Rooting and plant coverage determine greenhouse gas budget of water hyacinth (*Eichhornia crassipes*)

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Abstract

Water hyacinth occurs in numerous tropical and subtropical countries, either as a native or as an invasive exotic species, where it can establish large and dense mats. The plant is also frequently used for water purification and bioremediation purposes. Although it is a free-floating species, the plant roots into the sediment of shallow waters, tapping into the sediment nutrient pool. Its long and extensive root system strongly increases nutrient absorption, resulting in high growth rates and concurring high carbon sequestration rates. On the other hand, the plants may also fuel methane (CH_4) production as dense mats may deplete oxygen in the surface water and sediment below, which in combination with the high production of organic matter creates favorable conditions for methanogenesis. We hypothesize that water hyacinth vegetation acts as a strong greenhouse gas (GHG) sink due to its high growth rates, especially when (sediment) nutrient availability is high. Still, this sink may be counterbalanced by CH_4 release, which will be most pronounced when the plants are rooting in the sediment due to potential CH_4 shuttling from the sediment through the roots and leaves into the atmosphere (plant-mediated transport). To mechanistically unravel the influence of water hyacinth on nutrient dynamics and greenhouse gas fluxes, we performed an aquarium experiment in which plant coverage and root access to the sediment were manipulated. Although plant cover led to lower concentrations of dissolved total phosphorus (DTP) and phosphate, there were no effects of coverage or rooting. We found no vegetation effect on the ebullition of CH_4,

but its diffusion was 4.5 times higher at high plant coverage. Rooting increased CH_4 diffusion by 1.3 (high coverage) and 4 times (low coverage), demonstrating the plant-mediated transport that we hypothesized. Independent of rooting, however, water hyacinth at high coverage sequestrated less carbon compared to low coverage, possibly due to space limited growth and self-shading. Overall, water hyacinth enhanced CH_4 emissions, especially when rooted. Due to water hyacinth's high CO_2 sequestration rates, the overall GHG budget in terms of CO_2 equivalents still resulted in water hyacinth mats being near-neutral or even a GHG sink, depending on water hyacinth coverage. Our results show that the effect of water hyacinth mats on GHG fluxes strongly depends on both plant coverage and contact with the sediment. This indicates that, when making regional GHG balances, not only plant presence but also its coverage and water depth – regulating sediment-root contact – should be taken into account.

Key words: floating plant, nutrient dynamic, CH_4 emission, carbon dioxide sequestration, greenhouse warming potential.

1. Introduction

Water hyacinth (*Eichhornia crassipes*) is notorious at a global scale because of the problems it poses to economy, society and ecology when occurring at high coverage (Villamagna and Murphy, 2010; Malik et al., 2007). Its high tolerance range for environmental conditions including pH, temperature and nutrients (Gutierrez et al. 2001; Wilson et al. 2005) provides an ample spectrum of colonization, and explains its wide-spread occurrence and nuisance around the world. Its fast growth rates and rapid dispersal through asexual reproduction explain its ability to form large floating mats comprising high biomass (Pinto-Coelho and Greco, 1999). Water hyacinth is

also frequently used for water purification and bioremediation purposes because of its high nutrient uptake rate (Aoyama and Nishizaki, 1993; Mandi, 1994; Polprasert and Khatiwada, 1998).

Nutrient availability strongly determines *Eicchornia*'s growth rate as well as nutrient allocation (Xie et al., 2004). Maximum nutrient uptake efficiency is typically reached in the early growth stage (Reddy, Agami & Tucker, 1989; Reddy, Agami & Tucker, 1990), explaining the formation of big mats in a few days (Tellez et al., 2008). Water hyacinth's high growth rate results in high carbon dioxide (CO_2) uptake at rates of 3.4 - 5.4 g C-CO_2 m^{-2} day^{-1} as reported for tropical lakes (Peixoto et al., 2016). In these lakes, the vegetation even sets off open water CO_2 emissions, turning the system into CO_2 sink. While water hyacinth growth will decrease CO_2 emissions, its presence may simultaneously increase the emission of methane (CH_4) (Banik, Sen, and Sen, 1993), having a global warming potential (GWP) of 34 times CO_2 over a 100 year time scale (Myhre, Shindell and Bréon, 2013). Therefore, even relatively low rates of CH_4 emissions could offset the high CO_2 assimilation, turning water hyacinth mats into a greenhouse gas (GHG) source. The high coverage of water hyacinth suppresses light penetration and therefore photosynthetic activity in the water below. In combination with reduced O_2 diffusion from the atmosphere into the water by its cover, this can result in anaerobic conditions below the water hyacinth mat (Reddy and DeBusk 1991) as has also been described for other floating plants (e.g. Caraco and Cole, 2002; Grasset et. al, 2016). Research performed in ditches and tanks showed that the combination of decreasing O_2 concentrations and high organic matter production by water hyacinth favors CH_4 emission. This effect was strongest after multiple years, probably due to organic matter accumulation (Banik et al., 1993).

