Snails promote methane release from a freshwater lake ecosystem

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Benthic fauna, as ecosystem engineers, can strongly affect microbial-driven ecosystem biogeochemical cycling. However, the effects of benthic fauna, especially epifauna, on CH_4 cycling remain still elusive. In this study, CH_4 effluxes were both measured along a gradient of snail density in a freshwater lake ecosystem in China, and monitored in manipulated laboratory microcosms with and without snails. Field CH_4 efflux was significantly increased with snail density. Likewise, the stimulating effects of freshwater snails on CH_4 effluxes were evident in the homogenized indoor microcosms. These results show that snails can stimulate CH_4 efflux in the freshwater lake ecosystem. Moreover, the average efflux of CH_4 emitted from snails' habitats has reached 15.33 mg CH_4-C m^{-2} d^{-1}. By comparing with those emitted from vegetated coastal marsh and alpine wetland, this data indicates that snails' habitats are strong sources of CH_4 in a freshwater ecosystem. This study suggests identifying and modeling epifauna activity as a function of CH_4 cycling could improve the mechanistic understanding of wetland biogeochemical cycling responses to climate change.

Keywords: aquatic fauna, invertebrate-microorganism interaction, methanogens, CH_4 oxidizing bacteria, CH_4 cycling, freshwater ecosystem

INTRODUCTION

Benthic fauna are known as ecosystem engineers, whose activities can create unique microniches for microorganisms to inhabit (Laverock et al., 2011), and increase resource availabilities for microbial metabolism (Mermillod-Blondin and Rosenberg, 2006). Consequently, altered microbial community structure and composition due to fauna activities could largely influence microbial-driven ecosystem biogeochemical cycling (Mermillod-Blondin and Rosenberg, 2006; Laverock et al., 2011).

Wetlands are the largest natural source of CH_4 to the atmosphere, contributing 217 Tg annually (IPCC, 2013). Wetland CH_4 effluxes are primarily driven by net effects of two microbial processes, the CH_4 production and oxidation (Whalen, 2005). However, the effects of benthic fauna, especially epifauna, on CH_4 cycling are very limited (Weyhenmeyer et al., 2009). Hence, present study was planned to establish the effects of epifauna on CH_4 cycling in freshwater ecosystems. Understanding the potential roles of epifauna in regulating CH_4 cycling can not only fully reveal the dynamic processes of CH_4-cycling in freshwater ecosystems, but also throw a new light on their ecosystem processes in the process-based CH_4 models (Cao et al., 1996).

Snails are known as a common epifauna that live on the surface sediment in aquatic ecosystems, including lakes and ponds. They can forage on attached microorganisms such as algae, and sediment organic carbon within the upper few millimeters of the sediment (Zheng et al., 2011). They were also found to enhance

Oxygen (O_2) consumption (Zheng et al., 2011). Therefore, snails have potentially profound effects on microbial activities, thus may affect CH_4 efflux. In this study, both field and microcosm experiments were performed to elucidate the potential roles of snails in CH_4 cycling. CH_4 effluxes were measured along a gradient of snail density in the largest freshwater lake in China. Sediment properties and two microbial-mediated CH_4 processes (CH_4 oxidation and production potential rates) of the top layer of sediments were characterized. Furthermore, sediment sample as a common growth substrate was incubated with and without snail addition at the constant conditions to determine microbial capacity to produce and oxidize CH_4.

MATERIALS AND METHODS
STUDY SITE AND FIELD EXPERIMENTAL DESIGN
Poyang Lake (28°24′–29°46′ N, 115°49′–116°46′ E), the largest freshwater lake in China, is located in the northern part of Jiangxi Province along the southern bank of the middle reaches of the Yangtze River. Poyang Lake exhibits a large seasonal water level fluctuation, with a maximum and water depth of 17.6 m in July and a minimum water depth of 10.5 m in January on average (Wang et al., 2007). In winter, more than 90% of its water can be lost (Wang et al., 2007). As the water level declined, the large inland sea could be divided into numerous sub-lakes, and vast areas of flat grass-covered marshlands become exposed. This lake has a monsoon climate of subtropical zone. The mean

annual temperature is 17.2°C. The mean annual precipitation is 1482.3 mm (46% falls in April-June), and the mean annual evaporation is 1184 mm (Wang et al., 2007). The sediment texture is mainly hard clay and fine sand (Wang et al., 2007).

