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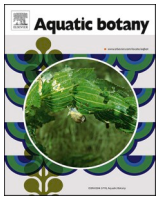

Short-term effects of macrophyte removal on emission of CO_2 and CH_4 in shallow lakes

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ABSTRACT

Mass development of macrophytes in freshwater ecosystems is today considered a worldwide problem and substantial resources are spent on macrophyte removal each year. By removing the dominant primary producer, however, this management practice radically changes the ecosystem overnight. Here, we studied short-term effects of the removal of a mass development of free-floating (*Pontederia crassipes*), submerged (*Elodea nuttallii*) and emergent (mix of *Ludwigia grandiflora* and *L. peploides*) macrophytes on fluxes of CH_4 and CO_2 in three lakes. In our field experiment, we assigned an impact site where macrophytes were removed, and a control site where vegetation remained. Before and after removal, diffusive fluxes of CO_2 and CH_4 were determined in lakes dominated by *P. crassipes* and *E. nuttallii*, whereas total emission of CH_4 was determined in all three case study lakes. Additionally, plant biomass, and physical and chemical parameters were measured before and after removal. While removal of emergent *Ludwigia* spp. showed no clear effect on total CH_4 emission, removal of submerged *E. nuttallii* reduced both CO_2 fixation and total CH_4 emission. Removal of free-floating *P. crassipes*, on the other hand, increased CH_4 fluxes and stimulated phytoplankton blooms. The lack of a universal response across our case study lakes suggests that both macrophyte life forms and environmental parameters can be important factors determining effects of removal. Additionally, indirect effects of macrophyte removal on temperature and dissolved oxygen can help to explain carbon emissions. Long-term effects should be studied to allow development of sustainable management practices.

1. Introduction

Mass developments of macrophytes frequently occur in freshwater ecosystems (Hussner et al., 2017). These mass developments not only hinder human recreational activities such as boating or swimming (Verhofstad and Bakker, 2019), but may also increase the risk of flooding of adjacent land (Boerema et al., 2014) and strongly reduce vegetation diversity (Hilt et al., 2006). Therefore, considerable resources are spent on their removal, using either chemical, biological or mechanical approaches (Hussner et al., 2017).

Although mass developments are generally monocultures that may have replaced or threaten a more diverse vegetation, they are still likely to fulfil important functions within the ecosystem. High nutrient uptake by aquatic macrophytes and their periphyton - and in some cases allelopathy - reduces the abundance of phytoplankton (van Donk and van de Bund, 2002), creating clear water conditions. Dense macrophyte stands also promote sedimentation and carbon burial (Hilt et al., 2017), thus contributing further to water clarity. Increased surface area for biofilm growth ensures higher nitrogen (N) removal through coupled nitrification and denitrification by the associated microbial community (Körner, 1999). The high surface to volume ratio of submerged macrophytes provides a large surface area for periphyton, while radial oxygen loss from rooted macrophytes can influence the sediment microbiota. This microbial community also uses root exudates and decomposing plant biomass as important sources of organic carbon and nutrients for biogeochemical reactions. Furthermore, macrophyte stands provide

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both shelter and food to many macroinvertebrates and fish species and support high biodiversity (Hilt et al., 2017).

In freshwater ecosystems with dense aquatic vegetation, macrophytes are expected to have a strong impact on the carbon (C) cycle (Reitsema et al., 2018). Mechanical removal of macrophytes, a common management practice in shallow lakes with dense aquatic vegetation, could therefore affect the fluxes of carbon dioxide (CO_2) and methane (CH_4) in the ecosystem. Macrophyte dominated lakes are often sinks for CO_2 (Kosten et al., 2012). Macrophyte removal could therefore increase CO_2 emission due to reduced primary production, possibly turning the lake into a net source of CO_2. The effect on CH_4 emission seems less straightforward and may depend on macrophyte life form. Rooted macrophytes can oxygenize the sediment, thereby reducing methanogenesis and promoting methane oxidation (Laanbroek, 2010). Their roots may, however, also form a direct pathway for CH_4 emission (via the so-called chimney effect; Bhullar et al., 2013). In systems dominated by dense mats of floating aquatic macrophytes, on the other hand, the gas exchange across the water-atmosphere interface is strongly reduced (Attermeyer et al., 2016). While this reduces the oxygen availability in the water column (Morris and Barker, 1977), thereby creating ideal conditions for methanogenesis, the release of this CH_4 may be reduced as floating leaves can 'capture' the gas bubbles (Kosten et al., 2016), while radial oxygen loss may promote CH_4 oxidation (Yoshida et al., 2014). During removal of floating vegetation, sudden release of accumulated CH_4 bubbles may therefore be expected.

A recent review by Thiemer et al. (2021) suggests that mechanical macrophyte removal can have severe negative impacts on ecosystem functioning and structure. Studies on the influence of mechanical macrophyte removal on greenhouse gas emissions, however, are lacking. In this study, we determined the short-term effects of macrophyte removal on fluxes of CO_2 and CH_4 in three shallow lakes infested with invasive macrophytes using a Before-After-Control-Impact (BACI) design. The three lakes were each dominated by macrophytes with a different life form: floating *Pontederia crassipes* (Mart.) in Hartbeespoort Dam (South Africa), submerged *Elodea nuttallii* ((Planch.) St. John) in Lake Kemnade (Germany) and a mix of emergent *Ludwigia grandiflora* and *L. peploides* at Lake Grand-Lieu (France). For each lake, we analysed the effect of macrophyte removal and local environmental conditions on the fluxes of CO_2 and CH_4. We hypothesised that net carbon emission will increase following removal and that the margin of effect will be different between lakes. In addition, we expect that removal of floating vegetation results in a stronger increase in CH_4 emission than that of submerged or emergent plants. Determining these short-term effects will be an important start to understanding how the common management practice of macrophyte removal impacts C-fluxes and ecosystem functioning in freshwater systems.

2. Material and methods

2.1. Studied lakes

Three lakes or reservoirs with mass developments of invasive macrophytes were used as case studies (Fig. 1). Hypertrophic reservoir Hartbeespoort Dam (−25° 44' 30.59"N, 27° 52' 0.59" E; area: 1850 ha; mean depth: 9 m) in South Africa has been infested by the floating macrophyte *Pontederia crassipes* (formerly known as *Eichhornia crassipes*) since the 1960s. It is considered a nuisance for recreational activities such as boating. Approximately 10 % of *P. crassipes* is removed manually each year on private initiatives along the shoreline. Additionally, biological control has been used since the early 1990 s with the following arthropods being introduced: *Neochetina eichhorniae*, *N bruchi*, *Eccritotarsus catarinensis, Niphograpta albiguttalis* and *Orthogalumna terebrantis* (Coetzee et al., 2021). The introduction of *Megamelus scutellaris* in 2018 was followed by a reduction in cover from 47 % to 5 % in the summer of 2019–2020 (Coetzee et al., 2021). Lake Grand-Lieu in France (47° 04' 60.00" N, 1° 39' 59.99" E) is a 3500 ha (6300 ha in winter) shallow lake (mean depth 0.7 m and 1.6 m in summer and winter, respectively), which is a protected bird habitat and natural reserve. The lake and its surrounding area have been invaded by two species of the emergent genus *Ludwigia* (*L. grandiflora* subsp. *hexapetala* (Hook. & Arn.) G.L. Nesom & Kartesz and *L. peploides* subsp. *montevidensis* (Spreng.) P.H. Raven) since the 1990s. To reduce the impact on native vegetation,