Aquatic plants rooting in the sediment tend to enhance CH_4 emissions by transporting CH_4 directly from the sediment to the atmosphere. This plant-mediated CH_4 emission is an important pathway, which may even explain more than 50% of the total emission of inland waters (Dacey and Klug 1979; Grosse, Armstrong & Armstrong 1996). Plant-mediated CH_4 transport may take place through convective flow (pressurized flow), a

common mechanism for aquatic plants, or by passive molecular diffusion (Cronk and Fennessy 2016; Grosse, Armstrong & Armstrong 1996; Konnerup et al. 2011). Although water hyacinth is generally reported as a floating plant, the species can root in the sediment when the water level is sufficiently low (less than 50 cm; personal observation). Plant-mediated CH_4 emission is expected to increase when the plant is rooted in the sediment as transport rates are determined by concentration differences between compartments, and CH_4 concentrations tend to be much higher in sediment pore-water than in surface water. Possibly, the increase in plant-mediated transport of pore-water CH_4 to the atmosphere may inhibit the formation of bubbles in the sediment, thereby decreasing ebullition.

On the other hand, metanotrophic microbial communities associated with plant roots (Yoshida et al. 2014) may oxidize a considerable portion of the CH_4 dissolved in the surface water or pore-water (Kosten et al., 2016). The overall effect of floating plants on CH_4 emissions is therefore not straightforward (compare, for instance, findings of Bolpagni et al. (2007) and Ribaudo et al. (2012) who found that floating plants increase CH_4 emissions with Bharati et al. (2000) who found a reduction of CH_4 emissions; see also Kosten et al. (2016) for a review of field observations of the effect of floating plants on CH_4 emissions). Even when aquatic plants increase CH_4 emissions, their overall GHG budget may still be counterbalanced by their high growth rates - and therefore CO_2 uptake rates. CO_2 uptake rates can be expected to be highest when plants have access to nutrients in both sediment and water.

All in all, the effects of water hyacinth mats on GHG emissions are not at all straightforward. Only few studies have investigated the effects of water hyacinth on total GHG (CH_4 and CO_2) emissions (Banik et al., 1993; Peixoto et al., 2016; Attermeyer et al., 2016), and none have included the effects of plant coverage or rooting. Moreover, the few studies that investigated the effect of water hyacinth on GHG balance showed contrasting results (Banik et al., 1993; enhanced CH_4 emissions; and Attermeyer, et al., 2016; decreased CH_4 emissions).

We therefore used an experimental approach under controlled conditions, to elucidate the roles of water hyacinth coverage and rooting on GHG dynamics. We hypothesize that differences may be 1) due to variation in coverage, and 2) related to whether or not the plants are rooted in the sediment. An increase in coverage may either increase or decrease CH_4 emissions, depending on the dominant process: enhanced methanogenesis due to lower oxygen (O_2) concentrations below the plant layer or enhanced CH_4 oxidation due to a higher root biomass and associated metanotrophic communities. With respect to hypothesis 2) we expect CO_2 uptake rates to be highest when plants have access to nutrients in the sediment, also leading to lower pore-water nutrient concentrations and C:N and C:P ratios. In addition, we expect diffusive CH_4 emissions – including plant-mediated emissions to increase when plants are rooted. To elucidate the roles of coverage and rooting of water hyacinth vegetation on GHG fluxes and nutrient dynamics, and to study the directions of their effects, we used a full-factorial, controlled indoor aquarium experiment.

2. Materials and methods

2.1. Experimental set-up

The experiment was conducted in 24 glass aquaria of 24 L (20 × 20 × 60 cm; length × width × height) filled with a layer of 7 cm of fresh sediment, and a layer of 38 cm of demineralized water. The sediment was collected from a eutrophic drainage ditch (Ede, The Netherlands; 51°59'43.58"N, 5°38'38.91"E) in September 2014, and was sieved with a 5.0 mm sieve to remove stones and vegetation remnants. Sediment characteristics were determined at the beginning of the experiment (Table 1). The aquaria were placed in a water bath at 23°C in the greenhouse facilities of the Radboud University (Nijmegen, The Netherlands). A light frame of 220 µmol m^{-2} s^{-1}

PAR (16h light/8h dark) was provided by Philips Green Power 400V/1000 WE lamps in a New E-Papillon 1000 W armature, to provide sufficient light in case of cloudy conditions.

Water hyacinth was collected from a commercial breeder (Nijmegen, the Netherlands) and cultivated in the greenhouse for approximately 10 months prior to the experiment, on organic sediment to which slow-release phosphorus granules were added. The experiment lasted for 59 days, from October to December 2014. The aquaria were randomly assigned to controls without plants, low coverage (50% of water hyacinth coverage) or high coverage (100% coverage) (n = 8 for each treatment). In half of the treatments, a mesh (1.0 mm mesh size) was placed just above the sediment (n=12) to prevent the plants from rooting in the sediment, dividing the plant treatments into rooted and non-rooted treatments. There were 4 controls without, and 4 with a mesh (jointly referred to as 'controls'). We added individual water hyacinths to each aquarium: 1) 160 g (fresh weight - FW) to the low coverage treatment with mesh (non-rooted – 50%nR) or without mesh (rooted – 50%R); and 2) 413 ± 2.63 g (FW ± SD) to the high coverage treatment with mesh (non-rooted – 100%nR) or without mesh (rooted – 100%R) (Fig. 1). The fresh weight of the total biomass was measured using paper towel to carefully blot the plants dry and remove water attached, and then place the plants on a digital scale. To maintain low coverage, water hyacinth was harvested partially at day 31 and 45.

