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# Widespread dominance of methane ebullition over diffusion in freshwater aquaculture ponds

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An ever-increasing demand for protein-rich food sources combined with dwindling wild fish stocks has caused the aquaculture sector to boom in the last two decades. Although fishponds are potentially strong emitters of the greenhouse gas methane (CH<sub>4</sub>), little is known about the magnitude, pathways, and drivers of these emissions. We measured diffusive CH<sub>4</sub> emissions at the margin and in the center of 52 freshwater fishponds in Brazil. In a subset of ponds ( $n = 31$ ) we additionally quantified ebullitive CH<sub>4</sub> fluxes and sampled water and sediment for biogeochemical analyses. Sediments ( $n = 20$ ) were incubated to quantify potential CH<sub>4</sub> production. Ebullitive CH<sub>4</sub> emissions ranged between 0 and 477 mg m<sup>-2</sup> d<sup>-1</sup> and contributed substantially (median 85%) to total CH<sub>4</sub> emissions, surpassing diffusive emissions in 81% of ponds. Diffusive CH<sub>4</sub> emissions were higher in the center (median 11.4 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) than at the margin (median 6.1 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) in 90% of ponds. Sediment CH<sub>4</sub> production ranged between 0 and 3.17 mg CH<sub>4</sub> g C<sup>-1</sup> d<sup>-1</sup>. We found no relation between sediment CH<sub>4</sub> production and *in situ* emissions. Our findings suggest that dominance of CH<sub>4</sub> ebullition over diffusion is widespread across aquaculture ponds. Management practices to minimize the carbon footprint of aquaculture production should focus on reducing sediment accumulation and CH<sub>4</sub> ebullition.

## KEYWORDS

greenhouse gases, fishponds, diffusion, tilapia, sediment, mitigation, food production, fish farming

## 1. Introduction

With the current growth in global population and improving living standards, the demand for high-protein food sources is rapidly increasing (Henchion et al., 2017). Fish has historically played an essential role as an animal food source, and is rich in protein, essential fatty acids, and micronutrients (Arts et al., 2001; Kawarazuka, 2010; Hicks et al., 2019). Until the 1990s, nearly all demand for fish was supported by capture fisheries (FAO, 2020). However, wild fish stocks are dwindling rapidly due to overfishing, and capture fishery production has plateaued over the past two decades (FAO, 2020). Accordingly, the increasing demand for fish has been supported by meteoric growth in aquaculture production. Globally, the contribution of aquaculture to total fish production has risen from 26% in 2000 to 46% in 2016–2018 and is expected to increase to 53% by 2030, surpassing capture fisheries production (FAO, 2020). However, this massive increase comes with environmental costs, such as substantial greenhouse gas (GHG) emissions (Kosten et al., 2020).

Most aquaculture production is freshwater based (63% in 2018), and the majority of freshwater aquaculture facilities use earthen ponds, which are shallow, excavated structures consisting solely of soil materials (FAO, 2020). To optimize production, these ponds often receive large amounts of fish feed and fertilizers. This external input of organic matter and nutrients could lead to environmental pollution, including high emissions of the greenhouse gas (GHG) methane (CH<sub>4</sub>) (Vasanth et al., 2016; Wu et al., 2018; Yuan et al., 2019). CH<sub>4</sub> is a strong GHG with a 27 times higher global warming potential than carbon dioxide (CO<sub>2</sub>) on a 100-year time horizon (Canadell et al., 2021). CH<sub>4</sub> production takes place during the breakdown of organic matter, mostly under anoxic conditions. High levels of nutrients, such as nitrogen (N) and phosphorous (P), can exacerbate CH<sub>4</sub> emissions by increasing primary production, and, therefore, substrate availability for methanogens (e.g., Whiting and Chanton, 1993; DelSontro et al., 2016; Beaulieu et al., 2019). Fishpond CH<sub>4</sub> emissions may be minimized by climate-smart management, enabling sustainable protein production for biodiversity and the climate. However, as little is known about the magnitude, pathways, and drivers of CH<sub>4</sub> emissions, it is uncertain what climate-smart management targeting fishpond GHG emissions entails (Kosten et al., 2020).

CH<sub>4</sub> emission values from semi-intensive fishponds reported in the literature range from 0.2 to 480 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> (Yuan et al., 2019). Although the published emission rates tend to be substantial, they are prone to be underestimated due to a lack of insight in spatial and temporal dynamics (e.g., hotspots and hot moments) and in CH<sub>4</sub> emission pathways (Kosten et al., 2020). Notably, most studies focus only on the diffusion of dissolved CH<sub>4</sub> from the surface water to the atmosphere, neglecting CH<sub>4</sub> emissions through the episodic release of bubbles that build up in the sediment (ebullition). The few studies that have considered CH<sub>4</sub> ebullition in aquaculture ponds show that it can be substantial. A recent eddy covariance study in an aquaculture complex (including clams, fish fry, and crayfish), for instance, found that ebullition contributed 70% on average to total CH<sub>4</sub> emissions (Zhao et al., 2021). A similar contribution (67%) was found in temperate fishpond, with very high ebullition rates near feeding zones (Waldemer and Koschorreck, 2023). In an experimental tambaqui (*Colossoma macropomum*) monoculture, ebullition contributed 84% to total CH<sub>4</sub> emissions (Flickinger et al., 2020), similar to what has been described for a mixed Prussian carp (*Carassius auratus gibelio*) and silver carp (*Hypophthalmichthys molitrix*) pond (83%) (Fang et al., 2022). Other aquaculture studies including organisms other than fish found high ebullition contributions as well: ~80% in Chinese mitten crab (*Eriocheir sinensis*) ponds (Yuan et al., 2021) and >90% in mariculture shrimp ponds (Yang et al., 2020, 2023; Tong et al., 2021). The exclusion of ebullitive CH<sub>4</sub> fluxes has significant implications for current aquaculture emission estimates. Due to the low number of aquaculture ponds studied, however, it remains uncertain whether the substantial contribution of ebullition can be extrapolated to fishponds in general and across many management styles.

By assuming a 42–77% contribution of CH<sub>4</sub> ebullition to the total CH<sub>4</sub> flux based on experimental work (Davidson et al., 2018; Oliveira Junior et al., 2019), Kosten et al. (2020) estimated that consideration of ebullition could drastically shift GHG emissions per kg of protein, increasing the carbon footprint of farmed fish

from below chicken to the range of pork. The IPCC emission factor of 183 kg CH<sub>4</sub> ha<sup>-1</sup> yr<sup>-1</sup> for freshwater ponds (including fishponds) also does not consider ebullitive emissions (IPCC, 2019). Moreover, as insufficient data were available, this estimate does not include the effects of climate, management strategies, pond characteristics, and environmental variables. Quantifying CH<sub>4</sub> emissions and potential drivers in a large variety of fishponds is essential to arrive at more accurate estimates of fishpond emissions and climate-smart management strategies.

