RESEARCH ARTICLE

Direct and indirect effects of native plants and herbivores on biotic resistance to alien aquatic plant invasions

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Abstract

1. Biotic resistance to alien plant invasions is mainly determined by ecological interactions in two layers of the food web: competition with native plant species and herbivory by native herbivores. While the direct effect of native plants on alien plant performance via competition has been well documented across ecosystems, less is known about the direct and indirect effects of herbivores in providing biotic resistance. Our main aims were to determine whether temperate native aquatic plants and herbivores can provide biotic resistance to plant invasions, understand the underlying mechanisms and search for potential interactive effects of competition and herbivory on invader performance (i.e. growth).

2. We mimicked natural temperate mesotrophic and eutrophic freshwater lakes in mesocosms, by growing three native submerged plant species in monocultures (*Ceratophyllum demersum*, *Myriophyllum spicatum* and *Potamogeton perfoliatus*) at three competition levels (no, low and high) without and with the native aquatic generalist snail *Lymnaea stagnalis*. We subsequently simulated an early stage of establishment of the South American highly invasive alien plant species *Egeria densa*.

3. We found that competition by native plant biomass significantly reduced invader performance but depended on native species identity. Herbivory had no direct negative effect on invader performance as the snails fed mainly on the available filamentous algae, which are commonly found in meso- and eutrophic systems, instead of on the plants. However, the consumption of filamentous algae by herbivores indirectly had positive effects on the invader total biomass, thus facilitating the invasion by *E. densa*. Nonetheless, these indirect effects worked through different pathways depending on the native plant identity.

4. *Synthesis.* We found evidence for biotic resistance through competition by native plant species. However, we show that herbivores can indirectly facilitate South American plant *E. densa* invasion promoting its growth through selective feeding on filamentous algae, but this effect depends on the native plant species involved. Our experiment illustrates the important role of indirect interactions to understand the potential of biotic resistance in natural ecosystems.

1 | INTRODUCTION

With the intensification of trade, transport and tourism, the number of alien species introductions has significantly increased in the last centuries (Meyerson & Mooney, 2013). However, only few of these introduced species truly establish, become invasive and cause negative impacts (Caley, Groves, & Barker, 2008; Meyerson & Mooney, 2007; Williamson & Fitter, 1996). This can partly be explained by the concept of biotic resistance (Elton, 1958; Levine, Adler, & Yelenik, 2004), which states that interactions with native species can reduce the establishment or spread of alien species. Biotic resistance to plant invasions is mainly determined by ecological interactions in two layers of the food web: competition with native plant species, and herbivory by native herbivores (Levine et al., 2004). Previous studies have shown that native plant competition can have strong negative direct effects on the performance of invading plant species in grasslands (te Beest, Mpandza, & Olff, 2018; Corbin & D'Antonio, 2004), forests (Fine, 2002), saltmarshes (Amsberry, Baker, Ewanchuk, & Bertness, 2014), marine (Balestri, Vallerini, Menicagli, Barnaba, & Lardicci, 2018; Bando, 2006; Britton-Simmons, 2006) and freshwater systems (Chadwell & Engelhardt, 2008; Michelan, Thomaz, & Bini, 2013; Petruzzella, Manschot, van Leeuwen, Grutters, & Bakker, 2018). Similarly, native herbivores can provide direct biotic resistance across various ecosystem types by foraging on alien plant species, such as in mangroves (Zhang, Meng, Wang, & He, 2018), grasslands (Pearson, Potter, & Maron, 2012), coastal dunes (Cushman, Lortie, & Christian, 2011), saltmarshes (Ning, Xie, Liu, Bai, & Cui, 2019), marine (Cebrian, Ballesteros, Linares, & Tomas, 2011) and also freshwater ecosystems (Parker, Caudill, & Hay, 2007). Although most studies to date have focused on the individual direct effects of native plants or herbivores on alien plant species success, the interaction between these factors still remains poorly understood (Alofs & Jackson, 2014; Kimbro, Cheng, & Grosholz, 2013; Levine et al., 2004).

Besides the direct effects that native plants and herbivores can have on alien plant establishment success, these plants can also be affected by indirect effects which is defined as the effect of one species on another via a third species (White, Wilson, & Clarke, 2006). Indirect effects can have a negative or positive impact on alien plant species. However, these more complex interactions have so far received little attention. The role of herbivores on invasion resistance may be more complex than simply reducing alien plant biomass, as they can also indirectly interact with alien plant species. For example, native herbivores can selectively feed on native plants and therefore release alien plants from top-down control, a concept known as the enemy release hypothesis (Keane & Crawley, 2002). Herbivores foraging on native plant species can also reduce native plant competition abilities (Le Bagousse-Pinguet, Gross, & Straile, 2012; Li, Xiao, Zhang, & Dong, 2013). Furthermore, herbivores can also indirectly affect alien plant establishment through non-trophic effects, including alteration of disturbance regimes and/or resource availability, for example by increasing nutrient recycling (Ribas et al., 2017). The impact of herbivores on alien plant invasions therefore varies by their feeding preferences, and how they may otherwise interact with species in the food web.