Freshwater snails are dominant epifauna in Poyang Lake, account for 24.7% and 29.2% in total density (659 ind m^{-2}) and biomass (187.3 g m^{-2}) of benthic fauna (Wang et al., 2007). According to the hydrologic/weather conditions and snail growth, field sampling was only conducted in autumn. Due to habitats covering by water, few snails can be found during flooded seasons (from April to July). Freshwater snails generally start to reproduce in late summer and reach maximum values of density and biomass in autumn because of accommodative growth temperature.

To avoid disturbances from agricultural and grazing activities, the *in situ* experiment was conducted in Banghu Lake, the isolated sub-lake located in the core area of Poyang Lake, in September, 2011. Eight sampling sites were carefully selected along the lakeshore within 1 km based on the gradient of epifaunal density. None of infauna was found in the upper 5 cm sediment of these sampling sites. The mean distance between each two sampling sites is about 18 m. All these sites have similar sediment textures (hard clay) and hydrologic regime (a water depth of 3-5 cm and similar water flow velocity). The only epifauna observed at these sites were freshwater snails. Over 80% of the total freshwater snails belonged to *Viviparidae* and *Melaniidae*. All snails had similar size (length ∼12 mm) across study sites. To ensure that the samples were representative of study sites, each site (1 m wide × 5 m long) was evenly divided into three sub-sites for sampling. Vegetation zones with the mean straight-line distance of 3 m from snail habitats were dominated by a C_3 grass *Carex lasiocarpa*. No visible litter fall occurred at any of the sites during sampling.

CH$_4$ EFFLUX

CH$_4$ efflux measurements from each sub-site were made using a static chamber technique. The Plexiglas chamber consisted of two compartments. The top compartment (40 × 40 cm wide and 50 cm high) was equipped with a sampling port and a 12 V fan was located on the top panel inside the chamber for mixing air. The bottom compartment (40 × 40 cm wide and 20 cm high) was inserted carefully into the sediment to a depth of about 10 cm with as little disturbance as possible before sampling, and then mounted the top compartment and sealed gastight. Headspace gas samples (50 ml) were taken at time zero and after periods of 20 min in polypropylene syringe equipped with three-way stopcocks. Gas samples were then injected into 100-mL -evacuated gas bags (Delin Inc, Dalian, China).

CH$_4$ concentrations were determined with a gas chromatography (Agilent HP5340 equipped with FID and a 2-m Porapak Q (60/80 mesh) column, Agilent Technologies Inc., Santa Clara, CA, USA). The oven, injector, and detector temperatures were set at 80, 200, and 330°C, respectively. The carrier gas (N_2) flow rate was 30 mL min^{-1}, and the flow rates of flaming gases (H_2 and synthetic air) were set at 40 and 400 mL min^{-1}, respectively. Calibration was performed with a standard CH$_4$ gas of 9.9 ppm.

SEDIMENT SAMPLING

After measuring CH$_4$ efflux, three cores in each sub-site were randomly taken from the top 5 cm sediment using a 2.5-cm-diameter auger, and then thoroughly mixed. The mixed samples were handpicked to remove stones and snail shells. Samples were stored at 4°C for determining potential rates of CH$_4$ oxidation (PMO) and production (PMP), and chemical properties.

