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A long-established invasive species alters the functioning of benthic biofilms in lakes

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Abstract

1. Invasive species often transform environmental conditions, exclude native species and alter ecosystem functioning, including key ecosystem processes underpinning nutrient and energy cycles. However, such impacts have been most documented during periods of invasive species dominance; their influences on functioning at lower relative abundances and after long-term establishment are less well-known.
2. We investigated the effects of *Elodea canadensis*, a macrophyte native to North America with a long invasion history in many regions of the world, on the biomass accrual and metabolism of littoral zone biofilms growing on organic and inorganic substrates.
3. We deployed nutrient diffusing substrates (NDS) in 18 replicate transects distributed across six lakes, comprising three invaded by *E. canadensis* and three uninvaded reference lakes. NDS were amended with nitrogen (N), phosphorus (P) or N + P together, or were deployed as unamended controls. *E. canadensis* relative abundance varied widely in the invaded transects, ranging from 13% to 93% of all macrophyte cover.
4. On control substrates, algal biomass, quantified as Chlorophyll-a, and gross primary production (GPP) were 42% and 78% greater in the invaded compared to uninvaded lakes, respectively. Respiration rates, attributable to responses of both autotrophs and heterotrophs, were 45% greater on control substrates in invaded lakes. By contrast, N-limitation of both biofilm GPP and respiration was 25% and 35% greater in uninvaded compared with invaded lakes.
5. There was no evidence for differences in nutrients, light availability or grazing pressure between invaded and uninvaded transects. Rather, the observed differences in metabolism suggest that the presence of *E. canadensis* increases availability of N at local scales, reducing N-limitation of biofilms and resulting in elevated rates of biofilm productivity.

6. Our results demonstrate that invasive elodeids might have significant impacts on biofilms and processes associated with the cycling of nutrients, even when long-established and present at lower relative abundances.

KEYWORDS

biomass accrual, GPP, invasive dominance, N-limitation, nutrient response ratio

1 | INTRODUCTION

Massive invasion by nonindigenous species is one of the main drivers of current global change, notable not only for impacts on biodiversity but also for causing substantial shifts in ecosystem functioning, with consequences for nutrient and carbon (C) cycling, ecosystem services and economic activity (Ehrenfeld, 2010; Mack et al., 2000; Marbuah et al., 2014). Impacts on ecosystem functioning are often strongest when invasive species attain high biomass, dominating habitat and resource use (Hladyz et al., 2011; Yelenik et al., 2004). Extreme levels of dominance by invasive species often occur cyclically or transiently (Simberloff & Gibbons, 2004; Strayer et al., 2006), suggesting that the most marked impacts on ecosystem functioning are likely to be temporary, or fluctuate in time and space (Strayer, 2012). However, there is a need for more research focussed on the impacts of invasive species on ecosystem function when they have been established over longer time periods (>100 years) and are present at lower relative abundances (Jeschke et al., 2014).

Invasive species might impact ecosystem functioning even when not dominant if they have attributes of foundation or keystone species, or specific traits with strong influences on ecosystem processes (Creed & Reed, 2004; Strayer, 2012). Many of the most widespread invasive species in fresh waters are macrophytes––foundational plant species in aquatic food webs (Carboneras et al., 2018; Jeppesen et al., 1998). Invasive macrophytes can alter habitat architecture (increases in frequency of stemmed or floating vegetative structures, or novel leaf and root morphologies), which in turn can transform local environmental conditions through dense shading, reduction of flows, and/or alteration of local oxygen and nutrient dynamics (Mack et al., 2000; Urban et al., 2006). Implications of such changes for other organism groups and for ecosystem processes require urgent investigation, given the potential for invasive macrophytes to spread rapidly in river and lake catchments, either transported by water, attached to dispersing birds, for example, or inadvertently transported by humans, attached to boats or clothing (Mack et al., 2000; Riis & Sand-Jensen, 2006; Urban et al., 2006).

Elodea canadensis Michx is a highly invasive macrophyte that has broad environmental tolerances, and which has particularly strong potential to alter local environmental conditions (Carey et al., 2016; Tattersdill et al., 2017). *E. canadensis* occurs in every continent outside of its native range in North America, except Antarctica, and continues to expand in northern Arctic and subarctic regions (Carey et al., 2016; Hussner, 2012; Marbuah et al., 2021). When at peak dominance, *E. canadensis* has extensive impacts on biodiversity and ecosystem functioning (Rørslett et al., 1986; Simpson, 1990; Zehnsdorf et al., 2015), with the sheer level of biomass attained leading to altered nutrient cycling, reduced light, changed water clarity and exclusion of native species (Carpenter & Lodge, 1986; Kornijów et al., 2005; Mjelde et al., 2012; Rørslett et al., 1986; Tattersdill et al., 2017; Zehnsdorf et al., 2015). However, such periods of dominance are typically transient, and in some regions *E. canadensis* appears to have stabilised at lower population densities, with little apparent ongoing impact on biodiversity (Boiché et al., 2010; Kolada & Kutyła, 2016). Nevertheless, *E. canadensis* has several attributes which might cause it to impact ecosystem functioning even at lower population densities, including allelopathic chemicals (Erhard & Gross, 2006; Mohamed, 2017), the capacity to use bicarbonate (HCO_3) as a C source during photosynthesis (Pokorny & Kvet, 2004), and a relatively high efficiency of absorbing nutrients from both water and sediment (Madsen & Cedergreen, 2002; Ozimek et al., 1993). These attributes are not necessarily unique to *E. canadensis* – some native macrophyte species also exploit HCO_3 and/or have allelopathic chemicals to varying degrees (Adamec & Ondok, 1992; Mohamed, 2017) – but taken together are likely to underpin many of its impacts on ecosystem functioning.

Autotrophic and heterotrophic microorganisms inhabiting benthic biofilms might be particularly susceptible to the local environmental effects of *E. canadensis* (Gette-Bouvarot et al., 2015; Jones et al., 1999). Autotrophic organisms, including diatoms, cyanobacteria and green algae, are more likely to dominate biofilms on inorganic substrates, such as stones, gravel and sand, which are key sites for autotrophic productivity in shallow lake habitats (Battin et al., 2016; Mora-Gómez et al., 2016). Heterotrophic bacteria and fungi are more likely to dominate on organic detritus, and play a major role in decomposing detritus such as leaf litter and wood (Jabiol et al., 2013; Johnson et al., 2009).These organisms play a fundamental role in the biogeochemical pathways regulating nutrient cycling in aquatic ecosystems, while also representing an important energetic resource to consumers (Battin et al., 2016; Vadeboncoeur & Power, 2017). The efficiency with which autotrophic and heterotrophic microorganisms are able to produce biomass, cycle nutrients and decompose detritus depends on several environmental parameters potentially affected by macrophyte community composition, and by the presence of *E. canadensis* in particular, including dissolved nutrient availability, pH and calcium (Ca) availability, allelopathic chemicals, and shading (Jones et al., 1999; Mora-Gómez et al., 2016).

Here, we investigated the effects of the presence of *E. canadensis* on metabolism (gross primary production [GPP] and respiration) of biofilms, based on quantification of biofilm oxygen dynamics in the light and the dark, as well as on biomass accrual of Chlorophyll-a (Chl-a), as a proxy for algal biomass. We additionally investigated how the presence of *E. canadensis* affects biofilm nutrient limitation, by conducting an experiment using nutrient diffusing substrates (NDS; Johnson et al., 2009) with both inorganic and organic substrates. We predicted that the presence of *E. canadensis* would increase nutrient limitation of metabolism for both autotrophic and heterotrophic biofilms, which typically dominate inorganic and organic substrates respectively, and decrease algal biomass accrual, in line with the capacity of *E. canadensis* for absorbing nutrients from the water column. However, the strength of these responses might further depend on other potential environmental changes associated with dense growth of *E. canadensis*, including changes in the light environment or effects on populations of algal grazing invertebrates (both expected to affect GPP more) or pH (expected to affect heterotrophs more due to the requirement of heterotrophic fungi for Ca as a cofactor; McKie et al. (2006)).