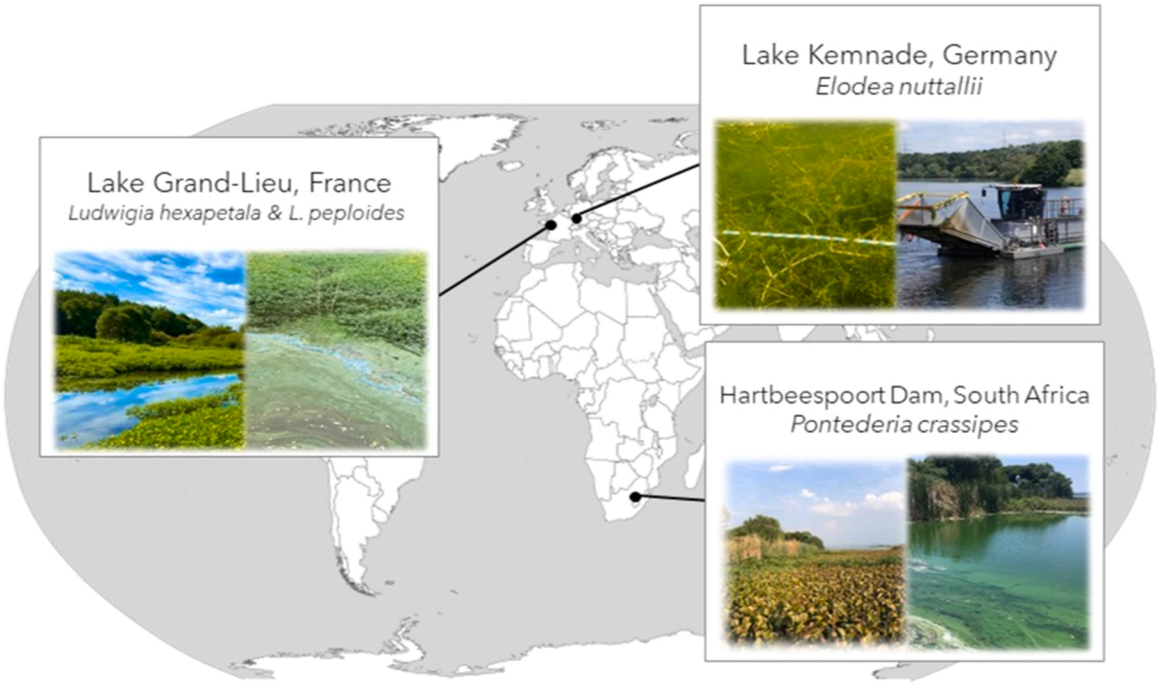

Fig. 1. Map indicating the locations of the three lakes with mass developments of invasive macrophytes. After removal of both *Ludwigia* spp. (Lake Grand-Lieu) and *P. crassipes* (Hartbeespoort Dam), blooms of cyanobacteria occurred. At Lake Kemnade, a specialised mowing boat was used to remove *E. nuttallii* throughout the summer months.

Ludwigia is manually removed every year (2020: 64 m^2; (Pierre, 2020)). Lake Kemnade in Germany (51° 25' 13.825" N 7° 15' 41.674" E) is a reservoir in the river Ruhr, with a surface area of 125 ha and a mean depth of 2.4 m. Since the early 2000s, the reservoirs in this area have seen mass development of *Elodea nuttallii,* an invasive submerged macrophyte that severely impacts recreational activities (boating, fishing, swimming) in the lake. At Lake Kemnade, *E. nuttallii* is removed annually using a specialised mowing boat, which is continuously deployed by the local water authorities between May and September. During 2020, approximately 1500 m^3 of *E. nuttallii* was removed from the lake (Ruhrverband, 2020). To prevent damage to this mowing boat, the bottom 50 cm of the lake are not mowed, thus leaving part of the mass development behind.

2.2. Experimental design

Our sampling of the three lakes was carried out in the summer of 2020 (Jan–March in South Africa; June–August in Europe), using a standardised BACI design. In each location, two plots were created in a section of the lake with homogenous, dense vegetation. In one of these plots, macrophytes were removed either mechanically or manually (impact site). Meanwhile, a similarly sized plot, located at approximately 5 m, 100 m and 30 m from the impact plot at Hartbeespoort Dam, Lake Kemnade and Lake Grand-Lieu, respectively, was assigned as a vegetated control (control site). Plot size differed between lakes, reflecting the current management practices. Plots measured 625 (depth 1.2–1.8 m), 5000 (depth 1.3–1.5 m) and 500–550 m^2 (depth 0.3–0.5 m) for Hartbeespoort Dam, Lake Kemnade and Lake Grand-Lieu, respectively. Removal took place over 2–3 days. Fluxes of CO_2 and CH_4 and environmental conditions were measured one week before and one week after macrophyte removal. Additional measurements were conducted during the 24 h immediately after removal (to determine the disturbance effects) and six weeks after removal.

2.3. Emission of methane and carbon dioxide

Diffusive fluxes of CO_2 and CH_4 (including plant-mediated CH_4 transport) were determined in-situ in Lake Kemnade and Hartbeespoort Dam, using an opaque, closed chamber connected to a portable greenhouse gas analyser (LGR-MGGA; cavity enhanced absorption greenhouse gas analyser; Los Gatos Research-ICOS, U.S.A.). Opaque rather than transparent chambers were used to avoid problems with condensation at the relatively high ambient temperatures at our lakes. While photosynthetic activity of submerged *E. nuttallii* could be approximated with this method, carbon uptake by floating *P. crassipes* could have been underestimated as its uptake of atmospheric CO_2 would be limited by shading. Diffusive fluxes could not be measured at Lake Grand-Lieu due to Covid-19 travel restrictions. Chambers had circular bases with a diameter of 40 and 30 cm and total volumes of 16 and 24 L at Lake Kemnade and Hartbeespoort Dam, respectively. Due to low water flow in Lake Kemnade, the closed chambers were not anchored and therefore free to drift next to the boat as recommended by Lorke et al. (2015). In Hartbeespoort Dam, dense cover of *P. crassipes* prevented the chambers to drift. Each chamber was therefore carefully placed over a *P. crassipes* plant and allowed to equilibrate for 10 min before connecting the GHG analyser. Chambers were aired between measurements. Measurements were repeated in 3–4 locations within the impact and the control site, and repeated multiple times a day (generally early morning, noon and late afternoon), and 1–3 times in each period (before, immediately after and one and six weeks after removal). During measurements, chambers were kept on until a clear ($R^2 > 0.9$) linear increase had been observed for approximately 5 min. The linear increase of CO_2 and CH_4 concentrations inside the chamber (in ppm) were then converted to diffusive fluxes per m^2 using the following formula

$$F_{dif} = \frac{\Delta C_i}{\Delta t} * \frac{P}{R*T} * M * \frac{V_i}{A_i} * 1000 \quad (1)$$

in which F_{dif} is the diffusive flux (mg C m^{-2} h^{-1}), $\Delta C/\Delta t$ is the change in CH_4 or CO_2 concentration (in ppm $\bullet 10^{-6}$) in the headspace of chamber *i* over time (h), P is atmospheric pressure (in atm.), R is the gas constant (L * atm / mol * K), T is temperature (K), *M* is the molar mass of carbon (g mol^{-1}) and V_i (L) and A*i* (m^2) are the volume and area of chamber *i*, respectively.

Total daily fluxes of CH_4 (including diffusion, ebullition and plant-mediated CH_4 transport) were determined at all lakes by placing opaque closed chambers ($n = 4$ at Lake Grand-Lieu and Hartbeespoort Dam; $n = 5$ at Lake Kemnade; same dimensions as described above) in the impact and control sites, before and after vegetation removal. Chambers rather than commonly used funnels (but see (Cole et al., 2010; Peixoto et al., 2016)) were used to be able to cover the vegetation, and thus include plant-mediated CH_4 transport. Since some emergent species switch from convective to diffusive gas transport during dark periods (Chanton et al., 1993), using opaque chambers may have underestimated plant-mediated CH_4 transport by *Ludwigia*, although Brix et al. (1992) could not detect convective flow in *Ludwigia peploides*. Chambers were placed with open valves for 30 min to equilibrate before a background sample was collected. Valves were then closed, and after 24 h, a final headspace sample was collected. Before sampling, a 30 mL syringe was used to flush the headspace several times to ensure mixing before the actual sample was collected. The headspace samples were transferred into 3 mL gastight vials with a septum lid (Labco, High Wycombe, UK), by displacing a known amount of demineralised water from the vial. Samples were stored upside down to prevent leaking and were analysed by injection into the portable greenhouse gas analyser (described above). For this, a closed loop was created by connecting the inlet and outlet of the analyser by gastight tubing with a glass injection port in between. Samples were collected with a glass gastight syringe (Hamilton 250 μL RN syringe with 26 G removable needle) and injected into the custom-build injection port through a 12.7 mm septum (premium-non-stick BTO septum, Restek), which was replaced after every 50 samples. Samples collected at Lake Kemnade and Hartbeespoort Dam were analysed on-site within one week, while samples collected at Lake Grand-Lieu were analysed after approximately 1 month. Total CH_4 emission rates were calculated with the following formula:

$$F_{tot} = \left(\frac{C_{i,\,24} - C_{i,\,0}}{\Delta t} * \frac{P}{R*T} * M * \frac{V_i}{A_i} * 1000\right) + (k*(\overline{C}_h - C_w)*\alpha) \quad (2)$$