CH$_4$ OXIDATION AND PRODUCTION POTENTIALS

To determine the PMO and PMP rate, sediment-water slurries were prepared using 30 g fresh sediment and 30 mL sterile deionized water, and then shook (150 rpm) for 30 min at room temperature. PMO was determined with a method modified from Bodelier and Frenzel (1999). Four Labco exetainers (12 mL, Labco Limited, Buckinghamshire, UK) were added with 5 mL of the slurry samples, and were purged with CH$_4$-free gas (20% O_2, 80% N_2) for 10 min. To activate the CH$_4$-oxidizing bacteria, 0.3 mL of pure CH$_4$ gas (99.9%) was injected into each exetainer through a rubber septum and the exetainers were pre-incubated in dark at 30°C for 2 days. All exetainers were then purged again with CH$_4$-free gas, and injected 0.3 mL of pure CH$_4$ gas, and incubated for 24, 48, 72, and 96 h, respectively. At the end of incubation 0.1 mL of ZnCl$_2$ solution (50%, w/v) was injected into each exetainer, which was shaken to stop CH$_4$ oxidation. The decrease in CH$_4$ concentration in the headspace was monitored with the gas chromatograph (GC) at 24-h intervals. The PMO rate was calculated from the slope of linear regression of CH$_4$ concentration against incubation time ($R^2 > 0.85$).

PMP was determined with a method modified from Bodelier et al. (2006). Five mL of the slurry was also transferred to four Labco exetainers. The exetainers were flushed with pure N_2 (99.999%) for 10 min. To activate methanogens, the slurry was pre-incubated in dark at 30°C for 2 days. All exetainers were flushed again with pure N_2, and the slurry sample in each exetainer was incubated for 24, 48, 72, and 96 h, respectively. At the end of incubation 0.1 mL of ZnCl$_2$ solution (50%, w/v) was injected into each exetainer, which was shaken to stop methanogenesis. Accumulation of CH$_4$ in the headspace was monitored with the GC at 24-h intervals. The PMP rate was calculated from the slope of linear regression of CH$_4$ concentration against incubation time ($R^2 > 0.85$).

CHEMICAL ANALYSIS

Sediment samples were oven-dried at 50°C to constant weight and then ground and passed through 100-micron meshes. Sediment pH was measured with a glass electrode in the sediment supernatant (1:5, w/v). NH_4^+ and NO_3^- ($NO_3^- + NO_2^-$) were extracted with 2 M KCl (1:4, w/v) and determined colorimetrically with discrete auto analyzer (SmartChem200, WestCo, America). Dissolved total nitrogen (DTN) and organic carbon (DOC) were extracted from sediment samples using 0.5 M K_2SO_4 (1:4, w/v) and analyzed with a TOC analyzer (vario TOC cube, Elementar, Germany). DON was calculated as DTN - ($NH_4^+ + NO_3^-$). Total carbon (TC) and nitrogen (TN) were determined with an N/C analyzer (Flash EA 1112Series, Thermo, Italy).

MICROCOSM INCUBATION EXPERIMENT

Individual snails as well as sediments were also collected from the sampling sites with highest snail density for indoor incubation. Sediments were air-dried, ground, gently sieved through a 2 mm mesh, and thoroughly homogenized. 3.2 kg of the defaunated sediment and 1.6 L sterile deionized water were transferred into each of eight microcosms (25×16 cm inner diameter (i.d.) PVC cylinders) to a depth of about 15 cm. Microcosms were stabilized at room temperature ($22 \pm 2°C$) for 7 days and the overlying water was maintained at a depth of 3–5 cm. Snails had been washed and acclimatized in a larger container with the same experimental conditions for 1 week before being introduced to these microcosms. After the stabilization period, 10 snails were added to each of 4 microcosms, equivalent to a density of 500 ind m^{-2}, while 4 microcosms were kept as defaunated controls. CH$_4$ effluxes were measured every 7 days during a 49-day incubation. During CH$_4$ efflux measurements, the microcosms were sealed with 15×16 cm i.d. PVC lids. The center of the top lid was equipped a 4 mm i.d. sampling port for sampling gas from the headspace. Efflux rates of CH$_4$ were calculated as the difference in headspace concentrations between initial and final samples during a 20 min incubation period. At the end of incubation, snails in the microcosms were re-collected for calculating survival rate. CH$_4$ concentrations were determined with the same method described above.

STATISTICAL ANALYSES

Polynomial regression analyses were used to determine the effects of snail density on CH$_4$ efflux and microbial processes. One-way ANOVA was performed to determine the effects of freshwater snail on CH$_4$ efflux in the indoor incubation. Pearson correlation analyses were performed to evaluate relationships between snail density, CH$_4$ efflux, microbial activities, and sediment properties. Regression analyses and Pearson correlation analyses were performed with the software of Sigmaplot 12.3 (Systat Software, San Jose, CA, USA). One-Way ANOVA was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Effects with $P < 0.05$ were considered statistically significant.