2 | METHODS

2.1 | Sites

We studied nutrient limitation of benthic biofilms in six lakes in the region of Uppland, south-central Sweden (c. 59°41′ N 18°34′ E; Table 1). Three lakes were invaded by *E. canadensis* and the other three served as uninvaded references (Table 1). All lakes were circumneutral to mildly alkaline, with moderate nutrient concentrations during the study period (Table 2). Modelled values for catchment loadings of N range from 2989 to 11,420 kg/year for the reference lakes, and 1793 to 8430 kg/year for the invaded lakes (Table S1). Modelled catchment nutrient loadings for P range from 44 to 243 kg/year for the reference lakes, and 42 to 243 kg/year for the invaded lakes (Table S1). Loadings standardised for lake surface area and volume are provided in Tables S2 and S3.

Within each lake, three independent bays were chosen, representing areas of more open littoral habitat separated by areas covered with dense growth of reeds. Within each bay, we established a 5-m-wide transect, extending perpendicular to the shoreline. Substrate composition and macrophyte cover of these transects were quantified in August 2013, as described in Tattersdill et al. (2017). The transects were dominated overall by finer substrate classes (mostly fine sediment and sand; Table 1). *E. canadensis* was present in the invaded lakes only, but percentage cover varied in the vicinity of our study transects (Table 1). By contrast, species of native *Myriophyllum* – like *E. canadensis* belonging to the macrophyte growth-form category of elodeids – were almost completely absent from the invaded lakes, despite being a prominent component of the macrophyte flora of the uninvaded reference lakes (Table 1, Tattersdill et al., 2017). Our nutrient-diffusing substrate (NDS) experiment was conducted in these transects during August 2014.

2.2 | Physicochemical data

Water samples were taken before and after the NDS experiment in August 2014, and sent to the Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment, for further analysis following international (ISO) or European (EN) standards (Fölster et al., 2014, and references therein). Analysed variables included pH, alkalinity and conductivity, along with Total N, ammonium (NH_4), nitrate, Total P and phosphate (PO_4-P) to assess nutrient status among the transects. Absorbance (at 420 and 436 nm) and total organic carbon (TOC) were additionally quantified for estimating water colour and C content. Dissolved oxygen (O_2) and turbidity were measured on site with a Manta™ multiprobe (Eureka Water Probes). One temperature and light sensor (Onset® Hobo Pendant® Temperature/Light 64K Data Logger, quantifying light as visible light illuminance, lux) was placed in each transect, and set to take readings every hour for the experimental duration. Visible light measurements were taken to quantify the extent of shading over the study period.

2.3 | Nutrient-diffusing substrates

Preparation of nutrient-diffusing substrates, and subsequent protocols and analytical approaches, followed Johnson et al. (2009). We filled 576 replicate polypropylene jars (80 mL volume to the rim) each with a 60 ml layer of 2% agar gel. The agar layer in each NDS was allocated to one of four nutrient amendment treatments, with 144 replicates of each: (i) controls (no nutrient amendment), (ii) N only (enriched with 0.5 M $NaNO_3$, VWR chemicals), (iii) P only (enriched with 0.5 M KH_2PO_4, Merck KGaA), and (iv) N + P combined (NP; enriched with both salts together at 0.5 M). Autotrophic and heterotrophic biofilms have the potential to differ in nutrient limitation status even within the same ecosystem, and this can be evaluated using these three different amendments which facilitate quantification of the extent of N, P or NP (where both nutrients are equally limiting) limitation of biofilms (Johnson et al., 2009; Tank & Dodds, 2003).

The NDS jars were closed with one of two filter types, representing two broad benthic substrate classes: inorganic and organic. This was done to facilitate colonisation by biofilms contrasting in relative proportion of autotrophs and heterotrophs, expected to differ in their responses to the environmental changes associated with *E. canadensis* invasion. We used glass fibre filters (thickness 1 mm, diameter 45 mm, pore size 1 μm; Pall Life Science) to represent inorganic substrata, whereas birch veneers (thickness 1 mm, diameter 45 mm) were used to represent organic substrata. The filters were held in place with a screw tip lid. The lid centre was bored to create a 4-cm-diameter hole. The nutrients diffused through the filters, where they could affect growth of biofilms growing on the surface of the filters, fully exposed to ambient water.

Our full sampling design, illustrating the deployment of NDS jars within bays within lakes, is depected in Figure S1. NDS jars were

TABLE 1 Selected characteristics of the studied transects, including invasion status, location, maximum depth, dominant substrate type, total macrophyte coverage and estimated cover of invasive *Elodea canadensis* as a percentage of total macrophyte cover.

						Dominant macrophyte species		
	Lake	Transect	Coordinates (WGS84)	Max depth (cm)	Dominant substrate[a]	Total (%)	*E. canadensis* (%)	Other abundant species[b]
Reference	Långsjön	1	59°36.292′; 18°22.607′	135	Fine sed.	45	0	Myrio Potam
		2	59°35.414′; 18°20.509′	145	Sand	25	0	Myrio
		3	59°35.633′; 18°20.899′	149	Fine sed.	59	0	Eleoc Myrio
	Ruggen	1	59°32.428′; 18°29.243′	106	Sand	2	0	Myrio
		2	59°32.285′; 18°29.078′	110	Sand	75	0	Myrio
		3	59°32.476′; 18°29.535′	133	Fine sed.	22	0	Schoe Myrio
	Ströjan	1	59°54.948′; 18°41.273′	119	Gravel	47	0	Myrio Potam
		2	59°54.981′; 18°41.074′	145	Fine rocks	35	0	Nupha
		3	59°55.042′; 18°41.340′	144	Fine sed.	38	0	Potam
Invaded	Lötsjön	1	59°51.181′; 17°55.850′	128	Fine sed.	80	25	Fonti Potam
		2	59°51.771′; 17°56.224′	113	Fine sed.	100	93	Fonti Potam
		3	59°51.915′; 17°56.532′	113	Fine sed.	100	56	Fonti
	Sparren	1	59°40.319′; 18°17.538′	121	Sand	100	20	Sparg
		2	59°42.543′; 18°21.020′	125	Fine sed.	95	13	Potam *Nupha*
		3	59°41.520′; 18°19.439′	113	Fine sed.	100	50	Potam Sparg
	Ubby-Långsjön	1	59°48.020′; 18°10.271′	91	Gravel	34	20	Elode Potam
		2	59°47.817′; 18°10.206′	120	Sand	42	24	Eleoc
		3	59°48.184′; 18°12.138′	93	Fine sed.	58	86	Potam

Note: Further details are given of other species commonly occurring in the transects. Total macrophyte cover was determined quantitatively as detailed in Tattersdill et al. (2017). Coverage of *E. canadensis* is based on a visual estimate within a 5 m radius of the transect length where the NDS plots were placed.

[a] Dominant substrate: Fine sediment (Fine sed.) ø < 0.2 mm. Sand ø = 0.2–2 mm. Gravel ø > 2–20 mm. Fine rocks >2–10 cm.

[b] Other common taxa: Fonti *Fontinalis* spp., Potam *Potomageton* spp., Sparg *Sparganium* spp., Nupha *Nuphar lutea*, Elode *Elodea nutall.*, Myrio *Myriophyllum* spp., Eleoc *Eleocharis acicularis*, Schoe *Schoenoplectus lacustris*.

TABLE 2 Temperature, light, nutrient, and selected water quality parameters for each of the study lakes pooling across sample dates and transects (mean ± SE).

