

BIOTIC RESISTANCE

to alien plant invasions in tropical and temperate freshwater ecosystems



ANTONELLA PETRUZZELLA

BIOTIC RESISTANCE TO ALIEN PLANT INVASIONS

ANTONELLA PETRUZZELLA

NIOO thesis 170

BIOTIC RESISTANCE

to alien plant invasions in tropical and
temperate freshwater ecosystems

ANTONELLA PETRUZZELLA

**Biotic resistance to alien plant invasions in
tropical and temperate freshwater
ecosystems**

Antonella Petruzzella

Thesis committee:

Prof. dr. E.M. Gross

Prof. dr. J.T.A. Verhoeven

Prof. dr. R. Pierik

Prof. dr. W.H. van der Putten

Dr. M. te Beest

Biotic resistance to alien plant invasions in tropical and temperate freshwater ecosystems

Biologische resistentie tegen invasies van uitheemse planten in tropische en gematigde zoetwater systemen

(met een samenvatting in het Nederlands)

Resistência biótica à invasões de plantas alienígenas em ecossistemas de água doce tropicais e temperados

(com um sumário em Português)

Cover illustration: Carlos Eduardo Vargas Grou

Cover design: Antonella Petruzzella and Máira N. T. Mucci

Printed by: Print Service Ede – www.proefschriftenprinten.nl

This thesis should be cited as:

Petruzzella, A. (2019) Biotic resistance to alien plant invasions in tropical and temperate freshwater ecosystems. PhD thesis. Utrecht University, Utrecht, the Netherlands.

The research presented in this thesis was conducted at the Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, the Netherlands.

ISBN: 978-94-92679-97-0

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

woensdag 12 juni 2019 des ochtends te 10.30 uur

door

Antonella Petruzzella

geboren op 11 maart 1989
te Macaé, Rio de Janeiro, Brazilië

Promotor:

Prof. dr. E. van Donk

Copromotoren:

Dr. E.S. Bakker

Dr. C.H.A. van Leeuwen

This thesis was financially supported by the Brazilian Science Without Borders Program and CNPq (Brazilian National Council for Scientific and Technological Development) through a grant no. 207514/2014-3.

"It always seems impossible until it's done."

Nelson Mandela

Contents

Chapter 1	General introduction	9
Chapter 2	Mechanisms of invasion resistance of aquatic plant communities	31
Chapter 3	Species identity and diversity effects on invasion resistance of tropical freshwater plant communities	67
Chapter 4	Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems	95
Chapter 5	Direct and indirect effects of native plants and herbivores on biotic resistance to alien plant invasions	147
Chapter 6	Synthesis	181
Chapter 7	References	201
Chapter 8	Summary	241
	Nederlandse samenvatting	247
	Sumário em Português	252
	Acknowledgements/Agradecimentos	257
	About the author	258



Chapter 1

General introduction

In 1958, Charles S. Elton noted in his classic book, which was also the first on invasion biology, *The Ecology of Invasions by Animals and Plants*. '*ecological resistance describes ignorance and not knowledge.*' (p. 116-117). Now, in 2019, ecologists still continue to search for a better understanding of the factors and underlying mechanisms regulating the establishment and subsequent spread of alien species. This is what this thesis is about. In particular, I focus on the potential of biotic interactions in providing resistance to invasions.

Biological invasions as a human-induced global change

Biological invasions represent a key component of human-induced global changes, together with habitat loss and degradation, species overexploitation, pollution and climate change (WWF 2018). Throughout the world, such invasions are threatening biodiversity (Lambertini et al. 2011, Lövei et al. 2012, Early et al. 2016). With the intensification of trade, transport and tourism, the number of introductions of alien species has significantly increased in the last centuries (Meyerson and Mooney 2013). It has been estimated that 37% of all recorded alien species have been introduced between 1970-2014 and yet there are no signs of saturation in the accumulation of these species (Seebens et al. 2017). We, scientists, are aware that species distributions and community structure are dynamic and change over time, and the idea to maintain pristine environments in this era of globalization is utopic. However, despite that alien species do not have to be problematic *per se*, they can lead to a wide range of problems when they become invasive (for a glossary with definitions see Box 1). Therefore, they deserve attention.

Invasive alien species have been considered one of the main drivers of biodiversity loss and have negative impacts on ecosystem services worldwide (Vilà et al. 2010, Butchart et al. 2010, Keller et al. 2018). They can also affect human life, health and cause economic damage to agriculture, forestry and fisheries. The costs related to the impacts of biological invasions in Europe alone are estimated to be at least EUR 12 billion annually, and are probably over EUR 20 billion (Kettunen et al. 2009, Keller et al. 2011). In Australia, Brazil, India, South Africa, the United Kingdom and the United States the annual losses are in the range of USD 300 billion (Pimentel et al. 2001, 2005). Invasions have been causing such profound ecological, economic and evolutionary changes in natural ecosystems that researchers have suggested that we are entering a new era called the 'Homogocene', which is creating in turn 'The New Pangaea' (Rosenzweig 2001). The term refers to biotic homogenization, i.e. increasing (genetic, taxonomic and functional) similarity in the composition of communities, which occurs when native species are replaced by already widespread alien species (Winter et al. 2009, Olden et al. 2018). Homogenization can have ecological and evolutionary consequences such as for example the loss of ecosystem functions due to reduced functional species diversity (Olden et al. 2004). The Homogocene is seen as a mere consequence of the Anthropocene, a proposed epoch dating from the start of significant human impact on Earth's geology and ecosystems.

Although the impacts of invasive alien species can be alarming, in fact only a small proportion of these introduced species establishes and becomes problematic. According to the tens rule hypothesis, about 10% of species successfully take consecutive steps during the invasion process (Williamson and Fitter 1996). Thus, most introduced species fail to establish or have their population size constrained, and never become invasive. This resistance to invasion can be due to abiotic or biotic

filters. In this thesis, I will focus on the biotic filters: the role of native species in providing biotic resistance to alien species establishment success.

Biotic resistance to alien species invasions

Invasion is a process that requires a species to overcome several barriers (Richardson et al. 2000, Kolar and Lodge 2001). Species invasions go through several stages before a species becomes invasive: the introduction, establishment, spread and impact phase (Fig. 1). Although the invasion process is a continuum and the stages are not discrete, separating the invasion process into stages allows us to discuss the relative importance of the filters and at which temporal-spatial scale they operate (Theoharides and Dukes 2007). For a plant species to arrive in a new region, it must be transported from its native range to that area overcoming the biogeographical barrier. After introduction, some alien species successfully establish by forming persisting populations after which we refer to them as naturalized species (see Box 1). Some naturalized species ultimately spread to a degree which strongly impacts the recipient systems, hence become invasive. However, most of the alien species either fail to establish, or naturalize at low densities, and never become invasive.

Box 1. A Glossary of Key Definitions

Native species (synonyms: indigenous)¹: Taxa that have originated in a given area without human involvement or that have arrived there without intentional or unintentional intervention of humans from an area in which they are native.

Alien species (synonyms: exotic, introduced, non-native, non-indigenous)¹: Taxa in a given area whose presence there is due to intentional or accidental introduction as a result of human activity or which have arrived there without the help of people from an area in which they are alien.

Naturalized species (synonyms: established)¹: Alien species that sustain self-replacing populations over many life cycles (at least 10 years) without direct intervention by humans by recruitment from fragments (seeds, larvae, ramets, tubers) capable of independent growth; it need not be invasive. Naturalized species include both invasive and non-invasive species.

Invasive species^{1,2}: A subset of naturalized species that produce reproductive offspring, often in very large numbers, at considerable distances from parent taxa, and thus have the potential to spread over a large area causing negative impacts on biodiversity, socio-economy or human health.

Biotic resistance³: Negative effect of native species on the performance of alien species during the establishment phase and after they have successfully established. Some researchers use a strict definition only using the term in situations where the native species completely prevent the establishment of the invader. I use the term throughout this thesis more broadly including any negative effect on alien species performance.

Invasibility⁴: Susceptibility of an environment to the establishment and proliferation of alien species.

Invasiveness⁴: The propensity of an alien species to become invasive.

Propagule pressure (synonyms: introduction effort)⁵: A composite measure of the number of individuals released into a new region which they are not native. It incorporates estimates of the absolute number of individuals involved in any one release event (propagule size) and the number of discrete release events (propagule number).

To establish, i.e. colonize, survive, grow and reproduce, alien species must overcome the ecological barrier imposed by the recipient ecosystem including biotic interactions with the native resident species, which can provide biotic resistance (see Box 1) (Shea and Chesson 2002). Biotic resistance is the ability of native species to reduce the success (establishment and performance) of alien species via species interactions such as competition, herbivory, predation or disease (Levine et al. 2004). If the abiotic conditions are suitable, biotic resistance can prevent the colonization, survival, growth and reproduction of the alien propagules (Figure 1 – stage A). Once the alien species have successfully naturalized, biotic resistance can regulate its spread and impacts (Figure 1 – stage B) (Levine et al. 2004). Biotic resistance is generally not effective in completely preventing invader establishment, especially not at high levels of propagule pressure (see Box 1) (Levine et al. 2004 and references therein). However, it has been recognized as a strong force in reducing the abundance, fecundity and/or spread of alien species once these are established (Theoharides and Dukes 2007).

