

Periphyton density is similar on native and non-native plant species

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Abstract

1. Non-native plants increasingly dominate the vegetation in aquatic ecosystems and thrive in eutrophic conditions. In eutrophic conditions, submerged plants risk being overgrown by epiphytic algae; however, if non-native plants are less susceptible to periphyton than natives, this would contribute to their dominance. Non-native plants may differ from natives in their susceptibility to periphyton growth due to differences in nutrient release, allelopathy and architecture. Yet, there is mixed evidence for whether plants interact with periphyton growth through nutrient release and allelopathy, or whether plants are neutral so that only their architecture matters for periphyton growth.
2. We hypothesised that (1) non-native submerged vascular plants support lower periphyton density than native species, (2) native and non-native species are not neutral substrate for periphyton and interact with periphyton and (3) periphyton density increases with the plant structural complexity of plant species.
3. We conducted an experiment in a controlled climate chamber where we grew 11 aquatic plant species and an artificial plant analogue in monocultures in buckets. These buckets were inoculated with periphyton that was collected locally from plants and hard substrate. Of the 11 living species, seven are native to Europe and four are non-native. The periphyton density on these plants was quantified after five weeks.
4. We found that the periphyton density did not differ between non-native and native plants and was not related to plant complexity. Three living plant species supported lower periphyton densities than the artificial plant, one supported a higher periphyton density and the other plants supported similar densities. However, there was a strong negative correlation between plant growth and periphyton density.
5. We conclude that the periphyton density varies greatly among plant species, even when these were grown under similar conditions, but there was no indication that the interaction with periphyton differs between native and non-native plant species. Hence, non-native plants do not seem to benefit from reduced periphyton colonisation compared to native species. Instead, certain native and non-native species tolerate eutrophic conditions well and as a consequence, they seem to host less periphyton than less tolerant species.

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KEYWORDS

epiphyton, invasive species, macrophyte, structural complexity, substrate

1 | INTRODUCTION

Aquatic plants are a crucial component of aquatic ecosystems through their provision of habitat structure and food to fauna, which increases biodiversity (Carpenter & Lodge, 1986), and their enhancement of water quality through nutrient retention (Burks et al., 2006; Jeppesen, 1998; Scheffer, Carpenter, Foley, Folke, & Walker, 2001). However, during the 20th century, many northwest European aquatic plants disappeared or became threatened due to eutrophication (Brouwer, Bobbink, & Roelofs, 2002; Gulati & Van Donk, 2002; Lamers, Smolders, & Roelofs, 2002; Sand-Jensen, Riis, Vestergaard, & Larsen, 2000). Under eutrophic conditions, submerged plants compete strongly with algae for light and nutrients (Scheffer, Hosper, Meijer, Moss, & Jeppesen, 1993). Especially epiphytic algae, which grow attached to plants, are a major cause of shade and contribute to the decline of native submerged vegetation under increasing nutrient loading (Hidding, Bakker, Hootsmans & Hilt, 2016; Phillips, Eminson, & Moss, 1978; Phillips, Willby, & Moss, 2016). Although native vegetation declines under these conditions, non-native plants typically grow excessively in eutrophic conditions and can dominate the vegetation (Hussner, 2012; Van Kleunen et al., 2015). Non-native plants can be ecologically or economically damaging (Hussner et al., 2017), and can be one of the factors that reduces the diversity of aquatic plants and fauna (Stiers, Crohain, Josens, & Triest, 2011). The success of non-natives has been attributed to many factors, including their rapid growth rate, release of enemies and ease of dispersion (Heger & Jeschke, 2014; Pyšek & Richardson, 2007; Schultz & Dibble, 2012). However, it is unknown whether non-native plants are less prone to colonisation by periphyton, which would grant non-natives a competitive advantage over native submerged plants, especially under eutrophic conditions. There are several plant traits that may differ between non-native and native plants, which may provide the mechanism through which non-native plants may potentially be less susceptible to periphyton.

There is no consensus on whether plant species differ in their suitability as periphyton hosts (Blindow, 1987), or instead might represent neutral substrate (Cattaneo, 1978; Eminson & Moss, 1980). Multiple factors control periphyton growth on plants and they can be split into environmental and plant-related factors. Environmental variables such as light availability, nutrient availability (Siver, 1978) and grazing pressure by macroinvertebrates strongly influence periphyton density (Bakker, Dobrescu, Straile, & Holmgren, 2013; Díaz-Olarte et al., 2007; Jones et al., 1999).

Of the plant-related factors, plant growth rate is a major factor controlling periphyton growth and it is negatively related to periphyton growth (Jones, Young, Eaton, & Moss, 2002; Sand-Jensen, 1977;

Sand-Jensen & Søndergaard, 1981). The effect of plant growth rate acts through multiple mechanisms. First, fast growth requires a high nutrient uptake, which reduces nutrient availability to periphyton and therefore reduces periphyton growth. Growing plants take up nutrients from the sediment (Chambers, Prepas, Bothwell & Hamilton, 1989) and this likely lowers the diffusion of nutrients from sediment to water. In addition, plants can take up nutrients and carbon directly from the water column using their leaves (Carignan & Kalff, 1980; Phillips et al., 1978, 2016), which lowers the nutrient availability for periphyton. Second, fast-growing plants have many young plant parts, which are less affected by periphyton than older plant parts (Blindow, 1987; Siver, 1978). The periphyton community on young plant parts is also young and requires time to become dense (Blindow, 1987; Siver, 1978). In addition, young plant parts may possibly excrete more allelochemicals or leave less nutrients for periphyton. Third, the plant surface area controls the availability of colonisation space to periphyton (Jones et al., 1999), and it is highly related to plant growth. The growth rate of many non-native plant species is high (Schultz & Dibble, 2012), and may be higher than that of native species (Umetsu, Evangelista, & Thomaz, 2012). Unfortunately no study has systematically compared growth rates between a large number of native and non-native macrophyte species.