In the context of neglected ebullitive CH<sub>4</sub> emissions from fishponds, we conducted measurements in 52 different fishponds in Southeast Brazil. We aimed to quantify the magnitude of CH<sub>4</sub> emissions from fishponds in this area and to assess the contribution of ebullition to total CH<sub>4</sub> emissions. We aimed to investigate potential drivers of CH<sub>4</sub> emissions by measuring a range of environmental variables (water quality, sediment quality including potential CH<sub>4</sub> production and pond characteristics) in these ponds.

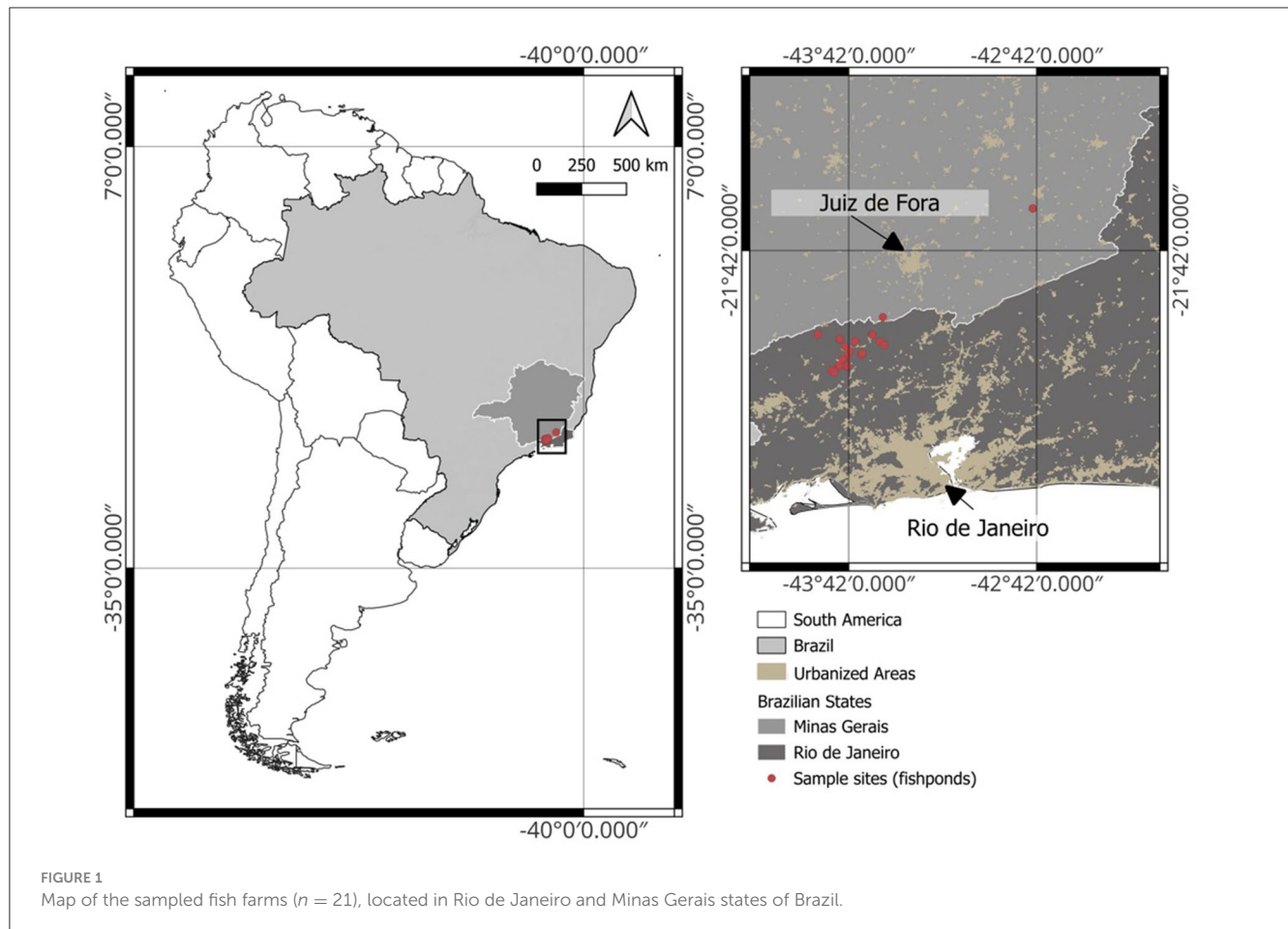
## 2. Materials and methods

### 2.1. Pond characteristics

We sampled 52 fishponds situated within 21 different farms in the states of Rio de Janeiro (43 ponds in 19 farms) and Minas Gerais (9 ponds in 2 farms), Brazil (Figure 1). These fishponds were selected as they were representative for the region and covered many management types. Ponds were used for commercial production, sustenance, recreation, breeding and research, or a combination. Nile tilapia (*Oreochromis niloticus*) was the most bred species in the studied fishponds, either as a monoculture ( $n = 22$ ) or mixed with other species ( $n = 19$ ). Commonly co-cultured species included *Hoplias* spp., *Astyanax* spp., and *Pterophyllum* spp. (for a complete description of the fishponds, see Supplementary Table S1). Several ponds contained wild or unknown species ( $n = 10$ ) and one pond contained only grass carp (*Ctenopharyngodon idella*). Most ponds were excavated earthen ponds ( $n = 46$ ), several were natural ( $n = 5$ ), and one was excavated and had a bottom layer of concrete. The areal extent of individual ponds ranged from 69 to 6,400 m<sup>2</sup>, with a median of 555 m<sup>2</sup>. Depth in the center of the pond varied between 0.6 and 3 m, with a median depth of 0.9 m.

### 2.2. Diffusive flux measurements

In all ponds, CH<sub>4</sub> diffusion was measured during daytime using a transparent floating acrylic chamber (29.2 cm inner diameter) connected with gastight tubing (0.4 cm diameter) to a microportable greenhouse gas analyzer (MGGA; ABB—Los Gatos Research, San Jose, CA, USA) in a closed, gastight circuit. Measurements were carried out consecutively in triplicates in the margin and the center of each pond (total  $n = 6$  measurements per pond) and lasted 180s. We used a dinghy with a paddle to navigate the ponds to prevent sediment disturbance. During each triplicate of GHG measurements, we measured air temperature, atmospheric pressure, and wind speed using a portable anemometer (Skymaster



Speedtech SM-28, accuracy: 3%).  $O_2$  concentrations, temperature, and pH were measured at 50 cm intervals in vertical profiles from the water surface down to the sediment using a Hach HQ40D portable multimeter (Hach, Loveland, CO, USA).  $CH_4$  and  $CO_2$  fluxes were calculated according to Almeida et al. (2016) using the following equation:

$$F = \frac{V}{A} * \frac{dC}{dt} * \frac{P * M * F1}{R * T}$$

Where  $F$  is the gas flux ( $mg\ m^{-2}\ d^{-1}$ ),  $V$  is chamber volume ( $m^3$ ),  $A$  is chamber surface area ( $m^2$ ),  $dC/dt$  is the change of the measured  $CH_4$  or  $CO_2$  concentration in time ( $ppm\ s^{-1}$ ),  $P$  is the atmospheric pressure (atm),  $M$  is the molecular mass of  $CH_4$  or  $CO_2$  ( $g\ mol^{-1}$ ),  $F1$  is a conversion factor of seconds to days (86,400),  $R$  is the gas constant ( $0.082057\ L\ atm^{-1}\ K^{-1}\ mol^{-1}$ ), and  $T$  is atmospheric air temperature (K).

