO_2$  fluxes by biofilms due to photosynthetic  $CO_2$  consumption at the sediment surface,<sup>49</sup> which is consistent with the results in rice paddies. However, different from in rice paddies, the  $CH_4$  flux was also reduced by biofilm on the mangrove soil surface. This might be attributed to the different biofilm biomass, microbial composition, tidal cycle (flooding and drainage), and flux measurement intervals compared with rice paddies.

#### Contribution of periphytic biofilm to carbon flux

The global  $CH_4$  emission factor of flooded rice fields was estimated to be  $1.19 \text{ kg ha}^{-1} \text{ day}^{-1}$  ( $4.96 \text{ mg m}^{-2} \text{ h}^{-1}$ ),<sup>50</sup> and the seasonal average  $CH_4$  flux in



**Figure 6. Effects of PB on the size and composition of methanogen and aerobic methanotroph communities in the surface and deep layer of paddy soil microcosms after incubation** Gene abundances of (A) *mcrA*, quantifying the methanogens, and (B) *pmoA*, quantifying the methanotrophs, and genus-level compositions of (C) methanogenic and (D) methanotrophic communities in the soil.

China is  $9.02 \text{ mg m}^{-2} \text{ h}^{-1}$ .<sup>51</sup> In this study, the seasonal  $\text{CH}_4$  fluxes from tropical, subtropical, and temperate experimental paddy fields were 4.12, 9.42, and  $1.48 \text{ mg m}^{-2} \text{ h}^{-1}$  on average in the presence of PB, respectively, and 3.82, 5.57, and  $1.32 \text{ mg m}^{-2} \text{ h}^{-1}$  from fields without PB, which are in the same range as other studies.

We quantified the contribution of PB to paddy carbon flux by comparing the fluxes induced by PB with the total flux from fields with PB. In the “with PB” field, the paddy was managed based on local practice, and PB grew normally without intervention, which is suitable for being a reference. The contributions of PB to the total  $\text{CO}_2$  sink and  $\text{CH}_4$  efflux are estimated as 7.2%–12.7% and 7.1%–38.5%, respectively, in the experimental paddy fields. Variations in the contribution of PB to carbon flux were found among the three climatic zones.

Soil TOC content (Table S2), DOC content (Figure S4B), and PB biomass (Figure S4A) were measured in the three experimental paddy fields. The PB biomass, as well as TOC and DOC contents, was highest in the subtropical field compared with tropical and temperate, indicating a stronger  $\text{CO}_2$  uptake of the subtropical PB. The increase in  $\text{CH}_4$  emission induced by PB is mainly attributed to the organic input from PB. The highest PB biomass led to maximum DOC accumulation and methanogen abundance and, as a result, the most active  $\text{CH}_4$  emission induced by PB. Further studies are still needed to investigate the factors driving PB biomass (e.g., soil and climate properties) and quantitatively explain the changes in  $\text{CH}_4$  and  $\text{CO}_2$  flux.

Despite the variations in contribution of PB to carbon flux, microbial aggregates on the soil surface of tropical, subtropical, and temperate rice paddies all increased  $\text{CH}_4$  emissions through  $\text{CO}_2$  fixation, carbon release, and associated increases in labile SOC availability. PB functioned as a biotic converter that transferred atmospheric  $\text{CO}_2$  into  $\text{CH}_4$ . The amount of  $\text{CO}_2$  fixed by PB was 2.1–57.3 times that of induced  $\text{CH}_4$  emissions, indicating a net flow of carbon from the atmosphere to the rice paddies. However,  $\text{CH}_4$  is a potent greenhouse gas, with a 25-fold greater greenhouse warming potential than  $\text{CO}_2$  on a 100-year time horizon.<sup>52</sup> The presence of PB reduced greenhouse gas emissions by  $11.8 \text{ kg CO}_2 \text{ equiv ha}^{-1} \text{ season}^{-1}$  in the temperate experimental paddy, and increased by 40.8 and  $2,318.0 \text{ kg CO}_2 \text{ equiv ha}^{-1} \text{ season}^{-1}$  in the tropical and subtropical experimental paddies, respectively.

Worldwide, the role of PB in  $\text{CH}_4$  emissions has not been recognized. PB is ubiquitously distributed across tropical, subtropical, and temperate rice paddies, and the assimilated  $\text{CO}_2$  by PB reached 79, 211, and  $118 \text{ kg ha}^{-1} \text{ season}^{-1}$ , respectively. The decay phase of PB (days 20–40 after flooding) exactly overlapped with the period when the paddy soils showed maximum  $\text{CH}_4$  emission potentials, and therefore, the PB exudations and debris provided abundant organic carbon for  $\text{CH}_4$  production. This suggests the possibility of developing mitigative strategies for greenhouse gas emissions through PB regulation. Decay of PB is due to the low availability of light and nutrients at the tillering stage of rice.<sup>19</sup> If its light and nutrient capture ability are improved, the lifespan of PB is expected to be prolonged, which on one hand helps the rice paddy to fix more  $\text{CO}_2$  and, on the other hand, staggers the times of PB carbon input and maximum  $\text{CH}_4$  emission potentials, hence enhancing the carbon sink of terrestrial ecosystems and achieving sustainable development and carbon neutrality.<sup>53,54</sup>

## Conclusions

This study provides proof-of-concept evidence for the discrimination of the contributions of surface microbial aggregates (i.e., PB) from those of soil microbes to  $\text{CO}_2$  and  $\text{CH}_4$  emissions throughout the rice growing season. Microbial aggregates (i.e., PB) on soil surfaces increased  $\text{CH}_4$  emission through  $\text{CO}_2$  fixation by autotrophs and were associated with increases in availability of labile SOC and changes in Eh and the soil microbiome. Thus, PB functioned as a biotic converter of atmospheric  $\text{CO}_2$  into  $\text{CH}_4$ . Overall, microbial aggregates at the soil surface play a pivotal role in carbon biogeochemistry of rice paddies, confirming that they should be accounted for in the development and optimization of predictive models of global carbon fluxes.

## MATERIAL AND METHODS

Material and methods are provided in the supplemental information.

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## AUTHOR CONTRIBUTIONS

S.W. and Y.W. designed the study. J.D., G.Z., N.G., and J.P. improved the experimental designs. S.W. performed the experiments with help from P.S. S.W., Y.W., S.E.-E., and J.P. wrote the paper with input from all coauthors.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xinn.2021.100192>.