(a) Temperature, light and nutrients

Invasion	Lake	Temperature (°C)	Light (lux)	Total N (µg L^{-1})	DIN (µg L^{-1})	Ammonium (µg L^{-1})	Total P (µg L^{-1})	PO4-P (µg L^{-1})
R	Långsjon	19.4 ± 0.1	2831 ± 2323	459.7 ± 14.6	9.5 ± 0.76	7.83 ± 0.79	26.22 ± 6.50	1.33 ± 0.21
R	Ruggen	21.5 ± 0.1	741.4 ± 338	605.7 ± 9.8	8.33 ± 1.09	7.33 ± 1.09	29.00 ± 3.37	1.50 ± 0.34
R	Ströjan	22.3 ± 0.0	616 ± 139.8	734.0 ± 20.5	12.83 ± 1.9	11.17 ± 1.66	39.58 ± 5.90	1.67 ± 0.42
I	Lotsjön	21.7 ± 0.0	442.6 ± 4.2	906.5 ± 14.1	10.33 ± 3.03	9.33 ± 3.03	18.55 ± 1.97	1.17 ± 0.17
I	Sparren	23.1 ± 0.2	659.7 ± 379.4	829.7 ± 17.8	13.67 ± 1.31	12.5 ± 1.23	37.13 ± 1.49	1.00 ± 0.01
I	Ubby-Långsjön	19.3 ± 0.1	503.7 ± 98.8	939.3 ± 18	20.83 ± 4.98	17.5 ± 4.77	34.22 ± 1.06	4.33 ± 1.20

(b) Other water quality variables

Invasion	Lake	Dissolved O_2 (% saturation)	Conductivity (µS cm^{-1})	pH	Alkalinity (mequ L^{-1})	TOC (mg L^{-1})	Chl-a (mg m^{-3})	Turbidity (NTU)
R	Långsjon	8.24 ± 0.13	8.76 ± 0.04	7.35 ± 0.06	0.50 ± 0.01	11.50 ± 0.34	9.02 ± 0.81	192.5 ± 189.1
R	Ruggen	8.48 ± 0.33	15.07 ± 0.06	7.66 ± 0.05	0.86 ± 0.01	16.05 ± 0.19	20.76 ± 8.06	17.5 ± 2.1
R	Ströjan	8.26 ± 0.82	20.87 ± 0.15	7.83 ± 0.15	1.72 ± 0.02	16.22 ± 0.25	11.32 ± 1.47	59.3 ± 18.5
I	Lotsjön	8.13 ± 0.12	21.62 ± 0.09	7.92 ± 0.08	1.53 ± 0.01	12.32 ± 0.11	7.04 ± 1.26	1128 ± 985
I	Sparren	8.48 ± 0.29	20.40 ± 0.07	8.24 ± 0.14	1.56 ± 0.01	16.18 ± 0.13	14.22 ± 0.72	128.5 ± 68.4
I	Ubby-Långsjön	7.93 ± 0.43	15.67 ± 0.05	7.52 ± 0.05	1.03 ± 0.01	26.35 ± 0.08	17.13 ± 2.95	9.2 ± 4.1

Note: All parameters were measured twice, at the commencement and conclusion of the study with the exception of dissolved O_2, turbidity and chlorophyll-a (chl-a), which were quantified at the conclusion of the study, only. Values for temperature and light are mean daily averages.

Abbreviations: DIN, dissolved inorganic N; PO_4-P, phosphate; TOC, total organic carbon.

deployed in the field in sets of eight, fastened to a plastic shelf in a single row (Wyatt et al., 2019, pictured in Figure S1). The rows were then placed in the transects at 50cm depth fasted to two metal poles, with the plastic shelf suspended between the poles 10cm from the lake bottom. Each row of eight jars comprised one replicate of each substrate (inorganic or organic) and nutrient (control, N, P, NP) treatment combination. Four rows were deployed in each transect (Figure S1), so that there were four replicates of each substrate by nutrient treatment combination in each transect, and 12 in each lake in total. Rows within transects were spaced at least 0.5m apart. In the invaded lakes, the rows were always placed within stands of *E.canadensis*, even when *E.canadensis* was otherwise rare in the transect as a whole (see Table 1 for *E.canadensis* density estimates over the transects).

In the field, we subjected our NDS jars to an additional barrier treatment with four levels, where we varied the degree to which the jars were protected from shading by macrophytes, and from grazing invertebrates (Figure S1). The treatments were randomly applied to entire rows within transects. One row was left completely exposed, a second had a fence around to prevent the macrophytes from shading the biofilm filters, a third had a Vaseline strip smeared around the edges of each substrate to hinder access by grazers, whilst both barrier treatments were applied to the final row in each transect.

The jars were retrieved after 21 days of exposure in the field. The substrates were temporarily removed from the jars to allow removal of the agar gel. Then the jar was filled with lake water, the filter replaced and the jar closed with an intact screw top lid for transportation back to the laboratory.

2.4 | Photosynthesis and respiration

In the laboratory, we followed a modified version of the light:dark bottle method for quantifying biofilm metabolism (after Johnson et al., 2009; Truchy et al., 2020). Biofilm metabolism measurements were taken within 24h of retrieval from the field. Firstly, NDS jars were emptied, and then refilled with unfiltered water of known oxygen concentration (always near 100% saturation, 9.2mg/L) from the same lake, to ensure that the biofilms were exposed to the same water in the laboratory incubations as previously in the field. Jars containing their original substrate filters were filled to the rim and sealed with transparent Parafilm "M"® (Bemis), with care taken to ensure no air bubbles were trapped under the parafilm. The jars were then placed in a growth cabinet, held at constant light emitted from growth lamps (200 μmol $m^{-2} s^{-1}$) and temperature (20°C). After 3h, the NDS jars were removed from the growth cabinet, with dissolved O2 (mg/L) immediately measured using a fibreoptic oxygen meter (FireString O2; Pyroscience). The water was then emptied and replaced with fresh lake water of known oxygen concentration, near 100% saturation. The steps mentioned above were then repeated, but this time the NDS jars were incubated in a dark growth cabinet, with temperature constant at 20°C for 3h, after which dissolved O_2 concentrations were again quantified.

Oxygen consumption during the dark incubation provides a measure of biofilm respiration, whilst the change in oxygen concentration during the light incubation provides a measure of net community metabolism (NCM; Johnson et al., 2009). GPP is estimated from O_2 production, calculated as the sum of respiration and NCM. During each incubation, three additional jars per lake also were placed in the growth cabinets and changes in O_2 quantified to determine O_2 dynamics mediated by planktonic organisms and organic material in the water. Data from these lake water only replicates were used to correct biofilm respiration and NCM measurements for variation in water metabolism among lakes. Following the second, dark incubation, the filters were frozen for further analysis.

2.5 | Biofilm Chlorophyll-a biomass

We estimated Chl-a biomass of biofilms using two approaches. For Lake Sparren, we estimated Chl-a in situ with a BenthoTorch (bbe Moldaeanke), which uses fluorescence to estimate Chl-a in mg/cm^2 (Kahlert & McKie, 2014). Owing to equipment failure, for all remaining lakes, we quantified Chl-a using a standard Chl-a extraction protocol (Kahlert & McKie, 2014). Briefly, biofilms were first vacuum-filtered over a GF/C glass microfibre filter (diameter 47mm, pore size 1.6μm; Whatman) to extract water. After this, Chl-a was extracted for 12h in 90% acetone, and the mass of Chl-a quantified using a spectrophotometer (TD-700; Turner Design) at wavelengths 664nm, 647nm, 630nm and 750nm. Finally, at Lake Långsjön, we were able to estimate Chl-a using both approaches to assess whether these estimates were comparable, as suggested elsewhere (Kahlert & McKie, 2014), and could thus be combined in a single analysis. Here, we found no significant difference in Chl-a concentrations quantified in Lake Långsjön from the same NDS units using both methods (paired Student's *t*-test $t=-1.21$, $p=0.23$, $n=47$).

2.6 | Data analyses

Before analysis, metabolism measurements (GPP and respiration) were converted to units of μg O_2 $h^{-1} cm^{-2}$, and algal biomass to units of μg Chl-a/cm^2. The area standardisation (per cm^2) was based on the area of substrate (glass fibre filter or wood veneer) on which the biofilms were growing, which for most replicates was 12.566 cm^{-2}. However, some (22 of 288) of the inorganic filters were damaged on retrieval, showing bite marks consistent with attempted consumption by large-bodied consumers (fish or crayfish). These filters were photographed, and the remaining area of these filters quantified with the aid of the image analysis software (Abramoff et al., 2004). Eight filters with less than 25% of the original filter surface area remaining were excluded from all analyses, as resulting estimates of metabolism per cm^2 were excessively large (five-fold greater than the average of all other replicates for GPP and seven-fold greater for respiration). The remaining 14 damaged filters had sufficient surface area (40%–90% remaining) for reliable calculation of metabolism and Chl-a quantities per cm^2, and these were retained in all analyses. Exclusion of these replicates had no effect on the outcomes of statistical tests.