¹Pyšek et al. (2004); ²CBD (2002); ³Levine et al. (2004); ⁴Richardson and Pyšek (2006); ⁵Lockwood et al. (2005)

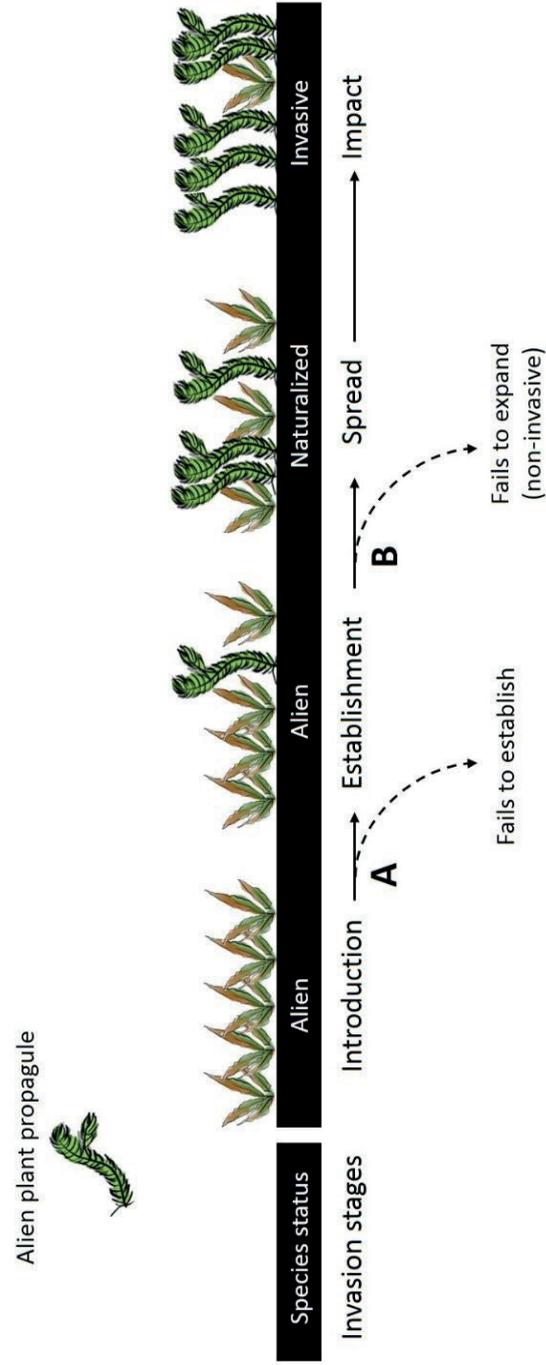


Figure 1. Schematic figure showing the stages that alien species propagules go through in the invasion process. **Introduction** – when propagules arrive in the system; **Establishment** – where those propagules become a reproducing population; **Spread** – where that population moves across the landscape; **Impact** – where the invader alters the ecosystem. Letters represent where in that process biotic resistance can influence the success of alien species: **(A)** acting as a barrier to invasion prior to establishment and **(B)** regulating the spread and its associated impacts once alien species have successfully established (Figure modified from Levine et al. 2004).

Mechanisms of biotic resistance

At finer spatial scales, native communities may resist invasion, i.e. reduce the establishment success of alien species through a diversity of niche-based mechanisms and interactions with other trophic levels. In this thesis, I investigated two main mechanisms of biotic resistance to alien plants: (i) competition from native species which can be mediated by species diversity and niche similarities between the alien and the native species, and (ii) herbivory by native herbivores (Fig. 2). These mechanisms are rarely mutually exclusive and can work synergistically (Mitchell et al. 2006).

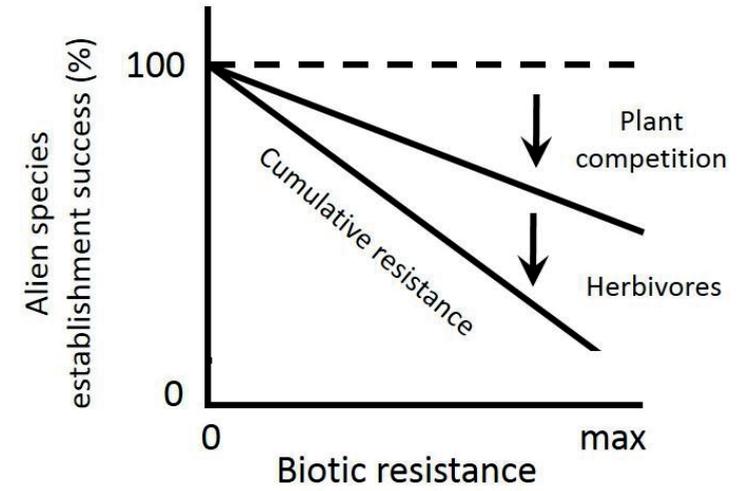


Figure 2. Conceptual figure showing the main biotic resistance mechanisms investigated in this thesis affecting alien plant species establishment success in the new environment. Establishment success is defined as the ability of the invader to colonize, survive, grow and reproduce (Figure modified from Theoharides and Dukes 2007).

i. Competition from native plants

Competition has been the most widely explored mechanism of biotic resistance in the plant invasion literature (Levine et al. 2004). Studies have found that native plant competition has a strong negative effect on alien species performance in grasslands (Corbin and D’Antonio 2004, te Beest et al. 2018), forests (Fine 2002), saltmarshes (Amsberry et al. 2014) and marine systems (Britton-Simmons 2006, Balestri et al. 2018). Competition, in particular mediated by native species diversity, has long been hypothesized to reduce community invasibility (see Box 1).

i.i. Species diversity

One of the earliest theories was Elton’s hypothesis, which predicts that more diverse communities are more resistant to invasions than less diverse communities, known as the biotic resistance hypothesis (Elton 1958). The diversity resistance effect has been attributed to the ability of more species and/or functional groups to occupy more space, produce more biomass and use more resources from the environment. A more diverse native community can provide resistance to invaders via two possible mechanisms: the complementarity effect and the sampling (i.e. selection) effect (Fargione and Tilman 2005).

Complementarity among multiple species (or functional groups) with non-overlapping resource use strategies could lead to a better use of the total available resources by the native plant community, leaving less resources available for a potential invader. Resource use complementarity is what researchers would consider to be a true diversity effect (Naeem et al. 2000). The second possible mechanism by which invasibility can be reduced with increasing native diversity is the sampling effect.

More diverse communities have a higher probability to have a superior competitor or a more productive species present in the community to potentially affect invading species (Wardle 2001).

The classical biodiversity publication of Naeem et al. (2000) demonstrated that – under experimental conditions – higher resident species richness increased crowding, decreased available light, and decreased available nutrients, all of which increased the competitive strength of diverse plant assemblages to the invader *Crepis tectorum* L. in grassland plots. In marine systems it has been shown that the most diverse macroalgal communities and those dominated by a superior competitor, a canopy-forming algal species, were the least invaded, which indicates that both complementarity and sampling mechanisms can operate simultaneously to reduce the invasion of the seaweed *Caulerpa cylindracea* Sond. (Bulleri et al. 2016). Several studies have provided further empirical support for negative relationships between native diversity and alien invasion success, although most studies were performed in terrestrial systems (Hector et al. 2001, Frankow-Lindberg et al. 2009, Brown and Rice 2010, Frankow-Lindberg 2012). However, the negative relationship between native diversity and invasion success found in empirical studies is in contrast with observational studies (Stohlgren et al. 2003, Jeschke et al. 2018).

Positive native-alien relationships have been reported at large spatial scale observational studies (Stohlgren et al. 2003, Jeschke et al. 2018, Peng et al. 2019). These contrasts between empirical and observational studies are referred to as the ‘invasion paradox’: i.e. the observation that species-rich communities are more resistant to invasions than species-poor communities, but at the same time species-rich ecosystems and regions are often hotspots for both native and alien species (Fridley et al. 2007). This ‘paradox’ is mostly attributed to extrinsic factors such as

environmental heterogeneity (e.g. climate conditions and nutrient availability) (Fridley et al. 2007, Tomasetto et al. 2013). Extrinsic factors that favor more niche opportunities would benefit both native and alien species (Shea and Chesson 2002). However, the debate as to whether there is an invasion paradox still continues (Peng et al. 2019).

i.ii. Niche similarities

Besides native species diversity, competition is also dependent on niche similarities between invading and native species. There are several theories explaining the success or failure of alien species establishment, of which many share the basic prediction that successful invaders are somehow different from species present in the native communities (MacDougall et al. 2009). Limiting similarity theory predicts that species and/or functional groups that are most similar to the invader should provide greater invasion resistance, because of similarity in morphology, physiology or resource use, resulting in niche overlap (MacArthur and Levins 1967).

Ecological similarities (or niche overlaps) among species can also be estimated based on phylogenetic relationships (under the assumption that closely related species require similar resources) (Daehler 2001, Strauss et al. 2006, Burns and Strauss 2011, Byun et al. 2013). Based on this assumption, Darwin's naturalization hypothesis predicts that alien species will face greater resistance in a native community if they are phylogenetic more closely related to native species (Darwin 1859, Yannelli et al. 2017). Some studies found support for this hypothesis (Strauss et al. 2006, Yannelli et al. 2017, Zheng et al. 2018) while others did not (as revised by Diez et al. 2008, Thuiller et al. 2010). These inconsistent results can partly be explained

by the use of different spatial (e.g. community plots, continent) and phylogenetic (e.g. genera, families) scales among studies (Thuiller et al. 2010).

ii. Herbivory by native herbivores

Interactions with other trophic levels can also affect the establishment success of alien species. Herbivores are well known for reducing plant performance and expansion in general (Maron and Crone 2006, Wood et al. 2017). The effect of interactions between herbivores and alien invasive plants can go in two directions (Maron and Vilà 2001). Herbivores can regulate alien plant success by foraging extensively on these species, which are often maladapted to deter them due to the lack of evolutionary history with these novel herbivores (Parker et al. 2006). In this case native herbivores may provide biotic resistance. For example, herbivory by a native rodent strongly suppressed the alien species *Spartina alterniflora* Loisel. survival and growth in a mangrove forest in southern China (Zhang et al. 2018b). However, herbivores can also neglect alien plants, and these plants can benefit from escaping herbivory by their coevolved specialist herbivores, called the enemy release hypothesis (Keane and Crawley 2002). Because most alien plant species escape some of the specialist herbivores from their original geographical regions (enemy release) but simultaneously encounter new generalist herbivores (biotic resistance), meta-analyses generally report both successful and unsuccessful biotic resistance of herbivores to plant invaders (Keane and Crawley 2002, Levine et al. 2004, Parker and Hay 2005, Parker et al. 2006, Jeschke et al. 2012a). The distinction between generalist and specialist herbivores is thereby especially critical (Parker et al. 2006), because native herbivore feeding preferences strongly determine how they may affect alien plant invasions (Grutters et al. 2017a, Christianen et al. 2019).

