

Impact of native and non-native aquatic plants on methane emission and phytoplankton growth

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Received: 1 November 2016 / Accepted: 5 August 2017 / Published online: 4 September 2017

Handling editor: Angela Boggero

Editor's note:

This study was first presented at the Centre for Wetland Ecology (CWE) symposium (24 June 2016, Wageningen, the Netherlands) on the role of exotic species in aquatic ecosystems (<https://www.wetland-ecology.nl/en/calendar/good-bad-or-bit-both-role-exotic-species-aquatic-ecosystems>). This symposium provided a venue to unravel how exotic plants and animals impact ecosystem functioning, find out whether they coexist or compete with native species and discover their impact on native flora and fauna.

Abstract

Freshwater plants affect the ecosystem functioning of shallow aquatic ecosystems. However, because native plants are threatened by environmental change such as eutrophication, global warming and biological invasions, continued ecosystem functioning may be at risk. In this study, we explored how the growth of native and non-native plant species in eutrophic, warm conditions impacts two plant ecosystem functions: regulation of phytoplankton growth and methane emission. We expected that plants would inhibit phytoplankton growth, while for methane emission both inhibition and stimulation are possible. We conducted an outdoor experiment using monocultures of four native and four non-native freshwater plant species planted at three different densities, as well as a no-plant control. Monocultures of each species were planted in 65 L mesocosms and after three weeks of acclimatisation each mesocosm was inoculated with phytoplankton. Subsequently, we added nutrients twice a week for eight weeks, before harvesting the plant biomass. During these eight weeks, we measured chlorophyll-*a* concentration thirteen times and the diffusive methane emissions once after four weeks. The mesocosms amplified the temperature of a warm summer so that plants were exposed to higher-than-average temperatures. We found that five plant species lost biomass, two species increased their biomass only at the highest initial plant density (native *Myriophyllum spicatum* and non-native *Lagarosiphon major*) and a single species increased its biomass at all densities (on average 14 times its initial mass; amphibious non-native *Myriophyllum aquaticum*). Overall, the mean biomass change of non-natives was positive, whereas that of natives was negative. This difference in biomass change between native and non-native plants did not relate to overall differences in phytoplankton mass or diffusive methane emissions. In mesocosms where submerged plant species gained biomass, chlorophyll-*a* concentration was lower than in the no-plant control and mesocosms with biomass loss. Diffusive methane emissions were highest in mesocosms where plants lost considerable biomass, likely because it increased substrate availability for methanogenesis. However, mesocosms where plant biomass increased had emissions similar to the no-plant control, hence we found no inhibitory effects of plant presence on diffusive methane emission. We conclude that plant growth in eutrophic, warm conditions varies strongly with plant identity. Our results furthermore suggest that plant identity determines whether the replacement of native by non-native freshwater plants will alter ecosystem functions such as regulation of phytoplankton growth and methane emission.

Key words: ecosystem functioning, eutrophication, greenhouse gas, invasive species, macrophyte species, phytoplankton blooms

Introduction

Climate change, eutrophication (Jeppesen et al. 2009; Moss et al. 2011) and biological invasions (Vilà et al. 2009) threaten the provisioning of ecosystem functions and services by freshwater ecosystems. Eutrophication and climate change act in synergy to shift freshwater ecosystems from plant- to algae-dominated systems (Smith et al. 1999; Jeppesen et al. 2009; Moss et al. 2011), which can result in larger and more frequent harmful algal blooms (Paerl and Huisman 2009; Michalak et al. 2013). Increases in water temperature, nutrient availability and CO₂ levels all favour phytoplankton, especially cyanobacteria, so that future temperate freshwaters will contain fewer submerged plants (Moss et al. 2011; Kosten et al. 2012; De Senerpont Domis et al. 2014). As submerged plants inhibit phytoplankton blooms and provide food and habitat for fauna this may result in a loss of ecosystem functions (Carpenter and Lodge 1986; Jeppesen 1998; Scheffer 2004). With continuing climate change and eutrophication, the provision of ecosystem functions likely depends on the growth of freshwater plants under these new conditions.

While many native plant species are in decline, non-native freshwater plants are becoming more abundant (Hussner 2012; van Kleunen et al. 2015) and often replace native freshwater plants (Stiers et al. 2011; Hussner et al. 2014). There are multiple reasons why invasive plants, defined as non-natives that cause negative environmental or economic impacts, are successful: they produce more shoots, are bigger, and attain a higher biomass than non-invasive plants (van Kleunen et al. 2010). Many non-native plants found in Europe originate from warmer regions (Hussner 2012). Adaptation to a warm climate enables non-native species to grow faster with increasing temperature (Hussner 2009) and to tolerate higher temperatures, which can provide them with a competitive advantage over natives (Hussner and Lösch 2005; Hussner et al. 2014). Some non-native plants can produce both submerged and aerial shoots, which yields a competitive advantage (Netten et al. 2010; Stiers et al. 2011; Hussner 2012; van Gerven et al. 2015). Other species tolerate low light availability resulting from phytoplankton and epiphyton shading (Hussner et al. 2010; Zefferman 2014), and a high proportion of non-native species found in Europe release oxygen to prevent anaerobic root damage (Lemoine et al. 2012). Because of these adaptations, non-native freshwater plants can perform better in warm, eutrophic conditions than natives, yet little is known about their provisioning of ecosystem functions in comparison with natives.