where F_{tot} is the total flux of CH_4 emitted to the atmosphere (mg CH_4-C m^{-2} h^{-1}), $C_{i,\,24}$ and $C_{i,0}$ are the concentrations (ppm $\bullet 10^{-6}$) of CH_4 in the headspace of chamber *i* at 24 and 0 h, respectively, Δ t is the exact time that the chamber was deployed (approx. 24 h), P is atmospheric pressure (atm.), R is the gas constant (L * atm / mol * K), T is temperature (K), *M* is the molar mass of carbon (g mol^{-1}) and V_i (l) and A_i (m^2) are the volume and area of chamber *i*, respectively. Fluxes were excluded (8 out of 123 measurements) when obvious disturbance had been noted in the field (e.g. chambers were not sealed properly on return). When headspace CH_4 concentrations in the floating chambers exceeded concentrations in the water layer, CH_4 may diffuse back into the water layer. The second term of Eq. 2 therefore applies a correction to account for the potential underestimation of the total fluxes (similar approach to (Oliveira-Junior et al., 2018), where *k* is the gas transfer velocity (set to 0.05 m d^{-1} as wind impact was strongly reduced within the floating chamber), C_h is the average CH_4 concentration in the headspace of the chamber and C_w is the dissolved CH_4 concentration in the water. The dissolved CH_4 concentration in the water (C_w) was determined in water samples that were collected separately by carefully filling 3 mL gastight vials completely with lake water before the start of the total flux measurements. After displacing 1 mL of water with N_2 gas and equilibrating, the CH_4 concentration in the headspace was measured by injecting into

the inlet port in the MGGA greenhouse gas analyser as described above, after which, the Bunsen coefficient (using the formula and constants from Yamamoto et al. (1976) at ambient temperature in K) was used to determine the dissolved CH_4 concentration. The loss of CH_4 by diffusion from the headspace into the water layer made up approximately 15 %, 23 % and 11 % of the total CH_4 flux at Lake Grand Lieu, Lake Kemnade and Hartbeespoort Dam, respectively. Finally, we estimated the contribution of ebullition to the total CH_4 emission from Lake Kemnade and Hartbeespoort Dam, by subtracting diffusive fluxes from total fluxes (assuming both fluxes included plant-mediated methane transport).

2.4. Dissolved CH_4 in the rhizosphere of P. crassipes

At Hartbeespoort Dam, acrylic dialysis chambers (Hesslein, 1976) with 20 equally spaced 10 mL sampling ports (one port per cm depth), were filled with demineralised water and closed off with a HT-Tuffryn 200 membrane (0.45 μm; GELMAN). The frames were installed just below the water surface at the impact and control sites and left for 24 h (before and after macrophyte removal), to allow equilibration of the concentrations of nutrients and elements across the membrane into the demineralised water. Samples were collected from sampling ports at 1, 6, 11, 16 and 20 cm depth by careful pipetting and transferred to gastight vials (filled completely and fixed with 15 μL 50 % $ZnCl_2$) for analyses of dissolved CH_4 concentrations (as described above).

2.5. Potential methane production

At Lake Kemnade and Hartbeespoort Dam, sediment incubations were carried out to determine potential CH_4 production rates of the sediment. For this, sediment was collected from the upper sediment layer (0–10 cm depth) and mixed, before being added to glass bottles (1 L DURAN GL 45 with bromobutyl rubber stoppers (DWK) at Lake Kemnade and 22 mL amber glass screwtop vials (Labsolute) fitted with magnetic screw caps with PTFE-sillicone septa (18 mm; 10 mil; Restek) at Hartbeespoort Dam). Bottles were incubated in the dark, at 20 °C at Lake Kemnade and 30 °C at Hartbeespoort Dam to reflect ambient temperature. Incubations were carried out with 150 mL and 15 mL sediment at Lake Kemnade and Hartbeespoort Dam, respectively. Bottles were filled with filtered (0.7 μm) lake water, leaving a headspace of 105 and 4 mL respectively (approximately 10–20 % in both experiments, which should minimise a lag phase in methanogenesis due to disturbance (Souto et al., 2010)), and closed off with a septum. At Lake Kemnade, additional bottles were set up containing similar amounts of sediment and 30 g FW of *E. nuttallii*. This treatment was added to study the effect of dense vegetation on net CH_4 production by either promoting (anaerobic) CH_4 oxidation or methanogenesis. After setting up the incubations, bottles were flushed with N_2 gas (OFN, grade 2; 5 mins for Hartbeespoort Dam and 20 mins for Lake Kemnade) to ensure anoxic conditions (DO concentrations <1 mg L^{-1} were measured in bottles at Hartbeespoort Dam). At Lake Kemnade, samples were collected from the bottles after 0, 2 and 20 h, using the same method as described above for total methane fluxes. To maintain constant pressure in the bottle, the extracted sample volume was simultaneously replaced by inserting anoxic, filtered (0.7 μm) lake water (obtained by flushing with OFN for 15 mins) through the septum. At Hartbeespoort Dam, the bottles were too small for repeated sampling. We therefore set up four parallel series of incubations, to allow bottles to be sacrificed after 0, 3, 22 and 46 h by injection with $ZnCl_2$ (50 %, 15 μL) to halt microbial activity after vigorous mixing. Methane concentrations were measured as described above, and potential methane production was determined from the increase in CH_4 over time and corrected for sediment dry weight. The following formula was used for this:

$$MG_i = \frac{\left(\frac{\Delta C_h * V_h + \Delta C_i * V_w * \alpha}{\Delta t}\right)}{M_s} \quad (3)$$

where MG_i representing potential methanogenesis (in nmol g DW h) in vial *i*, C_h representing the methane concentration in the headspace, V_h the volume of the headspace, V_w the volume of the water layer, α the Bunsen coefficient, t is time in hours and M_s is the dry weight of the sediment.

2.6. Environmental variables

At all locations, water samples (n = 5 per time point) were collected one week before and one week after macrophyte removal at the impact and control sites. At Lake Kemnade and Lake Grand-Lieu, additional samples were collected immediately after and six weeks after plant removal. At Lake Kemnade and Hartbeespoort Dam, sampling was repeated 2–3 times in the same week (between 9 and 11 am). At the time of sampling, pH, conductivity, water temperature and dissolved oxygen (DO) concentrations were recorded at the same locations. Water samples were fixed in the field with 2 N HCl and brought to the laboratory for analyses (samples from France and SA were transported while frozen; Lake Kemnade samples were kept at 4 °C during transport). Chlorophyll-*a* (chl-*a*) content was determined by filtering a known amount over a GF/F (Whatman; 0.7 μm) filter, which was frozen at −80 °C until analyses for content of chlorophyll-a using high-performance liquid chromatographic (HPLC, Shatwell et al., 2012). Additionally, temperature and DO concentrations (Minidot Logger, PME, U.S.A.) and relative light levels (HOBO Temperature/Light data logger, Onset, U.S.A.) were logged continuously at 20 cm below water surface and 20 cm above sediment surface at Lake Kemnade and Hartbeespoort Dam. Unfiltered water samples were analysed for total phosphorus (TP) and total organic carbon (TOC) concentrations. TP analyses were carried out photometrically after digestion with 10 N sulfuric acid and 30 % hydrogen peroxide. TOC concentrations were determined using a TOC analyser (Shimadzu TOC-LCPN with an TNM-L (Total Nitrogen Measuring unit)). Filtered samples (using 0.45 μm filters) were analysed colourimetrically for nitrate (NO_3^-) and ammonium (NH_4^+) using a continuous flow analyser (SEAL Analytical AutoAnalyzer AA3 with AACE Software 7.10).

2.7. Vegetation

At each of the three lakes, the macrophyte biomass was quantified one week before and one week after macrophyte removal. Biomass was collected from within a set quadrat (0.16 m^2) at 5 randomly chosen locations in both the impact and the control site. Harvested plant material was weighed (after shaking to remove excess water) to determine fresh weight, then oven-dried at 60 °C until stable weight. Using the quadrat size, biomass was then converted to g DW m^{-2}. At Lake Kemnade, vegetation cover and height were determined before, after and six weeks after removal. Using these data, the biomass (in g DW m^{-2}) could be estimated six weeks after removal of *E. nuttallii*.