Alien plant species invasions in freshwater ecosystems

Freshwater aquatic plant species tend to have broad distribution ranges (Santamaría 2002). Their distribution seems to be mostly limited by biogeographic barriers or different climatic regions (tropical-temperate) (Santamaría 2002). Alien aquatic species have been crossing these barriers by means of numerous human-mediated pathways, such as ship ballast water release, aquaculture and unintentional movement of propagules due to boating activity (Hulme 2007). With the e-commerce popularization, potential invasive species now are just a mouse click away from any home. For freshwater alien plants, especially the aquarium and ornamental trade are important introduction pathways (Padilla and Williams 2004b, Martin and Coetzee 2011, Peres et al. 2018). It has been estimated that one third of the world's worst aquatic invasive species are aquarium or ornamental species (Padilla and Williams 2004b). Therefore, freshwater ecosystems are under a great threat.

Alien species invasions are among the major threats to freshwater biodiversity (WWF 2018). Invasive aquatic plants can have a wide range of impacts in freshwater ecosystems (Gallardo et al. 2016), including changing the structure and composition of native zooplankton communities (Stiers and Triest 2017), communities of macroinvertebrates (Houston and Duivenvoorden 2002), other plant species (Michelan et al. 2010) and fish communities (Carniatto et al. 2013). Aquatic invasive plants can grow in dense mats that limit water flow and light penetration, cause hypoxia or anoxia in the water column and alter nutrient cycling (Urban et al. 2006, Thomaz et al. 2015). All these impacts can lead to a loss of ecosystem services, such as fishing, boating, recreational activities and aesthetic value of these ecosystems

(Keller et al. 2018). The dramatic case of the water hyacinth (*Eichhornia crassipes* (Mart.) Solms) invasion in Lake Victoria in Africa is a good example of a free-floating nuisance aquatic plant. It directly and indirectly affected approximately 30 million people through the loss of fisheries, water supply and transport, environmental health and power generation (Aloo et al. 2013). Submerged aquatic plants can also strongly impact ecosystem services. For example, *Hydrilla verticillata* (L. f.) Royle blocked the water intakes on the St. Stephen hydroelectric facility on Lake Moultrie, South Carolina, USA, forcing the shutdown of the dam for seven weeks resulting in loss of USD 2,650,000 of expenses due to repairs, dredging, and fish loss. In addition, during this repair period, there was an estimated \$2,000,000 loss in power generation for the hydropower plant (letter from Charleston District Engineer to Commander, South Atlantic Division, dated March 8, 1993).

The high potential of invasion of freshwater plants, in particular submerged species, is attributed to certain features that increase their invasiveness (see Box 1) such as vegetative growth by fragmentation, tuber and turion formation, rapid growth, long-distance spread, wide environmental tolerance and high phenotypic plasticity, and the ability to produce allelopathic compounds (Marko et al. 2008, Umetsu et al. 2012, Fleming and Dibble 2015). Notorious examples with these attributes are some plants of the family Hydrocharitaceae that I used as a model species in this thesis including tropical and subtropical submerged *Lagarosiphon major* (Ridl.) Moss, *Hydrilla verticillata* and *Egeria densa* Planch. (Fig. 3). An important characteristic of many invasive aquatic plants in relation to the work presented in this thesis is that they – after initial human-induced introduction – frequently disperse further via vegetative propagules. These propagules can travel long distances within connected and across unconnected water bodies to establish new populations. An important part of this thesis focuses on the establishment success of such free-floating

vegetative propagules, whereby I consider an alien species as successfully established once it has its roots attached to the sediment and grows.

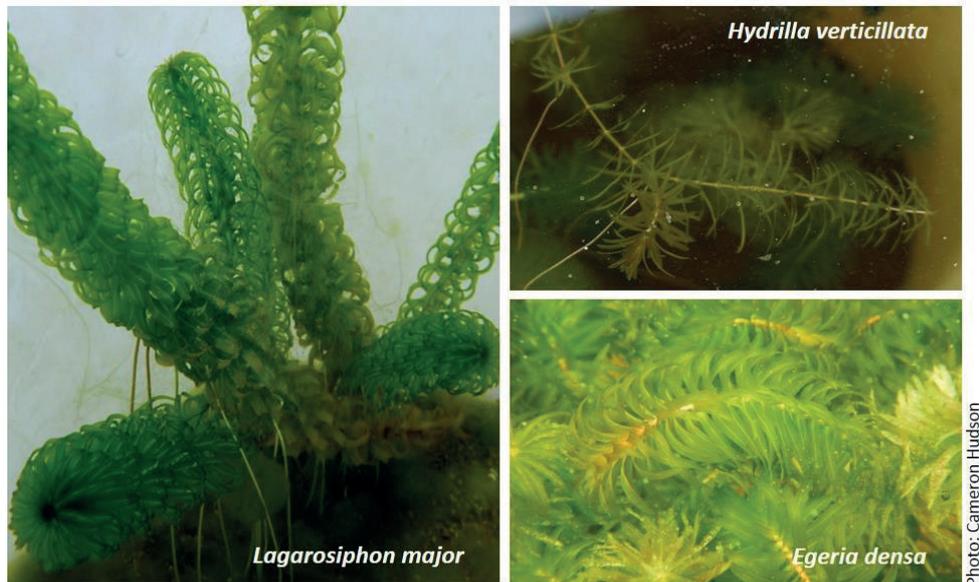


Figure 3. Alien submerged plants of the family Hydrocharitaceae used as a model species in this thesis. *Lagarosiphon major* is native to South Africa, *Hydrilla verticillata* is native to Asia and Australia and *Egeria densa* is native to South America. All three are reported as introduced from at least 9 countries in Europe (Hussner 2012).

Current knowledge on biotic resistance to establishment success of alien plant species is biased toward specific geographical regions and ecosystems. Most of our understanding about the process, patterns and mechanisms of invasions emerges from studies performed in temperate terrestrial systems (Lowry et al. 2013),

while only a few studies have been performed in tropical regions or have explored freshwater systems (Pyšek et al. 2008, Alofs and Jackson 2014). However, freshwater and terrestrial ecosystems differ in many ways including primary producer traits such as growth rate and nutritional quality, strength of trophic interactions and disturbance regimes (Shurin et al. 2006, Bakker et al. 2016). Also, in contrast to terrestrial systems, where many herbivores are specialists, most of the aquatic (freshwater and marine) herbivores are generalists. These differences may influence biotic resistance strength, leaving it unclear how research results for one ecosystem can be translated to another ecosystem. This knowledge gap is particularly problematic due to vulnerability of freshwater ecosystems to invasions and their ecological and societal importance.

Although freshwater ecosystems only occupy less than 1% of the Earth's surface they harbor greater biodiversity per surface area than terrestrial and marine ecosystems (Dudgeon et al. 2006). However, these systems are among the most endangered ecosystems globally due to the growing demand by human populations for services, water pollution, degradation of habitat and overexploitation (Dudgeon et al. 2006). They also show the strongest biodiversity decline (Sala et al. 2000, WWF 2018). The importance of alien species as a driver of biodiversity loss has led to an increased interest in understanding why some alien plant species establish as invaders when others fail is, or why some habitats are more susceptible to invasions than others. However, these questions remain surprisingly poorly understood in freshwater ecosystems, and especially in tropical regions. An increased understanding of biological invasions in these systems is crucial for possible prevention, control or management of alien species invasions in these systems.

Thesis outline

In this thesis, I focus on biotic resistance and the role of species interactions in reducing the success (colonization and performance) of alien species invasions. The major aim is to determine whether tropical and temperate native freshwater species communities can provide biotic resistance to alien plant invasions and to understand the underlying mechanisms in freshwater ecosystems. To study this, I used tropical and temperate submerged plant species and an aquatic generalist herbivore as a model system. My approach is a combination of mesocosm experiments and published evidence to answer my two main research questions:

- I-** Can native communities provide biotic resistance to alien plant invasions in freshwater ecosystems?
- II-** Which mechanisms are underlying biotic resistance to aquatic plant invasions?

Throughout the chapters I aim to improve our understanding of the biotic mechanisms underlying the establishment success of alien plant species in tropical and temperate freshwater aquatic ecosystems (Fig. 4). I consider native community susceptibility to invasion (invasibility) as well as alien plant attributes which increase their likelihood to establish and potentially become invasive (invasiveness).