The ability of aquatic plants to inhibit phytoplankton dominance and to limit greenhouse gas emissions are two major ecosystem functions. First, aquatic plants can prevent phytoplankton from dominating freshwaters through a variety of mechanisms, including competing for nutrients with phytoplankton, excreting allelochemicals that inhibit algal growth and facilitating grazers on phytoplankton (Scheffer et al. 1993; Scheffer 2004; Hilt and Gross 2008). Through positive feedback, the plants maintain a favourable environment for themselves as they reduce nutrient availability, increase light availability and control sediment biogeochemistry (Scheffer et al. 1993; Scheffer 2004). Second, aquatic plants may alter greenhouse gas emissions from freshwaters, especially methane, a potent greenhouse gas (Kosten et al. 2016). Methane emissions vary among wetland plant species (Ström et al. 2005; Koelbener et al. 2010), which is likely related to their differential effects on the processes driving emissions. Aquatic plants may affect the production, consumption and transport of greenhouse gasses (Pierobon et al. 2010; Ribauto et al. 2011). For example, aerial leaves can act as chimneys (Dingemans et al. 2011; Bhullar et al. 2013), submersed floral inflorescences can leak methane (Heilman and Carlton 2001a), and oxygen release into the sediment can hamper methane production and enhance methane oxidation thereby limiting its emission (Jespersen et al. 1998; van Bodegom et al. 2001; Bodelier et al. 2006; Ribauto et al. 2011). Methanotrophs growing on above-sediment plant tissue also contribute to methane oxidation (Heilman and Carlton 2001b). Furthermore, if all plants are lost and phytoplankton dominates, the decaying plants and dead phytoplankton are substrate for methane production, a process that thrives in anaerobic sediment (Dingemans et al. 2011; Ribauto et al. 2011).

In this study, we explored the growth of four native and four non-native aquatic plants in eutrophic, warm conditions, and investigated how plant growth affects phytoplankton growth and methane emissions. We hypothesised that (1) non-native plants grow better in eutrophic, warm conditions than natives, and that (2) plant growth influences phytoplankton mass and diffusive methane emissions.

Materials and methods

Experimental design

We planted monocultures of four native and four non-native plant species in cattle tanks (referred to as mesocosms; 34 cm height × 52 cm diameter, approx. 65 L; Supplementary material Figure S1) that

Table 1. Plant species information. The information on species native status and range is based on Hussner (2012) and the USDA ARS GRIN database (ars-grin.gov). Growth form indicates whether plants are submerged (underwater shoots) or amphibious (both underwater and aerial shoots). The initial plant mass (mean gram plant fresh mass) is provided for the three different densities (5, 35 and 80% plant volume infested).

Plant name	Status in NW Europe	Nativerange	Growth form	Rooting	Initial plant mass (g)
<i>Ceratophyllum demersum</i> (CD)	native	worldwide	submerged	unrooted	16; 109; 250
<i>Hottonia palustris</i> (HP)	native	worldwide	submerged	rooted	12; 84; 161
<i>Myriophyllum spicatum</i> (MS)	native	Europe, Asia	submerged	rooted	22; 127; 288
<i>Ranunculus circinatus</i> (RC)	native	Europe	submerged	rooted	23; 120; 256
<i>Cabomba caroliniana</i> (CC)	non-native	Americas	submerged	rooted	19; 147; 355
<i>Lagarosiphon major</i> (LM)	non-native	southern Africa	submerged	rooted	34; 149; 351
<i>Myriophyllum aquaticum</i> (MA)	non-native	Americas	amphibious	rooted	11; 66; 172
<i>Myriophyllum heterophyllum</i> (MH)	non-native	Americas	submerged	rooted	27; 147; 352

were placed outdoors at the Netherlands Institute of Ecology (coordinates: 51.987N; 5.671E). We also included a no-plant control to investigate the effect of plant presence. The experiment ran from 5 June to 28 August 2014 and started with a three-week acclimation period for plants to establish (see *Temperature data*). After acclimatisation, we exposed plant monocultures to severe eutrophication for 8 weeks. We measured two ecosystem functions: the inhibition of phytoplankton throughout the experiment and on a single date we quantified the diffusive methane emission from water to atmosphere.

Plant species

We selected four native species (*Ceratophyllum demersum* L., *Hottonia palustris* L., *Myriophyllum spicatum* L., *Ranunculus circinatus* Sibthorp; see Table 1) and four plant species non-native to Europe (*Cabomba caroliniana* A. Gray, *Lagarosiphon major* (Ridley) Moss, *M. aquaticum* (Vell.) Verdc. and *M. heterophyllum* Michx.) for the experiment. The four native species are common, show no decline in the Netherlands (FLORON 2015) and occur in meso- and eutrophic habitats (Bloemendaal and Roelofs 1988), hence are native species likely to persist during continued eutrophication. The chosen non-native species are increasingly abundant in Northwest Europe (Hussner 2012) and are expected to stay. *Lagarosiphon major* has its native range in southern Africa, whereas all of the other non-native species originate from the warm temperate or (sub)tropical Americas (USDA ARS GRIN database). Thus, all non-native species originate from warmer regions than the natives, as is common for non-native aquatic plants in Europe (Hussner 2012). Of all species, only *M. aquaticum* forms both underwater and aerial shoots and is an amphibious species (Stiers et al. 2011), whereas the others are strictly

submerged species (Table 1). *Ceratophyllum demersum* was the only tested non-rooting plant species.

We bought six species from an aquatic plant supplier (Zuurstofplantgigant, Hapert, the Netherlands) and collected *M. aquaticum* and *C. caroliniana* from the field, respectively from a pond at 51.347N; 6.127E and a canal at 52.169N; 5.062E in The Netherlands, where they occur as non-native species. All plants were thoroughly washed to remove fauna and debris before planting (for initial plant mass see Table 1). *Cabomba caroliniana* was planted two days prior to the end of the plant acclimatisation period because of logistical issues.