An increasing body of evidence shows that herbivores can have large impacts on freshwater plant abundances (Bakker et al., 2016; Wood et al., 2017). A limitation in our understanding of the potential of biotic resistance to alien plant invasions in these systems is that most of our knowledge comes from laboratory feeding trials, whereas studies that take into account the complexity of ecosystems including direct and indirect effects, in mesocosms or in the field, are scarce (Alofs & Jackson, 2014; Petruzzella, Grutters, Thomaz, & Bakker, 2017). In contrast to terrestrial systems, where many herbivores are specialists, most of the consumers of freshwater plants are generalist herbivores or even omnivores (Bakker et al., 2016; Wootton, 2017). Feeding trials have demonstrated that aquatic generalist herbivores can prefer native plants (Xiong, Yu, Wang, Liu, & Wang, 2008) or alien plants (Morrison & Hay, 2011; Parker & Hay, 2005) or have no preference for either (Grutters, Roijendijk, Verberk, & Bakker, 2017). In freshwater ecosystems, several generalist herbivores can also feed on algae instead of plant matter (Elger, De Boer, & Hanley, 2007). Filamentous green algae often grow on aquatic plants in mesotrophic and eutrophic conditions; their removal by herbivores can promote the growth of plants by reducing the negative effects of shading and nutrient competition which would have an indirect effect on either the invading, native or both native and invading plant species (no overall effect; Bakker, Dobrescu, Straile, & Holmgren, 2013; Brönmark, 1985, 1990; Hidding, Bakker, Hootsmans, & Hilt, 2016). Thus, this makes freshwater ecosystems excellent model systems in which to study this real-world complexity including both direct and indirect effects on biotic resistance. To our knowledge, only studies in the context of biological control—that is, using alien herbivores–have explored the interaction between competition and herbivory in submerged freshwater plants (Doyle, Grodowitz, Smart, & Owens, 2007; Van, Wheeler, & Center, 1998), whereas the role of indirect effects influencing invasion success has rarely been considered (White et al., 2006).

Here, we study the direct and indirect effects of native plants and herbivores on biotic resistance to alien aquatic plant invasions using a freshwater model system. Our aims were to determine whether temperate native aquatic plants and herbivores can provide biotic resistance to plant invasions, understand the underlying mechanisms and search for potential interactive effects of competition and herbivory on invader performance (i.e. growth). We used freshwater mesocosms in which we grew three common native submerged plant species in monocultures (*Ceratophyllum demersum* L.,

Myriophyllum spicatum L. and *Potamogeton perfoliatus* L.) at three competition levels (no plants, low and high competition) without and with the native aquatic generalist snail *Lymnaea stagnalis* L. We used three different plant species as native competitors to assess the role of competitor identity and palatability in these interactions. Then, we simulated an early stage of establishment by introducing the South American highly invasive alien plant species *Egeria densa* Planchon. The Brazilian waterweed *E. densa* (Hydrocharitaceae) is a rooted, submerged, freshwater perennial plant which has fast vegetative growth by fragmentation, can tolerate wide environmental conditions and spreads over long distances (Yarrow et al., 2009). This species already colonized 12 European countries and is listed in the European and Mediterranean Plant Protection Organization list of invasive plants as posing an important threat to plant health and/or the environment and biodiversity since 2005 (Hussner, 2012).

We predicted that competition from native plant species and herbivory by native generalist herbivores would interactively affect the performance of the invader *E. densa*. We hypothesized that (1) an increase in native plant biomass would provide biotic resistance by increasing competition with the invader, thereby reducing *E. densa* biomass (H1, solid arrow, Figure 1); (2) that herbivores would provide direct biotic resistance through selective feeding on the invader (H2, solid arrow, Figure 1). Alternatively, herbivores would indirectly affect the invader *E. densa* biomass by either (3) selective feeding on the native plants, thereby reducing the direct competitive effect of native plants on *E. densa* promoting its growth (H3, dashed and solid arrows, Figure 1) or (4) by selective feeding on the biomass of filamentous green algae, thereby reducing the competitive effect

of filamentous algae on *E. densa* (H4, dashed arrows, Figure 1) or indirectly increasing competition between the native and invading plant species (H4, dashed and solid arrows, Figure 1).

2 | MATERIALS AND METHODS

2.1 | Aquatic plants

We selected *E. densa* (Hydrocharitaceae) as invader. It is a popular aquarium plant in Europe and world-wide; the aquarium trade is considered its main introduction pathway (Yarrow et al., 2009). *Egeria densa* disperses mainly vegetatively for which fragments with only two nodes are enough to establish and develop new stands (Yarrow et al., 2009). The root system and shoots can break easily allowing plant fragments to be carried through the water to colonize new areas. This species can grow to over 3 m long and form monospecific stands with closed canopies, that can severely alter the structure of the native communities and local environmental conditions (Yarrow et al., 2009). It is well adapted to cold climates and can survive freezing winters by storing starch in its leaves and stems (Thiébaut, Gillard, & Deleu, 2016). *Egeria densa* has caused many problems throughout temperate regions including the United States of America and New Zealand, and has also become a nuisance species in its native range (Bini, Thomaz, Murphy, & Camargo, 1999).