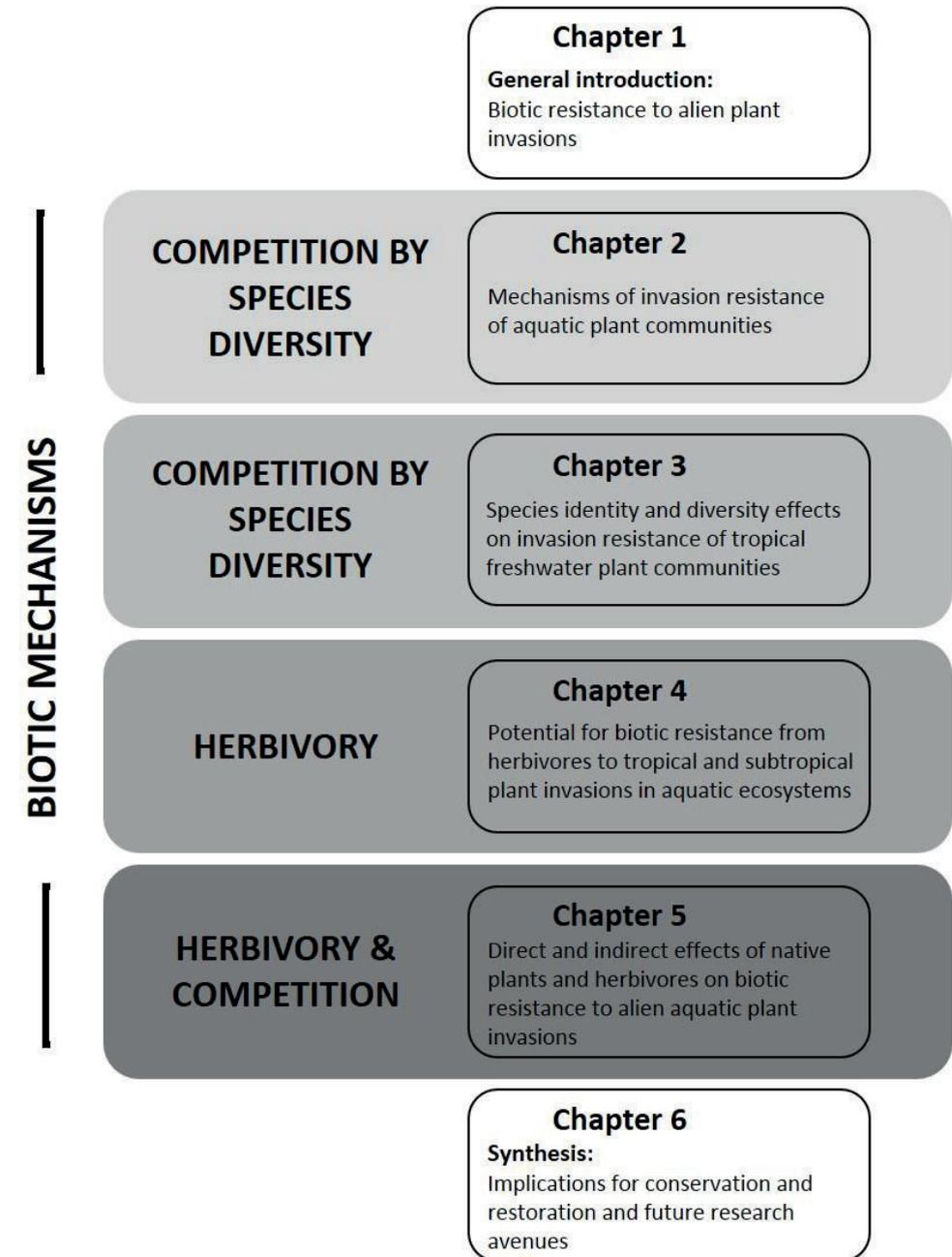


Figure 4. Schematic overview of the chapters of this thesis.

The biotic resistance hypothesis predicts that more diverse native communities provide more resistance to invasions than less diverse communities (Elton 1958). In the first two chapters of this thesis I tested this hypothesis by manipulating native plant species richness gradients in temperate (**Chapter 2**) and tropical (**Chapter 3**) freshwater ecosystems and experimentally assess whether native plant diversity can reduce the colonization and performance i.e. growth of alien submerged plant species. Increasing plant diversity, however, also increases the chance that a similar functional group (**Chapter 2**) or a phylogenetically closely related species to the invader (**Chapter 3**) is present in the native community. Thus, in these two chapters I also tested the principals of limiting similarity theory and the role of species identity affecting invasion resistance.

I subsequently included another mechanism affecting alien species invasion success, i.e. herbivory by native herbivores. In **Chapter 4**, I assessed whether native temperate herbivores can provide resistance to establishment success of tropical and subtropical alien aquatic plants. For this, I draw upon the literature to compile multiple lines of evidence presenting results from field studies and herbivore consumption in laboratory feeding trials to estimate their potential. In **Chapter 5** I tested how herbivory and competition by native plants interactively affect alien species growth after colonization.

In **Chapter 6** I synthesize my findings by discussing the role of biotic resistance and its underlying mechanisms in reducing the establishment success of alien aquatic plant species in freshwater ecosystems. I draw parallels and contrasts between the mechanisms in tropical and temperate systems, discuss possible implications of my findings for conservation and restoration of freshwater ecosystems, and suggest potential future research avenues.

A better understanding of biological invasions in understudied ecosystems and geographical regions will improve the robustness of generalizations of the potential for biotic resistance across ecosystems and latitudes. Hopefully this thesis will inspire the research community by filling the current gap of empirical evidence.

Chapter 2

Mechanisms of invasion resistance of aquatic plant communities

Antonella Petruzzella, Johan Manschot, Casper H. A. van Leeuwen,
Bart M. C. Grutters and Elisabeth S. Bakker

Frontiers in Plant Science (2018) 9:134

doi: 10.3389/fpls.2018.00134



Abstract

Invasive plant species are among the major threats to freshwater biodiversity. Few experimental studies have investigated whether native plant diversity can provide biotic resistance to invaders in freshwater ecosystems. At small spatial scales, invasion resistance may increase with plant species richness due to a better use of available resources, leaving less available for a potential invader (Complementarity effect) and/or the greater probability to have a highly competitive (or productive) native species in the community (Selection effect). In submerged aquatic plant communities, we tested the following hypotheses: (1) invader establishment success is greatest in the absence of a native plant community; (2) lower in plant communities with greater native species richness, due to complementary and/or selection effects; and (3) invader establishment success would be lowest in rooted plant communities, based on the limiting similarity theory as the invader is a rooted submerged species. In a greenhouse experiment, we established mesocosms planted with 0 (bare sediment), 1, 2 and 4 submerged plant species native to NW Europe and subjected these to the South-African invader *Lagarosiphon major* (Ridl.) Moss. We used two rooted (*Myriophyllum spicatum* L., *Potamogeton perfoliatus* L.) and two non-rooted native species (*Ceratophyllum demersum* L., *Utricularia vulgaris* L.) representing two distinct functional groups considering their nutrient acquisition strategy which follows from their growth form, with respectively the sediment and water column as their main nutrient source. We found that the presence of native vegetation overall decreased the establishment success of an alien aquatic plant species. The strength of this observed biotic resistance increased with increasing species richness of the native community. Mainly due to a selection effect, the native biomass of mixed communities overyielded, and this further lowered the establishment success of the

invader in our experiment. The strongest biotic resistance was caused by the two native plant species that were of the same functional group i.e. functionally most similar to the invader. These results support the prediction of Elton's biotic resistance hypothesis in aquatic ecosystems and indicate that both species richness and functional group identity can play an important role in decreasing establishment success of alien plant species.

Keywords: biotic resistance, diversity-resistance hypothesis, niche partitioning, functional group identity, limiting similarity, sampling effect, species diversity, species richness

Introduction

Aquatic plants have been crossing geographic barriers mainly due to the ornamental and aquarium trade, and have been intentionally or accidentally introduced to many new aquatic systems (Hussner 2012). Invasive species are among the major threats to freshwater biodiversity, strongly affecting the structure and functioning of these ecosystems (Dudgeon et al. 2006, Strayer 2010). As a consequence, there is a growing interest in understanding the factors regulating the success or failure of alien species and what makes plant communities more susceptible or resistant to invasions (Lonsdale et al. 1999, Davis et al. 2000, Chadwell and Engelhardt 2008, Thomaz et al. 2015 and references therein).

The biotic resistance hypothesis proposed by Elton (1958) predicts that species-richer communities are more resistant to invasions than species-poorer communities. At small spatial scales, this hypothesis has been largely supported (Kennedy et al. 2002, Lindig-Cisneros and Zedler 2002, Michelan et al. 2013). However, opposite patterns are observed at larger spatial scales, where species-richer communities are not more resistance to invasion (Stohlgren et al. 1999, Levine 2000). This 'paradox' has been attributed to the relative contribution of extrinsic factors to invasion resistance, such as resource heterogeneity, climate and disturbance, which can vary considerably with resident native diversity across broad spatial scales (Naeem et al. 2000, Davis et al. 2000). In contrast, at smaller scales, species interactions, such as competition, might play a major role in community assembly and invasion resistance (Fargione et al. 2003).

The diversity of native plant communities can influence the degree of competitive resistance through three different mechanisms. First, multiple species (or functional groups) with non-overlapping resource use strategies can complement

each other in the use of available resources, i.e. the complementarity effect. More efficient resource use leaves fewer resources available to potential invader species (Naeem et al. 2000, Britton-Simmons 2006, Stachowicz et al. 2007, Brown and Rice 2010, Byun et al. 2013). Second, more diverse communities have a higher probability to have a better competitor or more productive species present, a mechanism known as the sampling or selection effect (Wardle 2001). These superior competitive species would competitively suppress the invasiveness of alien species. A third alternative mechanism is that more diverse communities also increase the probability of including greater functional diversity and a functionally similar invader (i.e. limiting similarity) (Xu et al. 2004, Hooper and Dukes 2010). Limiting similarity theory predicts that the species that are most similar to an invader provide the greatest invasion resistance due to niche overlap in resource use. It is important to note that these three mechanisms are not mutually exclusive and can work synergistically (Fargione and Tilman 2005). Although the patterns and the underlying mechanisms have been widely debated in terrestrial and marine systems, few experimental studies have investigated biotic resistance or its underlying mechanisms in freshwater ecosystems (Michelan et al. 2013, Kimbro et al. 2013, Alofs and Jackson 2014).

Here, we performed a small-scale and full factorial experiment in which we manipulated native species richness and functional group identity to explore by which mechanisms the diversity of native plant species may decrease the establishment success of aquatic invasive species. Invader establishment success was measured as colonization ability and growth, defined as biomass increase. We used curly-leaved waterweed, *Lagarosiphon major* (Ridl.) Moss as our model species, which is a highly invasive submerged rooted macrophyte in freshwater submerged plant communities (Hussner 2012). We tested the following three hypotheses: (1) invader establishment success is greatest in the absence of a native plant community (i.e. no resistance

present); (2) invader establishment success is lower in plant communities with greater native species richness, due to complementary and/or selection effects; and (3) invader establishment success would be lowest in rooted plant communities, based on the limiting similarity theory as the invader is a rooted submerged species. Interspecific competition should be strongest between functionally similar species.

Material and Methods

Plant material

The experimental native plant communities were established using four co-occurring submerged aquatic macrophyte species native to, and widely distributed in, Northwestern Europe. We used two rooted (*Myriophyllum spicatum* L. (Haloragaceae), *Potamogeton perfoliatus* L. (Potamogetonaceae)) and two non-rooted species (*Ceratophyllum demersum* L. (Ceratophyllaceae), *Utricularia vulgaris* L. (Lentibulariaceae)). The two distinct groups (rooted and non-rooted) are functionally and morphologically different in their nutrient acquisition strategy which follows from their growth form, with respectively the sediment and water column as their main nutrient source. Most rooted species are able to take up nutrients from both the water and sediment with the relative contribution of each source depending on the relative nutrient availability (Fejoó et al. 2002 and references therein). For submerged rooted plants, the sediment becomes the main nutrient acquisition source once there are available nutrients in it. Non-rooted species absorb nutrients from the surrounding water. Whereas *C. demersum* forms anchoring adventitious roots, its nutrient uptake is almost entirely foliar (Denny 1972), as well as for *U. vulgaris*, which can absorb nutrients over the entire shoot surface (Friday 1992).