Mesocosm setup

We filled the 65 L mesocosms with 7 litres of organic sediment (Pokon Naturado BV, Veenendaal, the Netherlands) that we topped with 3 litres of sand and a 50 litre water column of tap water. Mesocosms were covered with 2 × 8 mm mesh (Mononet AR Hail, Rovero, the Netherlands) to prevent colonisation with macroinvertebrates and to prevent accidental escape of the non-native species. Each species was planted in three shoot densities: 5, 35 and 80% volume infested (PVI; see Table 1 for initial plant masses), because plant quantity can affect plant growth (van der Heide et al. 2010; Harpenslager et al. 2016). There were four replicates for each plant species × density treatment, so combined with the no-plant control treatment (n = 4), there were 100 mesocosms. Following the acclimatisation period, we recorded the PVI, before removing 30 litres of water and adding a phytoplankton inoculum to each mesocosm (30 litres of 85.4 ± 4.4 µg chlorophyll-*a* L⁻¹; mean ± SE; measured on PhytoPAM). This inoculum originated from an outdoor phytoplankton culture: sixteen 200 L cattle tanks in which phytoplankton communities were cultured using high doses of

nutrients twice a week (identical to additions described below). We left the plants and phytoplankton to grow and compete for 8 weeks, from 30 June to 28 August 2014. We added NH_4NO_3 and KH_2PO_4 twice a week to the mesocosms (each addition: $1.35 \text{ mg L}^{-1} \text{ N}$ and $0.22 \text{ mg L}^{-1} \text{ P}$, molar N:P ratio = 13.5) similar to that used by Bakker et al. (2010) and Declerck et al. (2011). The chosen nitrogen load is at the upper end of nitrogen loads in Northwest European lakes and rivers (Saunders and Kalff 2001; Harpenslager et al. 2016).

Temperature data

The mesocosms were not temperature controlled. As a consequence, they had higher-than-average water temperatures than natural freshwaters because of their limited size, dark colour and exposure to sunlight. In addition, the summer of 2014 was relatively warm (KNMI 2014), although it featured a big thermal contrast between July (6th warmest in the past century) and August (coolest in 20 years) (KNMI 2014). Temperatures frequently exceeded 25 or even 30 °C which is higher than the 20–25 °C range that is typical for temperate lakes (Wetzel 1983; Madsen and Brix 1997) (see Supplementary material Figure S2 for details). The temperature was logged every two hours (iButton, HomeChip, Milton Keynes, England) in 22 mesocosms, distributed evenly across treatments (excluding *C. caroliniana*) and confined to the centre of the mesocosm spatial arrangement. Four temperature loggers malfunctioned during the experiment (*H. palustris* 5%; *M. spicatum* 35% and 80%; *R. circinatus* 35%). The normalised minimum and maximum temperatures in the 18 mesocosms with loggers varied during the experiment (normalised as deviation from mean of 18 data points; Supplementary material Figure S3). It seemed that mesocosms with high plant biomass had slightly lower temperature maxima (i.e. *L. major* and *M. aquaticum* with PVI of 35 or 80%).

Measurements during the experiment

Because the initial phase was crucial for plant-phytoplankton dynamics, as superior competitors in the initial phase were expected to secure light and nutrient dominance, the strategy was to sample intensively at the beginning of the experiment and sample less frequently towards the end. Water samples for analysis were collected using 50 ml syringes (BD Plastipak, Franklin Lakes, New Jersey, USA) from the top five cm of the water column.

To quantify the phytoplankton biomass, we measured chlorophyll-*a* in 4 ml water samples using the

PhytoPAM (Waltz, Effeltrich, Germany), one day after adding nutrients, twice a week for the first five weeks, and then once a week for the remaining three weeks. To quantify filamentous algae and limit their effect, we carefully removed filamentous algae from infested plants and determined their dry weight (60 °C until constant dry mass), during the experiment and at the harvest (Figure S7). The removed material was not released back into mesocosms.

Multiple abiotic water parameters were determined: turbidity, alkalinity, pH, conductivity, oxygen, nitrate, nitrite, ammonium, orthophosphate and temperature. We measured the turbidity (Turb 430, WTW, Weilheim, Germany) one day after nutrient addition on five occasions during the experiment (1, 8, 14, 22 July and 20 August). The alkalinity and pH were measured using an auto-titrator (TIM840 with a PHC2401-a pH electrode, Hach, Düsseldorf, Germany) on four occasions (1, 8, 14 July and 20 August). On 1 July, we measured the temperature, pH, conductivity and oxygen concentration in all mesocosms using electrodes at 10 cm below the water surface (Multimeter 350i, WTW). Nitrate, nitrite, ammonium and orthophosphate were measured from GF/F filtered water sampled before adding nutrients (QuAAtro auto-analyser, Seal Analytical, Fareham, UK) on five occasions during the experiment (1, 8, 14, 22 July and 20 August).

Measurements of greenhouse gas emissions

We measured diffusive CH_4 emission on 29 July. Because 100 mesocosms had to be measured in one day, we sampled in replicate blocks: first, replicate 1 of all treatments was measured, subsequently all replicates 2, then 3 and lastly 4. For the measurement, we carefully placed an acrylic cylindrical chamber (headspace height: 230 mm; internal diameter: 292 mm) over the centre of each mesocosm (Figure S1). The chamber was connected in a closed loop to a greenhouse gas analyser (model GGA-24EP, Los Gatos Research, USA). This gas analyser uses the cavity enhanced laser absorption technique to measure methane partial pressure every second. Fluxes were calculated using the slopes of methane concentration and time based on measurement periods of one hundred seconds each (Supporting Information Datafile 1). We calculated the gas flux using Eq. (1) modified from Almeida et al. (2016):

$$F = \text{slope} \times \left(\frac{V}{Vm} \right) \quad \text{Eq. (1)}$$

where

$$Vm = \frac{P}{RT} \quad \text{Eq. (2)}$$

with F = gas flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$), slope = relationship between gas concentration and time (ppm h^{-1}), V = chamber volume (dm^3), V_m = molar volume ($\text{dm}^3 \text{mol}^{-1}$), A = chamber area (m^2), P = atmospheric pressure (Pa), T = air temperature at each hour from the nearest weather station: Deelen Air Base, the Netherlands, $52^\circ 03' 33'' \text{N}$; $005^\circ 52' 38'' \text{E}$, downloaded from: <http://knmi.nl/nederland-nu/klimatologie/uurgegevens> (degrees Kelvin).

Plant harvest

We harvested the 100 mesocosms over four consecutive days block-by-block (25–28 August). Aboveground plant biomass was clipped, washed and dried (60°C until constant dry mass) before weighing (dry mass; DM). Based on the initial and final plant dry mass, we calculated the net biomass gain or loss, which is the metric referred to as “plant growth” throughout the manuscript.