The three common native submerged species that we used in the experiments are widely distributed in Northwestern Europe and

FIGURE 1 Meta-model representing the hypothesized causal relationships among herbivores, native plant biomass, filamentous green algae biomass and the invader *Egeria densa* performance. Solid arrows denote direct effects and dashed arrows indirect effects on the invader performance. Red and black arrows respectively representing negative (biomass loss) and positive (biomass gain) relationships in the direction of the arrow. Blue arrow indicates both possibilities. Arrow numbers denote the corresponding hypotheses. The mechanism of biotic resistance is given along the arrows
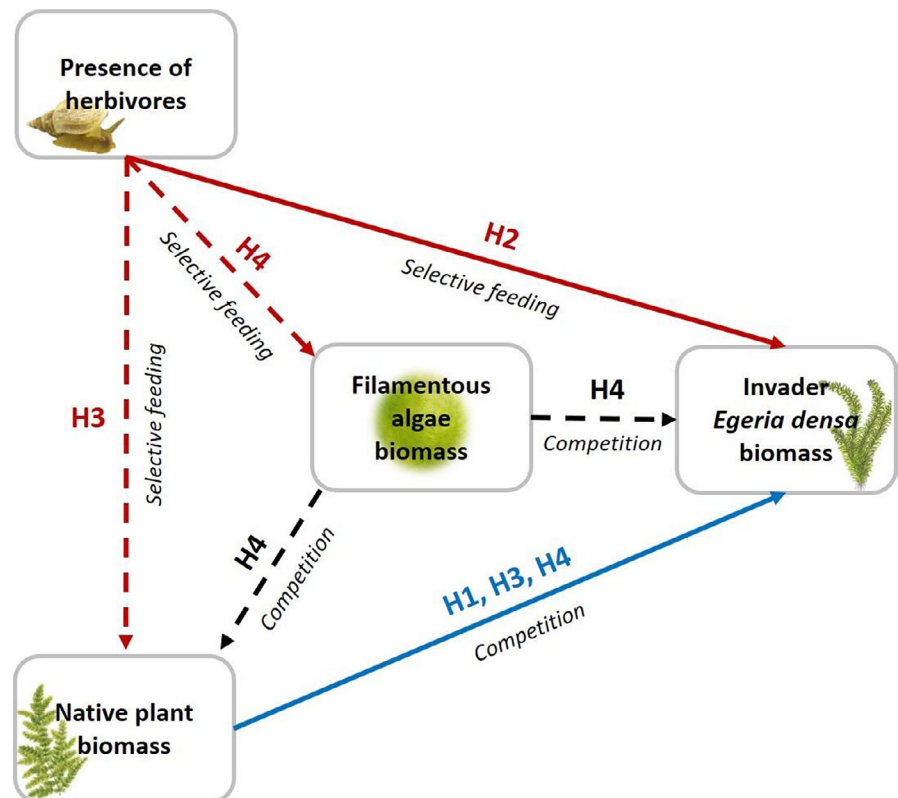
co-occur in temperate shallow lakes (Van De Haterd & Ter Heerdt, 2007). *Ceratophyllum demersum* (Ceratophylaceae) is a free floating submerged species and *M. spicatum* (Haloragaceae) and *P. perfoliatus* (Potamogetonaceae) are rooted species. All three species are perennial and capable of clonal growth. All the plants used in this study were acquired from a commercial plant trader (De Zuurstofplantgigant, Hapert, The Netherlands). The acquired plants were pre-cultivated in 200 L cattle tanks (diameter = 66 cm and height = 60 cm, two tanks per species) under controlled greenhouse conditions with a 16/8 hr light/dark cycle at a temperature of 21 ± 3°C during the day and 16 ± 3°C during the night (Figure S1). The tanks were filled with a 3.4 kg (~2 cm) bottom layer of artificial plant pond sediment (Plant soil Moerings—Velda, organic matter = 34.31%), 44.9 kg (~10 cm) of washed sand on top (0.8–1.0 mm grain size, organic matter content = 0.16%) and filled with water from freshwater Lake Terra Nova (52°12′55.2″N, 5°02′25.7″E). Lake Terra Nova is a shallow peat lake located in the centre of the Netherlands where all three native plant species used in the experiment co-occur (Van De Haterd & Ter Heerdt, 2007). The lake is characterized by high nutrient concentrations in the water (water used in the experiment: $M \pm SD$, $n = 6$ water samples, 0.14 ± 0.05 mg/L P-PO_4; 0.55 ± 0.46 mg/L N-NO_3). The plants were cultivated under the following conditions: water temperature 22.3 ± 0.8°C, dissolved oxygen 12.5 ± 1.3 mg/L, conductivity 263 ± 28 µS/cm, pH 9.8 ± 0.3 and alkalinity 2.37 ± 0.52 mEq/L. Plants were pre-cultivated for at least 20 days before the start of the experiment.