For each respiration, GPP and Chl-a variable, we also calculated the nutrient response ratio (NRR) as the ratio of each nutrient treatment to the control from each row. An NRR >1 for a given nutrient indicates a positive response to addition of that nutrient, and the size of the ratio provides a measure of the extent to which that nutrient is limiting. Finally, we also calculated the ratio of biofilm production to respiration (P/R) by dividing GPP by respiration for each replicate, as a measure of the level of autochthony relative to allochthony in our biofilms (Hall & Hotchkiss, 2017).

We used a mixed-effects model (MEM) to analyse data from our NDS experiment, which was executed using a split-plot design with fixed and nested random factors (Figure S1). Lake invasion status (replicated at the whole lake scale), barrier (applied to whole NDS rows) and nutrient treatments (applied to individual NDS jars) were all fitted as fixed factors. Lake identity, transect nested in lakes, and NDS rows nested in transects and lakes were fitted as random effects, to ensure that each fixed factor was tested at the appropriate level of replication in the design. Random effects were estimated using restricted maximum likelihood (REML).

After conducting our MEM analyses, we used two-sided paired Student's *t*-tests to assess *post hoc* whether the NRR of GPP, respiration and Chl-a for each nutrient treatment differed significantly from 1. Separate paired Student's *t*-tests were conducted for the invaded and uninvaded lakes, and for the organic and inorganic substrates. Finally, we also used bivariate correlation to assess the significance and strength of the relationships between Total N, DIN (dissolved inorganic nitrogen), Total P and PO_4-P, and each NRR ratio, averaged at the lake-transect scale (the scale at which our water chemistry measurements were obtained).

All analyses were conducted using JMP version 11.0 (SAS institute). Log or square-root transformations were applied where necessary to fulfil the assumptions of parametric analysis.

3 | RESULTS

3.1 | Physicochemical variables

We found no difference in temperature between invaded and uninvaded lakes (Table 2), whether analysed as mean daily averages or accumulated degree days (ANOVA both $F_{1,4} < 0.1$, $p > 0.8$). Likewise, we found no difference in light between invaded and uninvaded lakes (Table 2), whether analysed as mean daily averages, or accumulated light over the incubation (ANOVA both $F_{1,4} < 0.8$, $p > 0.5$). Among individual lakes, visible light intensities were markedly higher for the uninvaded Långsjön than the remaining lakes (Table 2), reflecting exceptionally high midday intensities (≤60622 lux) regularly recorded at transect 3.

The highest mean concentrations of DIN and NH_4 were observed in two of the invaded lakes, and the lowest in two of the reference lakes (Table 2). For Total P and PO_4-P, both the highest and lowest mean concentrations were observed in the invaded lakes. However, variation in nutrient concentrations among transects was high, and we found no clear differences in concentrations of Total P or N, nor of DIN, NH_4 or PO_4-P between invaded and uninvaded lakes (ANOVA all $F_{1,4} < 1.96$, $p > 0.23$; Table 2). There also was no evidence for differences in nutrient concentrations between the start and end of the study (all $F_{1,16} < 1.37$, $p > 0.26$), or for interactions between lake invasion status and sampling date (all $F_{1,16} < 2.00$, $p > 0.17$) for any nutrient. Finally, we did not find any difference in the remaining environmental variables between uninvaded and invaded lakes (all $F < 1.67$, $p > 0.14$) or between the start and end of the study (Table 2), with no interactions between sample date and invasion status apparent (all $F < 2.43$, all $p > 0.14$).

3.2 | Effects of the barrier treatment

The barrier treatment had no effect on any of our response variables, either as main effect or in interaction with another factors (all $F < 2.4$, $p > 0.15$). Since the barrier factor was not relevant for explaining variation in our models it was removed from all subsequent analyses.

3.3 | Gross primary production (GPP)

Mean GPP was 102% higher in the invaded (7.53 ± 0.41 μg O_2 $cm^{-2}h^{-1}$) than uninvaded (3.73 ± 0.16 μg O_2 $cm^{-2}h^{-1}$) lakes. Lake invasion status was not significant as a main effect, but interacted significantly with the nutrient and substrate treatment to alter GPP (Table 3), and hence these interactions are presented in our graphs (Figures 1 and 2). The greatest mean increase in GPP between invaded and uninvaded lakes was observed under the NP treatments (percentage effect size comparing invaded with uninvaded lakes: 177%), followed by the N treatment (84%), the controls (78%) and P (77%) treatment (Figure 1a). Average GPP was 21% higher on the inorganic than organic substrates in the controls and 9% higher under the N treatment, but differed little (<1% difference) under the P or NP treatments (Figure 1c; Table 3). The three way interaction between invasion status, nutrients and substrate type was not significant (Table 3).

The nutrient response ratio for GPP was well above 1 for the N addition overall (2.01 ± 0.19) but was close to and under 1 for the NP and P treatments, respectively (Figure 1b; Table 3). An interaction between invasion and nutrients (Table 2) was associated with a 26% higher GPP NRR for the N treatment in uninvaded than invaded lakes, whereas NRRs for the P and NP treatments did not differ between lake types (Figure 1b). The effect of nutrient addition on the GPP NRR was 22% greater for organic than inorganic substrates, with this difference strongest (at 36%) for the NP treatment, followed by the P (20%) and N (13%) treatments (Figure 1d).

TABLE 3 Output from separate mixed effects models testing effects of invasion (I), substrate (S), and nutrients (N) on biofilm gross primary production (GPP) and respiration, along with the nutrient response ratio (NRR) of GPP and respiration, and the ratio of GPP to respiration.

Factor	Num *df*	GPP			NRR GPP			Respiration			NRR respiration			GPP:Respiration		
		Est Ddf	*F*	*p*	Est Ddf	*F*	*p*	Est Ddf	*F*	*p*	Est Ddf	*F*	*p*	Est Ddf	*F*	*p*
Tested against LAKES as random blocks																
Invasion (I)	1	4	1.71	0.261	4	0.29	0.621	4	0.7	0.449	4	3.24	0.146	4	2.33	0.202
Tested against ROWS(TRANSECTS(LAKES)) as random blocks																
Substrate (S)	1	60	0.65	0.423	66.8	7.75	0.007	65.1	50.46	<0.001	67.2	45.96	<0.001	58.3	27.14	<0.001
I×S	1	60	1.73	0.194	66.8	1.59	0.212	65.1	0.87	0.356	67.2	0.53	0.469	58.3	3.22	0.078
Nutrients (N)	3	205.8	58.84	<0.001	139.2	62.91	<0.001	188.9	16.22	<0.001	136.7	12.67	<0.001	200	33.05	<0.001
I×N	3	205.8	6.96	<0.001	139.2	4.49	0.013	188.9	3.34	0.021	136.7	3.97	0.021	200	4.61	0.004
S×N	3	191.2	4.2	0.007	128.8	3.54	0.032	192.2	24.27	<0.001	132.4	15.17	<0.001	199.2	11.34	<0.001
I×S×N	3	191.2	1.63	0.183	128.8	1.2	0.304	192.2	2.2	0.09	132.4	2.15	0.121	199.2	1.79	0.15

Note: Lakes (the replicates for the invasion treatment) and rows nested in transects within lakes (the replicates for substrate and nutrient treatments) were fitted as random factors. The same data set was used for all analyses, but denominator degrees of freedom are estimated as part of restricted maximum likelihood estimation of random effects and consequently vary among analyses.

Abbreviations: Est Ddf, estimated denominator degrees of freedom; *F*, *F* statistic; Num *df*, numerator degrees of freedom; *p*, probability value.

We found no clear correlation between water column total N, DIN or Total P and the GPP NRR, averaged at the transect scale (linear correlation analysis, all $r^2 < 0.09$, $p > 0.1$). However, the GPP NRR had a negative correlation with PO_4-P ($r^2 = 0.17$, $p = 0.012$).

3.4 | Respiration

Biofilm respiration was affected by two-way interactions between nutrients and invasion status, and nutrients and substrate type (Table 3), and hence these interactions are presented in our graphs (Figures 2 and 3). Respiration was 34% higher in invaded than uninvaded lakes overall, but this difference was greater in the controls and P enriched treatments (both 45%) than under the N (25%) and NP (23%) enriched treatments (Figure 2a). Respiration was 55% and 58% greater on the organic than inorganic substrates under the N and NP treatments, respectively, but differed little between substrate types under the remaining treatments (Figure 2c). The three-way interaction between invasion status, nutrients and substrate type was not significant (Table 3).