While plant area provides colonisation space to periphyton, the suitability of plant area for periphyton growth varies among plant species. Shoots of aquatic plants differ in structural complexity (Ferreiro, Feijoó, Giorgi, & Leggieri, 2011; Grutters, Pollux, Verberk, & Bakker, 2015; McAbendroth, Ramsay, Foggo, Rundle, & Bilton, 2005), which can affect periphyton growth (Ferreiro, Giorgi, & Feijoó, 2013). It is thought that compared to simple plants, complex plants offer more microhabitats by creating heterogeneity in light, nutrient availability and grazing pressure (Cattaneo, 1978; Cattaneo & Kalff, 1980; Ferreiro et al., 2013). Plant complexity can be quantified using the fractal dimension, which is calculated from the relation of plant area or plant perimeter across multiple scales of measurement (McAbendroth et al., 2005). Native and non-native plants are not known to consistently differ in complexity (Grutters et al., 2015; Schultz & Dibble, 2012).

The surface area of plant species can also differ in suitability for periphyton development because aquatic plants are known to release compounds that inhibit algal growth: allelochemicals (Gross, 2003; Hilt & Gross, 2008). Allelochemicals can inhibit periphyton growth on plant shoots (Erhard & Gross, 2006), thus increasing nutrient and light availability for plant growth. The allelopathic strength of native and non-native aquatic plants has yet to be compared, but it is thought that successful non-native species typically possess strong allelochemicals (Schultz & Dibble, 2012). Because effects of allelochemicals are difficult to separate from other factors such as

nutrient competition, we will focus on differences in periphyton density among native and non-native plant species, not on the particular allelochemicals.

Non-native plant species may thus grow faster and possess stronger allelochemicals than natives, which would coincide with reduced periphyton growth. Yet, to our knowledge, there is no study that has compared the periphyton growth on natives and non-natives. Therefore, we conducted a controlled replicated experiment with seven native, four non-native freshwater plant species and one artificial plant analogue to test our hypotheses that (1) periphyton density is lower on non-native than native plant species. We also hypothesised that (2) plants will either suppress or stimulate periphyton growth, and are thus not neutral substrate, hence living plants would have a higher or lower periphyton density than artificial substrate of similar structure. Among plant species, we hypothesised that (3) periphyton density increases with plant structural complexity.

2 | MATERIALS AND METHODS

2.1 | Aquatic plants

Eleven aquatic plant species, of which seven are native to north-western Europe (Hussner, 2012), were selected for the experiment to include species varying in morphology and taxonomy (Table 1). On 16 May 2013, we collected plant fragments of each species from indoor or outdoor cultures at the Netherlands Institute of Ecology (Wageningen, the Netherlands). *Cabomba caroliniana* and an artificial plant analogue resembling *Cabomba* (Tetra Plantastics, Melle, Germany) were bought from an aquarium shop.

Plants were carefully rinsed to remove the majority of periphyton under running tap water, before they were cut into 5- to 7-cm-long fragments. Some firmly attached periphytic species, such as diatoms, were possibly still attached, but they could not be removed without damaging the plant species. The to-be-planted plant segments were blotted dry, weighed and kept in tap water until planting the same day. We prepared plastic *Cabomba* shoots, which acted as a structural control, similar to living plants. We planted a similar initial plant biovolume for each species (resulting in 0.4–1.3 g fresh weight per species).

2.2 | Experimental design

During 5 weeks, from 17 May to 20 June 2013, we tested 12 plant species (including the artificial plant), kept as monocultures, as substrate for periphyton in a fully randomised experiment ($n = 10$) using 120 black, polyethylene buckets (21 cm high, 22.5 cm diameter). To mimic the current state of many northwest European lakes (Lamers, Schep, Geurts, & Smolders, 2012), we aimed for a low nutrient availability in the surface water and a high nutrient availability in the sediment. On 16 May 2013, we filled the buckets with 4 L of tap water, which can be considered oligotrophic (pH: 7.7, 20.2°C, 8.6 mg/L O₂, conductivity: 175 µS/cm, 1.8 µM NO₃⁻, 0.0 µM NH₄⁺, 0.6 µM PO₄³⁻, alkalinity: 1.6 meq HCl L⁻¹). The water level was kept constant at 18.5 ± 0.5 cm (mean ± range) depth by a half-weekly tap water addition to compensate for evaporation. These buckets were placed in a controlled climate room with 16 hr of light (mean ± SD of 286 ± 38 µmol photons m⁻² s⁻¹ at 1 cm above the water surface measured for all 120 buckets on 17 May 2013), 80%–90% humidity and 20°C.