2.2. Chemical analyses

Dissolved oxygen (DO), pH and temperature were measured weekly at both the surface and bottom of the water column, using a portable multi-meter (HQ40d multi, HACH, Loveland, Colorado, U.S.A.). Surface and pore-water samples were collected anaerobically every week during the experiment using ceramic soil moisture samplers (SMS rhizons, Eijkelkamp, Giesbeek, Netherlands). Total inorganic carbon (TIC) of water samples was

measured with an Infra-red Gas Analyzer (IRGA; ABB Analytical, Frankfurt, Germany). Concentrations of PO_4^{3-}, NO_3^- and NH_4^+ in the water samples were measured colorimetrically on an Auto-Analyzer 3 system (Bran & Luebbe, Norderstedt, Germany) by using ammonium molybdate (Henriksen 1965), hydrazine sulphate (Kamphake et al. 1967) and salicylate (Grasshoff and Johannsen 1972), respectively. Concentrations of dissolved total P (DTP) were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Fisher Scientific, Franklin, MA, U.S.A.). Dissolved organic carbon (DOC) in water samples was measured with a TOC-L CPH/CPN analyzer (Shimadzu, Kyoto, Japan) at the end of the experiment.

Sediment samples were collected at the start and end of the experiment, and subsequently dried for 48h at 60°C. Dry samples were heated for 4 hours at 550°C and re-weighed to determine organic matter content. Dried sediment (200 mg) was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) using 4 ml 65% HNO_3 and 1 ml 30% H_2O_2 to determine total sediment Fe, Al, Ca and P concentrations. Digested solutions were analyzed by ICP-OES (see above). Olsen P extracts (plant available P) was determined by extraction according to Olsen (1954), whereas a NaCl-extraction (exchangeable NH_4^+ and NO_3^-) was performed as described by Tomassen et al. (2004).

2.3. Greenhouse gas flux measurements

2.3.1. *Diffusive flux*

After 30, 38 and 45 days greenhouse gas (CO_2 and CH_4) diffusive fluxes were measured during the day and night using a lid on top of the aquarium to establish a closed system connected to a Picarro G2508 Greenhouse Gas Analyzer (Picarro Inc., Santa Clara, CA, USA). The lid was sealed air-tight with paste (Terostat IX, Teroson GmbH, Heidelberg, Germany). Measurements were conducted until a clear linear increase in CO_2 and

CH_4 occurred (typically for 5 minutes). The slope of the relationship between gas concentration and time was used to calculate the gas flux as explained by Almeida et al. (2016). When the linear increase was interrupted by a sudden increase in gas concentration – due to bubbling – the lid was removed to restore ambient air concentrations, and the diffusive flux measurement was repeated.

2.3.2. Ebullitive flux

Total CH_4 fluxes (ebullitive + diffusive) were measured 3 times (on day 31, 39 and 46) during a period of 24 hours. During this time the glass lid (equipped with a rubber septum) was closed as described before. The increase in CH_4 concentration during 24 hours was determined by sampling the headspace (in duplicate) using a 1ml plastic syringe through the septum at the start and the end of the incubation and subsequent directly injecting 0.5 ml into the gas chromatograph (HP 5890 equipped with a Porapak Q column (80/100 mesh), a flame ionization detector (GC-FID, Hewlett Packard, USA) and oven temperature 120°C). The total amount of CH_4 emitted was calculated by multiplying the change in CH_4 concentration in the headspace between t=0 and at the end with the volume of the headspace. The ebullitive fluxes were calculated by subtracting diffusive CH_4 fluxes determined the day before from the total amount of CH_4 emitted. Diffusion back into the water, occurring when the concentration in the headspace becomes higher than the concentration in the water, may lead to an underestimation of the ebullitive flux. We calculated the CH_4 concentration in the water based on the diffusive flux and a gas transfer velocity of 0.05 m/d. We therefore calculated the flux into the water for these cases, using the same gas transfer velocity, the headspace concentration at the end of the 24 hour flux incubation, and the calculated concentration in the water. Finally, we added this flux to the ebullitive flux.

2.3.3. *Global Warming Potential*

To evaluate the net GHG effect we used a global warming potentials (GWPs) of 34 for CH_4 converting to CO_2-eq fluxes as described by Myhre et al. (2013).

2.4. Plant measurements

At the start of the experiment four extra plants were dried (148.37 ± 13.18 g FW ± SD and 9.00 ± 0.94 g DW ± SD) and used to analyze initial nutrient contents. At the end of the experiment all plants were collected. Water hyacinths were divided into leaves, petioles, and roots. The fresh plant samples were weighed and dried for 48h at 60°C, after which they were weighed again, grinded and homogenized. Subsequently, 200 mg of dry plant material was grinded and digested to determine total P concentrations in plants as described for the chemical analysis of sediment. An additional 3 mg of dry plant samples was combusted to determine C and N content with an elemental analyzer (Carlo Erba NA 1500, Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Statistical analyses

Shapiro-Wilk's test and Bartlett's test were conducted to test normality of residuals and equality of error variances, respectively. Non-normal or heteroscedastic data were log transformed to meet these two requisites. Linear mixed models were used to test the main effects and interactions of treatments over time on water characteristics, DO, GHG fluxes, and GWP. The effects on these variables, and on C:N, C:P and N:P ratios and N and P contents in different plant tissues were tested with aquarium number as a random effect, by using R

package *nlme*. Tukey tests were performed to find differences between treatments by using R package *multcomp*. The effects of treatments and differences between treatments were considered significant if $P < 0.05$. All statistical analyses were carried out using the software program R (version 3.2.1; R development Core Team, 2015). All graphs were plotted by using SigmaPlot (v.11 Systat Software Inc, 2008).