2.8. Statistics

Differences in water chemistry parameters (pH, concentrations of NO_3, NH_4, TP, TOC, DO, chl-*a*) and macrophyte biomass between lakes were determined by one-way ANOVAs, with Tukey HSD post-hoc tests for the control sites. For the TP concentrations at Lake Grand-Lieu, we ran a Rosner's Test to identify three outliers, which were removed from the dataset. Two-way ANOVAs were used to determine the impact of macrophyte removal on the same physical and chemical parameters within each lake. Linear mixed models were used to test whether macrophyte removal impacted diffusive CO_2 and CH_4 emission and total CH_4 emission in the three lakes. Before applying models, data were checked for normality and homogeneity by visual inspection of boxplots and histograms and log-transformed when needed. Rosner outlier analyses were run on visual apparent outliers, using the EnvStat package (Millard and Kowarik, 2020), and removed when found to be significant

outliers. Models were built with Site (control or impact) and Time (before or after removal) as fixed effects. Replicate ID for each lake was included as a random effect to account for repeated sampling. To determine the effect of removal, models including the interactive term between Site and Time were compared with models where this interaction was dropped using the log likelihood ratio (LLR). Estimated marginal means were used for pairwise comparison between timepoints when the interaction between Site and Time was significant and multiple timepoints were included. 'Time of Day' was added as an additional fixed factor but only improved model fit when determining effect on CO_2 fluxes. It was therefore dropped from models describing diffusive and total CH_4 fluxes.

To determine whether potential CH_4 production rates of the sediment differed between Lake Kemnade and Hartbeespoort Dam, a Student's *t*-test was used. Similarly, the difference in potential CH_4 production between the sediment-only treatment from Lake Kemnade and the treatment containing both sediment and *E. nuttallii* was tested with a student *t*-test. Differences between depth profiles of dissolved CH_4 concentrations in the rhizosphere at control and impact sites in Hartbeespoort Dam were determined using a Linear Mixed Model, with Depth (cm), Time (before and after removal) and Site (Impact and Control) as fixed factors and Replicate ID as random factor to account for repeated sampling (in depth profile, rather than time).

Boosted regression tree (BRT) models (De'ath and Fabricius, 2016) were used to identify environmental variables that best describe patterns in diffusive CO_2 and CH_4 flux in Hartbeespoort Dam and Lake Kemnade. The set of predictor variables consisted of macrophyte biomass (gDW m^{-2}), total phosphorus (TP; μmol L^{-1}), dissolved oxygen saturation (DO; %), water temperature (°C), pH, total organic carbon (TOC; μmol L^{-1}), and chlorophyll-*a* (μg L^{-1}). Moreover, time of day was also used as a predictor variable to account for photosynthetic activity. The variables time (before, during, after removal) and site (control and impact) were likewise included in the BRTs. In the BRTs for diffusive CO_2, DO and pH were initially included, but since these are collinear and a product of macrophyte photosynthesis, both variables were excluded in the final models. A detailed description of the BRT models and results (including figures) can be found in the Supplementary Information.

All statistical analyses and graphics were performed in R version 6.3.3 (R Core Team, 2020) using the following packages: lme4 (Bates et al., 2021, p. 4), gbm (Greenwell et al., 2020), dismo (Hijmans et al., 2021) emmeans (Lenth et al., 2022), EnvStats (Millard and Kowarik, 2020) and ggplot2 (Wickham et al., 2020).

3. Results

3.1. Effect of macrophyte removal on lake characteristics

The control and impact sites had comparable amounts of biomass per m^2 before macrophyte removal (Table 1) in each of the three lakes. Mowing removed 100 %, 73 % and 100 % of macrophyte biomass in Hartbeespoort Dam, Lake Kemnade and Lake Grand-Lieu, respectively. All remaining *E. nuttallii* biomass in the impact site at Lake Kemnade was present in the bottom 50 cm due to limitations of the mowing boat. Chemical composition of the lake water differed between the three lakes, but no effect of macrophyte removal was found. Similarly, most physical parameters did not change when macrophytes were removed, except for light availability, temperature and chl-*a* concentrations. Removal of *E. nuttallii* increased light attenuation from <1–10 % reaching 1.5 m depth (data not shown). At Hartbeespoort Dam, only about 1.3 % of global radiation penetrated the *P. crassipes* canopy (data not shown). After removal, however, light attenuation increased from 1.7 m^{-1} to 2.1 m^{-1} due to phytoplankton growth. Chlorophyll-*a* concentrations in the water layer were approximately 14 times higher in the impact compared to the control site after *P. crassipes* was removed (Table 1). At Lake Grand-Lieu, chl-*a* increased at both sites six weeks after removal (Table 1), while water temperature increased from 21.3 ± 1.7 °C to 27.0 ± 1.7 °C in the impact site only after removal (data not shown).

Table 1
Lake water characteristics and dominant macrophyte biomass at the three case study sites, presented as means ± standard deviation.

Lake	Site	Time	Biomass (gDW m^{-2})	pH	NO_3^- (μmol L^{-1})	NH_4^+ (μmol L^{-1})	TP (μmol L^{-1})	TOC (μmol L^{-1})	DO (% sat)	Chl-a (μg L^{-1})
Hartbeespoort Dam	Impact	Before	972 ± 137	6.9 ± 0.4	18.8 ± 2.3	72.6 ± 1.9	27.1 ± 11.1	1218 ± 1049	5.9 ± 6.5	NA
		After	0	6.9 ± 0.2	20.6 ± 12.3	41.4 ± 41.1	27.6 ± 8.6	3222 ± 7685	70.9 ± 53.9	4108 ± 8981
	Control	Before	937 ± 383	7.6 ± 0.7	20.5 ± 1.0	74.2 ± 2.3	18.7 ± 13.2	845 ± 520	4.8 ± 4.6	NA
		After	1279 ± 320	7.7 ± 1.1	18.5 ± 9.3	15.8 ± 14.6	28.7 ± 9.1	987 ± 797	66.2 ± 48.8	295 ± 465
Lake Grand Lieu	Impact	Before	183 ± 85	NA	1.2 ± 0.7	7.6 ± 2.3	21.6 ± 3.3	2320 ± 288	NA	177.4 ± 82.1
		After	0	8.0 ± 0.5	0.1 ± 0.0	3.6 ± 0.8	17.4 ± 7.1	1935 ± 111	36.2 ± 10.6	147.6 ± 18.0
		After 6	NA	8.9 ± 0.9	8.4 ± 14.1	12.9 ± 4.5	32.2 ± 1.2	3280 ± 198	115.7 ± 34.3	229.4 ± 118.9
	Control	Before	249 ± 54	NA	1.9 ± 1.6	14.3 ± 9.2	28.5 ± 8.8	2573 ± 841	NA	111.6 ± 10.1
		After	275 ± 101	7.4 ± 0.3	0.2 ± 0.3	4.0 ± 1.2	14.5 ± 2.7	1820 ± 80	59.6 ± 13.9	93.6 ± 24.3
		After 6	NA	7.4 ± 0.3	2.3 ± 0.9	10.0 ± 2.4	35.6 ± 5.5	2618 ± 412	35.8 ± 46.4	320.6 ± 43.7
Lake Kemnade	Impact	Before	421 ± 180	10.0 ± 0.1	35.3 ± 9.0	2.5 ± 0.3	1.7 ± 0.6	483 ± 79	200 ± 3.2	30.0 ± 20.1
		After	112 ± 135	9.4 ± 0.3	24.2 ± 2.4	2.8 ± 1.1	1.8 ± 1.0	316 ± 40	152 ± 33.2	12.5 ± 4.0
		After 6	242 ± 71	NA	80.8 ± 2.3	2.0 ± 0.3	1.1 ± 0.4	285 ± 18	NA	4.0 ± 1.7
	Control	Before	591 ± 165	9.7 ± 0.9	35.7 ± 14.4	3.0 ± 0.6	2.0 ± 2.3	441 ± 75	154.9 ± 48.8	20.5 ± 18.3
		After	972 ± 276	9.0 ± 0.9	21.9 ± 7.4	1.4 ± 0.3	2.2 ± 1.3	404 ± 121	139.6 ± 53.2	31.5 ± 11.6
		After 6	479 ± 43	NA	81.4 ± 1.4	3.9 ± 0.4	1.8 ± 0.8	307 ± 19	NA	9.8 ± 10.5