TABLE 1 Measurements on plant biomass and leaf complexity of the tested aquatic plant species

Plant name	Native status	Total area (cm ²)	Specific area (mm ² /mm)	Fractal dimension (D)	Final plant dry mass (g)
Artificial <i>Cabomba</i> (ARTCAB)	Artificial	48.58 ± 9.33	34.7	1.79 ± 0.03	2.58 ± 0.33
<i>Ceratophyllum demersum</i> (Ceratophyllaceae; CERDEM)	Native	33.49 ± 14.16	14.4 ± 1.3	1.76 ± 0.04	0.27 ± 0.07
<i>Chara vulgaris</i> (Characeae; CHAVUL)	Native	83.09 ± 38.01	2.2 ± 0.2	1.27 ± 0.09	0.50 ± 0.09
<i>Hottonia palustris</i> (Primulaceae; HOTPAL)	Native	12.51 ± 6.03	28.4 ± 7.2	1.71 ± 0.05	0.01 ± 0.02
<i>Myriophyllum spicatum</i> (Haloragaceae; MYRSP1)	Native	172.43 ± 74.62	15.5 ± 0.69	1.78 ± 0.03	0.56 ± 0.19
<i>Myriophyllum verticillatum</i> (Haloragaceae; MYRVER)	Native	19.68 ± 10.95	21.5 ± 5.2	1.76 ± 0.06	0.03 ± 0.02
<i>Potamogeton perfoliatus</i> (Potamogetonaceae; POTPER)	Native	59.20 ± 10.92	4.2 ± 0.38	1.47 ± 0.03	0.31 ± 0.07
<i>Ranunculus circinatus</i> (Ranunculaceae; RANCIR)	Native	308.90 ± 75.65	15.1 ± 2.4	1.78 ± 0.03	0.58 ± 0.09
Mean native plants		98.47 ± 107.51	15.0 ± 9.7	1.79 ± 0.08	0.32 ± 0.24
<i>Cabomba caroliniana</i> (Cabombaceae; CABCAR)	Non-native	32.44 ± 21.20	33.5 ± 0.73	1.77 ± 0.07	0.05 ± 0.03
<i>Elodea nuttallii</i> (Hydrocharitaceae; ELONUT)	Non-native	363.41 ± 150.16	10.5 ± 1.0	1.64 ± 0.06	0.84 ± 0.12
<i>Myriophyllum aquaticum</i> (Haloragaceae; MYRAQU)	Non-native	23.45 ± 17.23	12.7 ± 1.5	1.68 ± 0.01	0.05 ± 0.02
<i>Myriophyllum heterophyllum</i> (Haloragaceae; MYRHET)	Non-native	33.89 ± 11.69	21.5 ± 5.2	1.76 ± 0.06	0.14 ± 0.05
Mean non-native plants		113.30 ± 166.81	21.1 ± 10.3	1.78 ± 0.04	0.27 ± 0.38
Native versus non-native plants	t value	-0.13	-0.96	0.48	0.38
	p	.90	.37	.64	.71

On 17 May 2013, we planted each plant portion in a separate pot at a depth of 2–3 cm. These plastic pots (6.6 cm × 6.6 cm × 6.3 cm, L × W × H) were filled with 210 g of clean sand and contained 600 mg, that is, 2.67 g Basacote L⁻¹, slow-release fertiliser (Basacote 6 M Plus, 16-8-12 NPK, COMPO, Münster, Germany). Based on the manufacturer's specifications, the phosphorus release approximated that of sediment in the mesotrophic Dutch lake Loenderveen (Poelen et al., 2012), whereas the nitrogen release resembles eutrophic lake sediments (Poelen et al., 2012). This dosage was expected to provide the conditions of earlier experiments in which periphyton developed (Bakker et al., 2013). After planting, the pots were gently lowered into their experimental bucket, one per bucket.

On 18 May 2013, we inoculated the water in each bucket with a mix of periphyton that consisted of (1) a mixed sample of periphyton from all aquatic plant species used in the experiment collected from plant cultures in the greenhouse and (2) periphyton in the water used to store the plants prior to planting. This inoculum served to expose all aquatic plants to a similar community and high density of periphyton (i.e. high dosage initially added). The second inoculum component helped maximise the chance that all periphyton species were present in all treatments. Per 4 L water added to each bucket, we added 25.6 µg chlorophyll L⁻¹ as determined by spectrophotometry. Every week, we carefully replaced 95% of the tap water. These water replacements provided new dissolved inorganic carbon and limited phytoplankton growth. The water in the buckets was kept stagnant over the experiment.

We determined the periphyton density on two different surfaces: on the plants themselves (see Section 2.4) and on standardised substrate (glass slides). We attached a glass slide to each bucket, facing the middle of the climate room, to quantify the periphyton community composition in a standardised way that could be easily sampled.