Data analysis

The final plant dry mass was analysed using a two-factor ANOVA (plant species \times plant density) and Tukey post hoc comparisons. For the comparison among native and non-native species, we calculated group averages of all species \times density treatments ($n = 24$) and used these in a one-factor ANOVA (two levels: native versus non-native, with $n = 12$ for each level).

Phytoplankton concentration over time was tested using linear mixed models (LMM) with a three-factor fixed effect structure including all interactions (plant species, density and time), along with a random intercept (mesocosm) and an AR1 correlation structure (mesocosm). Inference on fixed effects was performed using type II Wald F tests. Post hoc comparisons were conducted by fitting a LMM for each species density treatment in addition to no-plant controls, followed by Wald F tests. P values were used with Bonferroni correction for three comparisons (5, 35 and 80% PVI for each species = three tests for each plant species).

We calculated the time-integrated phytoplankton mass (*area under the curve*) as a measure of the overall inhibition of phytoplankton and analysed it using a two-way ANOVA (factors: plant species \times plant density). The area under each individual phytoplankton curve was calculated with Simpson's rule (function *sintegral*) after converting chlorophyll-*a* from $\mu\text{g L}^{-1}$ to $\text{mg chl-}a$ (based on 50 litres of water per mesocosm). As post-hoc we used “Dunnett” comparisons within each plant species to test whether treatments differed from the no-plant control. For

the comparison among native and non-native species, we calculated group averages of all species \times density treatments ($n = 24$) and used these in a one-way ANOVA (two levels: native versus non-native).

Environmental parameters of interest were analysed using a two-way ANOVA tests (plant species \times plant density) and if these turned out significant, with Tukey post hoc comparisons.

CH_4 emissions were analysed using a two-way ANOVA (plant species \times plant density) blocked for timing of measurement on the day (four levels) followed by all pairwise post hoc comparisons with P values adjusted for the “false discovery rate” to reduce the number of false negatives while controlling for the number false positives (Benjamini and Hochberg 1995). Tests among groups of plants were performed using Kruskal Wallis tests because transformation did not help satisfy model assumptions. As an additional post-hoc, we used “Dunnett” comparisons to compare the three density treatments to the no-plant control within each species. To test the relation between net plant biomass change and methane emission, we performed linear regression, with the net biomass converted to positive values (by adding 50) and then transforming these data using the reciprocal. To test the relation between net plant biomass change and the integrated chlorophyll concentration, we performed a linear regression only on all strictly submerged species, thus excluding *M. aquaticum*.

Data were analysed in R version 3.3.2 (R Core Team 2013) using the *nlme* (Pinheiro et al. 2015), *multcomp* (Hothorn et al. 2008), *Bolstad* (Curran and Bolstad 2016), *tidyr* (Wickham 2014), *dplyr* (Wickham and Francois 2015), *ggplot2* (Wickham 2011) and *car* packages (Fox and Weisberg 2011). Model assumptions were verified through residual analysis, e.g. for non-normality, heteroscedasticity, and when necessary to meet assumptions, data were square-root or log transformed. Data are available from the Dryad Digital Repository: <https://datadryad.org/resource/doi:10.5061/dryad.6hf6b>.

Results

Plant growth and survival during acclimatisation and under eutrophication

Plant species differed in their response to the three-week acclimatisation period (Two-way ANOVA; plant species: $F_{6,63} = 14.3$, $P < 0.001$; plant density: $F_{2,63} = 1.79$, $P = 0.17$; interaction: $F_{12,63} = 1.89$, $P = 0.053$, Supplementary material Figure S4). Three species increased their percent volume infested (PVI): *L. major* (+22% across all treatments), *M. spicatum* (+49%) and *M. heterophyllum* (+14%), whereas one

Figure 1. Plant dry mass at the end of the experiment for native (green) and non-native (blue) aquatic plant species. Each data point represents mean \pm SE (if no error bars are visible it is because they are small, $n = 4$). Different letters indicate statistically significant differences among treatments (post-hoc tests of all pairwise comparisons using false discovery rate). For full species names see Table 1.

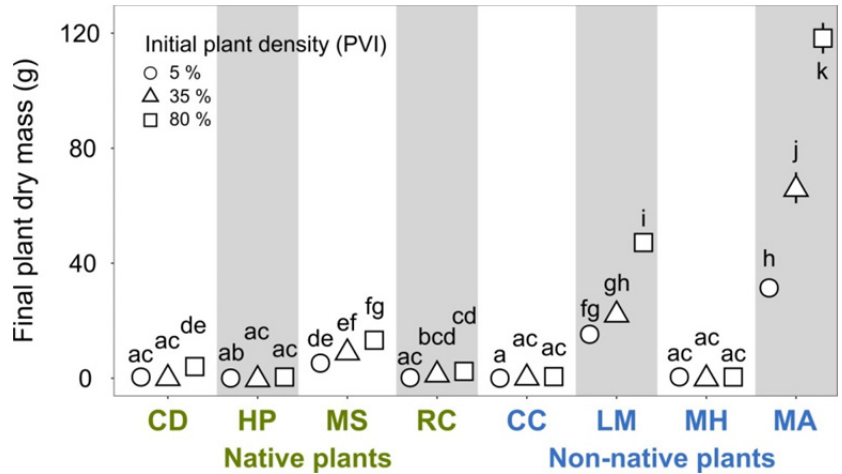
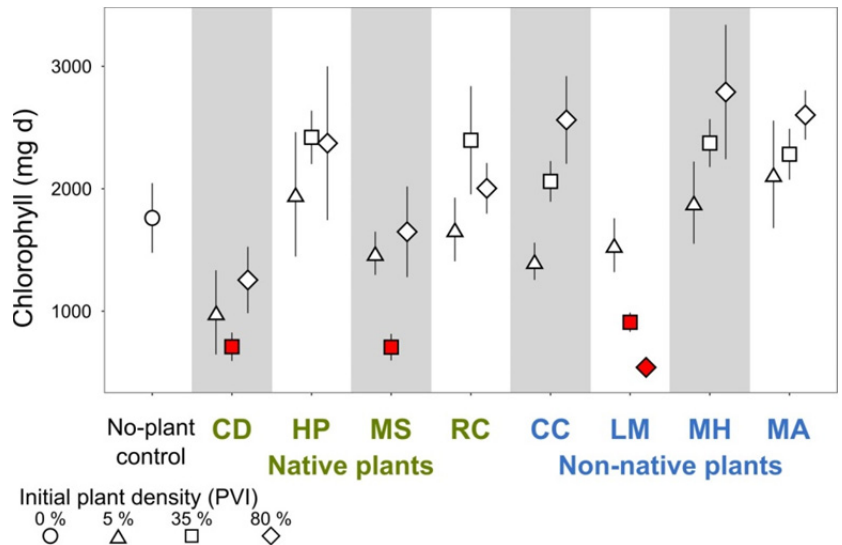