The respiration NRR was affected by substrate and nutrients (Table 3), being greater than 1 on organic (1.13 ± 0.04) substrates, but less than 1 on inorganic (0.75 ± 0.05) substrates, and greater on N (1.06 ± 0.07) than either P (0.77 ± 0.02) or NP (0.90 ± 0.06) amended substrates (see Figure 2d). Additionally, the respiration NRR was affected by interactions between nutrients and invasion status, and nutrients and substrate (Table 3). The NRR for N enrichment was 35% greater in uninvaded but not invaded lakes, and also was clearly above 1 in the uninvaded lakes only (Figure 2b). All other NRRs were less than 1, except for under the NP treatment in uninvaded lakes, where the NRR was 1 (Figure 2b). Nutrient enrichment caused NRR >1 on the inorganic substrates for the N and NP treatments (Figure 2d). All NRRs for the inorganic substrates were <1, (Figure 2d).

We found no clear correlation between water column total N, DIN, PO_4-P or Total P and the respiration NRR, averaged at the transect scale (all $r^2 < 0.02$, $p > 0.4$).

3.5 | Biofilm P/R ratio

P/R was affected by the main effects of substrate type and nutrients (Table 3; Figure 3). Overall, P/R was 195% greater on inorganic (6.26 ± 1.57) than organic (2.12 ± 0.09) substrates, and was higher on the N-amended (7.53 ± 2.23) than remaining nutrient treatments (NP: 5.85 ± 2.17, controls: 1.81 ± 0.05, P: 1.55 ± 0.08). However, additional interactions between nutrients and invasion, and nutrient and substrate, were apparent (Table 3). P/R was stimulated 221% and 170% more strongly by the N and NP amendments in invaded than uninvaded lakes, respectively (Figure 3a). By contrast, the difference between invaded and uninvaded lakes was 20% and 13% under the control and P-amendment treatments. Likewise, P/R was stimulated 355% and 270% more strongly on the inorganic than organic substrates under the N and NP amendments, respectively, in

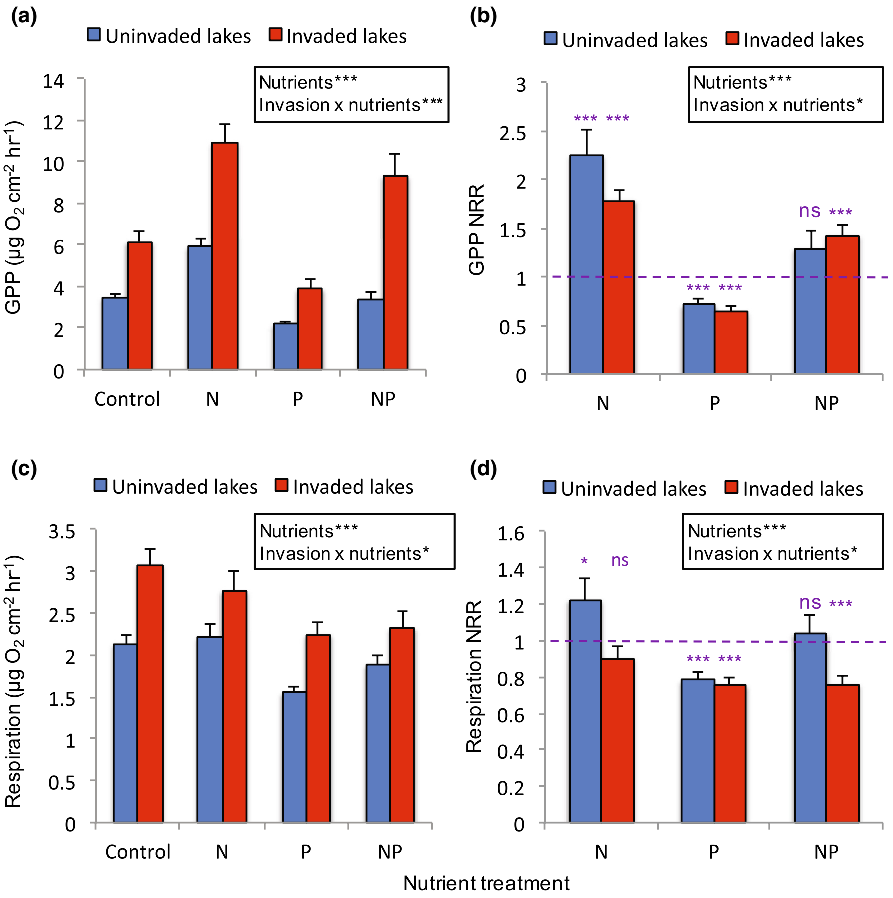


FIGURE 1 Mean effects (± *SE*) of nutrients and lake invasion status on biofilm gross primary production (GPP; a) and respiration (c), and on the nutrient response ratio (NRR) of GPP (b) and respiration (d). Significant factors from MEM analyses are listed in inset boxes on each panel (see Table 3 for details). Additionally, the dotted line on (b) and (d) plots the 1:1 line for the NRR, with significance levels from paired Student's *t*-tests assessing the difference between the mean and the 1:1 line overlaid above the columns in purple text. NRR means significantly higher and lower than the 1:1 line indicate increased and decreased rates of GPP respectively, relative to the controls. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

contrast with the 11% and 14% difference between substrate types under the control and P-amendment treatments (Figure 3b).

3.6 | Algal biomass

Algal biomass, quantified as Chl-a, was highest in the N-amended (3.69 ± 0.31 µg/cm^2) than the remaining treatments (NP: 2.91 ± 0.31, control: 0.86 ± 0.05, P: 0.62 ± 0.03 µg/cm^2). The effect of nutrients on algal biomass further varied with lake invasion status. The N and NP treatments increased algal biomass by 67% and 73%, respectively, in invaded than uninvaded lakes (Figure 5a). The algal biomass response to nutrients also varied depending on substrate status, with algal biomass 4%–11% higher on inorganic than inorganic substrates in the control, N and P treatments, but not under the NP treatment (Figure 5c). Finally, algal biomass responded to substrate differently depending on invasion status. Biomass was 126% higher on inorganic substrates in the invaded compared with uninvaded lakes, but only 20% higher on organic substrates (Figure 4, Table 4).

The algal biomass NRR was overall greater on N-amended (5.39 ± 0.64 µg/cm^2) than P- (0.98 ± 0.07 µg/cm^2) or NP- (4.08 ± 0.53 µg/cm^2) amended substrates. In addition, the algal biomass NRR response to nutrients varied depending on substrate type. The NRR for the N and NP treatments were always greater than 1 (Figure 5d), but the NRR to N amendment was 40% greater on inorganic than inorganic substrates. The P NRR was lower than 1 on organic substrates, and 47% lower on organic than inorganic substrates (Figure 5d). Nutrients and invasion status interacted (Table 4), with the algal biomass NRR greater than 1 for the N and NP treatments in both invaded and reference lakes, but lower than 1 for the P treatment in invaded lakes (Figure 5b).

We did not find any clear correlation between Total N, DIN, PO$_4$-P or Total P and the algal biomass NRR, averaged at the transect scale (all $r^2 < 0.02$, $p > 0.5$).