RESULTS

CH$_4$ EFFLUX AND MICROBIAL ACTIVITIES

Field CH$_4$ efflux significantly increased with snail density (**Figure 1**). In support of data on CH$_4$ effluxes, snail density was positively correlated with PMP rate (**Figure 2A**), and negatively correlated with PMO rate (**Figure 2B**). Likewise, snails [$F_{(1, 4)} = 6.919$, $P < 0.05$, **Figure 3**] significantly promoted CH$_4$ efflux compared to defaunated control during the 49-day incubation. At the end of indoor microcosm incubation, the survival rates of snails reached >90%.

SEDIMENT PROPERTIES

Sediment properties were listed in Table S1 (please see supplementary material in Data sheet 1). Sediment pH was positively correlated with snail density (**Table 1**). The concentrations of NH$_4^+$ and DON were significantly increased and decreased with
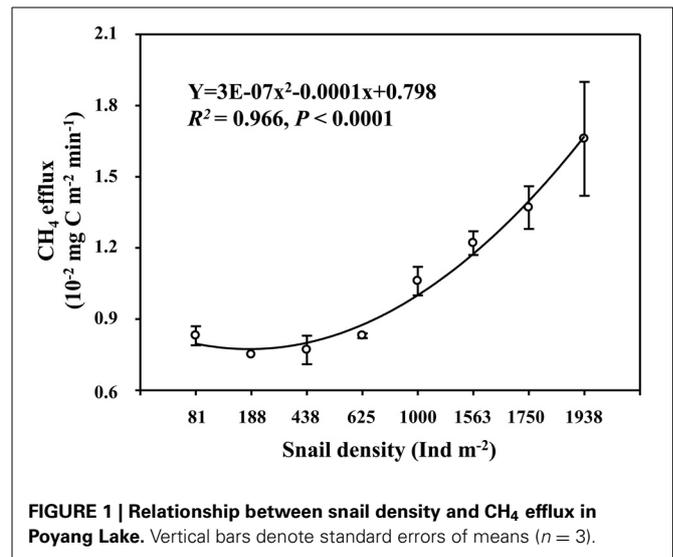
FIGURE 1 | Relationship between snail density and CH$_4$ efflux in Poyang Lake. Vertical bars denote standard errors of means ($n = 3$).

snail density, respectively (**Table 1**). Snail density had no significant Pearson relationships with DOC, NO$_3^-$, TC, and TN, respectively (**Table 1**).

RELATIONSHIPS BETWEEN FIELD CH$_4$ EFFLUX, MICROBIAL ACTIVITIES, AND SEDIMENT PROPERTIES

CH$_4$ efflux was positively correlated with NH$_4^+$ concentration (**Table 1**). NH$_4^+$ concentration was positively correlated with PMP rate and negatively correlated PMO rate (**Table 1**). Furthermore, sediment pH and DON was negatively and positively correlated with PMO rate (**Table 1**), respectively.

DISCUSSION

A higher CH$_4$ efflux in the habitat with a greater snail density suggests that snail could stimulate CH$_4$ efflux in the largest freshwater lake in China (**Figure 1**). Promoted potential of CH$_4$ production and suppressed potential of CH$_4$ oxidation across the gradient of snail density (**Figure 2**) suggest that snail-induced changes in microbial functioning relative to CH$_4$ cycling are expected to result in more greenhouse gas effects, thus potentially contributing to a positive climate feedback.