Figure 2. Chlorophyll-*a* – as a proxy for phytoplankton – integrated over time (area under the curve; for 50 L mesocosms; see Supplementary material Figure S8 for chlorophyll-*a* curves over time) for native (green) and non-native (blue) plant species at three initial plant densities (PVI). Data points represent mean \pm SE. Red filled data points indicate treatments that differ significantly from the no-plant control. For full species names see Table 1.



species remained unchanged: *M. aquaticum* (-2%). On the contrary *C. demersum* had a slightly decreased PVI (-20%), but *H. palustris* (-36%) and *R. circinatus* (-44%) lost respectively a third and almost half of their initial plant PVI. Overall, we found that non-native species (mean: $+11.5\%$) had a higher PVI increase than natives (mean: -13% ; One-way ANOVA: plant species $F_{1,19} = 5.01$, $P = 0.037$).

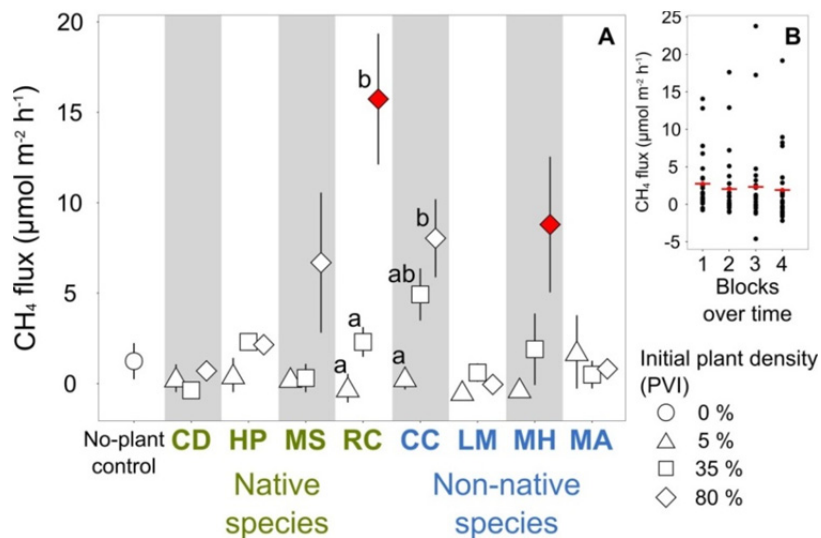
At the end of the experiment the plant monocultures differed substantially in their dry mass (Figure 1; two-way ANOVA: plant species: $F_{8,75} = 468$, $P < 0.001$; plant density: $F_{2,75} = 110$, $P < 0.001$; interaction: $F_{14,75} = 17$, $P < 0.001$). Both the non-natives *L. major* and *M. aquaticum* grew during the experiment and the native *M. spicatum* sustained itself at relatively low biomass, whereas the other species had only little biomass left at the end of the experiment (Figure 1). The initial planting density affected

the final biomass in four species (*C. demersum*, *L. major*, *M. aquaticum*, *M. spicatum*, see Figure 1) as plant biomass was higher in the highest compared to the lowest initial planting density in these species. Among all plant species, non-native species (33.6 ± 2.6 g; mean \pm SE) had a higher final dry mass than natives (3.1 ± 2.5 g; One-way ANOVA: plant species $F_{1,19} = 5.61$, $P = 0.029$).

Inhibition of phytoplankton

The time-integrated phytoplankton mass (area under the curve) varied among plant species and for some species it showed an effect of planting density (Two-way ANOVA; plant species: $F_{7,72} = 13.0$, $P < 0.001$; plant density: $F_{2,75} = 1.98$, $P = 0.15$; interaction: $F_{14,72} = 2.21$, $P = 0.015$; Figure 2). PhytoPAM measurements suggested that green algae dominated

Figure 3. Diffusive water-atmosphere methane fluxes ($\mu\text{mol m}^{-2} \text{h}^{-1}$) for (A) each of the tested native (green) and non-native (blue) submerged plants varying in initial densities (percent volume infested in %) and the no-plant control, and (B) each of the experimental design blocks over time. Positive values represent emission to the atmosphere. Letters indicate statistical differences among density treatments within each species and are omitted if there were no statistical differences. Red data points in panel A indicate statistically different treatment means compared to the no-plant control (Dunnett test). Each black data point in panel B represents one replicate. Red data points with lines represent the mean. See Table 1 for full species names.



the phytoplankton community (proportion green algae > 0.5 in 1039 cases, blue algae > 0.5 in 1 case, brown algae > 0.5 in 260 cases; $n = 1300$). Tested within plant species, we found that phytoplankton mass in *C. demersum*, *L. major*, *M. spicatum* at 35% initial PVI and *L. major* at 80% initial PVI were significantly lower than in the no-plant control (Figure 2). Tests on phytoplankton concentration over time provided similar results (see Supporting Information Figure S8; Table S1). On average, native and non-native plant species did not differ in their integrated phytoplankton mass (One-way ANOVA; plant species: $F_{1,22} = 1.15$, $P = 0.30$).