4 | DISCUSSION

The invasion of freshwater ecosystems by *E. canadensis* outside its native distribution range has a history of more than 150 years, and in many regions the species has become so well established that large-scale eradication or even control of further spread are considered unfeasible (Carboneras et al., 2018; Hussner, 2012; Zehnsdorf et al., 2015). Nevertheless, "maintenance" management might still be required to limit population sizes and minimise impacts on biodiversity and ecosystem functioning in invaded ecosystems (Simberloff, 2021). However, this requires an improved understanding of the ongoing ecosystem-level impacts of *E. canadensis*––especially beyond the establishment phase when relative abundances might be lower. Our investigation of littoral biofilm functioning suggests that the presence of *E. canadensis* in lakes increases availability of N locally, resulting in a 177% mean increase in GPP in invaded

FIGURE 2 Mean effects (± *SE*) of nutrients and substrate on biofilm gross primary production (GPP; a) and respiration (c) and on the nutrient response ratio (NRR) of GPP (b) and respiration (d). Significant factors from MEM analyses are listed in inset boxes on each panel (see Table 3 for details). Additionally, the dotted line on (b) and (d) plots the 1:1 line for the NRR, with significance levels from paired Student's *t*-tests assessing the difference between the mean and the 1:1 line overlaid above the columns in purple text. NRR means significantly higher and lower than the 1:1 line indicate increased and decreased rates of biofilm respiration respectively, relative to the controls. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

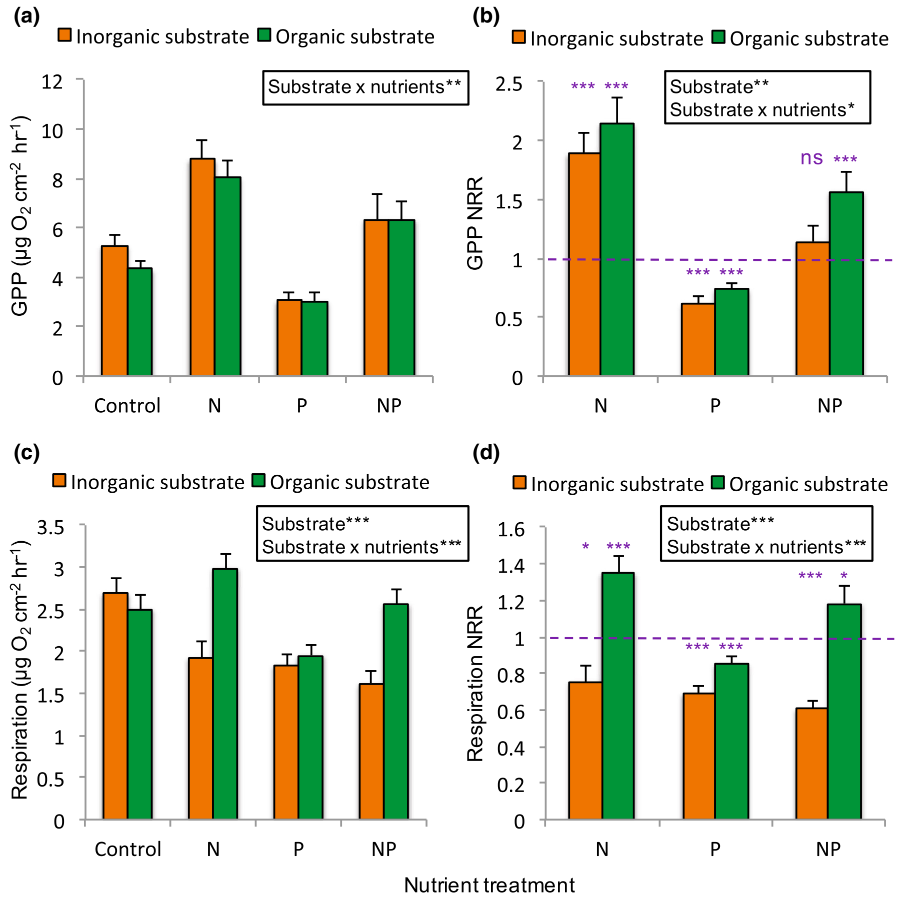
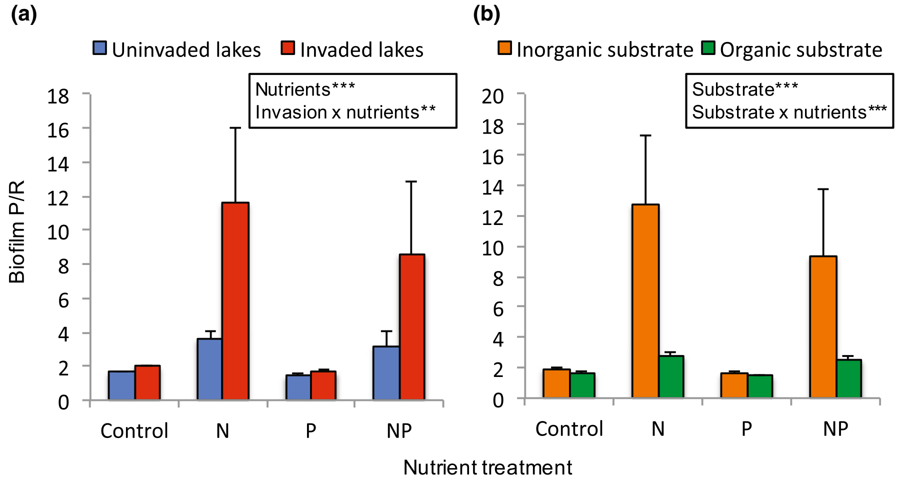


FIGURE 3 Mean effects (± SE) of nutrients and (a) lake invasion status and (b) substrate on P/R ratio (i.e the ration of biofilm GPP:respiration). Significant factors (see Table 3 for details) from MEM analyses are listed in inset boxes on each panel. **$p < 0.01$; ***$p < 0.001$.



lakes, and 126% increase in algal biomass on inorganic substrates. Respiration rates, attributable to the responses of both autotrophs and heterotrophs, were also greater on control substrates in invaded lakes. Previous studies have regularly documented increases in rates of nutrient and C flux in ecosystems affected by invasive species, and especially invasive plants, albeit with many exceptions (Ehrenfeld, 2010; Liao et al., 2008). Our results suggest that the presence of *E. canadensis*, even at lower relative abundance, might in a similar way be associated with shifts in the functioning of biofilms on organic and inorganic substrates in littoral macrophyte beds of Swedish boreal lakes.

Overall, biofilm GPP and algal biomass accrual were N-limited. This result is in line with previous investigations of nutrient limitation of phytoplankton in Swedish and North American lakes (Bergström et al., 2008; Fork et al., 2020; Morris & Lewis, 1988), which found that N-limitation dominates when the ratio of DIN:P_{tot} is less than 1.5 (this ratio was <1 in all of our lakes). Additionally, however, our N-amendment treatment indicates that the degree of N-limitation was reduced in lakes invaded by *E. canadensis* when compared to uninvaded references. It is unlikely that this finding reflects differences in terrestrial nutrient loading among lakes, given that modelled nitrogen inputs tended to be higher in the reference lakes (Table S1),

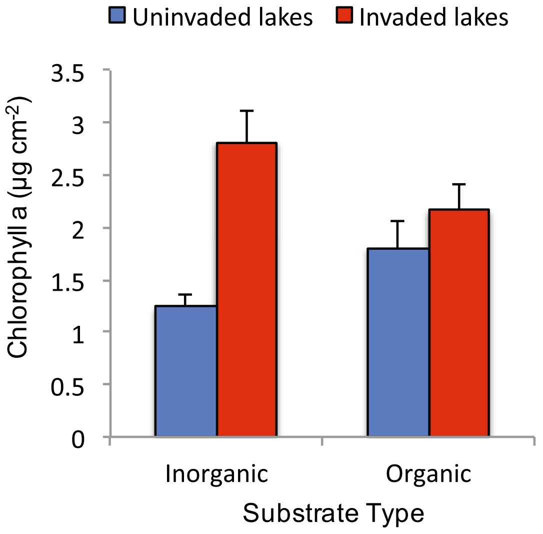


FIGURE 4 Mean effects (± SE) of substrate type and lake invasion status on algal biomass (as chlorophyll-a). Mean ± SE plotted. Invasion × substrate interaction $p < 0.001$ (see Table 4).

where nutrient limitation was greatest. Our spot measures of nutrient concentrations revealed high within lake variation, with no overall significant difference between invaded and uninvaded lakes. Furthermore, there were no significant relationships between the nutrient response ratios of GPP, respiration, or algal biomass and either Total N or DIN. It also is unlikely this change reflects the loss of a notably nutrient-demanding native species, given that the main native macrophytes missing from the invaded lakes are Myriophyllum spp., which remove N from the water at a similar rate to *E. canadensis* (Choudhury et al., 2022). Rather, our NDS results point towards differences in the local nutrient environment adjacent to biofilms, influenced by the presence of *E. canadensis*. These differences were evidently not detected in our water chemistry analyses, which were based on samples collected above the macrophyte bed on two occasions per transect. However, previous research demonstrates that the presence of macrophytes generally, and *E. canadensis* in particular, can have strong influences on the physicochemical environment at local scales, with knock-on effects on other organisms living within the macrophyte bed (Adamec & Ondok, 1992; Carpenter & Lodge, 1986; Gette-Bouvarot et al., 2015; Pfender, 1973; Pokorny & Kvet, 2004).