We selected the South African species *L. major* (Hydrocharitaceae) as invader. *L. major* is a rooted, submerged plant species recognized as a noxious aquatic weed (Hussner 2012). According to the European and Mediterranean Plant Protection Organization (EPPO) it is present in 13 countries in Europe and listed on the 'EPPO¹ List of invasive alien plants'.

M. spicatum, *P. perfoliatus*, *C. demersum* and *L. major* were acquired from a commercial plant trader in the Netherlands (De Zuurstofplantgigant, 51°22'00.7"N, 5°15'0.7.2"E). *U. vulgaris* was collected from a private pond in the Netherlands (52°09'5"N, 5°00'16"E). All plants were pre-cultivated under controlled greenhouse conditions with a 16/8 h light/dark cycle at an average temperature of 21°C during the day and 16°C during the night. We used 200 L cattle tanks (two tanks per species) filled¹ with 20 L of artificial plant pond sediment (Plant soil Moerings - Velda), 20 L of washed sand on top and filled with tap water (mean \pm SD, n = 6: 20 \pm 3.2 $\mu\text{g L}^{-1}$ P-PO₄; 37.7 \pm 3.1 $\mu\text{g L}^{-1}$ N-NO₃; 1 \pm 1.7 $\mu\text{g L}^{-1}$ N-NO₂; 13 \pm 31.8 $\mu\text{g L}^{-1}$ N-NH₄, pH 8.20 \pm 0.02). Plants were cultivated for at least two weeks.

Experimental design

To evaluate the effects of native community diversity on the invader establishment success of *L. major* we established seventy-two mesocosms in a greenhouse under controlled conditions (with a set regime of 16/8 h light/dark, 16-21°C night/day) at the Netherlands Institute of Ecology (NIOO-KNAW) during the

summer of 2016 (July-September) in the Netherlands. The experiment ran for ten weeks including two weeks of establishment of the native plant community.

For the experiment, 147 unrooted apical shoots without lateral shoots were collected from the cultivation tanks for each of the species *M. spicatum*, *P. perfoliatus* and *C. demersum*; for *U. vulgaris* the entire plant was used instead of only the apical part. The shoots were cut to be 15 cm long and washed in running tap water to remove any material attached. Of the 147 shoot fragments of each species, 15 were randomly selected, dried to a constant mass at 60°C for at least 48h, and weighed for initial biomass measurements. The plant density in the mesocosms was kept constant across treatments at 8 shoots per mesocosm, which is 254.78 plants m⁻². Thus, mesocosms containing two species contained four individuals of each species, whereas those with four species contained two individuals of each species. Such shoot densities are within the range of the shoot densities of submerged macrophyte communities in natural conditions (Li et al. 2015). The plant shoots were planted 5 cm deep in the sediment.

The mesocosms consisted of 10 L white plastic buckets (32.5 cm height and 20 cm diameter) filled with a bottom layer of artificial plant pond sediment (Plant soil Moerings - Velda) (1 cm/ 200 g, organic matter content=34.31%) with a top layer of washed sand (0.8-1.0 mm grain size) (5 cm deep, 2650 g sand per bucket, organic matter content=0.16%). Then the bucket was filled up with tap water (mean \pm SD, n = 6: 20 \pm 3.2 $\mu\text{g L}^{-1}$ P-PO₄; 37.7 \pm 3.1 $\mu\text{g L}^{-1}$ N-NO₃; 1 \pm 1.7 $\mu\text{g L}^{-1}$ N-NO₂; 13 \pm 31.8 $\mu\text{g L}^{-1}$ N-NH₄). The water level was maintained constant during the whole experiment by refilling once a week to compensate for evapotranspiration. The physical and chemical parameters of the water were measured weekly in all mesocosms and the growing conditions were found to be suitable for the fragments (mean values at

¹ <http://www.eppo.org>

daytime throughout the experiment, mean \pm SD, n = 792: water temperature $22 \pm 0.9^\circ\text{C}$, dissolved oxygen concentration $7.9 \pm 2.0 \text{ mg L}^{-1}$, pH 9.6 ± 0.6 , n=432: alkalinity $0.8 \pm 0.5 \text{ mEq L}^{-1}$).

Treatments were applied in a full factorial design (see Table 1). We established four levels of native plant species richness, 0 (bare sediment), 1 (monocultures), 2 and 4 species (see Table 1). To test limiting similarity, we grouped our treatments according to plant growth form, assuming this parameter is also related to the major nutrient acquisition sources (the sediment and the water column) and therefore intensity of nutrient competition. Our plant species represented two different functional groups: a rooted group containing only rooted submerged macrophytes and a non-rooted group containing only non-rooted ones. When both functional groups were combined in one bucket we defined these as mixtures. Each of the twelve treatments (four species richness levels, three functional group combinations) was replicated six times, using a block design, yielding a total of seventy-two mesocosms (Table 1 and Supplementary Figure 1).

After the establishment of the native plant community (determined by the growth of new shoots of at least one individual of each species, which took two weeks), we introduced two *L. major* propagules per mesocosm. Each propagule consisted of a 15 cm long shoot with apical tip, but no lateral branches, to minimize variation in the size of the initial shoot material. Fifteen *L. major* fragments were randomly selected, dried to a constant mass at 60°C for at least 48h, and weighed for initial biomass measurements. Fragments with an apical tip have higher regeneration and colonization abilities and higher growth rates than fragments without apical tips (Riis et al. 2009). Invader fragments were not planted in the sediment but were dropped into the mesocosms simulating how alien species arrive

in a new area where a native community is already established (Riis and Sand-Jensen 2006). Pilot trials showed that the fragments float for days to weeks first growing side branches. Gradually they start forming aerial roots and growing downwards to sink, thus, reaching the sediment (Supplementary Figure 2).

Table 1. Overview of the 12 treatments used in the mesocosm experiment.

Diversity treatment	Community composition	Species	Functional group(s)	Species richness
1	Bare sediment	Bare sediment		0
2	M	<i>Myriophyllum spicatum</i>	Rooted	1
3	P	<i>Potamogeton perfoliatus</i>	Rooted	1
4	C	<i>Ceratophyllum demersum</i>	Non-rooted	1
5	U	<i>Utricularia vulgaris</i>	Non-rooted	1
6	M + P		Rooted	2
7	C + U		Non-rooted	2
8	M + C		Mixture	2
9	M + U		Mixture	2
10	P + C		Mixture	2
11	P + U		Mixture	2
12	M + P + C + U		Mixture	4

Functional groups are classified based on the main nutrient acquisition strategy which follows from their growth form.

Plant harvest and data collection

We measured invader establishment success in terms of the ability of the fragments to colonize and grow. We defined successful invader colonization as at least one fragment of the invader having its roots attached in the sediment (Supplementary Figure 2). At the end of the experiment, we counted the number of mesocosms that was successfully colonized. Invader growth was defined as biomass increase (dry weight, DW). At the end of the experiment (after 8 weeks), the invader was harvested. Total root and shoot DW were determined summing values from both introduced fragments. From these values we calculated the root:shoot ratio and relative growth rate (RGR). The RGR was calculated considering the total biomass of the invader (including both fragments and roots + shoots) in the mesocosms as follows:

$$\text{RGR} = (\ln W_f - \ln W_i) / \text{day};$$

where, W_f = final DW; W_i = initial DW.

Additionally, the native plant community biomass was harvested and sorted by species at the end of the experiment. All the harvested plants were dried to a constant mass at 60°C for at least 48h, and weighed.

Data analyses

We analyzed the ability of invader fragments to colonize, defined as the invader having at least one fragment with roots attached in the sediment, using Fisher's-exact test, which is used to assess the significance of a difference between the proportions in two groups.

To test the effects of the native community on the invading species, we used a mixed-modeling approach. Response variables of interest were the root DW, shoot DW, root:shoot ratio and RGR of the invading species *L. major*. We used General Linear Mixed-effects Models (General LMMs) in case the response variables were normally distributed, i.e. all data for *L. major* shoot DW and RGR, and the data for *L. major* root DW and root:shoot ratio when analyzed in combination with only native species monocultures. We used Generalized Linear Mixed-effects Models (Generalized LMMs) in case of non-normal distributions, i.e. when including all species richness treatments for *L. major* root DW and root:shoot ratio. Fixed factors that were included in the models were (1) the presence of native communities (bare sediment and the 4 species monocultures), (2) species richness (0, 1, 2 or 4 species) or (3) functional group identity (bare sediment, non-rooted, rooted, or a mixture of non-rooted and rooted native species present). Block (the 6 replicates) was included as random factor in all models. Normality of residuals and homoscedasticity were checked with plots of residual *versus* fitted values, and qqplots of residuals. Response variables were natural log-transformed when necessary for satisfying assumptions of normality. We assessed statistical significance of fixed factors in the models by performing likelihood ratio tests between models including and excluding these factors (F-tests for General LMMs and Chi-squared tests for Generalized LMMs). All statistical analyses were carried out using RStudio version 3.4.2 (R Core Team 2017).

To test our first hypothesis that *L. major* establishment success would be greatest in absence of a native community, we used a General LMM [*lme* function in the R package *nlme* (Pinheiro et al. 2018)] to test whether all four invader response variables differed between the treatment with only bare sediment (as intercept) and each of the four monocultures. Root DW and root:shoot ratio were natural log-transformed for satisfying assumptions of normality.

To test our second hypothesis that invader establishment success would be lowest in richer native plant communities, we tested the effect of species richness on all four invader response variables. Possible effects of species richness on *L. major* root DW and root:shoot ratio were analyzed following the procedure described by Fletcher et al. (2005). This method is applied for ecological data that has a substantial proportion of zeros and is positively skewed which often makes assumptions for linear analysis (e.g. normality of errors) invalid. First, we analyzed whether invader root formation (presence/absence) was affected by species richness by fitting a binomial Generalized LMM (*glmer* function in the R package *lme4* (Bates et al. 2015)) using presence or absence of roots as response variable. Second, for those mesocosms in which the invader developed roots, we subsequently analyzed possible effects of species richness on the amount of root biomass produced using the *lmer* function of the same package. We natural log-transformed root DW and root:shoot ratio to ensure normality of model residuals. Possible effects of species richness on normally distributed *L. major* shoot DW and RGR were assessed using General LMMs. To test the effects of species richness on the native community biomass we also used a General LMM, in which species richness was a fixed factor, native community biomass the response variable and block as random factor.