Through regression analyses on plant biomass and chlorophyll-*a*, we found that net biomass gain correlated with decreased time-integrated chlorophyll-*a* during the experiment, tested with only strictly submerged species (Figure 4A; linear regression: $R^2 = 0.38$; $F_{1,19} = 18.1$; $P = 0.002$). Regression with amphibious *M. aquaticum* showed the opposite: mean integrated chlorophyll increased with increasing mean net biomass gain (Figure 4A; $R^2 = 0.99$; $F_{1,1} = 190.1$; $P = 0.046$), although sample size was limited.

Methane emissions

Diffusive CH₄ emissions varied among plant species and initial plant density, with no significant block effect (Figure 3A and 3B; Two-way ANOVA with blocks; plant species: $F_{7,69} = 4.83$, $P < 0.001$; plant density: $F_{2,69} = 20.7$, $P < 0.001$; interaction: $F_{14,69} = 3.74$, $P < 0.001$; block: $F_{3,69} = 0.40$, $P = 0.75$). Emissions were especially high for *R. circinatus* and *C. caroliniana* at the highest initial PVI. There was no

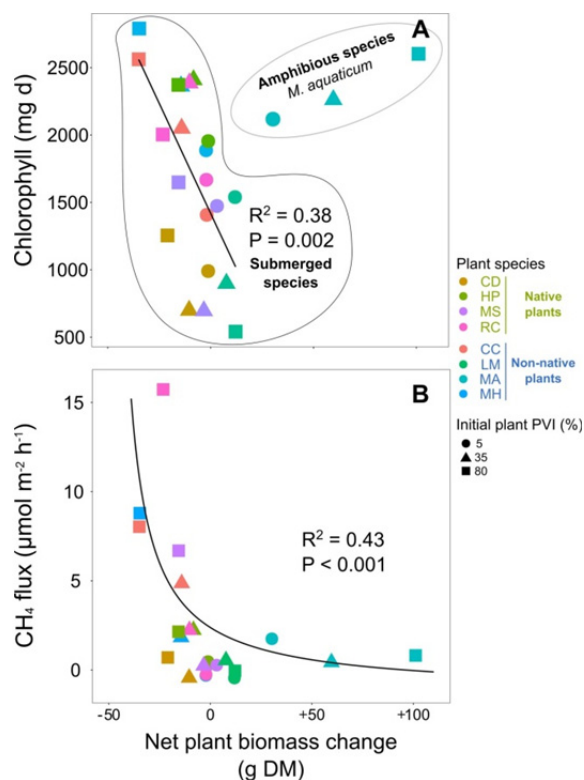


Figure 4. Correlation between mean net plant biomass change (g DM) versus (A) time-integrated chlorophyll concentrations (mg d; see Supplementary material Figure S8 for the curves) and (B) mean diffusive methane fluxes ($\mu\text{mol m}^{-2} \text{h}^{-1}$), for all eight tested plant species at three initial densities (circle: 5 %, triangle: 35 % and square: 80 % PVI).

difference in mean CH₄ emission of native and non-native plants (Kruskal Wallis test: $\chi^2 = 0.06$; df = 1; P = 0.81).

CH₄ emissions were positively correlated with net plant biomass loss, but net plant biomass gain did not reduce CH₄ fluxes compared to the no-plant control (Figure 4B; linear regression: R² = 0.43; F_{1,22} = 18.1; P < 0.001).

Environmental parameters and filamentous algae

At the end of the experiment, the nitrate concentration ranged from 0 to 61.6 $\mu\text{M L}^{-1}$ and was highest in mesocosms with *C. demersum*, *M. spicatum* and *L. major* (Supplementary material Figure S5). The mean ammonium concentration did not differ among plant species nor among initial planting density (Two-way ANOVA; plant species: F_{8,75} = 1.48, P = 0.18; plant density: F_{2,75} = 0.57, P = 0.37; interaction: F_{14,75} = 0.50, P = 0.92; Supplementary material Figure S5). Phosphate concentrations varied strongly among plant species treatments (Two-way ANOVA; plant species: F_{8,75} = 9.2, P < 0.001; plant density: F_{2,75} = 1.01, P = 0.37; interaction: F_{14,75} = 0.57, P = 0.88; Supplementary material Figure S5), with low concentrations in the no-plant control (0.02 ± 0.01 $\mu\text{mol L}^{-1}$; mean ± SE) and *M. aquaticum* (0.02 ± 0.01) and high concentrations in *C. demersum* (0.46 ± 0.09) and *M. spicatum* (0.33 ± 0.07). Nitrite was not detected in the water samples.

Alkalinity decreased steadily with ongoing nutrient additions for *C. demersum*, *L. major*, *M. aquaticum* and *M. spicatum*, less so for all other species, and in the no-plant control the alkalinity stabilised at 1 meq L⁻¹ (Supplementary material Figure S6).

Plant species monocultures differed in how much filamentous algae biomass they accumulated (Two-way ANOVA: plant species: F_{8,75} = 6.11; P < 0.001), but the filamentous algal biomass did not vary with initial plant density (F_{2,75} = 0.05; P = 0.96) nor with the interaction of density with plant species (F_{14,75} = 0.87; P = 0.60). Post hoc tests showed that *M. aquaticum* had significantly less filamentous algae biomass than *C. demersum* (Supplementary material Figure S7), whereas all other species accumulated intermediate biomasses of filamentous algae.

Discussion

Native and non-native aquatic plant species varied strongly in their response to eutrophication, and in their inhibition of phytoplankton and diffusive methane emissions. Non-native plants gained on average a higher biomass than the natives. Two non-natives in particular were capable of sustaining a high, positive

growth in eutrophic conditions under warmer-than-average temperatures, thus partially supporting our first hypothesis. Furthermore, plant growth, which was negative in case of biomass loss, was related to both phytoplankton mass and methane emission, supporting our second hypothesis. Plants with a strong positive growth inhibited phytoplankton, and those with a negative growth increased methane fluxes compared to the no-plant control. Despite their higher growth as a group, i.e. an increase in biomass, non-native plants did not affect phytoplankton mass or methane emissions differently compared to native plants.