Evidence for a stronger degree of N limitation in the uninvaded lakes suggests that native macrophyte assemblages and their associated periphyton utilise a greater fraction of the available N, leaving less for benthic biofilms. Several mechanisms potentially explain this result. Firstly, greater macrophyte diversity has been associated with higher nutrient uptake rates (Choudhury et al., 2018, 2022; Engelhardt & Ritchie, 2001), and increasing dominance by *E. canadensis* might disrupt mechanisms underlying these effects (e.g., efficient partitioning of space or facilitative interactions among species). This could reduce nutrient uptake rates by the entire macrophyte assemblage, increasing availability of those nutrients to biofilms. Secondly, although *E. canadensis* often occurs in constructed wetlands, both by design and as a vagrant weed, its uptake efficiency is only moderate when compared to other species, especially emergent species with more extensive root systems (Gumbricht, 1993; Kadlec & Wallace, 2008; Ozimek et al., 1993). Accordingly, increasing dominance by *E. canadensis* might suppress overall uptake by the macrophyte community if it occurs at the expense of more efficient nutrient accumulators. Thirdly, *E. canadensis* supports lower abundances of denitrifying bacteria, capable of converting DIN into N gases (N_2 and N_2O), than most native species of macrophyte (Choudhury et al., 2022), and studies have documented very low denitrification rates associated with *E. canadensis* in southern Sweden (Gumbricht, 1993; Kadlec & Wallace, 2008). Such microbes are especially associated with the roots, which are relatively simple in *E. canadensis* (Kattge et al., 2011), but also occur on leaves (Zhang et al., 2016). It is possible that these microbes may be suppressed by the allelopathic chemicals which *E. canadensis* is known to produce against epiphytes (Erhard & Gross, 2006). Any reduction in denitrification rates also might increase availability of DIN at local scales for biofilms to exploit. Further research is required to assess these potential explanations, but most likely a combination of mechanisms explains the reduction in N limitation of biofilms growing in the invaded transects.

A further factor potentially influencing the availability of nutrients within macrophyte beds are the forms of N favoured by different macrophyte species, with some known to prefer ammonium as an inorganic N source, and others nitrate (Schuurkes et al., 1986). Benthic algal biofilms readily utilise nitrate as an inorganic N source (Ribot et al., 2013), and it is therefore possible that the relative abundance of nitrate-preferring macrophytes might explain some variation in biofilm responses to our nutrient addition treatments. However, the macrophyte species known to preferentially use nitrate are generally characteristic of nutrient-poor soft waters (Schuurkes et al., 1986). Our lakes were instead characterised by species, including *E. canadensis* itself, that preferentially use NH_4 (Boedeltje et al., 2005; Nichols & Keeney, 1976; Ozimek et al., 1993; Schuurkes et al., 1986). Ammonium was the dominant source of inorganic N in our lake littoral water samples, collected from within the transects. Thus, it is unlikely that differences in macrophyte N preferences caused differences in the availability of different forms of inorganic N that might explain the reduced N-limitation of algal biofilms in our invaded lakes.

Reduced nutrient limitation is likely to explain much of the increased metabolism and autotrophic biomass of biofilms growing in the presence of *E. canadensis*. However, other potential influences of *E. canadensis* on local environmental characteristics might also explain differences in the performance of biofilms on the unamended substrates, and the size of the biofilm response to N enrichment. Apart from nutrients, biofilms also are strongly regulated by light, temperature, and grazing by herbivores (Battin et al., 2016; Mora-Gómez et al., 2016). However, there was no evidence that these variables differed between the invaded and uninvaded transects, based either on data from the light and temperature loggers, or from our barrier treatments. There also was no effect of our grazer exclusion treatment, suggesting that grazing pressure was not intense in

TABLE 4 Output from separate mixed model ANOVAs testing effects of invasion (I), substrate (S), and nutrients (N) on biofilm algal biomass along with the nutrient response ratio (NRR) of biomass.

		Algal biomass			NRR algal biomass		
Factor	Num *df*	Est D*df*	*F*	*p*	Est D*df*	*F*	*p*
Tested against LAKES as random blocks							
Invasion (I)	1	4	1.59	0.276	3.994	0.3009	0.6125
Tested against ROWS(TRANSECTS(LAKES) as random blocks							
Substrate (S)	1	68.5	2.97	0.089	71.9	1.63	0.206
I×S	1	68.5	12.3	<0.001	71.9	0.78	0.381
Nutrients (N)	3	208.9	60.16	<0.001	140.1	66.45	<0.001
I×N	3	208.9	4.62	0.004	140.1	5.36	0.006
S×N	3	204.6	4.2	0.007	137.2	6.08	0.003
I×S×N	3	204.6	1.42	0.237	137.2	1.67	0.193

Note: Random effects and denominator degrees of freedom calculated as for Table 2.

Abbreviations: Est D*df*, estimated denominator degrees of freedom; *F*, *F* statistic; Num *df*, numerator degrees of freedom; *p*, probability value.

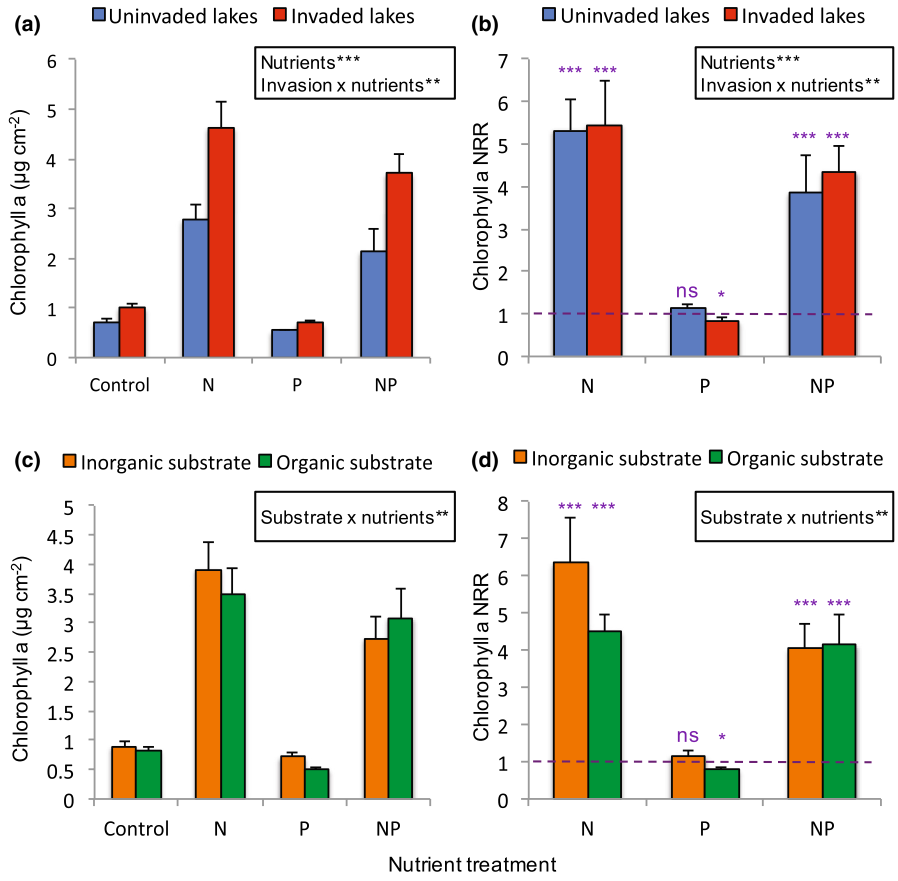


FIGURE 5 Mean effects (± SE) of nutrients and either lake invasion status (a, b) or substrate (c, d) on algal biomass (chlorophyll-a) (a, c), and on the nutrient response ratio (NRR) of algal biomass (b, d) Significant factors from MEM analyses are listed in inset boxes on each panel (see Table 4 for details). Additionally, the dotted line on panels b and d plots the 1:1 line for the NRR, with significance levels from paired *t*-tests the difference between the mean and the 1:1 line overlaid above the columns in purple text. NRR means significantly higher and lower than the 1:1 line indicate increased and decreased rates of algal biomass accrual, respectively, relative to the controls. $^*p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.001$; ns, not significant.

our lakes, at least from snails, which are the organisms most effectively excluded by the petroleum jelly barrier method (e.g. Hladyz et al., 2011).