3. Results

3.1. *Biogeochemistry in water column and sediment*

Dissolved oxygen (DO) concentrations did not vary in time ($P > 0.05$ for all treatments; data not shown), and were below saturation value (8.3 mg L^{-1} at 22 ^{0}C). Average DO concentrations in the water layer were significantly lower in aquaria with plants (3.5 ± 0.2 mg L^{-1}) compared to the control aquaria without plants (5.7 ± 0.4 mg L^{-1}, $X^2 = 9.20$, $P < 0.05$). The effect of coverage ($X^2 = 4.15$; $P < 0.05$) was also seen on DO concentration where rooted treatments presented higher values than non-rooted (Fig 2).

The treatments including water hyacinth had about 10-50 % lower concentrations of DTP and phosphate (PO_4^{3-}) in the surface water compared to the controls (X^2=79.82, $P < 0.001$ and X^2=84.03, $P < 0.001$ for DTP and PO_4^{3-} respectively; Table 2). In addition, they had lower NO_3^- concentrations in the surface water (X^2=69.38, $P < 0.001$)

3.2. *Nutrient concentrations in different plant tissues*

Plant coverage or rooting did not show effects on nutrient concentrations in different plant tissues ($P > 0.05$). For low coverage, P concentrations in petioles were higher in rooted plants than in not-rooted plants ($P < 0.01$; Fig S1A). In addition, only low-coverage treatments showed higher P concentrations in petioles at the end of the

experiment compared to the start ($P < 0.01$; Fig S1A). For all treatments P concentrations in roots were significantly higher at the end of the experiment than at the start of the experiment ($P < 0.001$; Fig S1B). Furthermore, only the high coverage rooted plants had higher N concentrations in petioles at the end of the experiment compared to the start ($P < 0.001$; Fig S2). N concentrations in petioles were higher in the treatment with high coverage rooted plants than all other treatments ($P < 0.001$; Fig S2).

In general, nutrient did not change in time or differ between treatments. There were some exceptions however (Fig 3). Rooted plants growing at high coverage had a higher N: P ratio in the petioles (1.72, compared to 0.85) (X^2=38.75, $P < 0.001$), and the N: P ratio in the roots was lower at the end than at the start (X^2=24.44, $P < 0.001$).

3.3. *Greenhouse gas fluxes*

Total CH_4 fluxes were highest at high density (ANOVA for density effect; $X^2 = 9.27$, $P < 0.05$). This was mostly due to the high diffusion rates in the high coverage. Diffusion rates showed statistical difference between the treatments ($X^2 = 68.30$ $P < 0.05$). At low coverage, diffusive CH_4 emissions were significantly higher in aquaria with rooted water hyacinth ($X^2 = 9.59$, $P < 0.01$) (Fig. 4A). On average, CH_4 ebullition was 34.8 ± 23.2 mg CH_4 m^{-2} d^{-1} and differed for high coverage compared to low coverage and control treatments ($X^2 = 9.46$; $P < 0.05$) (Fig 4A). As diffusion back into the water might have caused an underestimation of the ebullitive fluxes in the controls and the 50%nR treatments, this was corrected for (see Materials and Methods). Maximum loss of CH_4 from the headspace, i.e. assuming ebullition took place at the beginning of the 24 hour measurement, was 14% on average. In terms of CO_2, the controls without water hyacinth functioned as a source, whereas the treatments with water hyacinth functioned as a CO_2 sink (X^2=14.70, $P < 0.001$) (Fig 4B). Day and night CO_2 fluxes for the treatments with

plants were found to be opposite, showing CO_2 uptake during the day and emission during the night (Fig. S3). Differences in time were not found for GHG fluxes (X^2=0.87; $P > 0.05$ for CH_4; and X^2=4.99; $P > 0.05$ for GWP) except for CO_2 ($X^2 = 30.80$; $P > 0.05$)

4. Discussion

We found that water hyacinth presence significantly increased diffusive CH_4 fluxes. These fluxes increased with plant coverage, especially when plants were rooting in the sediment. The latter confirms our hypothesis regarding the role of plant-mediated CH_4 fluxes. It also stresses the importance of water depth for CH_4 emissions. Contrary to our expectations, however, we found no effect of rooting on CO_2 uptake rates. Due to water hyacinth's high CO_2 sequestration rates, the overall GHG budget in terms of CO_2 equivalents still resulted in water hyacinth mats being near-neutral or even a GHG sink, depending on water hyacinth coverage.

4.1. The effect of water hyacinth on oxygen and CH_4 emissions

Water hyacinth's cover lead to lower oxygen concentrations in the water column (Fig. 2). Low oxygen concentrations below other floating plant species have been reported in field and laboratory studies (Masifwa, Twongo and Denny, 2001; Nahlik and Mitsch, 2006) and have been attributed to the suppression of O_2 diffusion across the air-water interface, decrease of primary production in the water column due to lower light availability and the high oxygen demand of decomposing plant material (Reddy and DeBusk 1991).