3.2. Effect of removal on diffusive fluxes of methane and carbon dioxide

Removal of *E. nuttallii* and *P. crassipes* did not affect diffusive CH_4 emission in Lake Kemnade and Hartbeespoort Dam (Fig. 2), which ranged from 0.2 to >10 mg C m^{-2} h^{-1} (median 1.07) and from 0.1 to >15 mg C m^{-2} h^{-1} (median 1.24), respectively. The BRTs showed that diffusive CH_4 emissions from both lakes were best explained by water temperature (both 29%) and DO concentrations (23–30 %) (Supplementary Information 1). At Lake Kemnade, both impact and control site showed net CO_2 fixation during daytime, with fluxes of approximately −10 to −80 mg C m^{-2} h^{-1} (Fig. 3). Fixation was higher in the control (median −59 mg C m^{-2} h^{-1}) than in the impact (median −38 mg C m^{-2} h^{-1}) site. Time of day had a strong influence on CO_2 fluxes ($p = 0.008$, LLR = 7.0, df = 1), with fluxes becoming more negative from morning to late afternoon in both control and impact site (indicating increased C-fixation). Immediately after removal of *E. nuttallii*, CO_2 fluxes increased rather than decreased during the day. This 3-way interaction was only a trend ($p = 0.075$, LLR = 6.9, df = 3), and 1 week after removal, no differences were observed in daily CO_2 patterns between impact and control site. This effect of removal contrasts with observations at Hartbeespoort Dam (Fig. 3). Here, daytime CO_2 fluxes were very high before removal (100–300 mg C m^{-2} h^{-1}). After removal of *P. crassipes*, the impact site showed negative daytime fluxes (median −9.4 mg C m^{-2} h^{-1}), indicating photosynthetic activity of phytoplankton, while the control site remained a net CO_2 source (105 mg C m^{-2} h^{-1}; $p < 0.001$, LRR = 18.9, df = 1). The BRTs also showed contrasting results for the two lakes. At Lake Kemnade, CO_2 fluxes were best explained by water temperature (23 %), time-of-day (14 %) and macrophyte biomass (13.5 %), whereas in Hartbeespoort Dam water temperature (33 %), TOC (30 %) and chl.*a*. (22 %) explained most variation in CO_2 fluxes.

3.3. Effect of removal on total fluxes of methane

Removal of *Ludwigia* from Lake Grand-Lieu showed no clear effect on total CH_4 emission (Fig. 4). Compared to the Lake Kemnade and Hartbeespoort Dam, total emission of CH_4 was high, with rates between 3.5 and 48 mg C m^{-2} h^{-1}. Although fluxes were lower in the impact site compared to the control site, this difference already existed before removal and could not be attributed to presence or absence of *Ludwigia*. After removal, the total CH_4 flux seemed to decrease in the control site while remaining the same in the impact site, whereas at six weeks after removal, fluxes had increased in both impact and control site.

At Lake Kemnade, total CH_4 emission in the impact site was reduced following the removal of submerged *E. nuttallii* ($p = 0.01$, $LLR_{interaction}$ = 10.9, df = 3; Fig. 4). Fluxes dropped from 2.6 to 1.1 mg C m^{-2} h^{-1} (a decrease of 58 %) immediately following removal, and were lower than in the control site both immediately ($p = 0.009$; *estimated marginal means*) and one week ($p = 0.09$; *estimated marginal means*) after removal. While total emission in the impact site returned to approximately 2.6 mg C m^{-2} h^{-1} one week after removal, the control site meanwhile showed an increase from 8.3 to 10.4–12.2 mg C m^{-2} h^{-1} (an increase of 24–47 %). This increase at the control site was likely correlated with an increase in average water temperature from 22° to 26 °C in this period. Six weeks after removal, in early autumn, rates had dropped again to approximately 1.4 and 2.0 mg C m^{-2} h^{-1} at the impact and control site, respectively. At the control site, the contribution of ebullition to the total flux was 62–85 % (Table 2). At the impact site, ebullition accounted for 84 % before removal, but immediately and one week after, this contribution was brought down to 0 %. After six weeks, ebullition again constituted about 80 % of the total flux at the impact site. The lowest total CH_4 fluxes were recorded at Hartbeespoort Dam (Fig. 4). Here, fluxes in *P. crassipes* mats ranged from 0.01 to 2.20 (median 0.8) mg C m^{-2} h^{-1}. Macrophyte removal increased the total flux to approximately 0.6–9.0 (median 2.2) mg C m^{-2} h^{-1} ($p = 0.023$, LLR = 5.2, df = 1), while fluxes in the control site remained unchanged. This increase in total flux was mainly due to ebullition, which did not add to the CH_4 emission before removal but accounted for about 60 % of the flux one week after *P. crassipes* was removed (Table 2). Simultaneously, removal of *P. crassipes* reduced the concentration of dissolved CH_4 along a depth gradient in the top 20 cm of the water layer ($p = 0.006$, F= 8.1, df=1). With an intact floating mat, dissolved CH_4 ranged from 159 ± 112 nmol L^{-1} in the top 5 cm to 106 ± 137 nmol L^{-1} around 20 cm depth, whereas after *P. crassipes* removal, concentrations ranged from 28 ± 26–65 ± 32 nmol L^{-1} at 5 and 20 cm depth, respectively (Supplementary Information, Fig. S-3).

3.4. Potential methane production

Potential CH_4 production rates in sediments were higher at Hartbeespoort Dam (4.5 ± 2.0 nmol g DW^{-1} h^{-1}) than at Lake Kemnade (1.1 ± 0.5 nmol gDW^{-1} h^{-1}; $p = 0.008$, F = 7.84, df = 2). (Fig. 5). At Lake Kemnade, presence of *E. nuttallii* doubled the potential CH_4 production ($p = 0.015$, F = 9.55, df = 1). Using the potential CH_4 production (per L sediment used in the incubations) and assuming an active

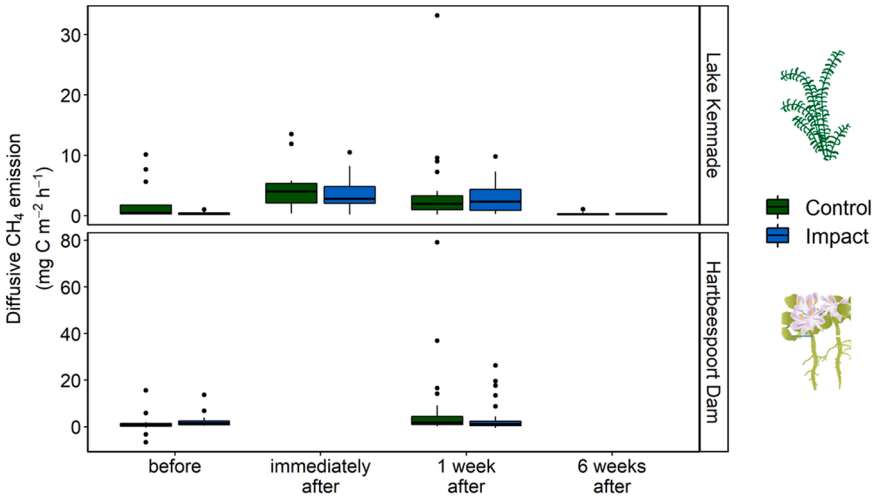

Fig. 2. Diffusive flux of methane from Lake Kemnade (top; n.s.) and Hartbeespoort Dam (bottom; n.s.), before and after removal of macrophytes (*Elodea nuttallii* and *Pontederia crassipes*, respectively). At Lake Kemnade, fluxes were also measured immediately after removal and six weeks after. Mind the different scales on the y-axis. Horizontal bold lines indicate the median, boxes the 25 % and 75 % percentiles, and whiskers the minimum and maximum values. Points represent outliers.

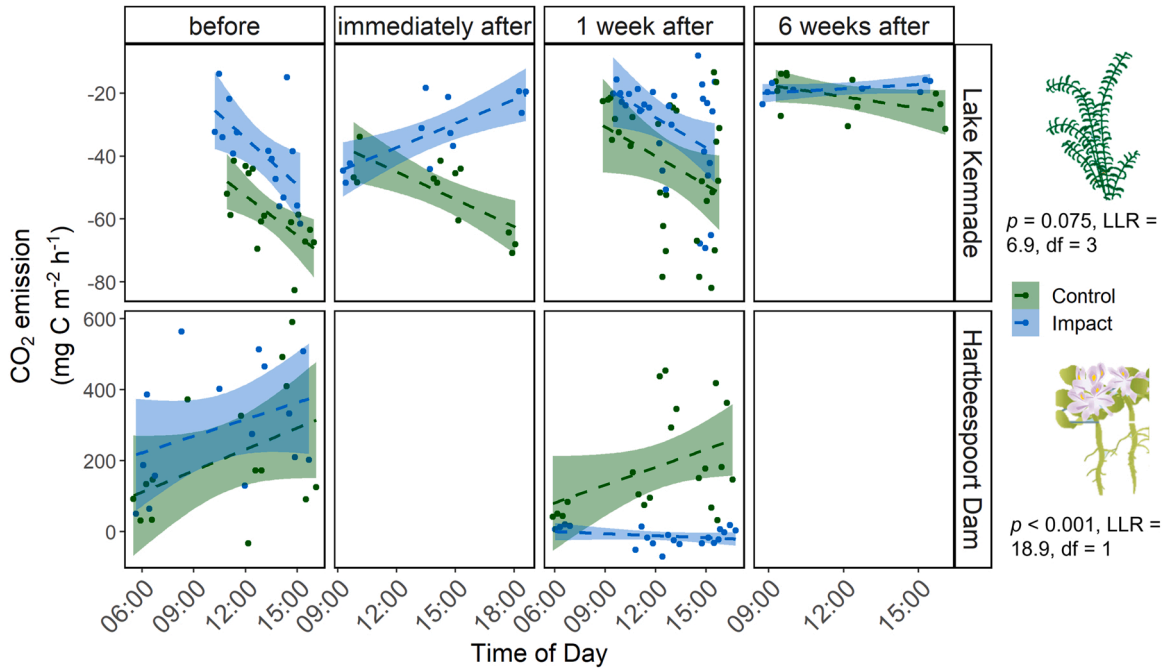

Fig. 3. Diffusive fluxes of CO_2 plotted against time of day, measured in the impact and control sites at Lake Kemnade, before, immediately after, one week after and six weeks after removal of *Elodea nuttallii,* and at Hartbeespoort Dam before and one week after removal of *Pontederia crassipes*. Mind the different scales on the y-axis. Statistical information is given on the interactive effect of Site, Time and Time-of-Day.