2.3 | Plant trait analyses

To measure plant fractal complexity, we scanned five independent shoots, similar to the shoots that were planted, of each plant species used (Epson Perfection 4990 Photo, Suwa, Japan) and analysed the scans to calculate the plant area per cm of stem and fractal dimension (referred to as plant complexity) using ImageJ adapted from (Grutters et al., 2015; McAbendroth et al., 2005). Calculating both parameters using intact fragments facilitated the analysis of the different plant species. The fractal dimension (area occupancy as in McAbendroth et al., 2005) was determined with the box counting method (boxes of 0.26–16.3 mm in ImageJ; Schneider, Rasband, & Eliceiri, 2012), while the plant area and shoot length were calculated by converting pixels to lengths in millimetres. The plant area was calculated using scans of intact shoots, not using completely dissected plant material. While imperfect for the total area, the method using intact shoots approximates the total area rather well (based on $n = 3$ plant species tested, R^2 of at least 0.86 within species of $n = 5$). For *Myriophyllum verticillatum*, there was not enough material to make scans. Given its similarity to *M. heterophyllum*, we used the area and complexity of that plant for *M. verticillatum*. The plant area per cm

of stem was used to estimate the surface area of the plant fragments of which we extracted the periphyton.

2.4 | Plant harvest

From 20 to 23 June 2013, the aquatic plants were harvested following a randomisation scheme and their total fresh mass was weighed. We then sampled and separately analysed two plant parts within one shoot: the apical plant fragment (fragment length 2–5 cm, depending on the plant species, referred to as the *young* part) and the lower basal fragment (fragment length 2–8 cm, depending on the plant species, referred to as the *old* part) excluding 1 cm of shoot closest to the sediment to prevent sampling periphyton growing on the sediment. These two types of fragments were sampled, because periphyton density typically decreases towards the apex (Blindow, 1987; Siver, 1978). For plants with low periphyton density, we sampled multiple plant fragments (up to three) and pooled them for analysis, typically species that grew rapidly during the experiment. The remaining plant material was analysed for plant biomass, but not for periphyton density.

We extracted the periphyton growing on each plant part by shaking for 60 s in 100 mL tap water (Zimba & Hopson, 1997), which has a removal efficiency of 90% (Zimba & Hopson, 1997) and can remove firmly attached periphyton (Jones, Moss, Eaton, & Young, 2000), before drying the plants (60°C to constant dry mass) and determining their dry mass. The extracted periphyton was quantified by filtering a known volume that saturated GF/F glass filters (3–30 mL; Whatman, Maidstone, England) before adding the filter to 90% ethanol, boiling this substance for 10 min, resting it for 24 hr at 6°C in the dark and, finally, spectrophotometrically measuring absorbance value at 665 and 750 nm (Lambda 800 Spectrometer, PerkinElmer, Waltham, USA) (Sartory & Grobbelaar, 1984; Wasmund, Topp, & Schories, 2006). We used these values to calculate the chlorophyll-*a* content corrected for phaeopigments. Periphyton was expressed as µg chlorophyll per cm² of plant surface (referred to as periphyton density). The periphyton density per area (µg/cm²) was strongly correlated with periphyton density per plant mass (µg/g; Pearson's $r = .90$, $p < .001$; $n = 110$).

2.5 | Glass substrate harvest

The glass slides were collected from 26 June to 1 July. After collection, we scraped off the periphyton growing on the open water side of each slide (5 × 2.6 cm) into tap water using a scalpel. The periphyton was quantified through spectrophotometry (see Section 2.4) and expressed as µg chlorophyll per cm². Besides quantifying chlorophyll-*a*, we checked which algal species were most frequent in the periphyton. The most frequently observed periphyton species were the green algae *Chlorella* sp. and *Acutodesmus* cf. *obliquus* and the cyanobacteria *Gloeotrichia echinulata* and *Chroococcus turgidus*.

2.6 | Water quality parameters

In the second (3 days after water change) and fourth week (4 days after water change) of the experiment we recorded water

temperature, O₂, pH and conductivity in each experimental bucket (WTW 350i, Weilheim, Germany) and also the concentration of nitrate, nitrite, ammonium and orthophosphate in GF/F-filtered water (QuAAtro auto-analyzer, Seal Analytical, Fareham, UK). We also determined these parameters (five replicates) and the alkalinity of tap water (meq/L HCl to pH of 4.2; TitraLab, Radiometer Analytical, Villeurbanne, France). Furthermore, the phytoplankton density (μg chlorophyll L⁻¹) in all experimental buckets was quantified using the Phyto-PAM (Walz, Effeltrich, Germany) at the end of the experiment on 19 June 2013 just before the plant harvest.

2.7 | Data analysis

We compared the periphyton density among plant species using one-way ANOVA and tested the relation between periphyton density and plant complexity using linear regression. Because plants and glass slides were harvested over multiple days due to logistic constraints, periphyton density was standardised to the first day of harvest, for which we assumed that periphyton grew linearly. The standardised periphyton density was unrelated to harvest date (one-way ANOVA, with day as a four-level factor; for plants, $F_{3,116} = 0.74$; $p = .53$; for glass slides $F_{3,113} = 0.23$; $p = .87$). The periphyton density on native and non-native plants was compared with t tests. Because periphyton density was expected to differ between young and old leaf tissues, we compared the periphyton density of young and old leaves of different plant species using a two-way ANOVA and subsequent post hoc contrasts to test within species. We tested for differences in environmental variables (pH, nitrate, ammonium, phosphate, conductivity, phytoplankton biomass, oxygen content) and chlorophyll on glass slides among plant species, and also between native versus non-native species, separately for each variable and using one-way ANOVAs or t tests respectively.