### 2.3. Ebullitive flux measurements

Ebullitive  $CH_4$  fluxes were assessed during 24 h in a subset of 33 ponds. Three bubble traps were installed in the center of each pond. Traps consisted of a funnel (60 cm height, 50 cm diameter) attached to two buoys (empty plastic 500 mL bottles) and to a concrete block

that served as an anchor. A 215 mL glass bottle was screwed upside-down on top of the funnel. The traps were filled with water through submersion to capture bubbles in the glass bottles. After  $\sim 24$  h, the glass bottles were unscrewed and a stopper with a three-way valve was inserted in the bottle while holding it vertically upside down underwater. Subsequently, the volume of the gas phase was determined by adding the required amount of water to fill the bottle. The ebullitive  $CH_4$  flux was calculated by multiplying the total gas volume with a  $CH_4$  concentration of 48% [based on recent measurements in fish ponds in the same area ( $n = 22$ ) (Barbosa et al., in preparation<sup>1</sup>)] and dividing it by the area of the funnel and time of deployment.

### 2.4. Water sampling and processing

Water samples were taken in triplicate from the water surface in both the margin and the center of each pond. Thirty milliliter of water and 10 mL of atmospheric air were collected in a syringe,

<sup>1</sup> Barbosa, I., Kosten, S., Muzitano, I. S., Nasário, J., Almeida, R. M., Mendonça, R., et al. (in preparation). Greenhouse gas ( $CH_4$ ,  $N_2O$ , and  $CO_2$ ) emissions from fishponds in Brazil: factors determining spatial and temporal variation.

which was closed and shaken vigorously for 1 min to equilibrate gas and water CH<sub>4</sub> concentrations. Subsequently, 3 mL of gas from the headspace was transferred to a second syringe. This sample was injected into the MGGGA in a custom-made setup. This setup was composed of a CO<sub>2</sub> filter (a tube containing soda lime) followed by a three-way valve used as the sample inlet, which was then connected with gastight tubing to the MGGGA inlet. The CH<sub>4</sub> concentrations (*c*<sub>tot</sub>) in the water phase were calculated as follows:

$$c_{tot} = (c_{gas} * V_{gas} - c_{atm} * V_{gas} + c_{water} * V_{water}) / V_{water}$$

Where *c*<sub>gas</sub>, *c*<sub>water</sub>, and *c*<sub>atm</sub> are the CH<sub>4</sub> concentrations (mg L<sup>-1</sup>) in the gas and water phase, and in the atmosphere during water sampling (as measured by the GGA) and *V*<sub>gas</sub> and *V*<sub>water</sub> are the volumes (L) of the gas and water phase, respectively. The concentration in the water phase was calculated according to Sander (2015):

$$c_{water} = c_{gas} * k_H^{cc}$$

Henry's volatility *k*<sub>H</sub><sup>cc</sup> is calculated accordingly:

$$k_H^{cc} = k_H * R * T$$

Where *k*<sub>H</sub> (mol L<sup>-1</sup> atm<sup>-1</sup>) is calculated as follows:

$$k_H(T) = k_H^\theta * e^{\frac{-D \ln k_H}{d(T-1)}} * \left( \frac{1}{273+T} - \frac{1}{294.15} \right)$$

Where *k*<sub>H</sub><sup>θ</sup> is Henry's constant (for CH<sub>4</sub>: 1.4\*10<sup>-3</sup> mol L<sup>-1</sup> atm<sup>-1</sup>),  $\frac{-D \ln k_H}{d(T-1)}$  is 1,600 K.

One additional water sample was taken at the water surface of both the margin and the center of each pond to determine Chlorophyll (*Chl a*) on the same day. A PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany) was used to determine cyanobacteria and green and brown algae concentrations. We used the sum of cyanobacteria and green algae *Chl a* in our analyses, as the signal for brown algae *Chl a* is easily distorted by the presence of humic acids or dead algae (Jakob et al., 2005).

In the center of the ponds in which ebullition was determined (*n* = 31 ponds), we took a sample of the entire water column using a custom-made integrated water sampler consisting of PVC tube with a valve at the bottom, which could be closed underwater by pulling a rope. Water samples were divided into two 10 mL subsamples and stored at -20°C and, after adding 0.1 mL of 65% nitric acid for preservation, at 4°C, until further analysis.

## 2.5. Sediment sampling and processing

Sediment was taken from the center in all ponds where ebullition was assessed, in case sediment was present (*n* = 27). A Van Veen sediment grab (Eijkelkamp Soil & Water, Giesbeek, The Netherlands) was used to sample the upper 5–10 cm of sediment, which was placed in vials and stored at 4°C until further processing.

To determine bioavailable nutrients, extractions were carried out using 17.5 g of fresh sediment and 50 mL of demineralized water. After 120 min of incubation on a shaker at 105 rpm,

fluid was extracted into a pre-vacuumed syringe connected to a rhizon sampler (Rhizosphere Research Products, Wageningen, The Netherlands). After extraction, samples were divided into three 10 mL subsamples and were either stored at -20°C or stored at 4°C or acidified [adding 0.1 mL of 65% nitric acid (HNO<sub>3</sub>)] and stored at 4°C, until further chemical analysis (see below). Subsamples of a known volume of fresh sediment were dried at 70°C for 48 h to determine water content and bulk density.

## 2.6. Sediment incubations

Sediment incubations were carried out to quantify sediment CH<sub>4</sub> production potential. Based on the measured *in-situ* CH<sub>4</sub> emission rates, a subset of 20 ponds was selected to cover the entire range of emissions, including the most heterogeneous values. For each of the selected ponds, ~10 g of surface sediment and 30 mL of demineralized water were inserted in a 100 mL glass bottle, using four replicates per pond (*n* = 80 bottles in total). In one replicate per pond, we glued a non-intrusive planar oxygen-sensitive spot (PreSens Precision Sensing GmbH, Regensburg, Germany) to the inner bottle wall, which could be read out using a compact fiber optic oxygen meter (OXY-1 SMA, PreSens Precision Sensing GmbH, Regensburg, Germany). After closing with a rubber stopper, bottles were flushed with nitrogen gas (N<sub>2</sub>) for 1 h to create anoxic conditions. We confirmed anoxia by reading out the O<sub>2</sub> sensor spots. Bottles were covered in aluminum foil to prevent algal growth and were kept at room temperature. Twice a week, we measured the CH<sub>4</sub> concentrations in the headspace of the bottles. Prior to each measurement, bottles were gently swiveled to equilibrate CH<sub>4</sub> concentrations in the headspace and water phase. In each bottle, we took a 3 mL sample from the headspace using a syringe, which was injected in the MGGGA according to the method described above. Subsequently, we added 3 mL of N<sub>2</sub> to the bottles to restore atmospheric pressure. O<sub>2</sub> concentrations were assessed in the bottles containing a sensor spot. After measurements on days 15 and 33, bottles were flushed with N<sub>2</sub> for 1 h to prevent methanogenesis inhibition by the accumulation of CH<sub>4</sub> or other gaseous metabolites in the headspace (Magnusson, 1993; Guérin et al., 2008). The incubations were continued for 43 days.