Effects of eutrophication

Five aquatic plants performed poorly in the eutrophic experimental conditions: only the native *M. spicatum* and the non-natives *L. major* and *M. aquaticum* had substantial biomass (~ 10 g dry mass) left at the end of the experiment. The natives *H. palustris* and *R. circinatus* had mostly disintegrated by the end of the experiment. The non-native *M. aquaticum* was most successful, probably because of its amphibious growth form, which can facilitate a high growth rate, improve its tolerance to eutrophication, and ease light, carbon and nutrient competition with phytoplankton (Hussner 2009). The non-native *L. major* and native *M. spicatum* also persevered until the end, but with lower biomass. These two submerged species are commonly associated with eutrophic conditions and being elodeids, they are expected to handle warming and eutrophication well (Mckee et al. 2002).

Before the eutrophication phase, *H. palustris*, *R. circinatus* and *C. demersum* were already in decline. The non-native plants had increased their density or remained unchanged compared to their density at planting. The three declining natives may have succumbed to the higher-than-average temperatures, although we cannot be certain because there was no control treatment for temperature. The non-native species can grow at temperatures up to 30 °C or above (Mckee et al. 2002; Hogsden et al. 2007) and so does the native *M. spicatum* (Van et al. 1976; Barko and Smart 1981). On the contrary, the three other natives generally have lower temperature tolerance (Van et al. 1976; Hyldgaard and Brix 2012). All of these freshwater plants faced challenging higher-than-average water temperatures, often exceeding 25 °C or even 30 °C. These summer temperatures exceed the range of 20–25 °C that is common in temperate lakes. Thus, species were exposed to a future temperature scenario for temperate areas, which is known to exacerbate eutrophication stress (Smith et

al. 1999; Jeppesen et al. 2009; Moss et al. 2011). The nutrient level in the experiment was at the high end of the combined atmospheric deposition (Morris 1991), external loading (Saunders and Kalff 2001), and internal mobilisation (Geurts et al. 2010). These are not unrealistic however, as many wetlands receive very high nutrient inputs, especially in Africa and Asia (Hecky et al. 2003; Odada et al. 2004; Solanki et al. 2010; Jafari et al. 2015). Land run-off is predicted to increase in response to climate change which will further increase nutrient availability in freshwaters worldwide (Jeppesen et al. 2009; Strayer and Dudgeon 2010; Moss et al. 2011). Finally, *C. caroliniana*, and possibly some native species, might have performed better on a different sediment.

We found that the tested non-native plants performed better than natives in the eutrophic conditions, as indicated by the net biomass change. However, it should be noted that there was largely interspecific variation in plant growth: only two of four non-native species performed well: *L. major* and *M. aquaticum*, as did the native *M. spicatum*. Interestingly, the native *M. spicatum* is highly invasive in North America (Aiken et al. 1979; Patrick et al. 2012). All three successful plant species in our experiment are therefore highly invasive outside their native range, which illustrates that plant invasiveness seems linked to plant growth in disturbed conditions, such as high nutrient loads and higher temperatures (Hussner and Lössch 2005; Ali and Soltan 2006; Chase and Knight 2006; Hussner 2009; Patrick et al. 2012).

Ecosystem function: inhibiting phytoplankton growth

So, how did plant growth under eutrophication affect phytoplankton growth? We found contrasting results among the different plant species. The single amphibious species tested, *M. aquaticum*, with mostly emergent biomass, failed to inhibit algal growth and interestingly, it even stimulated phytoplankton growth at higher biomass. Perhaps *M. aquaticum* released organic compounds that enhanced phytoplankton growth. The nutrient levels in mesocosms with *M. aquaticum* were low, but we cannot be certain whether the plants or the phytoplankton depleted the nutrients. On the other hand, for submerged species, we found that a net biomass gain inhibited phytoplankton growth, whereas a net biomass loss stimulated phytoplankton growth compared to the no-plant control. The submerged species *M. spicatum* and *L. major* managed to inhibit phytoplankton, likely through a combination of their growth strategy of rapidly growing towards the water surface, their rapid growth rate and ability to take up nutrients, carbon and light. The inhibition of phytoplankton by these species was

restricted to high initial planting density, 80% plant volume infested (PVI), which makes it likely that the increased nutrient uptake by all this plant biomass played an important role in outcompeting the phytoplankton. In addition, allelopathic substances may have been excreted: *M. spicatum* contains allelopathically active polyphenols such as tellimagrandin (Gross et al. 1996; Leu et al. 2002; Gross 2003) and while no tests have yet been performed for *L. major*, its relatives *Elodea* spp. contain potent allelochemicals (Erhard and Gross 2006). However, we collected no direct evidence to support or reject the possibility that excreted allelochemicals helped these species outcompete phytoplankton. Biomass losses stimulated phytoplankton growth, which may be due to increased nutrient availability. Overall, plant growth and the inhibition of phytoplankton are intricately related. The inhibition of phytoplankton will generally improve plant growth, and vice versa.

Ecosystem function: inhibiting methane emissions

We found that diffusive methane atmosphere-water fluxes varied widely among plant species, with high emissions found especially in treatments with *R. circinatus* and *C. caroliniana* at high initial densities. Daytime diffusive methane emission was negatively related to net plant biomass change: mesocosms with plants that grew hardly emitted any methane. Unfortunately, emissions in the no-plant control group were so low that potential stimulatory or inhibitory effects of plant cannot be determined. On the other hand, mesocosms with plants that lost biomass emitted more methane with increasing biomass loss. The availability of carbon in organic matter, such as decaying plants, is a major determinant of methane production (Bodelier et al. 2006; Bastviken 2009; Duc et al. 2010). The loss of plant biomass had a strong effect, despite the sediment already having a high organic matter content. Possibly the freshly decomposing plant matter was a better carbon source than the organic sediment (Duc et al. 2010), which was also buried under a two cm layer of sand and thus less accessible. Decomposing algae may also have offered substrate for methanogens, but we observed no relation between phytoplankton mass and methane emission and cannot investigate further because there is no data on the turnover of algae or plants. These results represent only a single daytime measurement. Furthermore, fluxes can differ over the season and can show day-night variation (Heilman and Carlton 2001b).