Low oxygen concentrations in the water may, however, result in a lower O_2 penetration depth in the sediment, in turn increasing CH_4 emissions (Huttunen et al. 2006) which may, at least partially, explain why the

diffusive CH_4 emission was up to 17 times higher in aquaria with water hyacinth compared to the controls. Water hyacinth has previously been reported as a CH_4 enhancer (2 to 5 times more CH_4 emissions from water hyacinth mats compared to open waters) (Banik et al., 2013). Other studies, in contrast, showed 2.6 times higher CH_4 fluxes from open waters compared to from water hyacinth mats (Attermeyer et al., 2016). We postulate that this discrepancy may well be driven by different underlying mechanisms. For one, the coverages in these two studies might have differed, with higher coverages leading to higher methanogenic rates. Additionally, along the roots of water hyacinth, CH_4 oxidation takes place due to the metanotrophic activity (Yoshida, et al., 2014), and due to a radial oxygen loss provided by this plant (Kosten, et al., 2016). Variation in root biomass and exudate loss, the composition and activity of microbial communities, and water and sediment composition can be expected to affect CH_4 oxidation rates and hence CH_4 emission rates. In our study, we found that rooting led to 1.3 and 4 times higher diffusive CH_4 emissions at high and low plant coverage, respectively, most possibly caused by the direct transportation of the CH_4 produced in the sediment to the atmosphere thereby escaping CH_4 oxidation (Bastviken, 2009; Thomas, et al., 1995). Previous studies also found that shallow systems might show more effective plant-mediated CH_4 transport due to relative short distance between the sediment and the floating parts (Hamilton et al., 2014), resulting in higher emission rates.

Higher coverage led to higher diffusive CH_4 fluxes, presumably due to the production of (dissolved) organic matter substance and further lowering of O_2 concentrations. If the plant-mediated transport indeed occurs, rooting in the sediment might avoid the formation of bubbles in the sediment, thereby decreasing ebullition and enhancing the proportion of CH_4 emitted by the plant tissue. We did, however, not find a significant effect of rooting on ebullition in our study, only for plant coverage.

4.2. The effect of water hyacinth on nutrient dynamics and carbon dioxide emissions

The percentage coverage by plants and their access to the sediment did not change nutrient uptake and allocation in a consistent way, although the rooting plants grown at high coverage showed higher N: P ratios (Fig. 3), due to higher N concentration (Fig S2). Relatively high N concentrations under high-coverage conditions have been found in the field as well and have been related to N supply to the plant (Reddy et al., 1989). The general absence of a strong effect of root access to the sediment on plant nutrient contents suggests that the plants are capable of mobilizing nutrients from the sediment even without direct contact. This has also been demonstrated for the floating macrophyte *Stratiotes aloides*, for which the lowering of O_2 levels due to high coverage can promote P release from the sediment by weakening the bonds of Fe-P complexes (Harpenslager, et al., 2016). The fact that water column nutrient concentrations tented to be higher in the treatments where the plants are rooted in the sediment (Table 2) suggests, however, that the plants preferably tap into the rich sediment nutrient pools directly. Pore-water concentrations of N and P were 220 and 30 times higher than in the surface water (results not shown). *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum* have been reported to only take up P from the sediment (Barko and Smart, 1980).

More efficient nutrient uptake when rooted in the sediment could lead to higher growth rates and concomitant CO_2 sequestration. We did not find, however, higher CO_2 sequestration in our rooted treatments. We only found a clear difference between the coverages, with on average 1.6 times lower CO_2 sequestration rates at high coverages, which we attribute to the limited space for growth.

On average our plant treatments sequestrated -3.4 ± 2.2 g CO_2 m^{-2} day^{-1}, regardless of coverage and the position of the roots. This is notably higher than sequestration rates of other rooted aquatic plants, such as *Typha domingensis* and *Eichhornia azurea*, showing sequestration rates around -0.09 g CO_2 m^{-2} day^{-1} (Gripp et al. 2013).

Our results match with the range found previously for water hyacinth between 3.4 and 5.4 g CO_2 m^{-2} day^{-1}, in field conditions (Peixoto et al. 2016; Attermeyer et al. 2016).

In aquaria without water hyacinth, CO_2 fluxes took place leading a 24h net emission of, on average, 0.3 g CO_2 m^{-2} day^{-1} (Fig. 4). The net emissions from the non-vegetated aquaria contrast the net CO_2 sequestration in the plant treatments indicating that the plants offset the CO_2 emissions from the systems without plants. Clearly, net emissions from both vegetated and non-vegetated systems strongly depend on sediment CO_2 production rates related to the input of decaying plant material.

4.3. Effects of water hyacinth on the overall GHG balance

Under the experimental conditions of our study in the absence of water hyacinth, CH_4 emissions were modest and net CO_2 emissions took place, leading to an overall emission of GHGs (Fig. 5). At low coverage, however, water hyacinth was a net sink of GHGs, regardless of the position of the roots. At high coverage CO_2 sequestration only partially counterbalanced CH_4 emissions, thereby making the system become a small GHG source.

Whether water hyacinth acts as a GHG sink or source depends on the balance of its effects on CH_4 emissions and CO_2 uptake rates. We here show that this balance strongly depends on plant coverage and rooting, with partly opposite effects with respect to CO_2 and CH_4. The plants tend to enhance CH_4 emissions especially at high coverage and when rooting in the sediment, whereas CO_2 uptake rates are highest at low coverage where growth was not space-limited and nutrient availability per plant is higher.

Ebullition plays an important role in the overall GHG balance, since it accounted, on average, for 58% of the total CH_4 emissions for all treatments and even reached 82% at low coverage. This underlines once more that ebullition is one of the most important pathways of CH_4 emission from wetlands to the atmosphere (Bastviken et al, 2008; Sawakuchi et al. 2014; Segarra et al. 2013).