To test our third hypothesis that invader establishment success would be lowest in rooted submerged species based on limiting similarity theory, we analyzed whether the four invader response variables were affected by functional group identity. We fitted four mixed models with functional group identity as fixed factor and block as random factor. For the root and root:shoot ratio data, we also analyzed following the procedure described by Fletcher et al. (2005) but we are only interested in the results of those mesocosms in which the invader developed roots. Root DW and root:shoot ratio given the presence of roots were natural log-transformed for

satisfying assumptions of normality. For models with normally distributed shoot DW and RGR as response variables we used General LMMs. We used Tukey's *post hoc* test to detect pairwise differences between functional group identity treatments. We applied a Bonferroni correction for adjusting the significance levels to control for Type I error in a multiple testing situation.

Additive partitioning of diversity effects

To determine the mechanisms of possible biodiversity effects, the net diversity effect (ΔY) in 4 species mixture treatment was partitioned into a complementarity effect and selection effect using the additive partitioning of biodiversity effects method proposed by Loreau and Hector (2001). This method compares the total mixture yield (Y_O) with the expected yield of the mixtures based on monoculture yields of component species (Y_E).

$$\Delta Y = Y_O - Y_E$$

The complementarity effect for a specific number of species (M) was calculated as:

$$N \overline{\Delta RY} \overline{M}$$

where $\overline{\Delta RY}$ is the average change in expected relative yield of all species in the mixture and \overline{M} is the average monoculture yield. A positive complementarity effect occurs if species yields in a mixture are on average higher than expected based on monoculture yields of component species (Loreau and Hector 2001).

The selection effect was calculated as the covariance between monoculture yields of component species (M) and their change from expected relative yield in the mixture of all species in the mixture (ΔRY) multiplied by N of the mixture.

$$N \text{ cov}(\Delta RY, M)$$

A positive selection occurs if species with higher than average monoculture yields dominate the mixtures (Loreau and Hector 2001).

Results

Overall, the presence of native plants strongly decreased the ability of the invader to colonize. The invading species *L. major* had fragments with roots attached to the sediment in only 5 out of 66 mesocosms in which native plants were present (7.6 % colonized). Whereas in the treatment with bare sediment invader fragments attached with roots to the sediment in 4 out of 6 mesocosms (66.7% colonized, which was significantly higher than when native plants were present, odds ratio=0.045, $p=0.002$). When native plants were present, in particular treatments with *U. vulgaris* species were colonized by *L. major* fragments. In *U. vulgaris* monocultures, 50% of the mesocosms were colonized and this rate decreased when *U. vulgaris* grew in combination with other species to 16.7% when grown with either *P. perfoliatus* (PU) or *C. demersum* (CU), whereas no colonization was observed when *U. vulgaris* was grown with *M. spicatum* (MU).

Generally, *L. major* formed significantly more shoot biomass in the absence of a native plant community (Table 2). Contrasts between the bare sediment treatment and monocultures of the native plants showed that *L. major* root biomass was significantly larger in the bare sediment treatment than in monocultures of the

two rooted species *M. spicatum* and *P. perfoliatus*, but not in non-rooted *C. demersum* and *U. vulgaris* (Table 2). Similarly, we also did not find significant differences between root:shoot ratio and RGR of the invader in the bare sediment treatment and monocultures of *C. demersum* and *U. vulgaris* (Table 2), i.e. invader growth was as great in the non-rooted species treatments as in the absence of native plants. In contrast, monocultures of the rooted species *M. spicatum* and *P. perfoliatus* significantly lowered the invader's root:shoot ratio and RGR (Table 2). It is important to point out, at the start of the experiment, all the native plants were growing well, whereas during the course of the experiment *U. vulgaris* plants started dying. These results likely indicate the invader may have benefited from this to colonize and grow.

Table 2. Results of general linear mixed effects model (General LMM) using contrasts between the bare sediment mesocosms (set as intercept) and the effects of native species monocultures of *Myriophyllum spicatum* (M), *Potamogeton perfoliatus* (P), *Ceratophyllum demersum* (C) and *Utricularia vulgaris* (U) on root dry weight, shoot dry weight, root:shoot ratio and relative growth rate (RGR) of the invader *Lagarosiphon major*.

Invader response variable	Treatment	Mean \pm SD, $n = 6$	t	$p(> t)$
Root DW (g)*	Bare sediment	59.42\pm77.3		
	M	0.05\pm0.1	-3.64	0.001
	P	3.37\pm2.5	-3.04	0.006
	C	14.37 \pm 10.2	-1.72	0.100
	U	87.57 \pm 118.4	-0.03	0.968
Shoot DW (g)	Bare sediment	1.71\pm0.25		
	M	0.91\pm0.21	-6.65	<0.001
	P	1.00\pm0.16	-5.93	<0.001
	C	1.42\pm0.18	-2.38	0.026
	U	1.44\pm0.28	-2.25	0.035
Root:shoot*	Bare sediment	0.033\pm0.039		
	M	0\pm0	-3.08	0.005
	P	0.003\pm0.002	-2.38	0.026
	C	0.011 \pm 0.01	-1.35	0.189
	U	0.058 \pm 0.071	0.33	0.741
RGR (g g ⁻¹ DW day ⁻¹)	Bare sediment	0.026\pm0.003		
	M	0.014\pm0.004	-6.81	<0.001
	P	0.016\pm0.003	-5.77	<0.001
	C	0.022 \pm 0.002	-2.08	0.050
	U	0.023 \pm 0.004	-1.60	0.123

Bold numbers indicate significant differences ($p < 0.05$). *natural log-transformed variables for satisfying assumptions of normality.

With increasing species richness of the native plant community, the growth of *L. major* decreased. Floating *L. major* fragments were less likely to form roots when native species richness was higher, and when roots were formed, their biomass was lower (Fig. 1a, b). With increasing native species richness shoot biomass production, root:shoot ratio (given the presence of roots) and RGR of the invader also decreased (Fig. 2a, b, c). Native species richness enhanced the biomass of the native community (Fig. 3). Native biomass at the highest species richness level (4 species) was 29.2% more than the average biomass of monocultures, indicating overyielding. The positive net diversity effect was mainly caused by a selection effect (Fig. 4), while the complementarity effect was slightly negative. This positive selection effect was most likely caused by the biomass production of the two rooted species, *M. spicatum* and *P. perfoliatus*. These species produced more biomass (mean \pm SD, $n=12$: 3.54 \pm 0.43 g) than the two non-rooted *C. demersum* and *U. vulgaris* (mean \pm SD, $n=12$: 0.48 \pm 0.52 g, Fig. 3) in monocultures. Their presence in higher proportion in the mixtures was the main reason for the larger native plant productivity in richer communities.

Figure 1. Effect of native plant species richness on the invader *Lagarosiphon major* (a) root formation and, given their presence, on (b) log-transformed root biomass production. Data points were jittered in the graph (a) so the binomial (presence/absence) of roots at many of the treatments could be seen. Significance level at $p < 0.05$.

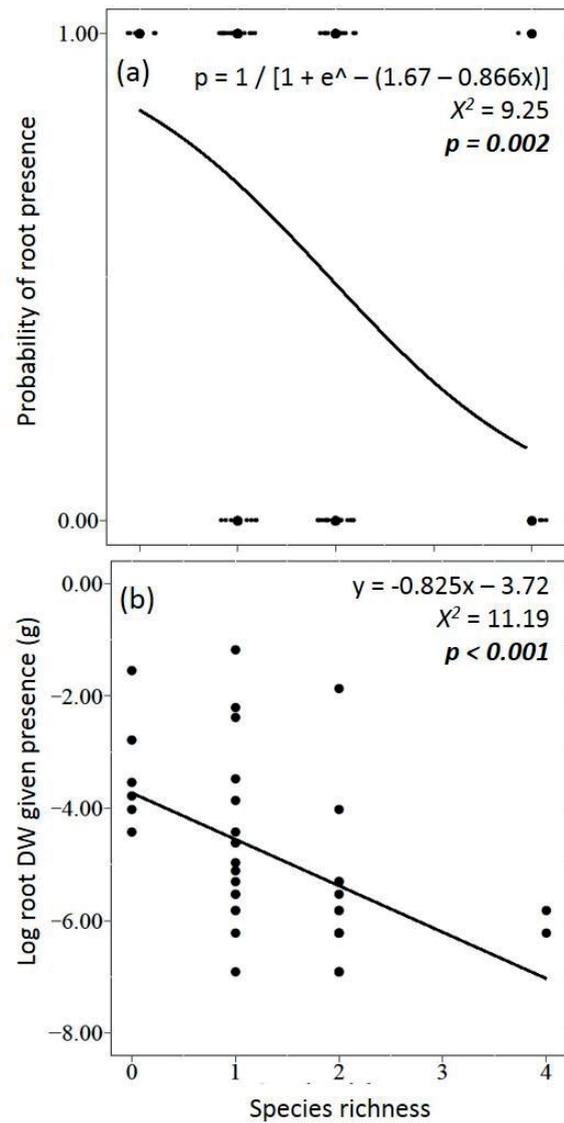


Figure 2. Effects of native plant species richness on the invader *Lagarosiphon major* (a) shoot biomass, (b) log-transformed root:shoot ratio given their root formation and (c) relative growth rate (RGR). Significance level at $p < 0.05$.