Post hoc tests were conducted with Tukey's contrasts and the Benjamini–Hochberg procedure, which controls the false discovery rate (Benjamini & Hochberg, 1995). To conform to model assumptions, plant and periphyton biomass were log transformed. Statistics were performed using R version 3.2.3 (R Core Team, 2013) and the packages *multcomp* (Hothorn, Bretz, & Westfall, 2008), *MASS* (Venables & Ripley, 2002), *ggplot2* (Wickham, 2011), *nlme* (Pinheiro, Bates, Debroy, Sarkar, & Team, 2015) and *car* (Fox & Weisberg, 2011). Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d4k51>.

3 | RESULTS

The mean periphyton density was not statistically different for native and non-native plants (Figure 1b; t test: $t_9 = -1.64$; $p = .14$). Among plant species, we found large differences in the mean periphyton density (Figure 1a; one-way ANOVA: $F_{11,108} = 19.6$; $p < .001$). Plants with a high periphyton density were the natives *Hottonia palustris* and *M. verticillatum*, the non-natives *M. heterophyllum*, *M. aquaticum* and *C. caroliniana*, and the artificial *Cabomba* (Figure 1a). To the contrary, the natives *Myriophyllum spicatum* and *Ranunculus circinatus* and non-native species *Elodea nuttallii* supported the lowest periphyton density.

Comparing the periphyton density on young and old plant parts (Figure 2), we found a strong interaction between plant species and periphyton on top and bottom fragments (two-way ANOVA; interaction: $F_{11,198} = 4.2$; $p < .001$), with more periphyton on older fragments for *Ceratophyllum demersum* ($p < .001$), *Chara vulgaris* ($p = .016$), *E. nuttallii* ($p = .008$), *M. spicatum* ($p < .001$), *Potamogeton perfoliatus* ($p = .036$) and *R. circinatus* ($p = .021$). The other plant species supported a periphyton density that did not statistically differ between young and older plant parts. Also, on top and bottom

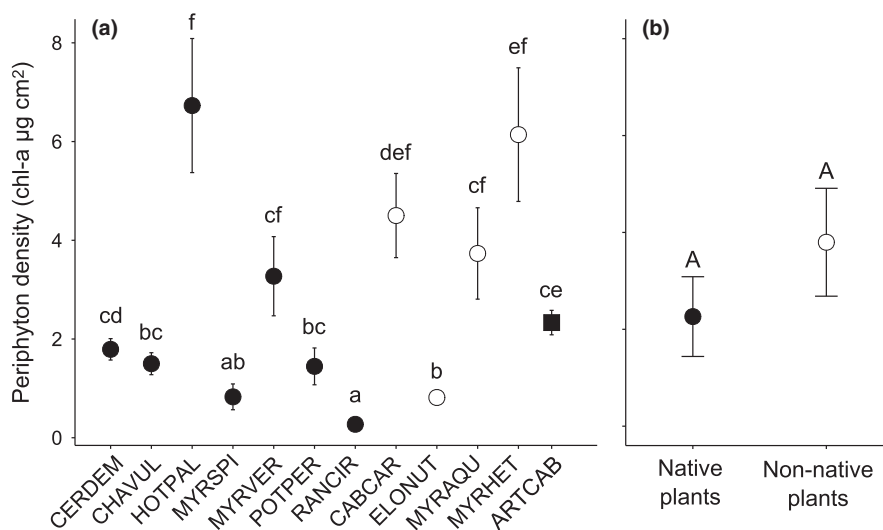


FIGURE 1 Left panels show periphyton density as chl-a in $\mu\text{g cm}^{-2}$ (a) on native (closed circles) and non-native (open circles) plant species (in mean \pm SE). Different letters indicate significantly different groups. Right panels show mean periphyton density (b) of grouped native ($n = 7$) and non-native plants ($n = 4$). 'ARTCAB' indicates the artificial plant analogue resembling *Cabomba caroliniana*, full plant names of living species are given in Table 1

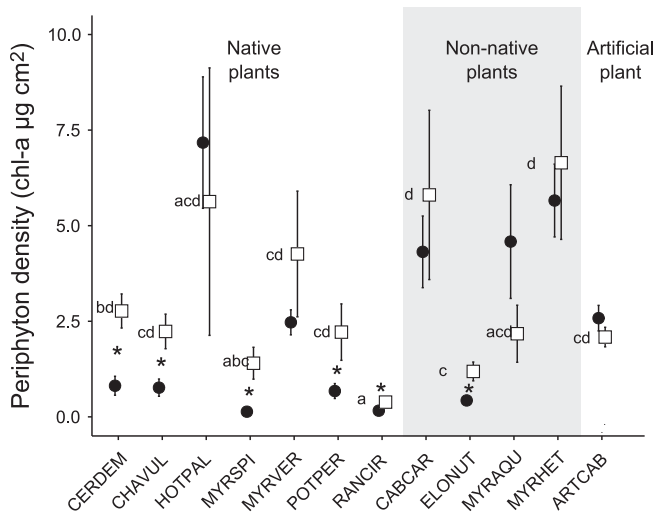


FIGURE 2 Periphyton density (mean \pm SE; as chl-*a* in $\mu\text{g cm}^{-2}$) on young (closed circles) and old (open squares) plant parts for each plant species. Asterisks indicate significant differences between both plant parts within a species. Full names of plant species are given in Table 1

fragments, the periphyton density was not statistically different between native and non-native plants (*t* tests of: $t_9 = -1.81$; $p = .10$ and $t_9 = -1.24$; $p = .25$ respectively).