2.2 | Generalist herbivore

Lymnaea stagnalis (Gastropoda, Pulmonata, Basommatophora), the great pond snail, is a common and widely distributed generalist herbivore native to the Holarctic region. Most freshwater gastropod species consume mainly algae, bacteria and detritus but large species such as *L. stagnalis* can consume considerable amounts of aquatic plants having a large impact on aquatic plant abundance (Brönmark, 1989, 1990; Wood et al., 2017). Densities of 10–40 *L. stagnalis* individuals/m² are commonly found under natural conditions (Elger et al., 2007), where it occurs in slow flowing and stagnant freshwater systems. This species has also been previous commonly used as model species in aquatic settings (Bakker et al., 2013; Elger & Barrat-Segretain, 2002, 2004; Grutters et al., 2017; Zhang, Liu, Luo, Dong, & Yu, 2018).

Adult snails were collected from a pond located at the Netherlands Institute of Ecology (NIOO-KNAW, 51°59′16.8″N, 5°40′24.7″E, Wageningen, The Netherlands). They were acclimated to laboratory conditions for at least 2 weeks in 15 L buckets filled with groundwater at 20°C and constant aeration and exposed to a 16:8 hr day:night cycle, before being experimentally used. The snails were fed butterhead lettuce (*Lactuca sativa* L.) 6 days a week. Once a week fish food pellets (Velda, Gold Sticks Basic Food) and chalk were provided to ensure enough nutrients and calcium for shell development (following Grutters et al., 2017).

2.3 | Experimental design and set-up

A greenhouse experiment was established at the Netherlands Institute of Ecology (NIOO-KNAW; 51°59′15.3″N and 5°40′14.8″E) during the summer of 2018 (July–October). The experiment was set up as a full factorial randomized block design, with 3 × 3 × 2 treatment combinations of monocultures of three native submerged plant species (*C. demersum*, *M. spicatum* and *P. perfoliatus*), three levels of competition (no native plants, low density and high density) and absence (no snails) or presence of herbivory (with snails). The 18 treatments were replicated six times using a block design, yielding a total of 108 mesocosms (Figure S1). The greenhouse controlled conditions consisted of a 16/8 hr light/dark cycle at a mean temperature of 21 ± 3°C during the day and 16 ± 3°C during the night.

The mesocosms consisted of 13 L glass cylinder aquaria (18.5 cm diameter and 48 cm height) filled with a bottom layer of artificial plant pond sediment (150 g resulting in a layer of ~1 cm depth) with a top layer of washed sand (2 kg resulting in a layer of ~5 cm). Each aquarium was filled with 8 L lake water (resulting in 27 cm depth), leaving the upper 15 cm free to prevent snails from escaping. The water level was maintained constant during the whole experiment by refilling once a week with lake water to compensate for evapotranspiration. Abiotic parameters were monitored throughout the experiment and the growing conditions were found to be suitable for the plants ($M \pm SD$, $n = 1{,}166$, water temperature 23.3 ± 1.0°C, dissolved oxygen 12.9 ± 1.9 mg/L, conductivity 283 ± 30 µS/cm, pH 9.7 ± 0.7 and alkalinity 2.12 ± 0.47 mEq/L).

To establish native plant communities for the competition treatment, we cut 99 non-rooted apical shoots without lateral shoots from the cultivation tanks from each of the native species *C. demersum*, *M. spicatum* and *P. perfoliatus*. We cut 15 cm long apical shoots and washed them in running tap water to remove any material attached. We randomly selected 15 of the 99 shoots of each species, dried these individually to a constant mass at 60°C for at least 48 hr, and weighed them for initial biomass measurements (dry weight, DW). We established the competition levels by pairing the invader *E. densa* with a single native plant species at different native shoot planting densities. The planting densities of each native plant species versus *E. densa* were manipulated to be 0:2 shoots (no competition, invader growing alone), 1:2 (low competition) and 6:2 (high competition), corresponding to ~37 plants/m² (low competition) and ~222 plants/m² (high competition) respectively before the invader introduction. These shoot densities are within the range observed in natural conditions (Li et al., 2015). The plant shoots of the rooted species were planted 5 cm deep in the sediment while the shoots of the non-rooted submerged species *C. demersum* were dropped in the water.

The native plants were left to establish for 2 weeks (24 July to 6 August) to allow the growth of at least one new shoot. Then, we introduced the invader by planting two *E. densa* non-rooted apical shoots per aquarium (7 August), which is considered to represent medium propagule pressure (Li et al., 2015). We chose shoots with an apical tip because these have a higher ability to regenerate, colonize and grow than shoots without apical tips (Riis, Madsen, & Sennels, 2009).