Our results highlight that the presence of water hyacinth mats can alter GHG emissions. CO_2 sequestration rates are enhanced and hence can trigger a regional effect offsetting the greenhouse gas emissions for open waters. Using water hyacinth for nutrient-rich wastewater purification under a relatively low coverage (like 50% coverage) by regular harvest will likely reduce the emission of CH_4 and increase the sequestration of CO_2, especially when roots are prevented from reaching the sediment. As a main conclusion, we here showed that access to the sediment, as related to water depth, and plant coverage are crucial factors influencing both nutrient dynamics and GHG emissions, which may explain the discrepancies reported in literature and should be taken into account when making regional GHG balances.

5. Author contribution

The experiment was designed by Ernandes S. Oliveira Junior, Yingying Tang, Sarian Kosten and Leon P. M. Lamers, and executed by Ernandes S. Oliveira Junior, Yingying Tang and Sanne van den Berg. The manuscript was written by Ernandes S. Oliveira Junior and Yingying Tang with contribution of all co-authors.

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Tables

Table 1. Sediment characteristics at the beginning of the experiment (mean ± SD; n=3). All analyses were performed using fresh or dry sediment (see text 2.2).

Characteristic	Unit	
Organic matter content	%	3.32±0.42
Total-P	µmol g^{-1} DW	15.65±1.27
Olsen-P	µmol g^{-1} DW	0.85±0.10
Salt-extractable NH_4^+	µmol g^{-1} DW	0.25±0.08
Salt-extractable NO_3^-	µmol g^{-1} DW	0.02±0.00
Total-Fe	µmol g-1 DW	88.13±4.65
Total-Al	µmol g^{-1} DW	81.49±3.80
Total-Ca	µmol g^{-1} DW	100.43±6.63

Table 2. Water characteristics in surface water during the experiment. All concentrations are given in µmol L^{-1}. DOC concentrations were determined at the end of the experiment (mean ± SD; n=4), whereas other parameters were analyzed multiple times during the experiment (overall average are given, mean ± SD; n=4).

Characteristics	C w/o mesh	C with mesh	50%nR	50%R	100%nR	100%R
DTP	17.4±10.3[a]	11.1±2.7[a]	2.2±0.3[b]	7.8±9.2[b]	6.4±8.3[b]	3.7±2.6[b]
PO_4^{3-}	12.8±5.8[a]	9.5±2.7[a]	1.6±0.3[b]	7.8±10.8[b]	1.4±0.2[b]	2.8±2.0[b]
NH_4^+	16.9±12.4	6.2±1.1	6.8±0.9	60.6±108.4	39.8±67.5	6.2±1.1
NO_3^-	9.5±1.6[a]	1.2±1.1[b]	1.8±2.1[b]	0.4±0.2[b]	0.6±0.4[b]	0.4±0.3[b]
TIC	734.3±198.4[b]	1020.4±133.3[a]	1052.0±201.7[a]	1417.0±556.1[a]	1319.0±259.4[a]	1212.0±206.5[a]
DOC	944.0±271.8	890.7±663.1	855.5±396.0	469.3±548.5	997.2±547.3	629.0±290.5

Significant differences among treatments are indicated by different lower case letters.

Figure legends

Figure 1. Experimental design. C w/o mesh represents control without mesh; C w mesh represents control with mesh; 50% nR represents low coverage with mesh; 50% R represents low coverage without mesh; 100% nR represents high coverage with mesh; 100% R represents high coverage without mesh.

Figure 2. Mean dissolved oxygen concentrations (±SD) of the water layer at 20 cm depth for controls (C), low coverage (50%), and high coverage (100%) of water hyacinth with (R) or without rooting (nR) in the sediment. Different lower case letters indicate significant differences between treatments by post hoc test ($P < 0.001$).

Figure 3. Ratios (±SD) between C and P (light grey), between C and N (dark grey), and between N and P (black) in petioles (left panel) and roots (right panel) of water hyacinth for low coverage (50%) and high coverage (100%) with (R) or without (nR) roots in the sediment at the end and start of the experiment. All nutrient ratios are given in mol mol^{-1}. Different lower case letters indicate significant differences between treatments including the start of the experiment ($P < 0.05$). Note the log_{10} scale on the y-axis.

Figure 4. CH_4 (left) and CO_2 (right) fluxes (±SD) for controls (C), low coverage (50%), and high coverage (100%) of water hyacinth with (R) or without rooting (nR) in the sediment. Letters indicate significant differences between treatments ($P < 0.05$) indicating, from top to bottom: total fluxes, ebullitive flux, and diffusive flux. Note different scales for the y-axis. Negative numbers refer to sequestration.

Figure 5. Global warming potential (GWP; mean ± SD) for controls (C), low coverage (50%) and high coverage (100%) water hyacinth coverage with or without rooting in the sediment. Different lower case letters indicate significant differences between treatments ($P < 0.001$).