The plant fractal complexity differed significantly among plant species (ANOVA, $F_{10,54} = 67.4$; $p < .001$), ranging from 1.27 for *C. vulgaris* to 1.79 for the artificial plant analogue resembling *Cabomba*, and had an average of 1.67. However, the periphyton density among plant species was not explained by plant complexity (Figure 3; linear regression: $R^2 = -0.08$; $p = .71$). The highest periphyton density was found on plants of high complexity, but not

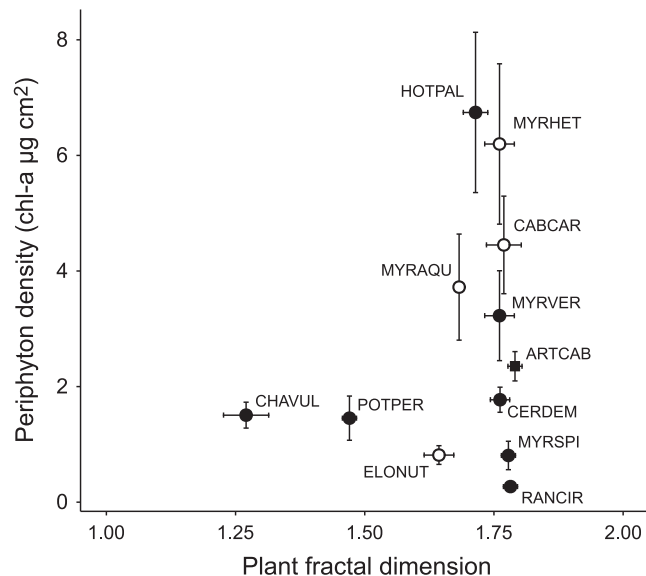


FIGURE 3 The relationship between plant fractal dimension (mean \pm SE, mean only for artificial) and periphyton density (mean \pm SE; as chl-*a* in $\mu\text{g cm}^{-2}$) on all tested plant species (native: closed circles, non-native: open circles). There was no significant relationship between these variables

all of them hosted a high periphyton density, for example, *C. demersum* and *R. circinatus* had a high complexity but supported a low periphyton density.

The periphyton chlorophyll on glass slides was $0.32 \pm 0.02 \mu\text{g/cm}^2$ (mean \pm SE; $n = 120$) and did not differ significantly among plant species treatments (ANOVA: plant species $F_{11,96} = 1.8$; $p = .06$; Table S1), whereas the periphyton density on the plants themselves was much higher at an average of $2.8 \pm 3.0 \mu\text{g/cm}^2$ (mean \pm SE; $n = 120$).

We found large differences in aquatic plant growth during the experiment (Figure 4). The species that accumulated the most biomass were the natives *M. spicatum*, *P. perfoliatus*, *R. circinatus* and the non-native *E. nuttallii*. Some plants showed little net growth: the native *M. verticillatum* and the non-natives *M. heterophyllum* and *M. aquaticum*, whereas native *H. palustris* and non-native *C. caroliniana* lost biomass during the experiment. Overall, the change in plant biomass during the experiment did not significantly differ between native and non-native plants (*t* test: $t_9 = 1.006$; $p = .34$). The periphyton density on plants was negatively related to plant final dry mass (Figure 5).

Dissolved oxygen concentrations, nitrogen, pH, temperature and conductivity were not significantly different among plant species treatments after either 2 or 4 weeks (Table S1). However, phosphate and phytoplankton concentrations in the water differed between some treatments. The phosphate concentration was higher in buckets with *E. nuttallii* than in buckets with *P. perfoliatus*, *C. demersum* and *M. verticillatum*. In addition, the phosphate concentration in buckets with *M. verticillatum* was lower than in those with *C. vulgaris*. The phytoplankton concentration in the water ($\mu\text{g chlorophyll L}^{-1}$) varied among treatments ($F_{11,108} = 2.4$; $p = .012$), with buckets containing *M. spicatum* having less phytoplankton than

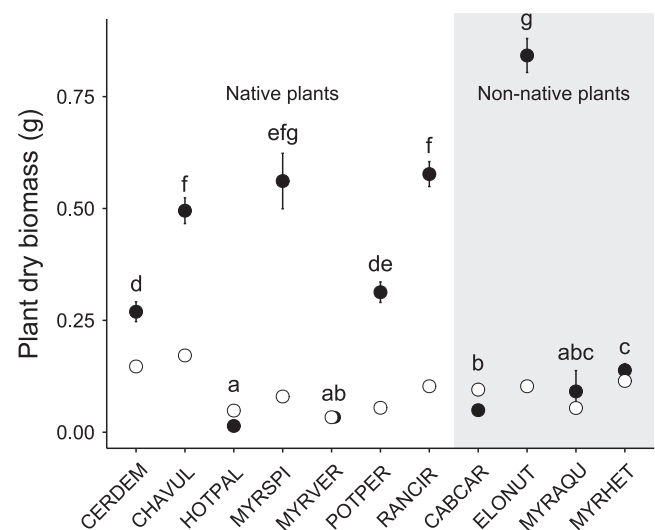


FIGURE 4 Plant biomass (mean \pm SE) of the 11 living plant species at the start (open) and end (closed circles) of the experiment. Different letters indicate significantly different groups. In some cases, the error bars are so small that they are hidden by the symbol