Additionally, *E. canadensis* can have strong effects on local pH and alkalinity. This species uses HCO_3 as a C source, resulting in precipitates of $CaCO_3$ that tends to raise pH overall and buffer diel pH fluctuations, especially by reacting with CO_2 produced at night (Pokorny & Kvet, 2004). Acidity clearly impacts diversity and productivity of periphyton, and alkalinity may be more limiting for biofilm productivity than nutrients below a threshold of around 100 µeq/L (Fairchild & Sherman, 1992; Wyatt & Stevenson, 2010). However, even above pH 7, increasing pH might alter biofilm growth. Indeed, a North American study found that increasing pH above 7 favoured greater autotrophic diversity and biofilm growth, and favoured a stronger response to the addition of additional N, but not P (Keithan et al., 1988), similar to our findings. Greater stability in pH regimes also may allow biofilms to more fully exploit additional nutrients, explaining the strong response to N-addition observed for biofilms from the invaded lakes.

In contrast to the effects of N, addition of P did not stimulate greater algal biomass, and generally resulted in negative nutrient response ratios for GPP and respiration. Phosphorus inhibition of biofilms has been observed previously in NDS and other nutrient assays, and attributed to potentially toxic effects arising from interactions between P and agar (e.g., production of growth inhibitors) and direct toxic effects of excessively high nutrient concentrations (Atkinson et al., 2013; Burrows et al., 2017; Tank & Dodds, 2003). Additionally, excess inorganic P can result in suppression of the synthesis and activity of key enzymes involved in algal nutrient uptake pathways, and suppress growth of some species in laboratory cultures (Cembella et al., 1982; Chu, 1943), and might favour heterotrophic bacteria over algae in some circumstances (Jansson, 1988).

Responses on the different substrate types were broadly as expected, with GPP and Chl-a generally greater on the inorganic substrates, and ecosystem respiration, reflecting activities of both auto- and heterotrophs, greater on organic substrates (Johnson et al., 2009). Overall though, the effects of N amendment on respiration were less marked and consistent than on GPP and algal biomass, with positive effects observed in uninvaded but not invaded lakes, and on organic but not inorganic substrates. These results are likely to reflect the influence of heterotrophic microbes on bulk respiration rates. Heterotrophs and autotrophs growing on the same substrates can differ in nutrient limitation status (Tank & Dodds, 2003). Our results suggest that heterotrophs were less limited by N overall than autotrophs, which weakened the net response to N, especially in the invaded lakes, where N overall was less limiting.

The ratio of production to respiration was slightly higher in the invaded than uninvaded lakes, suggesting that environmental effects arising from the presence of *E. canadensis* favours the activities of autotrophic over heterotrophic organisms (Alnoee et al., 2016; Duarte & Prairie, 2005). This ratio was shifted markedly by the addition of N, which caused large increases in GPP relative to respiration in the invaded lakes, especially on the inorganic substrates. Theoretical predictions (Thingstad & Pengarud, 1985) and some empirical observations (e.g., Myers et al., 2021; Rothhaupt, 1992) suggest that algae outcompete bacteria when C to nutrient ratios are lowered. This was especially likely to be the case on our inorganic substrates in the invaded lakes, if the presence of the invasive species increases nutrient availability at local scales, with C to nutrient ratios further reduced by the nutrient amendments. However, interactions between heterotrophs and algae are complex, encompassing not only competition but also parasitism and mutualism (Croft et al., 2005; Kim et al., 2008; Ramanan et al., 2016), and also can be affected by the presence of consumers (Hulot et al., 2001; Rothhaupt, 1992), pointing to the need for more research on mechanisms by which the presence of *E. canadensis* favours a shift towards greater autotrophy.

4.1 | Implications and future research

Our results suggest that the presence of *E. canadensis* might increase the biomass and activity of autotrophic biofilms, especially on epilithic substrates within the macrophyte bed. This adds to evidence from forest, grassland and wetland ecosystems that invasive species are often associated with increases in fluxes of nutrients and C through ecosystems (Ehrenfeld, 2010; Liao et al., 2008). For example, the combination of ecophysiological traits (inter alia rapid growth, high specific leaf area) characterising many species of invasive plants is associated with an average increase in net primary production of 83% in invaded compared with uninvaded ecosystems (Liao et al., 2008). Furthermore, these increased fluxes can be associated with longer term outcomes, including increased pools of C and nutrients in soils of invaded ecosystems, arising from increased productivity and stimulation of soil microbial biomass (Liao et al., 2008). In contrast, increased autotrophic productivity in the littoral zone of lakes is more likely to be initially transferred into greater productivity of secondary invertebrate consumers than into lake sediments, unless biofilms are dominated by less palatable taxa such as cyanobacteria (O'Neil et al., 2012; Weitere et al., 2018).

More research is required to assess whether the changes in biofilm activity observed here might drive longer term, cumulative changes in C and nutrient dynamics in invaded lakes. This includes assessment of the impacts of invasive macrophytes over a broader range of nutrient concentrations than studied here, given that potential effects on nutrient limitation of biofilms are likely to weaken as nutrient enrichment increases. It also remains unclear whether the observed reduction in N-limitation of autotrophic biofilms in our *E. Canadensis*-invaded stands is attributable to some combination of reduced nitrogen uptake by the macrophytes themselves, reduced activity by denitrifiers associated with macrophyte roots and leaves (Choudhury et al., 2022), and/or negative effects of allelopathic chemicals on N uptake by epiphytes (Engelhardt & Ritchie, 2001; Gumbricht, 1993; Kadlec & Wallace, 2008). Impacts on any of these N-processing pathways could point towards a reduction in the overall N-processing capacity of macrophyte beds, even when productivity of epilithic biofilms increases. Identifying which traits of *E. canadensis* explain the results observed here is crucial not only for managing the impacts of *E. canadensis* itself, but also evaluating the risk associated with other aquatic invasive species, including the closely related *E. nutalli* which is rapidly expanding its distribution in many regions of the world (Erhard & Gross, 2006; Zehnsdorf et al., 2015).

Significantly, our observed effects of species invasion on biofilm activity occurred even at the lower levels of *E. canadensis* relative cover that characterised most of our study plots, and despite the long time (>100 years) that has passed since *E. canadensis* first invaded the region. Aquatic invasive macrophytes typically become targets for active control measures when they begin to interfere with human recreational activities at very high biomasses (Verhofstad & Bakker, 2017). However, our findings demonstrate the potential for invasive species to have significant effects on ecosystem functioning at much lower levels of relative abundance, particularly for species characterised by a combination of traits that lead to a marked modification of local environmental conditions, as exemplified by *E. canadensis*. Once established, elimination of *E. candensis* and other invasive freshwater plants is extremely challenging, but proposals for harvesting invasive macrophyte biomass for feed and biofuels, for example, point towards potential economic incentives for controlling their density in individual water bodies (Zehnsdorf et al., 2015). Fully resolving economic and environmental cost–benefit equations associated with potential control and harvest measures requires an improved understanding of relationships between increasing invasive macrophyte relative abundance and negative ecosystem impacts (Marbuah et al., 2018), including on the key biofilm functions investigated here.

AUTHOR CONTRIBUTIONS

Conceptualization: Brendan G. McKie, Kristina Tattersdill, Frauke Ecke, Ryan A. Sponseller. Developing methods: Brendan G. McKie, Kristina Tattersdill, Ryan A. Sponseller. Conducting the research: Kristina Tattersdill, André Frainer. Data analysis: Brendan G. McKie. Data interpretation: Brendan G. McKie, André Frainer, Ryan A. Sponseller. Preparation figures & Tables: Brendan G. McKie. Writing: Brendan G. McKie, Kristina Tattersdill, André Frainer, Frauke Ecke, Ryan A. Sponseller.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

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SUPPORTING INFORMATION

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