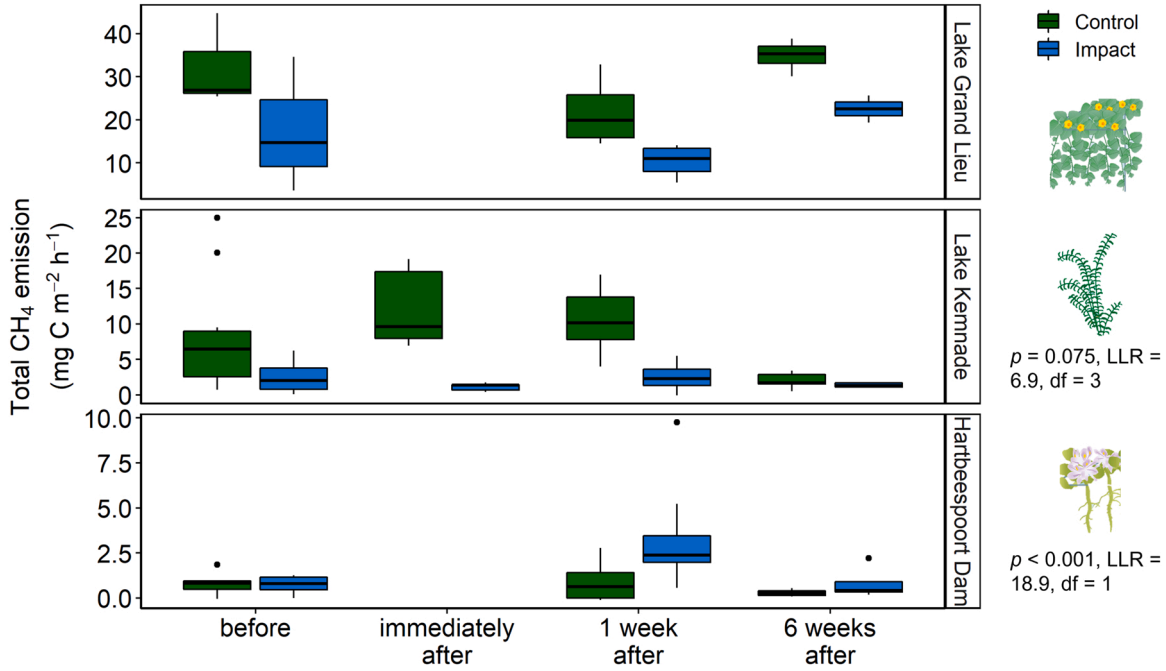

Fig. 4. Total flux of CH_4 determined in Lake Grand-Lieu (top), Lake Kemnade (middle) and Hartbeespoort Dam (bottom) before, immediately after and one after and six weeks after removal of invasive macrophytes (*Ludwigia* spp., *Elodea nuttallii*, *Pontederia crassipes,* respectively). Note different scale of the y-axis for the three lakes. Horizontal bold lines indicate the median, boxes the 25 % and 75 % percentiles, and whiskers the minimum and maximum values. Points represent outliers. Statistical information is given on the interactive effect of Site and Time.

sediment layer of 20 cm (Wilkinson et al., 2015), Lake Kemnade and Hartbeespoort Dam would see a sediment CH_4 production rate of approximately 0.64 ± 0.27 and 0.26 ± 0.31 mg CH_4-C m^{-2} h^{-1}, respectively.

4. Discussion

4.1. Short-term effect of macrophyte removal on CO_2 emission

Macrophyte removal had a different impact on CO_2 emission in Lake Kemnade and Hartbeespoort Dam, which are dominated by submerged *E. nuttallii* and free-floating *P. crassipes,* respectively (Fig. 6). Despite our

Table 2
Rates of total, diffusive and ebullitive CH_4 fluxes for Hartbeespoort Dam, Lake Kemnade and Lake Grand-Lieu. Average measured diffusive CH_4 fluxes (including plant-mediated CH_4-transport) were subtracted from the measured total fluxes to determine rates and relative contribution of ebullition. Fluxes are displayed as mean ± sd. Significant outliers (Rosner's Test) were excluded in this estimation of the contribution of ebullition.

Lake	Site	Time removal	Total (mg C m^{-2} h^{-1})	Diffusive (mg C m^{-2} h^{-1})	Ebullition (mg C m^{-2} h^{-1})	Ebullition (%)
Hartbeespoort Dam	Impact	Before	0.8 ± 0.5	1.9 ± 1.7	~0	~0
		After 1 week	3.4 ± 2.9	1.3 ± 1.9	2.1	61
	Control	Before	0.8 ± 0.5	1.5 ± 4.6	~0	~0
		After 1 week	0.9 ± 1.1	2.1 ± 2.1	~0	~0
Lake Kemnade	Impact	Before	2.6 ± 2.2	0.4 ± 0.3	2.2	84
						

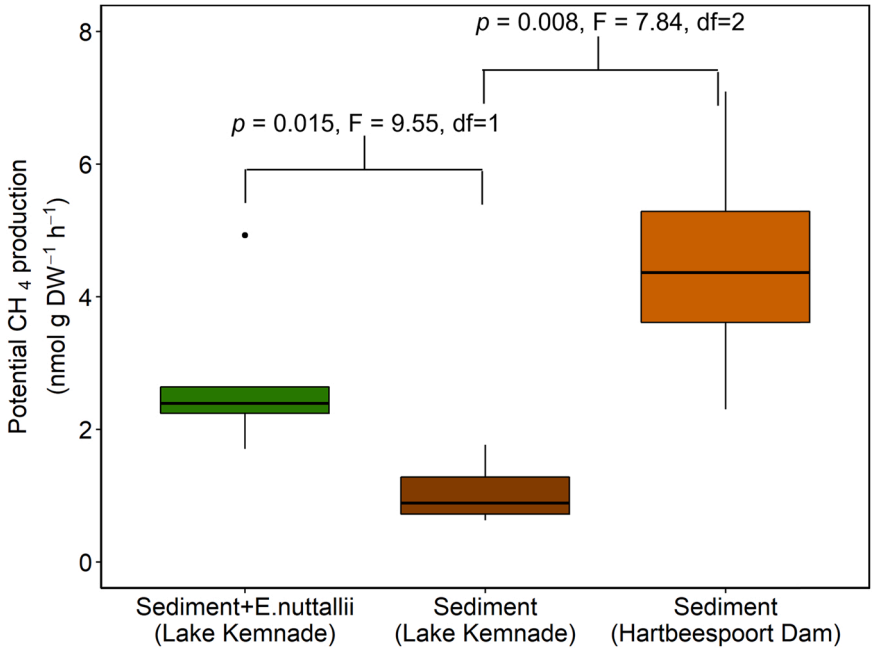

Fig. 5. Potential hourly methane production, derived from incubations of sediment with (left) or without (middle) *E. nuttallii* at Lake Kemnade, and sediment at Hartbeespoort Dam (right).

use of opaque chambers, significant daytime CO_2 uptake rates were measured at Lake Kemnade, which were reduced after removal of *E. nuttallii*. This was especially apparent at times when peak photosynthetic activity occurs, between noon and late afternoon, and the difference was strongest immediately following removal. As the mowing boat could not remove the bottom 50 cm of *E. nuttallii*, the remaining plants, possibly together with a modest growth of phytoplankton ensured that CO_2 was still being fixed during the day at reduced rates. Immediately after removal, photosynthetic activity was most likely limited by turbidity caused by disturbance of the sediment. One week after removal, daytime CO_2 fixation patterns had recovered to rates recorded before removal as sediment disturbance decreased and remaining *E. nuttallii* started to regrow. Average fixation rates one week after removal were about 25 % lower than before removal, which is still remarkable given that only 27 % of the vegetation biomass remained. *E. nuttallii* is known to be highly adapted to disturbance by both herbivory and removal and has a high relative growth rate (He et al., 2019). Six weeks after removal, *E. nuttallii* had already doubled its biomass compared to one week after removal, thus reaching an average growth rate of 3.7 g DW m^{-2} d^{-1}.