These results are similar with previous study which found infauna could increase CH$_4$ efflux in laboratory experiment (Weyhenmeyer et al., 2009). Furthermore, the effects of benthic fauna on CH$_4$ efflux were extended from laboratory incubation to an open freshwater lake ecosystem. Field CH$_4$ efflux is mediated by multiple biotic and abiotic factors (Dingemans et al., 2011). Influences of infauna (the benthic fauna have a habitat different from epifauna which live within the sediments rather than on the surface sediments) on CH$_4$ efflux could not be completely excluded in field sampling because infauna usually inhabit in their tubes deeper than 5 cm. Moreover, although no visible litter fall was found in these sampling sites, plant roots are known to stimulate CH$_4$ efflux by secreting organic substrates for methanogens in rhizosphere zone (Laanbroek, 2010). By using a common soil, microcosm incubations were conducted at constant conditions to homogenize influences from other biotic and abiotic factors,
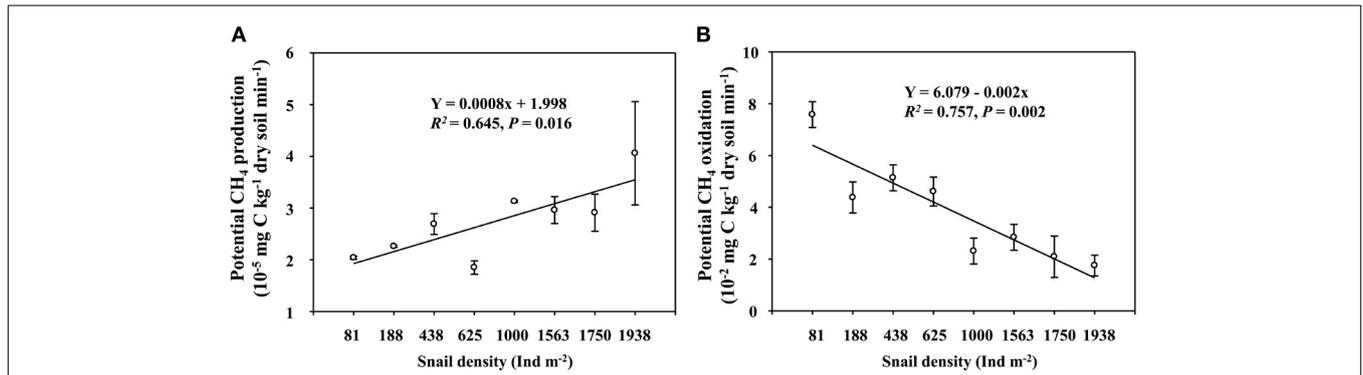
FIGURE 2 | The relationships of snail density with potential CH$_4$ production (A), and potential CH$_4$oxidation (B) rates in the upper 5 cm sediment of Poyang Lake. Vertical bars denote standard errors of means ($n = 3$).
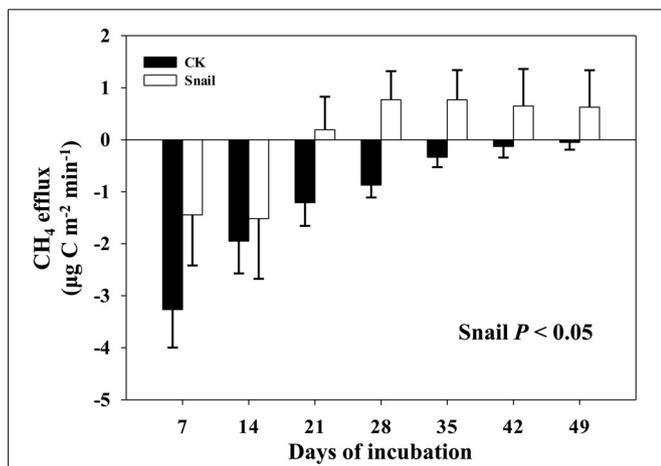
FIGURE 3 | CH$_4$ effluxes monitored in the microcosm headspace every 7 days of freshwater snail treatment and defaunated control (CK). Vertical bars denote standard errors of means ($n = 4$).

Table 1 | Coefficients of Pearson's correlations between snail density, CH$_4$ efflux, microbial activities and sediment properties across all 8 sampling sites.

	pH	NH$_4^+$	NO$_3^-$	DON	DOC	TC	TN
Snail density	0.71*	0.95***	−0.19	−0.74*	0.61	0.24	0.14
CH$_4$ efflux	0.70	0.89**	−0.24	−0.64	0.53	0.21	0.11
PMP	0.50	0.71*	−0.31	−0.47	0.21	0.19	0.16
PMO	−0.77*	−0.83**	−0.14	0.82*	−0.38	0.06	0.03

*PMP, potential CH$_4$ production rate; PMO, potential CH$_4$ oxidation rate; DON, dissolved organic nitrogen; DOC, dissolved organic carbon; TC, total carbon; TN, total nitrogen. Asterisks mark different levels of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*

and thus the positive effects of freshwater snails on CH$_4$ efflux (**Figure 3**) can be further confirmed.