To determine the introduced biomass in DW, we randomly selected 15 *E. densa* shoots, dried these to a constant mass at 60°C for at least 48 hr, and weighed them individually. *Egeria densa* was allowed to root for 2 days before we added the herbivore treatment, to simulate an early stage of establishment of *E. densa* in the new temperate native aquatic community.

In the herbivory treatment, we added two *L. stagnalis* snails per aquarium to half of our experimental units (10 August), representing intermediate snail densities observed in the field (Elger et al., 2007). We selected snails of the same size (shell length 30 ± 1 mm, wet weight 2.19 ± 0.27 g, *M* ± *SD*, *n* = 108) and starved the snails for 48 hr before adding them to standardize their appetite as is common practice in feeding trials (following Grutters et al., 2017).

2.4 | Harvest and data collection

At the end of the experiment (after 8 weeks, on 8 October), we removed the herbivores, harvested the alien and native plants and, as we observed the growth of filamentous green algae *Spirogyra* sp. in our mesocosms, we harvested its biomass present on the plants and in the water column (Figure S2). We washed all the plants from each aquarium in an individual container to ensure that all the filamentous algae were kept. Then, this remaining water together with the water left in the aquarium after plant removal was filtered over a sieve of 0.106 mm mesh size. The filamentous algae biomass on the sieve was washed and dried to a constant mass at 60° for at least 48 hr, and weighed to determine DW. We measured invader *E. densa* performance in terms of the following growth parameters: total root and shoot DW, summing values from both introduced propagules and total biomass summing total root and shoot DW. We also determined native plant biomasses. All plants were dried to a constant mass at 60° for at least 48 hr, and weighed to determine DW.

2.5 | Feeding trials

Herbivory consumption rates and preferences depend on plant palatability (Grutters et al., 2017). To determine plant palatability for the snails, we performed 24 hr no-choice feeding trials following established protocols (Elger & Barrat-Segretain, 2002, 2004; Grutters et al., 2017). Plant material for the trials was collected from the same cultivation tanks that provided plants for the greenhouse experiment, and washed to remove any attached material. Snails of similar size (shell length 28.9 ± 1.8 mm, *M* ± *SD*, *n* = 48) were selected for the feeding trials.

Ninety-six plastic cups (volume of 500 ml) were filled with 375 ml groundwater (20°C, pH 8, conductivity 212 μS/cm). Twenty-four cups were used per plant species, of which each received approximately 0.2 g (wet weight) of non-apical shoots of either *C. demersum*, *E. densa*, *M. spicatum* or newly grown leaves of *P. perfoliatus* (one species per cup). Half of the cups received one individual of *L. stagnalis* whereas the other half was kept snail free, to be used as control to correct

for autonomous changes in plant biomass due to growth. Snails were starved for 48 hr prior to the trial to standardize their appetite. All cups were covered with a mesh of size 1 mm to prevent snails from escaping. All cups were randomly placed on a rack in laboratory conditions at 20°C and exposed to a 16:8 hr day:night cycle (Figure S3). All snails were removed from their respective cup after 24 hr and euthanized by freezing at −20°C. Their soft body tissue was separated from their shells and dried in the oven at 60°C for at least 48 hr. The dry weights of plant fragments remaining in each cup were determined as described previously (see Section 2.4).

Plant palatability, indicated by relative consumption rate (RCR, mg g^{-1} day^{-1}) was calculated according to Elger and Barrat-Segretain (2002, 2004):

$$\text{RCR} = \left[\left(C_{fd}/C_{iw} \right) \times F_{iw} - F_{fd} \right] / S_d / 1 \, \text{day},$$

where C_{fd} is the final dry weight of the control plant, C_{iw} is the initial wet weight of the control plant, F_{iw} is the initial wet weight of the feeding trial plant, F_{fd} is the final dry weight of the feeding trial plant, and S_d is the snail dry weight without shell.

2.6 | Data analyses

To disentangle the direct and indirect effects of native plants and herbivores on biotic resistance, we used piecewise Structural Equation Modeling (piecewiseSEM, Lefcheck, 2016). SEM has been shown to be an important tool to describe complex natural systems (Grace, Michael Anderson, Han, & Scheiner, 2010). For each of the three native plant species (*C. demersum*, *M. spicatum* and *P. perfoliatus*), we fitted models to investigate whether the native plants, herbivores, filamentous algae and their possible second-order interactions affected the invader *E. densa* performance (measured as total biomass at the end of the experiment). We fitted GLMM with block (the six replicates) as a random factor in all models (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2018). We included these models in the *SEM* and performed model selection based on AICc criteria starting with the full model that included all second-order interactions among herbivory, filamentous algae biomass and native biomass. The best fitting models (lowest AICc) included only all main effects. Normality of model residuals, homoscedasticity and the influence of possible outliers were checked by visually inspecting plots of residual versus fitted values and quantile-quantile plots of model residuals. Native plant competition was evaluated using native plant species biomass as a continuous independent variable. PiecewiseSEM was performed in the software R (R Core Team, 2017) using the packages NLME and PIECEWISESEM (Lefcheck, 2016).