CH<sub>4</sub> concentrations in the bottles were calculated similar to concentrations in water samples (explained above), with the total amount of CH<sub>4</sub> per bottle per gram carbon determined as follows:

$$CH4_{tot} = (c_{gas} * V_{gas} + c_{water} * V_{water}) / TC$$

Where *V*<sub>gas</sub> and *V*<sub>water</sub> are the volumes (L) of the gas and water phase, respectively, and TC is the amount of TC in a specific bottle (g). The headspace volume was determined by subtracting the volume of the sediment, calculated from its weight and sediment-specific bulk density, from the total volume of the incubation bottle. CH<sub>4</sub> production rates were calculated as follows:

$$P = (CH4_{tot}(t) - CH4_{tot}(t-1)) / \Delta t$$

Where *P* is the production rate during a specific time interval (mmol g C<sup>-1</sup> d<sup>-1</sup>), CH<sub>4</sub><sub>tot</sub>(*t*-1) is the CH<sub>4</sub> concentration at the previous measurement, and Δ*t* is the time between measurements

(d). Maximum CH<sub>4</sub> production rates ( $P_{\max}$ ) were calculated according to Grasset et al. (2019), using a simple logistic model.

## 2.7. Chemical analyses

Ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), and phosphate (PO<sub>4</sub><sup>3-</sup>) concentrations in the sediment extraction and surface water subsamples stored at -20°C were determined by colorimetric methods (Auto Analyser III, Bran and Luebbe GmbH, Norderstedt, Germany). In the acidified subsamples, P and iron (Fe) were measured using inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo Fischer Scientific, Bremen, Germany).

Total carbon (TC) was measured using 150 mg of dry sample, by high-temperature catalytic oxidation in a Shimadzu TOC analyzer equipped with a solid combustion system (TOC/L ASI-L, SSM 5000).

## 2.8. Statistical analyses

Statistics were performed using R version 4.0.3 (R Core Team, 2020) and the package psych (Revelle, 2023). Figures were made using ggplot2 (Wickham, 2016). Replicates of CH<sub>4</sub> ebullition rates, diffusion rates and CH<sub>4</sub> concentrations were averaged for each pond per location within the pond (center or margin). Paired samples *t*-tests were performed to compute the difference between ebullition and diffusion (after log-transformation of both variables) and between margin and center diffusive emissions and CH<sub>4</sub> concentration. Relations between CH<sub>4</sub> diffusion, ebullition, and  $P_{\max}$  were tested using linear regression. Model assumptions were validated using Q-Q plots, variance plots, and Shapiro-Wilk tests. Principal component analysis (PCA) was used to explore relationships between environmental variables and CH<sub>4</sub> emissions from pond centers. As diffusion and ebullition datasets differed in size, and as we expected different drivers, with diffusion and concentration mostly driven by water phase parameters, and ebullition and  $P_{\max}$  by sediment parameters, we carried out two separate PCAs. The PCA covering CH<sub>4</sub> diffusion and concentration included explanatory variables surface water pH, O<sub>2</sub>, Chl *a* concentration, N (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>), PO<sub>4</sub><sup>3-</sup>, pond depth and pond surface area. For the model of ebullition and  $P_{\max}$ , we included sediment pH, N, and Fe concentrations, depth, and surface area. Variables were log-transformed if skewness exceeded 2 or if the min-max ratio was below 0.1 (Sobek et al., 2007). PCA results were visualized in biplots.

## 3. Results

### 3.1. Ebullition was the main CH<sub>4</sub> emission pathway

Ebullitive CH<sub>4</sub> emissions ranged between 0 and 477 mg m<sup>-2</sup> d<sup>-1</sup> (median: 76 mg m<sup>-2</sup> d<sup>-1</sup>, average: 115 mg m<sup>-2</sup> d<sup>-1</sup>), and exceeded diffusive emissions in 25 out of 31 ponds (Figure 2). Ebullitive emissions were significantly higher than diffusive

emissions (paired samples *t*-test,  $df = 29$ ,  $t = 4.21$ ,  $p < 0.001$ ). Ebullition contributed 0–99% to total CH<sub>4</sub> emissions (diffusion + ebullition), with a median of 85%, and an average of 72%. The highest ebullitive emissions were measured in the concrete pond, which was being drained during the measurements. Because of these conditions, this pond was excluded from further analyses. In the ponds where the sediment layer was too thin to be collected with our sediment grab, ebullition rates were low (median 13 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>,  $n = 4$ ). In several cases, within-pond spatial variation was high, with maximum 210 mg m<sup>-2</sup> d<sup>-1</sup> difference between the lowest and the highest measurement. Although the ebullitive CH<sub>4</sub> emissions in our ponds were generally high, some of the values are underestimated due to exceedance of the maximum gas volume (215 mL; nine bubble traps in three ponds, see Figure 2) during the 24 h sampling time. These ponds were excluded from further statistical analyses.

### 3.2. Diffusive CH<sub>4</sub> emissions were generally low and varied spatially

Diffusive CH<sub>4</sub> emissions from the ponds were highly variable, with average center emissions ranging from 2.2 to 86 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> (median: 11 mg m<sup>-2</sup> d<sup>-1</sup>, average: 19 mg m<sup>-2</sup> d<sup>-1</sup>,  $n = 48$ ) (Figure 3). Emissions at the margins were almost two times lower (paired samples *t*-test,  $df = 47$ ,  $t = -5.52$ ,  $p < 0.001$ ): ranging from 0.7 to 67 mg m<sup>-2</sup> d<sup>-1</sup> (median: 6.1 mg m<sup>-2</sup> d<sup>-1</sup>, average: 12 mg m<sup>-2</sup> d<sup>-1</sup>,  $n = 48$ ). Diffusive emissions were positively related to surface water CH<sub>4</sub> concentrations (linear regression,  $df = 91$ ,  $F = 50.19$ ,  $p < 0.001$ ), but not related to CH<sub>4</sub> ebullition (linear regression,  $df = 29$ ,  $F = 0.33$ ,  $p = 0.57$ ). Surface water CH<sub>4</sub> concentrations ranged between 0.002 and 15.63 μmol L<sup>-1</sup> (median: 0.42 μmol L<sup>-1</sup>, average: 1.46 μmol L<sup>-1</sup>), but did not differ between pond centers and margins (paired samples *t*-test,  $df = 45$ ,  $t = 0.74$ ,  $p = 0.46$ ).

### 3.3. Sediment CH<sub>4</sub> production

CH<sub>4</sub> production in fishpond sediments started immediately after incubation, with the highest increase in headspace CH<sub>4</sub> concentrations occurring between 22 and 33 days (Figure 4).  $P_{\max}$  ranged between 0 and 3.17 mg CH<sub>4</sub> g C<sup>-1</sup> d<sup>-1</sup>, with an average of 0.64 mg CH<sub>4</sub> g C<sup>-1</sup> d<sup>-1</sup>.  $P_{\max}$  did not correlate significantly with CH<sub>4</sub> ebullition (linear regression,  $df = 16$ ,  $t = 0.83$ ,  $p = 0.428$ ) or diffusion (linear regression,  $df = 16$ ,  $t = -1.61$ ,  $p = 0.127$ ).