At Hartbeespoort Dam, *P. crassipes* stands showed very high daytime CO_2 emission rates of 100–300 mg C m^{-2} h^{-1} before removal. Our findings contrast with previous studies that have found that *P. crassipes* can often offset CO_2 emissions in freshwater systems (Oliveira Junior et al., 2021; Peixoto et al., 2016), due to its high primary production under nutrient-rich conditions (Junk and Howard-Williams, 1984). A previous study in the Amazon and Pantanal has reported very high daytime CO_2 uptake rates of −1000 ± 500 mg C m^{-2} h^{-1}, which compensated for night-time emissions, resulting in a net CO_2 sink (Oliveira Junior et al., 2021). The contrasting findings in our study could result from using opaque chambers, as we exclude the direct uptake of CO_2 from the atmosphere by *P. crassipes*. However, as we observed a

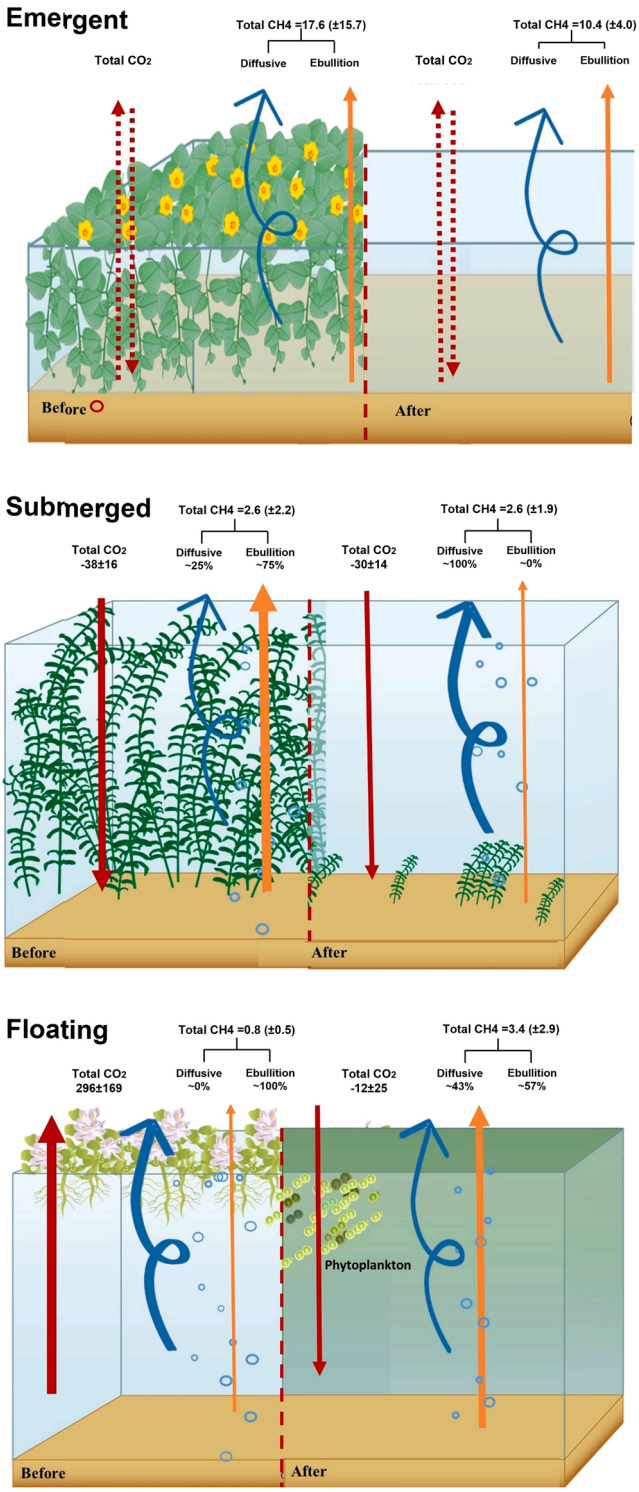

Fig. 6. Lake Grand-Lieu, Lake Kemnade and Hartbeespoort Dam (top to bottom) showed contrasting short-term responses in CO_2 and CH_4 fluxes after removal of their respectively mass developments of macrophytes. At Lake Grand-Lieu, the high total CH_4 fluxes did not seem to be impacted by the removal of macrophytes. Rather, a combination of high TOC availability, low DO and high temperatures most likely stimulated methanogenesis and limited methane oxidation in this shallow system. At Lake Kemnade, removal of the top layers of the *E. nuttallii* vegetation temporarily decreased CO_2 fixation (red arrows) but also CH_4 emission (blue and orange arrows). This was most likely caused by outgassing of CH_4 due to disturbance of the sediment by the mowing boat. At Hartbeespoort Dam, removal of the floating *P. crassipes* stimulated growth of phytoplankton, which resulted in net CO_2 uptake (red arrows). Simultaneously, the total CH_4 emission (blue and orange arrows) strongly increased after removal of the barrier of floating vegetation, which normally captures CH_4 and could stimulate CH_4 oxidation in the rhizosphere. Rates are based on measurements conducted at the impact sites before and one week after macrophyte removal, and are expressed in mg C m^{-2} h^{-1}. Width and direction of the arrows indicate proportion and direction of the CO_2 and CH_4 fluxes.

strong decrease in *P. crassipes* cover at Hartbeespoort Dam during the summer of 2019 and 2020, damage to the plants by the biological control agent *Megamelus scutellaris* most likely also played a role in the high CO_2 fluxes measured at this lake. After removal, the system showed net CO_2 uptake, due to the explosive growth of phytoplankton in the absence of light limitation. In addition, this cyanobacterial bloom may have benefitted from the removal of *P. crassipes*, since the species is known to produce allelopathic substances that inhibit cyanobacterial and algal growth (Pei et al., 2018).

The impact of macrophytes on diffusive CO_2 fluxes at Lake Kemnade and Hartbeespoort Dam were confirmed by the boosted regression trees, which showed that macrophyte presence explained 15.4 % and <5 % respectively. This is low compared to other factors that influence CO_2 emission, such as temperature (22–33 %), chl-*a*. (13–21 %) and TOC (30 %). These findings thus suggest a small direct effect of macrophytes on CO_2 fluxes. The environmental factors, such as temperature and chl-*a* content could, however, be affected by macrophyte presence themselves. At our lakes, we found that macrophyte removal raised water temperature at Lake Grand-Lieu and chl-*a* concentrations at Hartbeespoort Dam. Macrophyte presence could thus have direct and indirect effects on greenhouse gas emission.

4.2. Short-term effect of macrophyte removal on CH_4 emission

Although macrophyte dominated lakes are often sinks for CO_2 (Kosten et al., 2012), these systems can be important sources of CH_4 emission (Aben et al., 2017). Anoxic sediments, especially those with higher organic matter contents, provide ideal conditions for methanogens. The sediments of both Lake Kemnade and Hartbeespoort Dam showed potential CH_4 production rates, which roughly correspond with emissions of 0.3–0.6 mg CH_4 -C m^2 h^{-1}. This is slightly lower than the total fluxes of CH_4 that we determined at these lakes (but still in the same order of magnitude), which may have resulted from a lag phase in the incubation due to disturbance during set-up (Souto et al., 2010). Potential rates of methanogenesis in the incubations doubled when *E. nuttallii* was present. This indicates that the growth of dense macrophyte stands can substantially affect CH_4 dynamics in freshwater lakes, for example by providing easily degradable organic matter or through plant-mediated methane transport (see review by Joabsson et al., 1999).