3 | RESULTS

The Structural Equation Models revealed several direct effects. Snails were feeding strongly on the filamentous green algae that
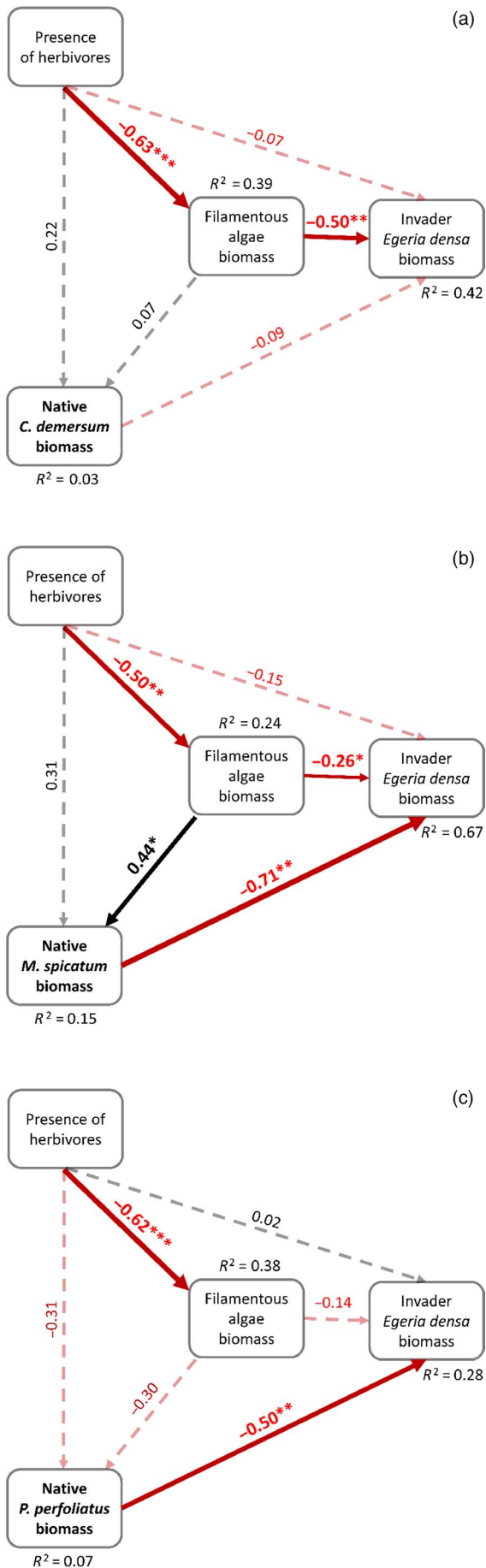
FIGURE 2 Structural equation models of herbivory by native herbivores and native plant species (a) *Ceratophyllum demersum*, (b) *Myriophyllum spicatum* and (c) *Potamogetum perfoliatus* competition on invader *Egeria densa* performance (total biomass). Boxes represent measured variables. Solid arrows denote direct effects and dashed arrows, indirect effects on the invader biomass with red and black arrows respectively representing negative (biomass loss) and positive (biomass gain) relationships. Semi-transparent arrows represent non-significant paths ($p ≥ 0.05$). Arrow width is scaled to the magnitude of the standardized regression coefficient, which is given alongside the arrows with their significance levels indicated by asterices (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The conditional R^2 for each component model is given on the box that contains its dependent variable. For all models, Fisher's $C = 0$ and $p = 1$, indicating there were no missing paths in any of the models

appeared in the aquaria, and significantly reduced their biomass in each of the native plant species treatments (Figure 2a–c). Native plants *M. spicatum* and *P. perfoliatus* biomass directly strongly reduced *E. densa* total biomass (Figure 2b,c). However, there was no direct effect of the native species *C. demersum* on the invader (Figure 2a). Herbivory also did not directly affect invader performance, that is, the snails did not significantly feed on *E. densa*. Filamentous algae affected invader biomass negatively only in the presence of *C. demersum* and *M. spicatum* as the native plant species (Figure 2a,b), but not in the presence of *P. perfoliatus* (Figure 2c).

The Structural Equation Models also revealed several indirect effects. The presence of herbivores reduced the filamentous algae, which indirectly had positive effects on the invader total biomass, thus facilitating the invasion by *E. densa*. However, these indirect effects of herbivory worked through different pathways depending on the native plant identity. In the presence of *C. demersum* and *M. spicatum*, herbivores had an indirect positive effect on the invader biomass because their consumption of the filamentous algae reduced the negative effects of the filamentous algae on the invader. In the *M. spicatum* treatments, herbivores seemed to indirectly decrease native biomass by feeding on filamentous algae in complex interactions between the invader, algae and native species. Algae possibly affected competition between the native and invading species.