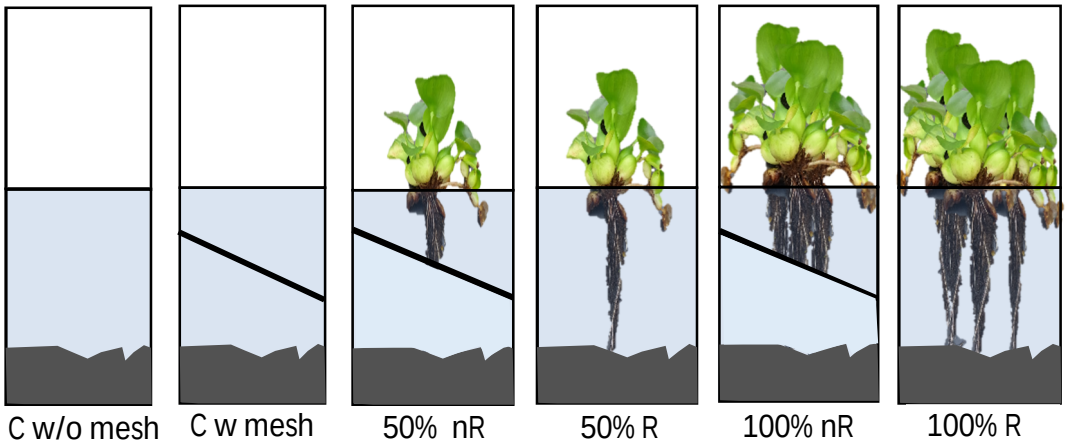

Fig 1

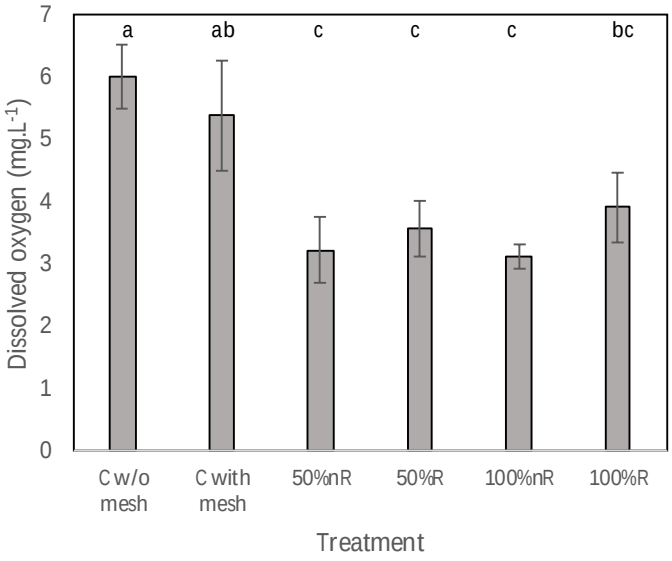
7
6
5
4
3
2
1
0
Dissolved oxygen (mg.L-1)
a
ab
c
c
c
bc
C w/o mesh
C with mesh
50%nR
50%R
100%nR
100%R
Treatment

Fig 2

5

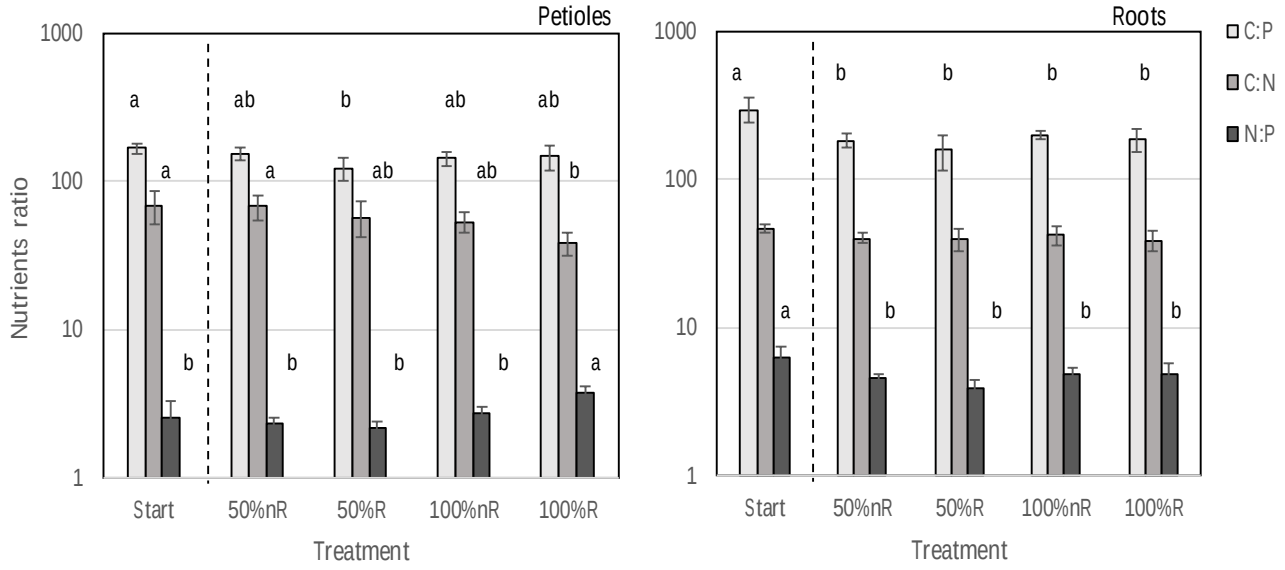
Petioles
Roots
C:P
C:N
N:P
Nutrients ratio
1000
100
10
1
a
ab
b
ab
ab
a
a
b
a
ab
ab
b
b
b
b
a
a
b
b
b
b
b
Start
50%nR
50%R
100%nR
100%R
Treatment