Lake Grand-Lieu, which experiences mass development by invasive, emergent *Ludwigia* species, showed a high total CH_4 emission that appeared unrelated to macrophyte presence. Therefore, it is implied that either plant-mediated CH_4 emission did not contribute significantly to the total flux during the investigated period, or that CH_4 oxidation in the rhizosphere counterbalanced the plant-mediated CH_4 transport. Another possibility is that plant-mediated CH_4 transport has been limited due to the use of opaque chambers lowering convective flow (Chanton et al., 1993), thereby underestimating CH_4 fluxes in *Ludwigia* dominated plots. Due to travel restrictions, we were unfortunately unable to determine diffusive fluxes in this system and can therefore not give an estimate of the relative contribution of the pathways of ebullition and diffusion. Lake Grand-Lieu is a very shallow system and our study sites had a water layer of 30–50 cm, low oxygen saturation and high TOC and TP concentrations. This high availability of organic carbon and TP could have resulted in a high biological oxygen demand, thus lowering the oxygen concentration in the water layer. Decaying mats of *Ludwigia* species have been known to cause anoxic conditions in shallow systems, with negative impact on fish and other fauna (Nehring and Kolthoff, 2011). Although *Ludwigia* was removed completely from our impact site, the high availability of TOC and TP remained and was possibly enhanced by sediment disturbance or phytoplankton growth. Both anoxic conditions and the availability of substrates for microbial metabolism could have stimulated the production of CH_4 at this lake, while the low oxygen concentrations would have limited CH_4 oxidation, resulting in high emission rates.

Removal of submerged *E. nuttallii* appeared to reduce CH_4 ebullition at Lake Kemnade but not diffusive CH_4 fluxes (Fig. 6). While ebullition contributed approximately 63–85 % to the overall CH_4 flux in vegetated control sites, it became negligible after removal of *E. nuttallii*. The most likely explanation for this is outgassing due to sediment disturbance during mowing. Although the mowing boat left approximately 50 cm of *E. nuttallii* growing on (and rooting in) the sediment, the physical removal and possibly shear stress caused by the large boat, will most likely have disturbed the upper sediment layers where bubbles had built up over time (Maeck et al., 2014). Simultaneously, while the control site showed an increase in total CH_4 emission over time, fluxes at the impact site remained low. This could indicate that methane production at the impact site had not yet returned to the pre-disturbance levels of bubble production. In a controlled laboratory study, Liu et al. (2016) observed a lag phase of approximately six days during which ebullition was negligible, with normal bubble production resuming after approximately 12 days (Liu et al., 2016). Our incubation experiment suggests a more direct effect of *E. nuttallii* on CH_4 fluxes, possibly by providing organic substrates for methanogenesis. Higher CH_4 fluxes from submerged vegetation than from non-vegetated zones have also been found in lakes (Zhang et al., 2019) and reservoirs (Cronin et al., 2006), and could be due to decaying biomass at the sediment surface providing organic substrate for methane production (Joabsson et al., 1999). This does not explain, however, why the difference in CH_4 emissions was only observed in total fluxes and not in diffusive fluxes.

At Hartbeespoort Dam, the diffusive fluxes of CH_4 were highly variable in both impact and control site and did not show an effect of macrophyte removal. Total CH_4 fluxes, however, showed a threefold increase when *P. crassipes* was removed. As *P. crassipes* did not root in the sediment at our study sites (Oliveira Junior et al., 2021), we assume plant-mediated methane transport did not play a substantial role and that the total flux is made up of diffusive fluxes and ebullition (Fig. 6). While the contribution of ebullition was negligible in *P. crassipes* mats, the total flux comprised of 60 % ebullition-derived methane and 40 % diffusive methane after removal. In dense floating mats, the gas exchange across the water-atmosphere interface can be strongly reduced and floating leaves can 'capture' the gas bubbles (Kosten et al., 2016), which then accumulate in the rhizosphere. Here, methanotrophs (Ávila et al., 2019) could oxidise this methane, thus further lowering emission to the atmosphere (Yoshida et al., 2014). This capturing of CH_4 is also illustrated by the higher dissolved CH_4 concentrations found in the rhizosphere of *P. crassipes* mats compared to the top 20 cm of the water layer after *P. crassipes* removal. By bringing the ebullition-pathway almost to zero, the mat of *P. crassipes* effectively reduced the emission of methane by an estimated 0.8–1.1 mg C m^{-2} h^{-1}, supporting the results of several studies reviewed by Kosten et al. (2016).

As with the diffusive CO_2 fluxes, the boosted regression trees indicated that the magnitude of direct effect of macrophytes on CH_4 fluxes was small, since macrophyte presence (in biomass) explained less than 5 % of the variation in CH_4 fluxes. Environmental variables such as temperature (28–29 %), dissolved oxygen (23–30 %) and pH (24 %) were the main factors explaining the patterns in CH_4 fluxes, as has also been reported in previous studies (e.g. Oliveira Junior et al., 2021). Again, the results of the BRTs may obscure the indirect effects that macrophytes have on the environmental factors that form the main explanatory variables.

4.3. Implication for management of shallow lakes with mass developments of macrophytes

Our three lakes each display their own unique combination of invasive macrophyte, environmental conditions and climate, and in each lake, macrophytes are removed for different reasons. As we hypothesised, macrophyte removal had contrasting short-term effects on the CH_4 and CO_2 emission from these lakes. Additionally, we had expected the overall C emission to increase following removal. At Lake Grand-Lieu, we could not determine the full effect of removal on C-

emission as the CO_2 fluxes could not be measured. We can assume, however, that the removal of invasive *Ludwigia* would lower CO_2 fixation. As there was no effect of removal on the CH_4 emission at this lake, we believe that removal overall would increase C emission at Grand-Lieu. At Hartbeespoort Dam, removal resulted in a strong increase in CH_4 emission, which fits with our hypothesis that the removal of floating vegetation has a greater impact on CH_4 fluxes than removal of submerged and emergent macrophytes. Although a cyanobacterial bloom caused net CO_2 fixation after removal, this would most likely not outweigh the C-uptake by a healthy stand of *P. crassipes* (~1000 mg C m^{-2} h^{-1}; Oliveira Junior et al., 2021). Application of biological control agents, as is the current management practice at Hartbeespoort Dam, could, however, have strongly reduced the net C-uptake by damaging the vegetation. While this biological control thus seems effective, it would be recommended that management at the lake focuses on reducing the nutrient input, since removal of *P. crassipes* most likely will result in recurring cyanobacterial blooms.

At Lake Kemnade, our measurements of CO_2 and CH_4 fluxes allow us to make a rough estimate of the effect of removal on the overall C emission. At the lake, approximately 47 of the 125 ha are covered by *E. nuttallii* (Ruhrverband, 2020). Without mowing, this area would see daytime CO_2 fixation of 340 kg C and a CH_4 emission of 70 kg C per day (using our average flux measurements from Table 2). This corresponds to a global warming potential (GWP) of 1367 kg CO_2 equivalents (using a GWP_{100} of 28 CO_2-eq. for CH_4) With a maximum capacity of 15.5 tons *E. nuttallii* removed per day by the mowing boat, approximately 9 ha can be mowed per week. Assuming that at any given time in the growing season, 9 ha is being mowed, 9 ha has just been mowed (1 week after) and 29 ha has regrown or remains vegetated, this lake would see daytime CO_2 fixation of 290 kg C and a CH_4 emission of 50 kg C per day, thereby reducing the GWP by ~40 % to 803 kg CO_2-eq. Although contradicting our hypothesis that C emission would increase after macrophyte removal, this rough calculation omits the probable outgassing of CH_4 due to disturbance of the sediment. These events may be included in future research, for example by using Eddy Covariance. In addition, for a full C-budget, night-time CO_2 measurements should be included, as well as the C emission of decomposing biomass after removal.

Macrophyte management in systems experiencing mass developments is carried out to relieve nuisance, usually for recreational activities. The consequences of macrophyte removal on ecosystem functioning, however, are rarely quantified. If macrophyte management is reviewed, it is often limited to determining effects on water quality and the occurrence of phytoplankton blooms. Given the current emphasis on reducing greenhouse gas emissions to reach the targets set by the Paris agreement, it is important to understand how management of freshwater systems impacts their contribution to the global greenhouse gas budget. Apart from the short-term effects here presented, there is a strong need to determine the long-term impact of macrophyte removal on whole lake carbon budgets to develop sustainable management strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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CRediT authorship contribution statement

Sarah Faye Harpenslager: Methodology, Formal analysis, Investigation, Writing – original draft. Kirstine Thiemer: Formal analysis, Investigation, Writing – original draft, Visualization. Constancia Levertz: Methodology, Investigation. Benjamin Misteli: Investigation, Writing – review & editing. Keneilwe Sebola: Investigation, Writing – review & editing. Susanne Schneider: Writing – review & editing, Funding acquisition. Sabine Hilt: Conceptualization, Writing – review & editing, Funding acquisition. Jan Köhler: Conceptualization, Writing – review & editing, Funding acquisition.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquabot.2022.103555.

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