### 3.4. Environmental variables differed substantially between ponds

Fishponds were highly variable in surface water O<sub>2</sub> concentration, pH and algae concentrations, indicating big differences in trophic state (Table 1). Surface water N was below 50 μmol L<sup>-1</sup> in 34 ponds, but 5 ponds had very high concentrations, up to 1,068 μmol L<sup>-1</sup>. Surface water PO<sub>4</sub><sup>3-</sup> concentrations were below 10 μmol L<sup>-1</sup> in all ponds. In the sediment, N concentrations

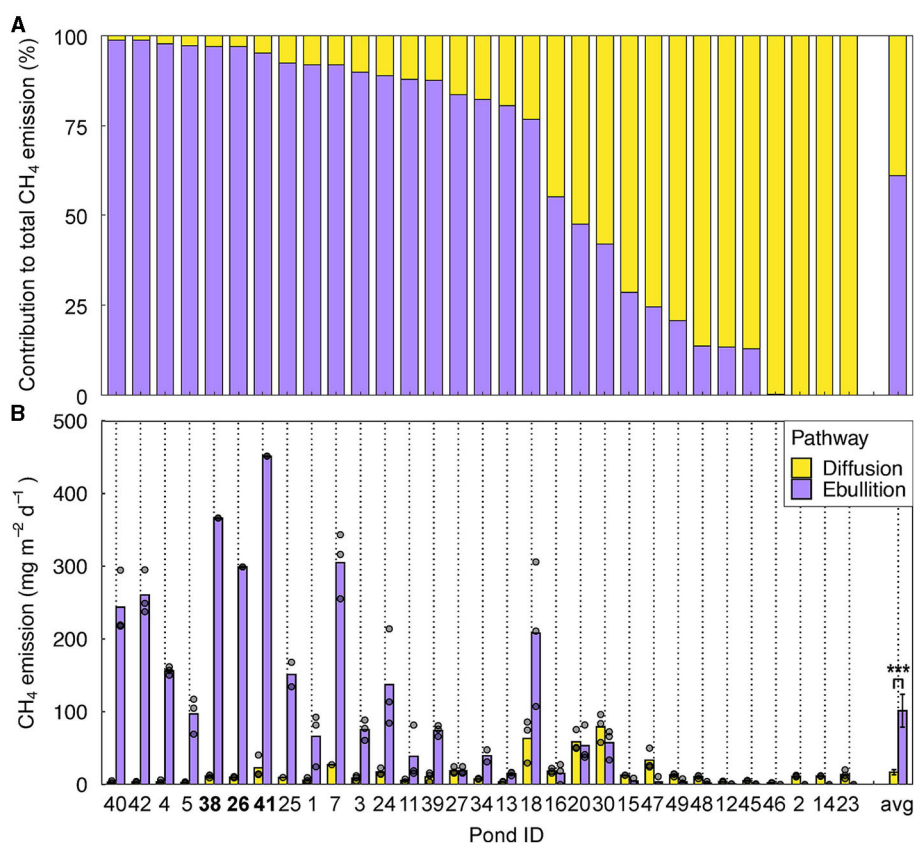


FIGURE 2

CH<sub>4</sub> diffusion and ebullition in 31 fishponds. (A) Contribution of diffusion and ebullition to total CH<sub>4</sub> emissions and (B) Diffusive and ebullitive CH<sub>4</sub> emissions measured in the center of the ponds. Bars represent average emissions. Gray dots represent individual measurements ( $n = 3$ ) and may overlap. Only ponds in which both emission pathways were quantified are depicted here ( $n = 31$ ). Bars are sorted from high to low ebullition contribution, with "avg" representing the averages over all ponds, error bars depicting standard errors, and \*\*\* representing significance level  $p < 0.001$  (paired samples  $t$ -test). The pond IDs printed in bold are the ponds where the ebullitive flux may have been underestimated due to exceedance of the maximum capacity of the bubble traps.

were generally high, up to  $4,526 \mu\text{mol kg dw}^{-1}$ . Sediment  $\text{PO}_4^{3-}$  concentrations were nihil in all but six ponds, which had concentrations up to  $47.2 \mu\text{mol kg dw}^{-1}$ . PCA analyses (Figure 5) indicate that CH<sub>4</sub> ebullition was positively correlated to sediment N concentration, and  $P_{\text{max}}$  was correlated negatively to sediment Fe content and positively to sediment pH. CH<sub>4</sub> diffusion and concentration were negatively correlated to surface water pH and O<sub>2</sub> and depth.