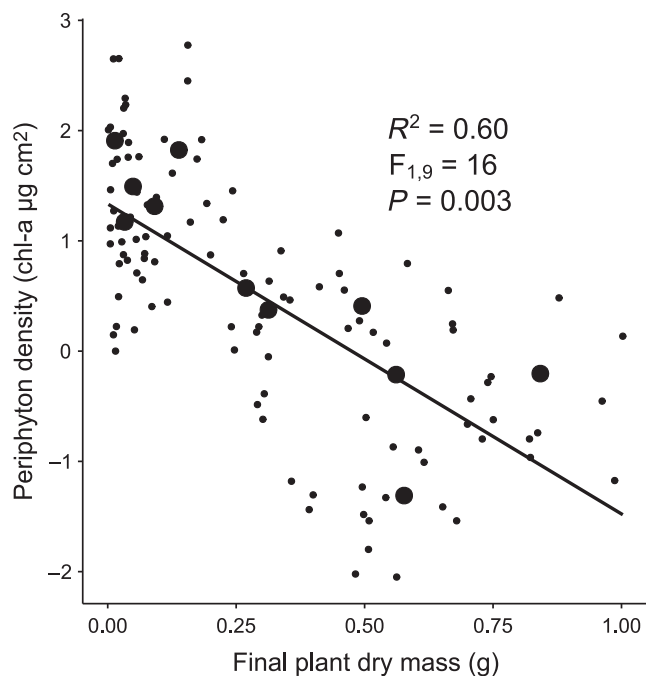


FIGURE 5 Relation between the periphyton density (as $\mu\text{g chl-}a\text{ cm}^{-2}$) and the final dry plant biomass of the tested living plant species. Small circles indicate values of individual replicates and big circles indicate plant species averages

buckets with *H. palustris* and *C. demersum*, and no differences among other plant species (Table S1).

4 | DISCUSSION

We found that periphyton density varied greatly among 11 tested living plant species and the artificial analogue, in a controlled laboratory experiment. The periphyton density on multiple living plant species differed from that on the artificial plant analogue. One living plant species hosted more and three species hosted less periphyton than the artificial plant. Some plant species thus did not act as neutral substrate for periphyton, which partly confirmed our second hypothesis. Yet, seven plants hosted similar periphyton densities as the artificial plant, indicating that many plant species appeared to be neutral substrate, hence we also partly reject our hypothesis. Contrary to our hypotheses, the periphyton density on native and non-native plant species was similar, and periphyton growth was not related to plant fractal complexity, thus we rejected our first and third hypothesis.

4.1 | Plant origin

Native and non-native plants supported similar periphyton densities, which matched the mean trait composition of the groups of species: native and non-native plant species were statistically similar in plant area, plant complexity as expressed by the fractal dimension and final plant dry mass. Overall, the same ecological processes appear to govern periphyton growth on native and non-native plant species,

resulting in differences among species, but not between natives and non-natives species overall.

4.2 | Factors related to periphyton growth on plants

The native species *M. spicatum*, *R. circinatus* and the non-native *E. nuttallii* supported significantly lower periphyton densities than the artificial plant analogue. These species also grew most during the experiment. Plant species that showed no net growth, such as the native *H. palustris* and the non-native *C. caroliniana*, supported denser periphyton than the artificial plant and plants that grew more. These results highlight the negatively related growth of plant and periphyton that we found in our study. A similar relationship has been commonly found in other experiments and in the field (Cattaneo, Galanti, & Gentinetta, 1998; Jones et al., 2002; Sand-Jensen, 1977; Sand-Jensen & Søndergaard, 1981). We cannot rule out that fast-growing plant species have high growth irrespective of periphyton, so that the periphyton densities on these plant species might be low because periphyton was spread over a larger area. However, it is also possible that fast plant growth reduces nutrients and time available for periphyton growth, resulting in reduced periphyton densities. In fact, fast plant growth may have occurred because periphyton failed to develop and could thus not inhibit plant growth.

The interaction between plants and periphyton may depend on the active release of allelochemicals or growth stimulants, or can be passive through competition for nutrients, light, surface area and time for colonisation (Blindow, 1987; Cejudo-Figueiras, Álvarez-Blanco, Bécares, & Blanco, 2011). It is difficult to disentangle these factors because plants and periphyton are intimately tied together. Plants can actively suppress periphyton through allelopathy. Two species supporting little periphyton in the experiment, *M. spicatum* and *E. nuttallii*, are known to possess allelochemicals that strongly inhibit algal growth (Erhard & Gross, 2006; Leu, Krieger-Liszkay, Goussias, & Gross, 2002). However, several other species used in the experiment such as the other *Myriophyllum* spp. (Gross, 2003; Hilt, Ghobrial, & Gross, 2006), *C. demersum* (Gross, 2003; Wium-Andersen, Anthoni, & Houen, 1983) and *Chara* spp. (Wium-Andersen, Anthoni, Christophersen, & Houen, 1982) are also known to be allelopathic, yet did not suppress periphytic algae strongly as they supported substantial periphyton densities. Thus, allelopathically active species did not clearly reduce periphyton density. Nutrient availability is another factor that can have affected periphyton growth. The sediment contained meso- to eutrophic levels of nutrients in the form of slow-release fertiliser (Bakker et al., 2013), whereas levels of dissolved nutrients in the water layer were relatively low ($<1\ \mu\text{M}$ total inorganic nitrogen as ammonium plus nitrite plus nitrate and $<0.30\ \mu\text{M}$ orthophosphate) compared to the average European concentrations in European lakes of $13.6\ \mu\text{M}$ total inorganic nitrogen and $0.65\ \mu\text{M}$ orthophosphate (Noges, 2009). It thus seems likely that plants and periphyton competed for nutrients. Slow-growing plants were likely poor competitors for nutrients and may also have released nutrients, stimulating periphyton growth,