During the feeding trials that we performed to determine plant palatability to the snails, the snails consumed all the plant species. The average consumption rates were higher when feeding on the native plants *M. spicatum* and *P. perfoliatus* than on *C. demersum*, but did not differ between the invading plant species and any of the three native plant species (Figure 3).

4 | DISCUSSION

Our experiment showed that competition by native plant species can directly have negative effects on invader performance, but also that these effects can vary with native species identity. Herbivory did not directly affect invader performance in our experiment, that
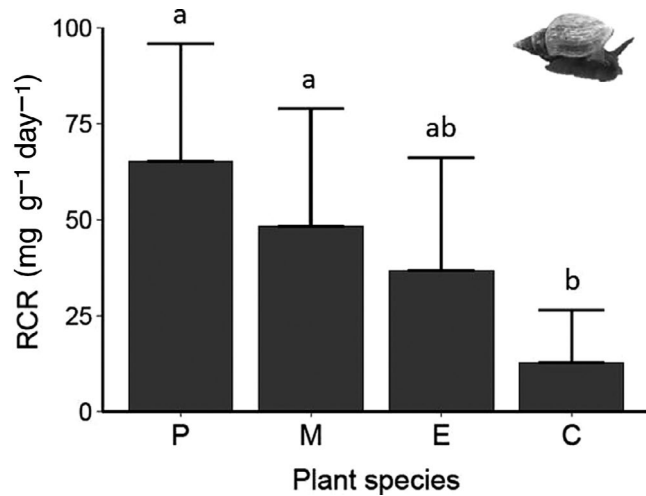
FIGURE 3 Relative consumption rate (RCR, DW) by the generalist herbivore *Lymnaea stagnalis*. Bars represent mean values ± CI (95% confidence intervals), n = 12. Different lowercase letters indicate statistically significant differences between plant species after contrast analysis at a significance level of p < 0.05. P = *Potamogeton perfoliatus*, M = *Myriophyllum spicatum*, E = *Egeria densa* and C = *Ceratophyllum demersum*

is, the snails were not feeding on *E. densa*. However, the herbivores did reduce the filamentous algae biomass, which had indirect positive effects on the invader total biomass. The herbivores reduced competition between algae and the invader, and therefore facilitated the invasion by *E. densa*. These indirect effects worked through different pathways depending on the native plant identity. Below we discuss our findings and the mechanisms that may underlie the observed invader facilitation.

In our first hypothesis we expected that increasing native plant biomass reduces *E. densa* performance. Competition by two (*M. spicatum* and *P. perfoliatus*) out of three native plant species directly reduced invader performance—thus partially confirming our first hypothesis that also in freshwater systems, native plant competition provides biotic resistance to alien plants. These differences are likely related to differential resource uptake of these species. Growth morphology has been recognized as an important factor in aquatic plant competition (McCreary, 1991). Both rooted submerged species *M. spicatum* and *P. perfoliatus* as well as the invader *E. densa* use the sediment as their main source for nutrient uptake while for non-rooted species (such as *C. demersum*) nutrient uptake is almost entirely foliar (Denny, 1972). Among the rooted species, *M. spicatum* was the strongest competitor with greatest negative effects on the performance of *E. densa*. *Potamogeton perfoliatus* plants also reduced *E. densa* performance, but less than *M. spicatum*. Both rooted submerged native species are from the same functional group as the invader, which is also rooted. The resulting overlap in their spatial resource use increases the competitive strength of both native species in reducing invader species success (Petruzzella et al., 2018). *Ceratophyllum demersum* is fully a floating plant, and can therefore only take up nutrients from the water column. Therefore, it does not strongly compete with the invader for nutrients, which likely

explains that it did not suppress invader growth. *Ceratophyllum demersum* has been observed to displace other aquatic plant species by shading due to closed dense canopy formation (Stiers, Njambuya, & Triest, 2011; Wells, de Winton, & Clayton, 1997). Generally, *C. demersum* accumulated less plant biomass in our study than the two rooted native species, indicating that nutrients may have limited its growth and its capacity to provide shading for the invader was limited (Figure S4). It is also important to note that the native plants selected for this experiment are also invasive in other parts of the world and are strong competitors (Daehler, 2003). This could potentially be affecting our results. If we would have selected native plant species that are weak competitors, we might have found a less strong effect of competition on biotic resistance.