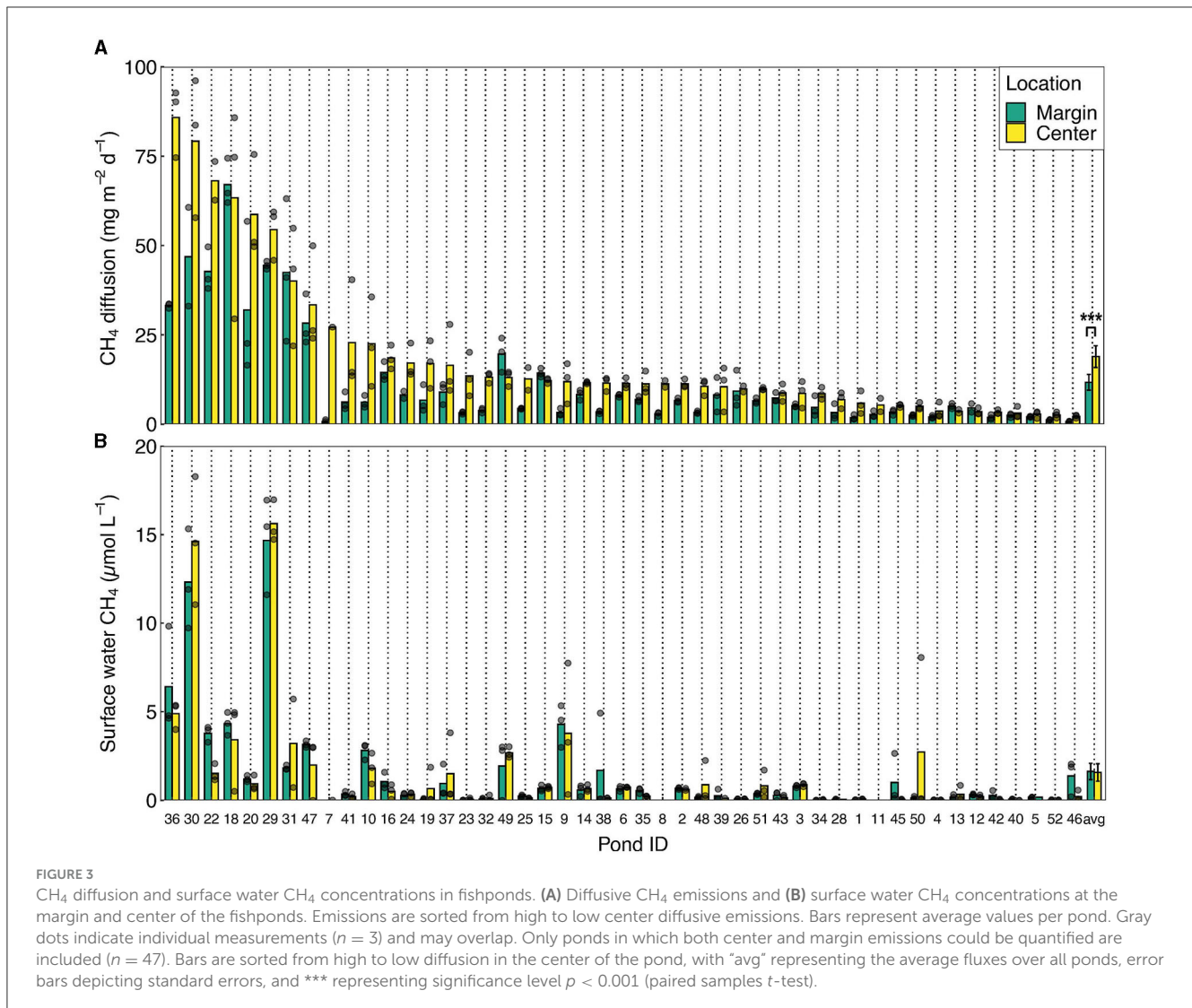
## 4. Discussion

### 4.1. Ebullition as a major CH<sub>4</sub> emission pathway in fishponds

Ebullition was the main CH<sub>4</sub> emission pathway in 81% of the fishponds in our study, contributing 85% (median) to the total CH<sub>4</sub> emission. In more than half of the ponds, the contribution of ebullition exceeded the 42–77% ebullition contribution previously found in mesocosm studies with fish (Davidson et al., 2018; Oliveira Junior et al., 2019), which were used in the recent aquaculture footprint estimate by Kosten et al. (2020). Our findings suggest that high contributions of CH<sub>4</sub> ebullition to total emissions identified

in previous studies on freshwater fishponds (Flickinger et al., 2020; Zhao et al., 2021; Waldemer and Koschorreck, 2023; Yang et al., 2023) are not exceptional, and dominance of ebullition over diffusion may indeed be widespread. However, we also found fishponds with very low ebullition rates, corresponding with very low total CH<sub>4</sub> emissions.

The contribution of ebullition appears to be higher in our fishponds than in most other freshwater ecosystems (40–60% contribution) (Bastviken, 2009). Although our temporal resolution was only 24 h per pond, our findings were consistent over a large number of ponds sampled during several weeks in locations with similar weather conditions. Our dataset therefore likely includes both hot and cold moments, making it representative for at least the summer months. Furthermore, recent studies found similarly high ebullition contributions in other aquaculture systems (Flickinger et al., 2020; Yuan et al., 2021; Fang et al., 2022). Ebullitive rates can be high in these shallow ponds due to low hydrostatic pressure facilitating bubble release and a short residence time for CH<sub>4</sub> oxidation in the water column (e.g., Bastviken, 2009; Natchimuthu et al., 2016). Furthermore, bubble formation is promoted by high sedimentation rates of reactive organic matter (Sobek et al., 2012) which often occur in fishponds due to the high feed input and



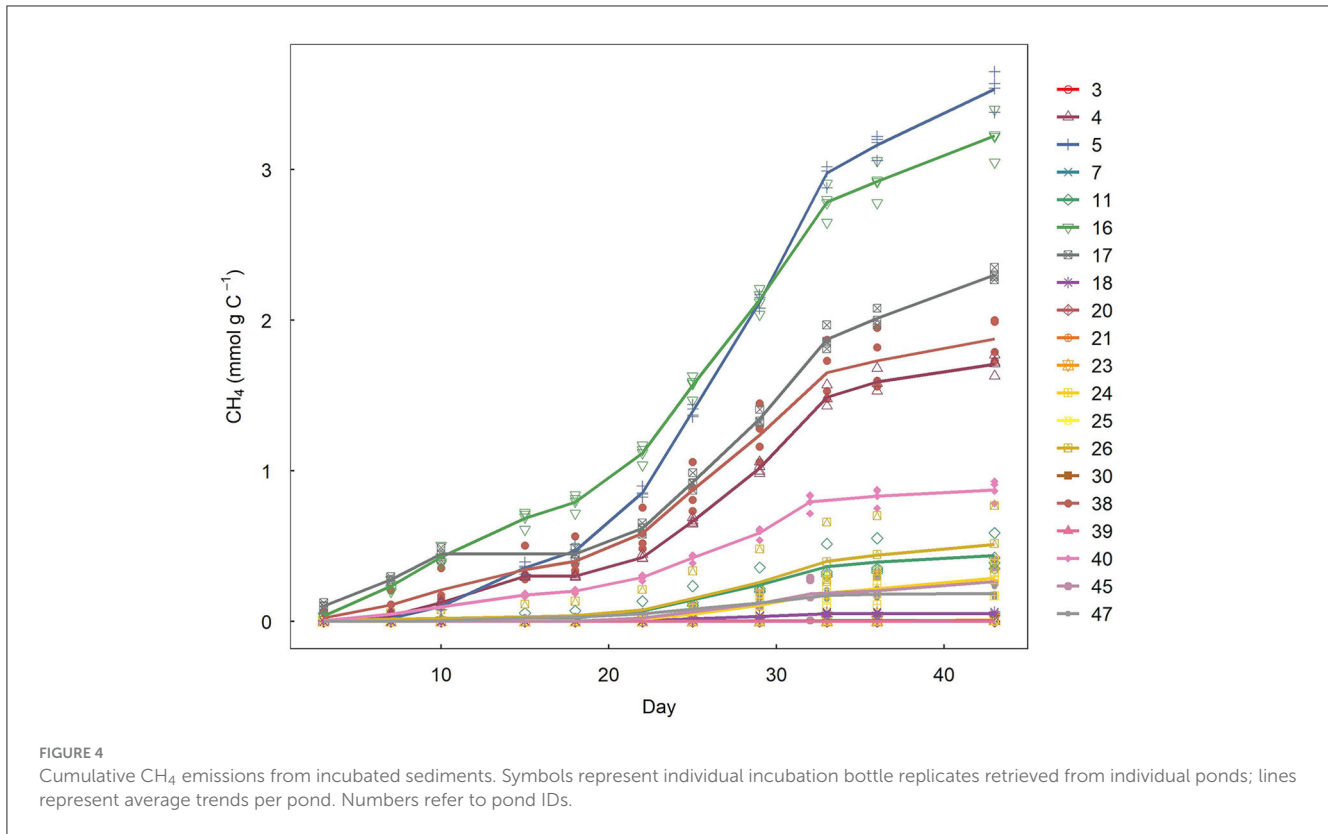
algae growth. Additionally, our ponds were situated in a tropical climate, where high temperatures, promoting microbial activity, sediment anoxia, and stratification, may even further exacerbate CH<sub>4</sub> ebullition (Holgerson et al., 2016). As ebullition has been observed to increase faster with temperature than diffusion, its contribution to total CH<sub>4</sub> emissions is expected to be higher in (sub)tropical regions (Aben et al., 2017).