especially plant species that lost mass (Granéli & Solander, 1988; Ozimek, Van Donk, & Gulati, 1993). In the experiment, the slow-growing plants indeed supported denser periphyton than the artificial or fast-growing plants, suggesting periphyton got a nutritional boost. On the contrary, fast-growing plant species hosted less periphyton than the artificial plant, which may indicate that these plants successfully competed for nutrients with the periphyton. Although it should be noted, as mentioned earlier, that we cannot establish whether this was due to competition for nutrients or effects of allelopathy. Besides plants and the attached periphyton requiring nutrients, the experimental buckets also contained substantial levels of phytoplankton, on average $104 \pm 82 \mu\text{g/L}$ (mean \pm SD), and periphyton on walls and the glass substrate. The periphyton on the bucket's walls and that on glass substrate was of much lower density than periphyton on plants, which matches the trend reported in literature that artificial substrate may underestimate green algae and cyanobacterial density, which were the most frequent phytoplankton groups in our experiment (Cattaneo & Amireault, 1992). With all these primary producers, the competition for dissolved inorganic carbon was likely intense, which is reflected by the average pH of 8.8. Although we replenished 95% of the water every week, dissolved inorganic carbon may have been a limiting resource, as it is in some eutrophic lakes (King, 1970). Especially *H. palustris* and *M. verticillatum*, which prefer CO_2 to HCO_3^- , may have been limited by CO_2 availability (Maberly & Madsen, 1998) and might have been poor competitors and thus better substrate for periphyton.

Plant complexity is another factor that is often linked to periphyton density (Cattaneo et al., 1998; Ferreira et al., 2013). We found that not all plant species of high fractal complexity supported a high periphyton density, which agrees with some studies (Ferreira et al., 2011; Taniguchi & Tokeshi, 2004), but contradicts others (Cejudo-Figueiras et al., 2011; Ferreira et al., 2013). We thus reject our third hypothesis that periphyton density increases with plant fractal complexity. A reason for this mismatch might be that we expressed periphyton per unit of leaf area to exclude the effect of area, which not all studies did. Furthermore, we measured the fractal complexity only at the start, not at the end of the experiment, which can have affected the outcome if the fractal complexity changed over time. A mechanistic factor for the lack of a link between fractal complexity and periphyton density might be found in the used scale (Ferreira et al., 2013). Plants are not truly fractal, but multifractal objects, with different fractal dimensions at different scales (Halley et al., 2004). At shoot scale, macroinvertebrate abundance often increases with plant complexity (Ferreira et al., 2011; McAbendroth et al., 2005; Taniguchi & Tokeshi, 2004; Thomaz, Dibble, Evangelista, Higuti, & Bini, 2008), however, at this scale periphyton was not linked to plant complexity in our study nor in the literature. Instead, at leaf scale, periphyton has been found to increase with the plant fractal complexity, reaching higher densities on plants bearing thorns or jagged edges (Ferreira et al., 2013). Diatoms grow more densely on complex leaf edges of both living and artificial plants (Cattaneo, 1978), which might be linked to increased nutrient or light availability. In addition, complex leaves have an

increased circumference per leaf area that may increase microhabitat availability to periphyton. Although in our study, plant species with jagged leaf edges such as *C. demersum* and *E. nuttallii* hosted fewer periphyton, instead of more (Ferreira et al., 2013), indicating that factors other than fractal complexity may have been more important in determining periphyton density. This is also indicated by a comparison among three plant species of similar architecture, all with hand-shaped finely dissected leaves: the artificial plant analogue, *R. circinatus* and *C. caroliniana*. Despite having a similar architecture, the periphyton density on these three species varied almost 20-fold, with values of 2.2, 0.28 and $5.4 \mu\text{g/cm}^2$, respectively, so that factors other than plant structure must be involved in determining the periphyton growth.

5 | CONCLUSIONS

We tested for the first time, to our knowledge, whether non-native plants are less prone to periphyton growth than natives, but we found no evidence for this. We found that the periphyton density on living plant species differed greatly among species, even when grown under similar conditions and for species of similar morphology. Periphyton density was not related to plant complexity, instead it was negatively related to plant growth. This may indicate that mechanisms such as nutrient competition and possibly allelopathy may have played an important role, but these could not be disentangled in our experiment. We conclude that similar processes drive the interaction of native and non-native plants with periphyton. Non-native plants do not seem to benefit from reduced periphyton colonisation compared to native species. Instead, those native and non-native species that tolerate eutrophic conditions host less periphyton because their fast growth permit them to limit the availability of resources (such as nutrient and light) required by periphyton, thereby limiting periphyton growth.

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