Besides the increased availability of substrate, the loss of plants may also have affected methane production via other pathways, although these

pathways were likely a minor factor given that emissions in the no-plant control were low. The presence of plants in the mesocosms may have greatly modified sediment and water conditions. Plants typically oxidise the sediment to prevent root damage due to anaerobic conditions (Lemoine et al. 2012), and by doing so they reduce diffusive methane fluxes (Ribaud et al. 2011; Soana and Bartoli 2013). This is because aerobic conditions stimulate methane oxidation, whereas anaerobic sediment typically has few favourable acceptors such as oxygen, nitrate and ferric iron, which stimulates methanotrophs (Bodelier et al. 2006; Bastviken 2009). Eutrophic freshwater plants grow better than the majority of oligotrophic plants in anaerobic sediment because they release more oxygen (Lemoine et al. 2012), amphibious and floating plants such as *M. aquaticum* and *Eichornia crassipes* in particular release much oxygen into the sediment (Teuchies et al. 2012; Shu et al. 2015). We found that *M. aquaticum* especially had a larger root-to-shoot ratio than the other species (personal observation), although we unfortunately have no quantitative data on root biomass. However, the porous stems that facilitate oxygen release into the sediment can also facilitate methane release through diffusion (Dingemans et al. 2011). We found that methane emissions for *M. aquaticum* were low, which together with the sediment's low methane production and emissions, suggests plant-mediated methane transport is unlikely. However, we cannot exclude this possibility because our data represent a single daytime measurement of diffusive methane flux.

The presence of plants can lower methane fluxes because more plant biomass increases the surface area for epiphytic methanotrophic microbes. Epiphytic methanotrophs oxidise methane and lower the overall emissions (Heilman and Carlton 2001b), which may have played a role in this experiment. Interestingly and contrary to our findings, in a field experiment sites with submerged macrophytes released more methane than sites with phytoplankton (Xing et al. 2006). Other studies have also reported that larger plant biomass correlates with higher CH₄ emissions in lakes (Thomas et al. 1996; Joabsson et al. 1999). In these natural sediments, plants boosted methane emission by supplying substrate and transport options rather than inhibiting its production or oxidising the product. The results of the mesocosm experiment might have been different if the sediment had produced more methane than it did.

The highest diffusive methane emissions that we measured (~ 24 μmol CH₄ m⁻² h⁻¹) come close to values of eutrophic tropical lakes (38–125 μmol CH₄ m⁻² h⁻¹) (Almeida et al. 2016), fall in the range of snail dominated ponds (54 μmol CH₄ m⁻² h⁻¹) (Xu et

al. 2014), but were lower than emissions from rice paddy fields (252 and 720 μmol CH₄ m⁻² h⁻¹) (Seiler et al. 1983; Schütz et al. 1989) and *Phragmites australis* stands in the littoral of Dutch lakes (252 μmol CH₄ m⁻² h⁻¹) (Dingemans et al. 2011). Our measurement was only a daytime measurement however, and night-time emissions of diffusive methane may have been different (Heilman and Carlton 2001b), which can affect the average daily methane flux (Heilman and Carlton 2001b; Natchimuthu et al. 2014). Moreover, the effect of high methane flux in treatments with few plants remaining may last only a short while and return to normal levels once the plants have decomposed. Future experiments on the greenhouse gas emissions of different freshwater plant species are required to gain more insight, allow among-species comparison and assess possible differential effects of native and non-native species.

Conclusions

In conclusion, plant identity strongly affected plant growth in eutrophic, warm conditions. Two of the four non-native and only one native species increased their average biomass during the experiment, causing non-natives to have a higher biomass at the end of the experiment than natives. However, this difference in plant origin did not yield a mean difference in phytoplankton mass or methane emission between natives and non-natives. The similarity of native versus non-native plant species in these two ecosystem functions is also seen for other ecosystem functions such as refuge provisioning (Grutters et al. 2015), interaction with periphyton (Grutters et al. 2017a) and food provisioning (Grutters et al. 2016; Grutters et al. 2017b).

More research is needed to better understand the both species-specific response of native and non-native plants to their environment, and their effect on the environment, but given our results these are expected to be strongly tied. Plant identity seems to control whether the replacement of native by non-native freshwater plants will alter ecosystem functioning.

Acknowledgements

The authors wish to thank Michael de Jong and Francesca Leggeri for general practical assistance, Annette Janssen for statistical advice, Erik Reichman for assistance with methane measurements and Nico Helmsing for help with nutrient measurements. BMCG and ESB acknowledge the Netherlands Organisation for Scientific Research (NWO) for financial support through Biodiversity works grant 841.11.011. SK was supported by NWO Veni grant 86312012. We thank three anonymous reviewers and the editor for their suggestions that helped improve the manuscript. This is publication 6345 of the Netherlands Institute of Ecology (NIOO-KNAW).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Full statistical data of three-way linear mixed model for the phytoplankton concentration over time.

Figure S1. Photograph of a mesocosm with *Lagarosiphon major*.

Figure S2. Temperature measurements at 120-minute intervals of all 18 temperature loggers in the outdoor mesocosm experiment.

Figure S3. Normalised minimum and maximum water temperature for the different treatments.

Figure S4. Plant percent volume infested (PVI) after three weeks of acclimatisation for native and non-native species as a percentage of the initial planted PVI.

Figure S5. The nitrate, ammonium and phosphate concentration in the water column at the end of the experiment aggregated for the three density treatments of each plant species.

Figure S6. Water alkalinity during the experiment for each of the no-plant, native and non-native species treatments.

Figure S7. Cumulative filamentous algae biomass collected weekly throughout the experiment from native and non-native species.

Figure S8. Chlorophyll-a concentration – as a proxy for phytoplankton density – during eutrophication in monocultures of native and non-native plant species with different initial densities.

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