## 4.2. Fishpond CH<sub>4</sub> emissions and environmental variables

The positive correlation of CH<sub>4</sub> ebullition with sediment N concentrations may point at an earlier reported (Deemer et al., 2016; DelSontro et al., 2016; Davidson et al., 2018; Beaulieu et al., 2019) link between eutrophication and ebullition rates. Eutrophication and a consequent increase in primary production can enhance CH<sub>4</sub> production through increased availability of organic substrate. The observation that ponds that had very little

sediment (those ponds where we were not able to collect sediment with our grab), had very low CH<sub>4</sub> ebullition is in line with the notion that that sediment accumulation could lead to enhanced CH<sub>4</sub> emissions.

Average CH<sub>4</sub> diffusive fluxes of  $19 \text{ mg m}^{-2} \text{ d}^{-1}$  in our pond centers were very low compared to the average of  $106 \text{ mg m}^{-2} \text{ d}^{-1}$  reported for semi-intensive aquaculture systems (Yuan et al., 2019), suggesting lower CH<sub>4</sub> production rates or higher oxidation rates in our systems. Daytime surface water O<sub>2</sub> concentrations in our ponds were indeed high, and negatively correlated to CH<sub>4</sub> diffusion and concentration, which would support the possibility of water column CH<sub>4</sub> oxidation as well as CH<sub>4</sub> oxidation in the upper layers of the sediment. The negative correlation of surface water CH<sub>4</sub> concentration and diffusion with water depth corroborates with previous findings in small ponds (Holgerson and Raymond, 2016). At a given gas exchange velocity, a shorter distance between the sediment (where most CH<sub>4</sub> is generally produced) and the water surface entails a shorter residence time of CH<sub>4</sub> in the water column and therefore less time for the CH<sub>4</sub> to be oxidized (Bastviken et al., 2008).



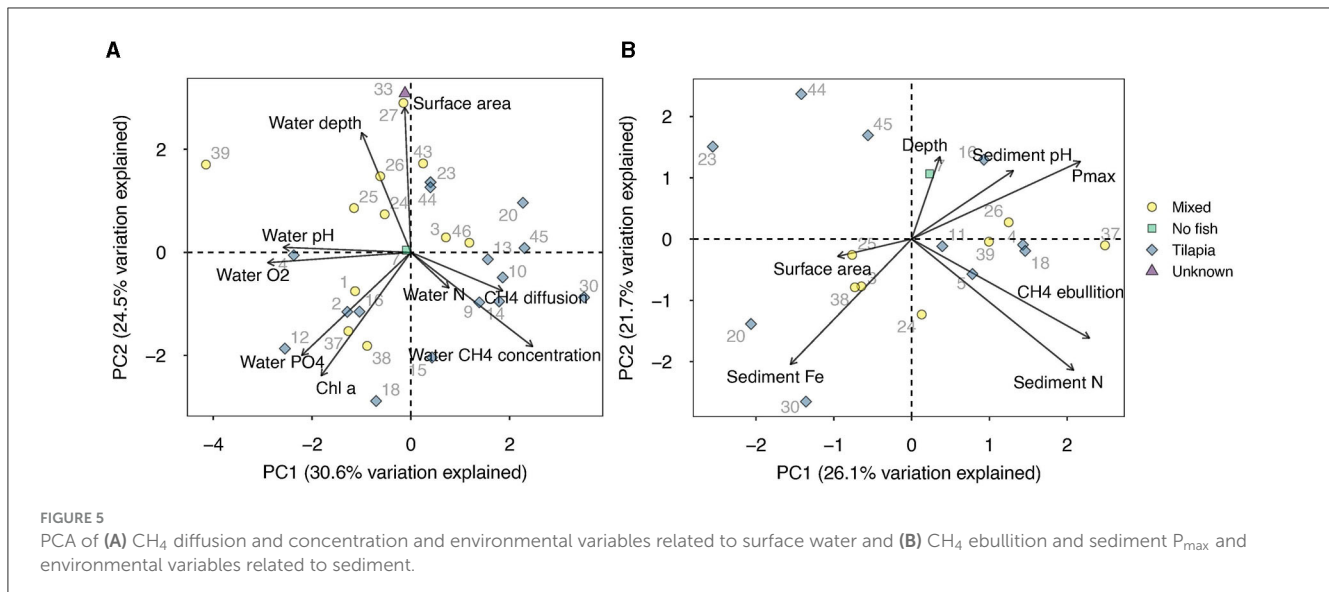
**TABLE 1** Summary of environmental variables measured in the fishponds.

Location	Variable	Unit	<i>n</i>	Min	Max	Median	Average	<i>SD</i>
Center	Surface water pH	-	51	5.78	9.26	6.6	7.07	0.99
	Surface water O <sub>2</sub>	mg L <sup>-1</sup>	45	0.65	15.19	8.15	8.05	3.27
	Surface water Chl <i>a</i>	μg Chl L <sup>-1</sup>	52	0	1,170	22.92	90.31	180.3
	Surface water N	μmol L <sup>-1</sup>	39	0	1,068	5.8	77.34	219
	Surface water PO <sub>4</sub> <sup>3-</sup>	μmol L <sup>-1</sup>	39	0	9.3	0.5	1.36	2
	Surface water temperature	°C	44	22.2	32.9	26.0	25.9	2.1
Margin	Sediment N	μmol kg <sup>-1</sup> dry weight	27	20.9	4,526	960.6	1,182	1,026
	Sediment PO <sub>4</sub> <sup>3-</sup>	μmol kg <sup>-1</sup> dry weight	27	0	47.2	0	3.15	9.64
	Sediment Fe	μmol kg <sup>-1</sup> dry weight	27	0	2,413	5	114	461.5
	Sediment TC	% of dry weight	27	1.2	6.73	3.41	3.28	1.13
	Surface water pH	-	49	6	9.52	6.6	7.04	1.01
	Surface water O <sub>2</sub>	mg L <sup>-1</sup>	39	1.67	15.22	7.85	7.66	3.01
	Surface water Chl <i>a</i>	mg Chl L <sup>-1</sup>	48	0	1,118	21.95	95.34	184.9
Surface water temperature	°C	40	22.0	32.0	25.8	25.8	2.3	

Sediment CH<sub>4</sub> production potential varied widely between ponds, but did not correlate with *in situ* CH<sub>4</sub> emissions. This underlines the importance of processes occurring *in situ*, such as

surface water characteristics (e.g., O<sub>2</sub> availability) and fish activity. A negative link between P<sub>max</sub> and sediment Fe concentration could be explained by the occurrence of Fe reduction in iron rich





sediments, which is energetically favorable over methanogenesis (Acht nich et al., 1995; Struik et al., submitted<sup>2</sup>).