Fig 3

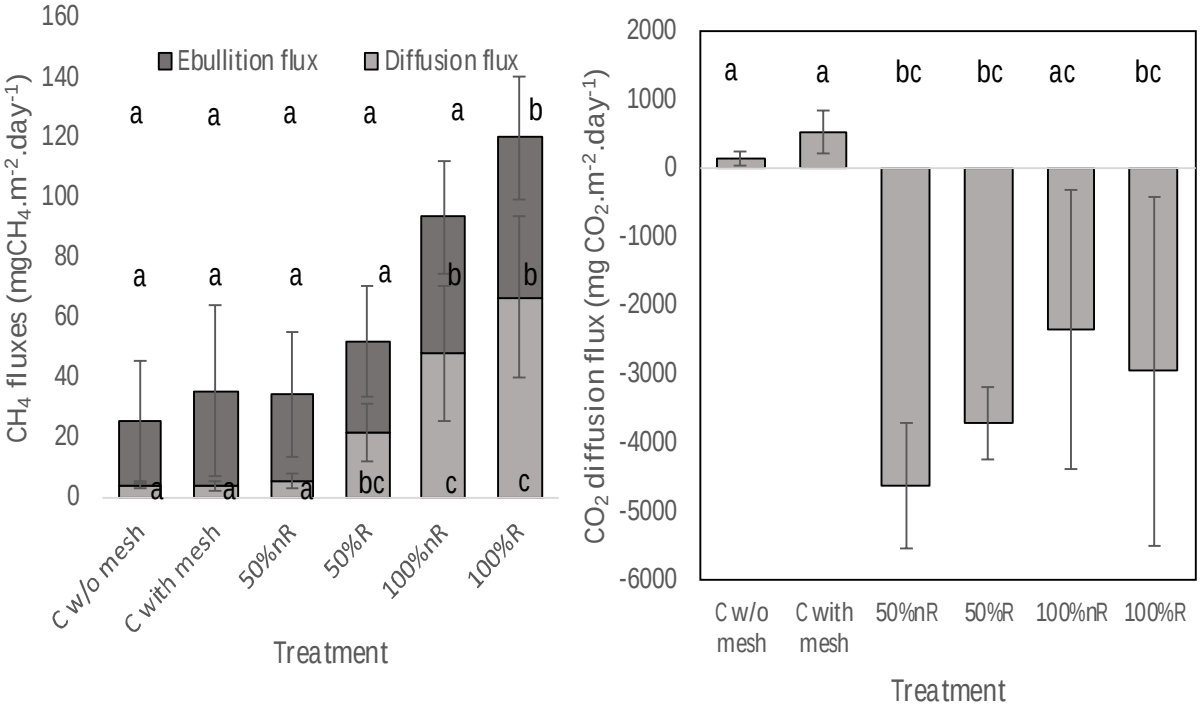
Ebullition flux
Diffusion flux
CH4 fluxes (mgCH4.m-2.day-1)
Treatment
C w/o mesh
C with mesh
50%nR
50%R
100%nR
100%R
CO2 diffusion flux (mg CO2.m-2.day-1)

Fig 4

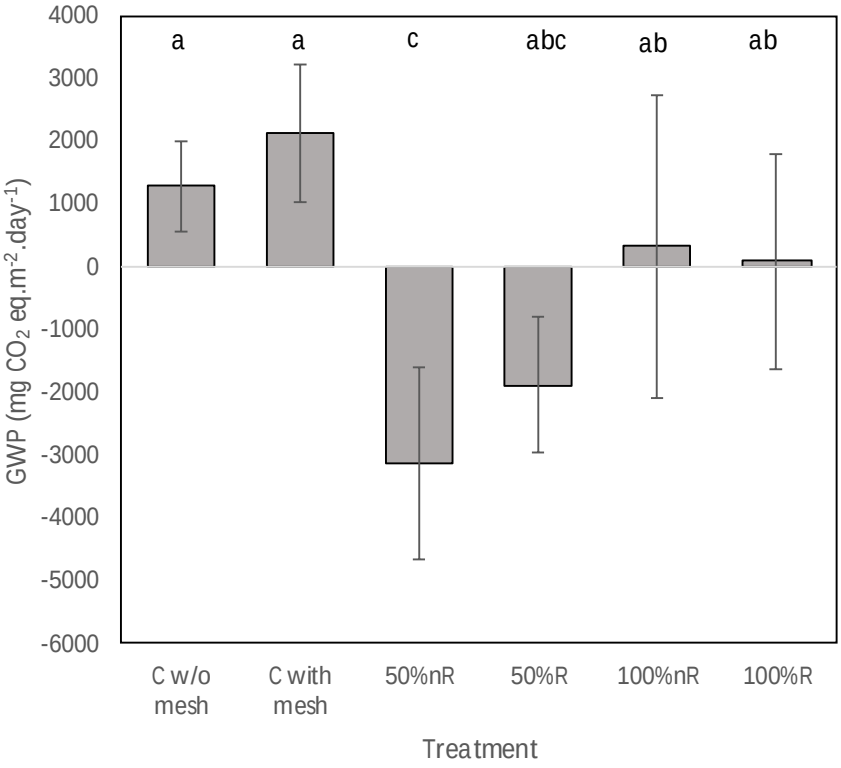
4000
3000
2000
1000
0
-1000
-2000
-3000
-4000
-5000
-6000
GWP (mg CO_2 eq.m^{-2}.day^{-1})
a
a
c
abc
ab
ab
C w/o mesh
C with mesh
50%nR
50%R
100%nR
100%R
Treatment

Fig 5