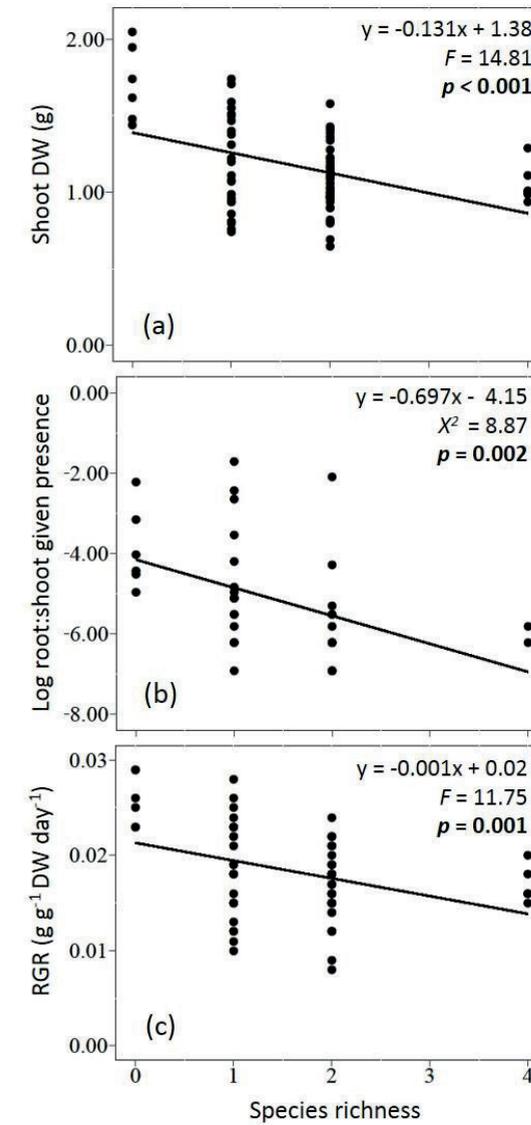


Figure 3. Effect of native plant species richness on total native community biomass production (g DW) per mesocosm.

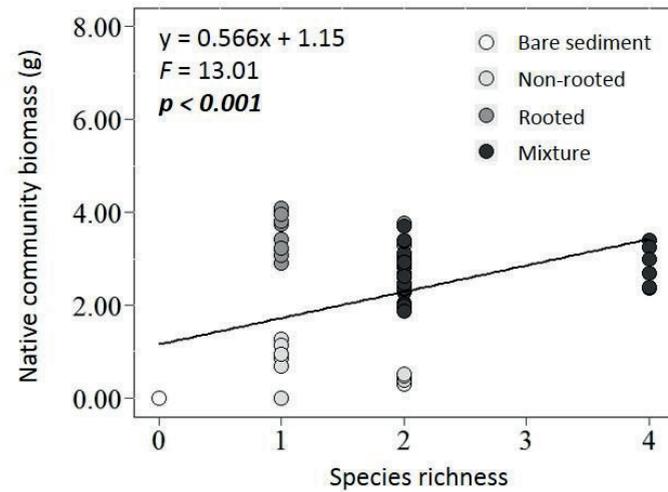
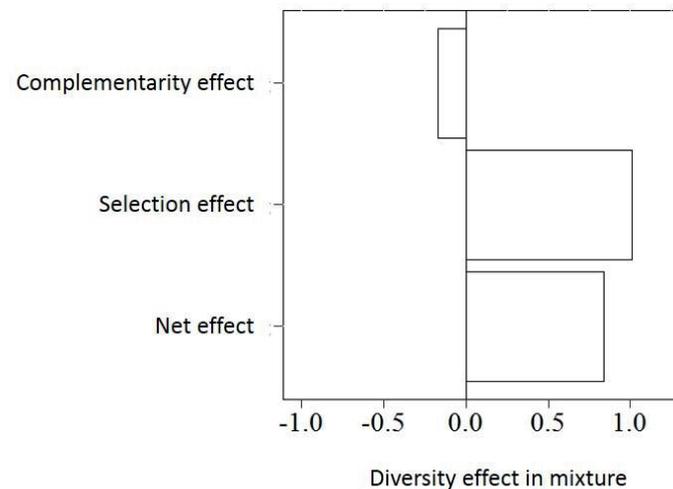
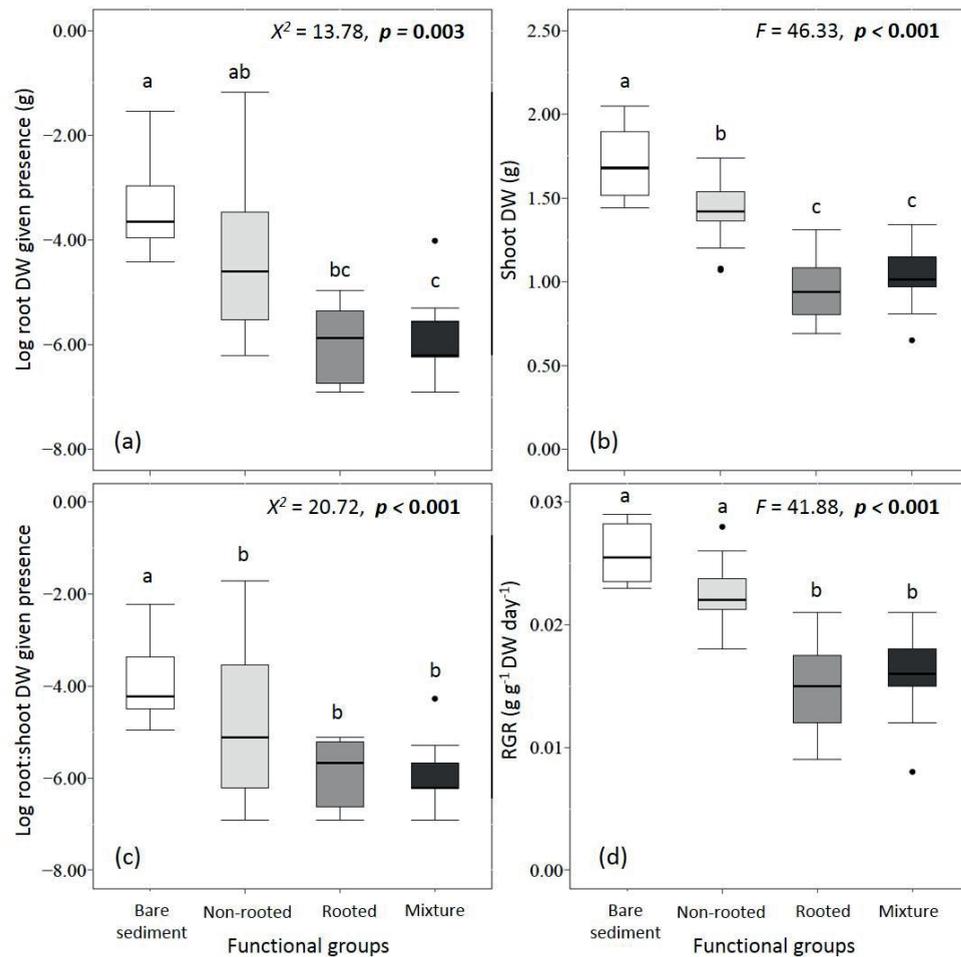


Figure 4. Partitioning of the diversity effect on native community biomass of the highest species richness level (4 species) into a complementarity effect and a selection effect by applying the additive partitioning diversity effect method proposed by Loreau and Hector (2001).



L. major establishment success did depend on which functional groups were present (Fig. 5a-d). Compared to the bare sediment treatment, *L. major* shoot biomass was significantly decreased in the presence of non-rooted native plants, and even more so in the presence of rooted native plants and mixed communities (Fig. 5b). The root biomass (given the presence of roots) and the RGR of the invader were most negatively influenced by rooted plants and mixtures, whereas the non-rooted plants did not significantly inhibit the invader's root biomass and RGR compared to the bare sediment treatment (Fig. 5a, d). The root:shoot ratio of the invader was significantly lower in all treatments containing native plants, which did not depend on the functional groups present (Fig. 5c). This may be due to the large variance of invader root biomass, and subsequently root:shoot ratio, observed in the non-rooted plant community treatment. This large variation probably results from contrasting responses of the invader to both non-rooted species, with more root production of the invader in *U. vulgaris* than in *C. demersum* treatments (Table 1).

Figure 5. Median, SE (boxes) and minimum and maximum (whiskers) values of (given the presence of roots), (a) log-transformed root biomass, (b) shoot biomass, (c) log-transformed root:shoot ratio and (d) relative growth rate (RGR) of the invader *Lagarosiphon major*. Different lowercase letters indicate statistically significant differences between functional group treatments (Tukey's *post hoc* test). Significance levels were determined after Bonferroni correction ($p < 0.008$).



Discussion

We found that the presence of native vegetation overall can decrease the establishment success of an alien aquatic plant species. The strength of this observed biotic resistance – as proposed by Elton (1958) – increased with increasing species richness of the native community. We could demonstrate that this was mainly due a selection effect, as a result of which the native biomass of mixed communities overyielded, which further lowered the establishment success of the invader in our experiment. The strongest biotic resistance was caused by the two native plant species that were of the same functional group i.e. functionally most similar to the invader. These results support the prediction of Elton's biotic resistance hypothesis and demonstrate that it can be applied to aquatic ecosystems. Moreover, our results show that both species richness and functional group identity can play an important role in decreasing establishment success of alien species.

We expected that invader establishment success, defined as the ability of invader fragments to colonize and grow, would be greatest in the absence of a native plant community. In line with our first hypothesis, the highest colonization rate of *L. major* fragments in bare sediment indicates that the ability of the invader to colonize was highest in the absence of biotic resistance. However, treatments with the species *U. vulgaris* were also colonized by *L. major* fragments to a certain extent, especially in the monoculture. As a result, *L. major* growth was as much in non-rooted species treatments as in bare sediment, with the only exception of shoot biomass. This is likely due to the limited growth of the two non-rooted native species in our experiment. When we started the experiment all the native plants were growing well, whereas during the course of the experiment the rooted plants performed better than the non-rooted ones. In particular, *U. vulgaris* plants started dying, which likely benefited

the chance of the invader to colonize and grow. The death of *U. vulgaris* shoots increased the chances of *L. major* fragments reaching the sediment surface due to removal of the physical barrier that these submerged plant biomasses imposed (Supplementary Figure 3).