Because NH$_4^+$ has a similar molecular size and structure to CH$_4$, it can act as more aggressive substrate than CH$_4$ and consequently competitively restrain CH$_4$ oxidation (Schimel, 2000).

Therefore, CH$_4$ oxidation might be inhibited by increased NH$_4^+$ concentration in this study (**Table 1**) supporting previous results that NH$_4^+$ concentration can mediate CH$_4$ oxidation (Smith et al., 2000). NH$_4^+$ concentration is mediated by the combined effects of bioturbation, microbial mineralization rate, and faunal excretion in the habitats with infauna (Papaspyrou et al., 2007). As bioturbation, the physical rearrangement of sediment particles by epifauna was shown to stimulate water solubility of nutrient exchange between sediment and overlying-water (Zheng et al., 2011). The bioturbation of epifauna can facilitate nutrient releases from sediment by molecular or eddy diffusion (Kristensen et al., 2012), possibly resulting in increases in NH$_4^+$ concentration with snail density increasing. Gilbertson et al. (2012) found that epifauna could increase N mineralization by shifting bacterial community structure due to grazing and/or mucus secretion. In the present study, DON was negatively correlated with snail density (**Table 1**) and NH$_4^+$ ($r = -0.84$; $P = 0.009$), respectively. These results suggest that freshwater snails may stimulate DON mineralization, thereby increase NH$_4^+$ concentration. Similarly, Jordan et al. (2009) found that microbial mineralization rate was positively correlated with the density of benthic fauna. In addition, macrofauna can excrete excess nitrogen (ammonia, urea and other nitrogen compounds) to avoid the toxicity of ammonia as it accumulates in body tissues (Wright, 1995).

Sediment pH as a secondary determinant is an important abiotic factor in regulating CH$_4$ cycling (Moore and Roulet, 1995). In the present study, CH$_4$ oxidation was significantly decreased with sediment pH increasing to neutral pH (**Table 1**). In addition, CH$_4$ efflux was marginally positively correlated with sediment pH ($P = 0.054$) (**Table 1**). This is consistent with previous studies which demonstrated CH$_4$ efflux decreased with pH decreasing from neutral pH because CH$_4$-oxidizing bacteria favor mild acidic environments (Amaral et al., 1998). These results suggests that sediment pH had a positive relationship with NH$_4^+$ concentration ($r = 0.76$, $P < 0.05$), possibly because released NH$_4^+$ from SOM was replaced by sediment dissociative H$^+$ based on the cation exchange. Therefore, increased pH due to enhanced NH$_4^+$ release by snail activities may restrain CH$_4$ oxidizability in this study.