#### 4.3. Spatial variation should be taken into account when measuring CH<sub>4</sub> emissions

Spatial variation in CH<sub>4</sub> diffusion, with higher emissions from the center than at the margin of ponds, may be explained by differences in sediment depth and sediment particle size distribution. Some ponds contained very little sediment, and these also tended to have low total CH<sub>4</sub> emissions. Our ponds were generally slightly deeper in the center than in the margins, which leads to higher sediment accumulation in the center. Furthermore, specifically the finer sediment particles tend to accumulate at the center of a pond (Boyd, 1995), resulting in a higher surface area for microbial biofilm formation (Sanchez et al., 1994). Similar spatial differences in CH<sub>4</sub> emissions from feeding zones, aeration zones and margins were found in other aquaculture studies (Yang et al., 2020; Waldemer and Koschorreck, 2023). Spatial variation in sediment depth may also be created by species-specific burrowing and nesting activities of fish, distinct feeding zones, and the location of the pond's water inlets and outlets (Boyd, 1995). Furthermore, sedimentation rates depend on pond age, trophic state, carbon content of the inlet water, drainage frequency, feeding, perimeter-to-surface area ratio and runoff intensity (Boyd, 1995; Brainard and Fairchild, 2012; Holgerson and Raymond, 2016). Measuring CH<sub>4</sub> diffusion from only the margin of a pond, which would be the easier option from a logistic viewpoint, could lead to a significant underestimation of whole-pond emissions. For instance, if a pond of 500 m<sup>2</sup> (a representative size for our dataset) has a 1-m margin, and the emission in the center is 60% higher than at the margin

(the average difference in our measurements), measuring diffusion exclusively at the margin would result in a ~50% underestimation of the whole pond's actual diffusive CH<sub>4</sub> emission.

#### 4.4. Implications and recommendations

Farmed fish are currently estimated to have a carbon footprint of 8.8–13.2 kg CO<sub>2</sub>-eq kg of protein<sup>-1</sup>, which is much lower than that of pork (43 kg CO<sub>2</sub>-eq kg of protein<sup>-1</sup>) or beef (140 kg CO<sub>2</sub>-eq kg of protein<sup>-1</sup>) (Pelletier and Tyedmers, 2010; Robb et al., 2017; Hilborn et al., 2018). This footprint estimate, however, is merely based on emissions from infrastructure, and does not include water-atmosphere GHG emissions. A recent conceptual paper added CH<sub>4</sub> diffusion and a hypothetical 42–77% contribution of CH<sub>4</sub> ebullition to total CH<sub>4</sub> emissions (Kosten et al., 2020). This resulted in an aquaculture fish footprint ranging between 48 kg CO<sub>2</sub>-eq kg of protein<sup>-1</sup> (similar to pork) and 111 kg CO<sub>2</sub>-eq kg of protein<sup>-1</sup> (80% of the beef footprint). Our ebullition results further widen the ebullition contribution range (0–99%), unveiling a lot of variation between ponds, regardless of their management type. On the one hand, this implies that a 42–77% contribution may be an underestimation. On the other hand, it shows that low carbon emission aquaculture is possible, which emphasizes the need to understand the drivers and management practices related to CH<sub>4</sub> ebullition in fishponds.

Our study captures only a snapshot of each pond, and detailed information about pond management was frequently lacking. Presumably, fish activity considerably influences carbon dynamics, and varies with fish species, density, size, and life stage (e.g., Rahman, 2015; Rutegwa et al., 2019). Furthermore, we may have captured specific hot or cold moments, due to the stochastic, weather-dependent nature of CH<sub>4</sub> ebullition. We therefore recommend GHG emission monitoring during the entire production cycle, including pond draining, dredging, and refilling events. Ebullition, the main pathway of fishpond CH<sub>4</sub> emissions, should be considered, if not focused on, in all future studies. As

<sup>2</sup> Struik, Q., Paranaiba, J. R., Glodowska, M., Kosten, S., Meulepas, B., AB, R.-M., et al. (Submitted). *Fe(II)Cl<sub>2</sub> Simultaneously Mitigates Eutrophication and Greenhouse Gas Production Through Iron-Dependent Anaerobic Oxidation of Methane.*

both ebullitive and diffusive emissions appear to vary spatially due to sediment dynamics, a sediment distribution map could be used as a basis for a stratified sampling design. Furthermore, eddy covariance techniques may be most suitable to capture both spatiotemporal variation and both emission pathways. Climate-smart management strategies should be studied by focusing on potential trade-offs between eutrophication, CH<sub>4</sub> emission and fish production to evaluate the carbon footprint per kg of fish yield. Additionally, energy costs of potential management strategies (e.g., use of aerators or excavators) should weigh up against the achieved GHG emission reduction. Finally, to create a full GHG budget, N<sub>2</sub>O emissions (Williams and Crutzen, 2010; Hu et al., 2012) and potential carbon burial as a result of photosynthesis (Boyd et al., 2010; Flickinger et al., 2020) should be taken into account. The contribution of carbon burial to the total carbon budget will depend on the ultimate fate of the sediment, as decomposition by drying or reuse as biofertilizer or biogas (Nhut et al., 2019; Drózd et al., 2020) releases stored carbon back into the atmosphere.

The reduction of GHG emissions from aquaculture by smart management practices is crucial to reduce the greenhouse gas footprint of fish as a sustainable protein source. Our study across many fishponds in a tropical system also shows that fish farming with low carbon emissions is possible. We found very low CH<sub>4</sub> emissions in some ponds, including ponds used specifically for commercial production and breeding. As several of these ponds contained very little to no sediment, the prevention of sediment accumulation appears to be an important management tool, as was also suggested by Zhao et al. (2021). Management options include the prevention of overfeeding and excess fertilization and removal of effluent from the bottom instead of the pond's surface. Furthermore, sediment could be collected in designated areas of the pond by creating depressions or by creating currents using aerators (Avnimelech and Ritvo, 2003). Sediment collection results in a lower sediment surface area and facilitates sediment removal. Aeration may further decrease CH<sub>4</sub> emissions by enhancing O<sub>2</sub> concentrations at the sediment-water interface (Oberle et al., 2019; Yuan et al., 2019), although the increased area of the water-atmosphere interface will enhance the gas exchange velocity of all GHGs (Kosten et al., 2020). In locations where fish farmers are often smallholders, the cost-effectiveness and effective dissemination of climate-smart management practices are crucial for their implementation. Implementing technologies that minimize greenhouse gas emissions and other environmental pollution may well pave the way for farmed fish as a low-footprint, economically sustainable animal protein source.

## Data availability statement

The datasets presented in this study can be found in the online repository Archiving and Networked Services (DANS) EASY at <https://doi.org/10.17026/dans-zhf-zx7m>.

## Author contributions

RV: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing—original

draft. SK: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing—review and editing. RA: Data curation, Formal analysis, Funding acquisition, Investigation, Writing—review and editing. RM: Methodology, Writing—review and editing. IM: Investigation, Resources, Writing—review and editing. IB: Investigation, Writing—review and editing. JN: Investigation, Writing—review and editing. EO: Conceptualization, Visualization, Writing—review and editing. AF: Funding acquisition, Writing—review and editing. NB: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frwa.2023.1256799/full#supplementary-material>

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