In our second hypothesis we hypothesized that herbivores could provide biotic resistance by directly consuming the invader, based on previous findings that there is potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems (Petruzzella et al., 2017). Most studies that test this hypothesis rely on feeding trials comparing the consumption rate of native and alien plants by herbivores (e.g. Grutters et al., 2017; Parker & Hay, 2005). In our experimental feeding trials (in which only the snails and plants were present) the herbivores were found to consume all plant species, with no distinguishable consumption rates on the invading and native species. This corresponds to the results of the mesocosm experiments, where we did not find any significant effect of herbivory alone on *E. densa* performance among the native species treatments. We therefore reject our second hypothesis, as we did not find selective feeding by the herbivore on the invader. This contradicts findings of previous studies, which have shown herbivory to reduce success of invasion of alien species (Parker et al., 2007; Ribas et al., 2017). Alternatively, the success of highly invasive aliens is often attributed to a release from their natural enemies (Enemy Release Hypothesis; Keane & Crawley, 2002), but these benefits could be lost over time since introduced species can acquire new enemies, depending on how long the invader has been present in the introduced range (residence time; Schultheis, Berardi, & Lau, 2015). The *E. densa* invasion is recent as it was first recorded in the Netherlands in 1944 but only after the year 2000 has started to be recorded every year (Matthews et al., 2014). In our study, we did not find evidence for the biotic resistance nor enemy release hypothesis, as the consumption rate of snails in the feeding trials was equal for the native and invasive species. This lack of support for either hypotheses can be explained by the fact that in our mesocosms more components of the ecosystem were present (including algae, native species and invading species), allowing the herbivores to feed selectively on other food sources than the native or invading species.

Our third and fourth hypotheses stated that the herbivore could indirectly affect the invader by feeding on the native plants or filamentous green algae respectively. Because our experimental system was mimicking natural situations found in temperate mesotrophic and eutrophic freshwater lakes as much as possible, we observed the growth of filamentous green algae *Spirogyra* sp. In our mesocosms the generalist snails were extensively feeding on the filamentous algae covering

the plants instead of on the plants. Therefore, we reject our third hypothesis. This grazing by the snails can have facilitated the growth of the invading species in two possible ways. First, herbivores feeding on filamentous algae could recycle nutrients to the water column, thus affecting native and invading plant growth by regulating nutrient concentrations in the water (Bakker et al., 2013; Brönmark, 1985; Feijoó, García, Momo, & Toja, 2002). Secondly, herbivores have previously been shown to decrease the effects of shading by reducing filamentous algae (Brönmark, 1989, 1990), which could lower the competition between the plants and algae for light. Our results showed that the herbivore presence was indirectly facilitating *E. densa* invasion (either via nutrients or via light competition) by consuming algae in two out of three native plant species treatments, providing support for our fourth hypothesis. These indirect effects of herbivory worked through different pathways depending on the native plant identity. Whereas in the presence of *C. demersum* the herbivory on algae facilitated *E. densa* invasion, in the *M. spicatum* treatments the filamentous algae also seemed to mediate possible competition between the native and invading plant species.

It is important to note that snails can have direct impacts on the plants, even in the presence of alternative food sources such as filamentous algae (Elger, Willby, & Cabello-Martinez, 2009). Therefore, we cannot completely rule out any direct herbivory effects on the vascular plants. We did observe grazed leaves of *P. perfoliatus* in the mesocosms, but it was not enough to affect its biomass. This may have resulted from the density of snails used combined with the duration of the experiment; when snails would have finished the filamentous algae they might have started to consume the vascular plants (Elger et al., 2009).

5 | IMPLICATIONS

Both competition and herbivory have been shown to decrease alien plant performance in terrestrial and marine systems (Kimbro et al., 2013; Levine et al., 2004). Although the role of biotic interactions in reducing invader success has been recognized (Levine et al., 2004), it remains poorly understood in freshwater ecosystems (Alofs & Jackson, 2014). Our experiment provided important insights into the mechanisms and their interactive effects that could lead to the success of alien aquatic plant invasions in temperate freshwater ecosystems. We predicted that competition from native plant species and herbivory by a native generalist herbivore interactively would affect the performance of the South American invader *E. densa*. We found evidence for biotic resistance through competition by native plants. Furthermore, we found that herbivory had indirect effects on the invader, resulting in invader facilitation, but this effect was not consistent among all native plant species involved. Future studies should consider communities dominated by different native plant species to evaluate how the dominance of different species and/or functional groups can either increase or reduce susceptibility of freshwater systems to invasion. Our study highlights the important role of indirect effects to understand the potential of biotic resistance in real ecosystems. Important indirect interactions by herbivores that may affect biotic resistance to invading plants including physical processes, such as bioturbation, trampling and chemical processes, such as nutrient recycling, deserve further investigation not only in freshwater systems but also in other ecosystems.

AUTHORS' CONTRIBUTIONS

A.P. and E.S.B. conceived the ideas and designed the methodology; A.P. collected the data; A.P., C.H.A.v.L. and E.S.B. analysed and interpreted the data; A.P. led the writing with the assistance of C.H.A.v.L., E.S.B. and E.v.D. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.mgqnk98wh (Petruzzella, van Leeuwen, van Donk, & Bakker, 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.