FIGURE 4 | Schematic presentation of the possible mechanisms of freshwater snail effects on the CH₄ cycling in the investigated freshwater lake ecosystem.
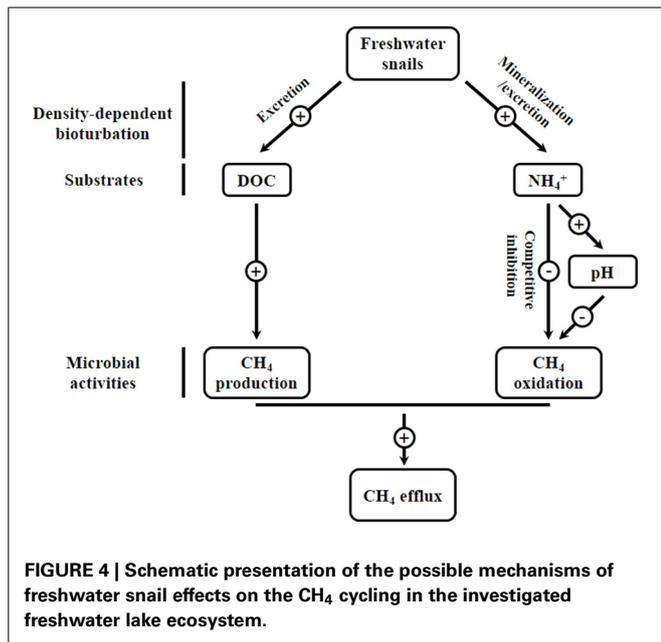
Methanogens use a limited number of substrates for methanogenesis (Rother, 2010), thus substrate availability also is an important factor in regulating CH_4 production (Le Mer and Roger, 2001). Methanogenesis is sensitive to the changes in DOC because DOC as available substrate can be rapidly utilized by methanogens or decomposed into the precursor substrates for CH_4 production (Liu et al., 2011). Bergman et al. (2000) found that the absence of DOC strongly suppressed CH_4 production rate in a peatland. Similarly, Liu et al. (2011) found that CH_4 production rate was significantly correlated with the DOC in freshwater wetlands. A higher DOC in the habitat with a greater snail density can supply more substrates for methanogenesis (**Table 1**) and may promote (marginally significant, $P = 0.10$) CH_4 efflux (**Table 1**) in this study.

O_2 availability largely detemines CH_4 cycling. In wetlands, CH_4 oxidation increased with O_2 concentration in the rhizospheres of emergent plants, because the plants enhance transportion of O_2 from atmosphere to their rhizospheres (Laanbroek, 2010). Similarly, infauna chironomid larvae can also stimulate CH_4 oxidation by increasing O_2 diffusion into flooded rice soil (Kajan and Frenzel, 1999). But this may not occur in the habitats of epifauna. As the limited horizontal activities, epifauna may have little effect on transport of O_2 into the sediments (Kristensen et al., 2012), suggesting that CH_4 oxidation and methanogenesis may not be stimulated and inhibited in the habitats of epifauna. O_2 availability was not measured in the sediments and water column due to the damage of O_2 electrode in the field. Further works are required to inverstigate whether epifauna can affect biogenochemical cycling through regulating O_2 availability.

As an intermittently inundated wetland, Liu et al. (2011) indicated that Poyang Lake might not be a strong source of CH_4 in comparison to other permanently inundated wetlands such as Sanjiang Plain marsh in China. In the present study, mean CH_4 efflux in snails' habitats (15.33 mg CH_4-C m⁻² d⁻¹) are comparable to 62% of those emitted from *Spartina alterniflora* dominated coastal marsh (24.72 mg CH_4-C m⁻² d⁻¹) (Ding et al., 2010) and 46% of those emitted from *Potamogeton*-dominated alpine wetland (33.1 mg CH_4-C m⁻² d⁻¹) (Hirota et al., 2004), respectively. In addition, numerous process-based models, such as wetland methane emission model (Cao et al., 1996), and Wetland-DNDC (Zhang et al., 2002), which depend on measurements at specific sites or from specific sources to determine efflux by various important parameters such as climate and vegetation (Cao et al., 1996) may underestimate freshwater ecosystem CH_4 efflux. For example, plant growth in terms of net ecosystem productivity plays a critical role in stimulating CH_4 efflux in the Wetland-DNDC model (Zhang et al., 2002). However, the results in this study suggest that snails' habitats might be a strong source of CH_4 in the non-plant-covered zones. Consequently, a better understanding of the impact of benthic fauna on CH_4 cycling will contribute to improved accuracy of model-based predictions of CH_4 effluxes from wetlands.

In summary, these results provide evidence that snails' habitats in a freshwater ecosystem are strong sources of CH_4. Increased CH_4 efflux may attribute to snail-induced stimulation in methanogenesis by supplying available substrates (DOC) and inhibition in CH_4 oxidation by increasing NH_4^+ concentration and sediment pH (**Figure 4**). Given the important role of freshwater epifauna in mediating CH_4 cycling, there is a clear need to enhance the understanding of the processes controlling CH_4 dynamics, a major greenhouse gas dynamics.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fenvs.2014.00012/abstract

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