The decrease in *L. major* growth observed with an increase of native species richness supports our second hypothesis. Our results are consistent with previous diversity-invasibility experiments performed in terrestrial and marine ecosystems which also showed a reduced invasibility in more species rich plant communities (Naeem et al. 2000, Fargione and Tilman 2005, Britton-Simmons 2006, Michelan et al. 2013, Byun et al. 2013, Zhu et al. 2015). We hypothesized that this species richness effect could be explained by a selection and/or complementarity effect (Naeem et al. 2000, Brown and Rice 2010, Byun et al. 2013). The partitioning of the biodiversity effect into these two mechanisms showed that the decrease of invader growth would not be explained by native species richness *per se*, but perhaps more specifically by competition between the invader and a particular native species and/or functional group which was indicated by the selection effect. In our study both rooted species, *M. spicatum* and *P. perfoliatus*, which produced more biomass in the monocultures, dominated the mixtures, leading to a high positive selection effect and high biomass and as a result, a lower growth of the invader. Other studies have also shown the effects of the presence of highly competitive species on reducing invader success in terrestrial and marine ecosystems (Wardle 2001 and references therein, Arenas et al. 2006). However, few studies have experimentally attempted to disentangle the effects of diversity in freshwater ecosystems (Michelan et al. 2013, Byun et al. 2013). In a greenhouse experiment, Michelan et al. (2013) found that the invasiveness of the wetland grass species *Urochloa arrecta* (Hack. ex T. Durand & Schinz) Morrone & Zuloaga was negatively affected by the species richness of native wetland plants, but

discarded a potential selection effect because *U. arrecta* growth did not differ in different native species monocultures.

Based on the mechanism of limiting similarity, we expected that invader establishment success would be lowest in rooted plant communities, as the invader is a rooted submerged species. Several studies performed in terrestrial systems have found evidence of functional similarity reducing invader success (Fargione et al. 2003, Pokorny et al. 2005, Mwangi et al. 2007, Young et al. 2009, Hooper and Dukes 2010), or partly reducing invader success (Price and Pärtel 2013). The results of *L. major* growth partly supported our third hypothesis. Invader shoot biomass and RGR, indeed, were both the lowest in rooted plant communities and in mixtures that were dominated by rooted plants. However, root biomass production of *L. major* was not consistently lower in rooted than in non-rooted native plant communities. This can be explained by the large variation in root production of *L. major* between the treatments with the two non-rooted native plant species. Although *C. demersum* performed less compared to the rooted species, the invader did not produce as much of root biomass as in the *U. vulgaris* treatments, causing large variation in the amount of root production by the invader in the presence of non-rooted species as a functional group.

Several reasons may underlie the negative effects on invader success by native plants. In general, suppression of *L. major* was particularly strong in native communities containing rooted submerged plants. These species produced the most biomass, which is in line with the rapid depletion of pore water nutrients over the sampling weeks (Supplementary Figure 4). Once the nutrients in the sediment are consumed, rooted submerged plants have the ability to take up nutrients also from the water column (Madsen and Cedergreen 2002 and references therein). This may

have caused depletion of nutrients in the water column first in the non-rooted plant treatments and then eventually also in the rooted plant treatments, leaving less available for the invader, thus, limiting its growth (Supplementary Figure 5). This has been demonstrated for the native rooted submerged aquatic plant species *Vallisneria americana* Michaux, which reduced the colonization success of the exotic invasive species, *Hydrilla verticillata* (L.F.) Royle through nutrient draw-down in the water column (Chadwell and Engelhardt 2008). Furthermore, due to their high productivity, densely growing native plants can also prevent colonization and decrease the growth of the invader by acting as a physical barrier, preventing the propagules to reach the sediment surface. Invader propagules might not allocate biomass for colonization, root production for example, once they are physically trapped in these dense canopies. Alternatively, when the native plants do not form a lot of biomass, such as *U. vulgaris* in our study, invasive fragments can reach the sediment and can start root development, using the excess of nutrients in the sediment (pers. obs. AP). This can thus allow the invader to have access an alternative nutrient source, strongly enhancing its subsequent performance. Additionally, we cannot rule out the possibility that allelopathic effects have occurred, influencing the performance of the invader. In particular *M. spicatum* and *C. demersum* are known to contain and excrete allelochemicals, which can inhibit the growth of other primary producers (Gross and Bakker 2012, Grutters et al. 2017b). This may have limited the general shoot growth of the invader, or more specific processes, such as the formation of roots. However, allelopathic effects are very difficult to establish (Hilt and Gross 2008), hence, the influence of allelopathy in our experiment remains speculative, but cannot be ruled out either.

Our experiment was designed to have the sediment as the main nutrient source, whereas nutrients were not directly added to the water column, but could

leak from the sediment into the water column. As such, our experiment represents a situation in which the water layer is transparent and relatively low in nutrients, whereas the sediment is more nutrient rich. This is a rather common situation in many restored shallow water bodies, which often contain nutrient-rich sediment, as a result of eutrophication in the past, whereas the water column is relatively low in nutrients, as a result of measures to reduce nutrient loading, in particular to remove excess phosphorus (e.g. Immers et al. 2015). In these systems, nutrients may be provided to the water column by leaking from the sediment, resulting in internal loading. These systems are sensitive to alien plant invasions, when there are no native plants present. However, native rooted submerged plants can grow very well under these conditions (Verhofstad et al. 2017), which may prevent invasion. If we would have provided nutrients in the water column in our experiment, and not in the sediment, the free submerged plants would have performed much better, and may have outcompeted the invader, whereas the rooted plants may have performed less well, even though nutrients would have precipitated on the sediment. Where the nutrients are more abundant will thus likely affect which functional plant group will dominate, but may not necessarily affect the result that invader establishment is reduced in more species rich native plant communities. Furthermore, it is important to highlight that aquatic plant communities are very often dominated by one or a few vascular species (Engelhardt and Ritchie 2002), suggesting species or functional group identity is likely to play an important role in biotic resistance to plant invasion in these systems, as we also found in our study.

At large spatial scales, the outcome of invasion success is not only determined by species interactions, but also by the interaction between co-varying factors such as propagule pressure and environmental conditions (including disturbance, climate and resource heterogeneity) (Naeem et al. 2000, Fleming and Dibble 2015). The

conflicting results of plant diversity effects on invasion resistance found at different spatial scales, i.e. 'paradox', have been attributed to the lack of control of these environmental factors (Naeem et al. 2000). Similarly, whereas Elton's hypothesis has been largely supported in experiments using pre-defined plant assemblages, conflicting results have been found in field experiments conducted at small spatial scales, which also likely result from the influence of co-varying, uncontrolled environmental conditions (Capers et al. 2007). Thus, it is difficult to determine whether the diversity effect on invasion resistance results purely from the species interactions or include the interaction with co-varying environmental factors. Controlled mesocosms experiments, such as we performed, allow us to disentangle the role of the effect of species richness, excluding such covariation.

Despite experiments manipulating plant diversity and testing invasion resistance being performed in marine and terrestrial systems (Levine et al. 2004, Kimbro et al. 2013), there are only very few performed in freshwater systems (Alofs and Jackson 2014). The most recent meta-analysis found only four studies of competitive resistance to an invasive freshwater producer and none of them explicitly addressed diversity (Wakeman and Les 1994, Dickinson and Miller 1998, Irfanullah and Moss 2004, Chadwell and Engelhardt 2008). We found that native plant diversity increases the biotic resistance of native communities to aquatic plant invaders. This is an important result. Alien species are currently spreading across the globe at unprecedented rates and this process is combined with rapid loss of our native biodiversity (Milligan et al. 2014) which we here show helps to prevent these alien species from becoming invasive. Our study therefore illustrates the importance of studying both invasions and loss of native biodiversity, and especially the novel interactions among native and invasive species. Furthermore, it stresses the

importance of the conservation of aquatic plants as a mean to increase the robustness of our aquatic ecosystems, mainly the restored ones.

Acknowledgements

We would like to thank all the technicians and colleagues of the Aquatic Ecology department of the Netherlands Institute of Ecology (NIOO-KNAW). None of this work could have been performed without their valuable help and assistance. AP specially acknowledges the Science Without Borders Programme and CNPq (Brazilian National Council for Scientific and Technological Development) for her Ph.D. scholarship. This is publication 6474 of the Netherlands Institute of Ecology (NIOO-KNAW).

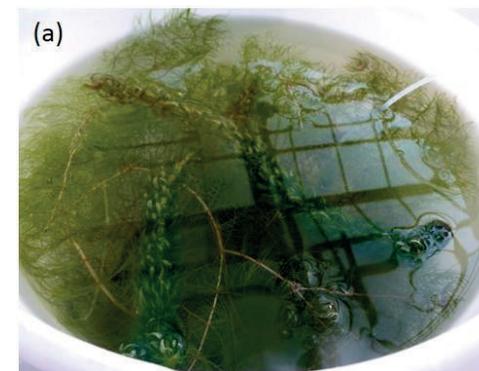
Supplementary material

Supplementary Figure 1. Overview of the experiment.

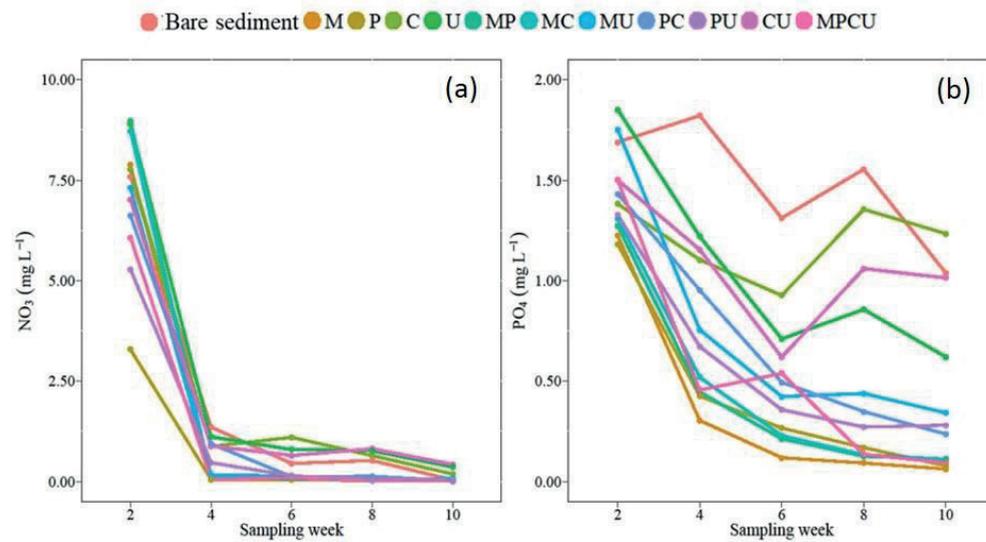


Supplementary Figure 2. Dynamic of *Lagarosiphon major* (Ridl.) Moss propagules settlement in bare sediment. These fragments float for days to weeks, first growing side branches. Gradually they start forming aerial roots and growing downwards to sink, thus, reaching the sediment.

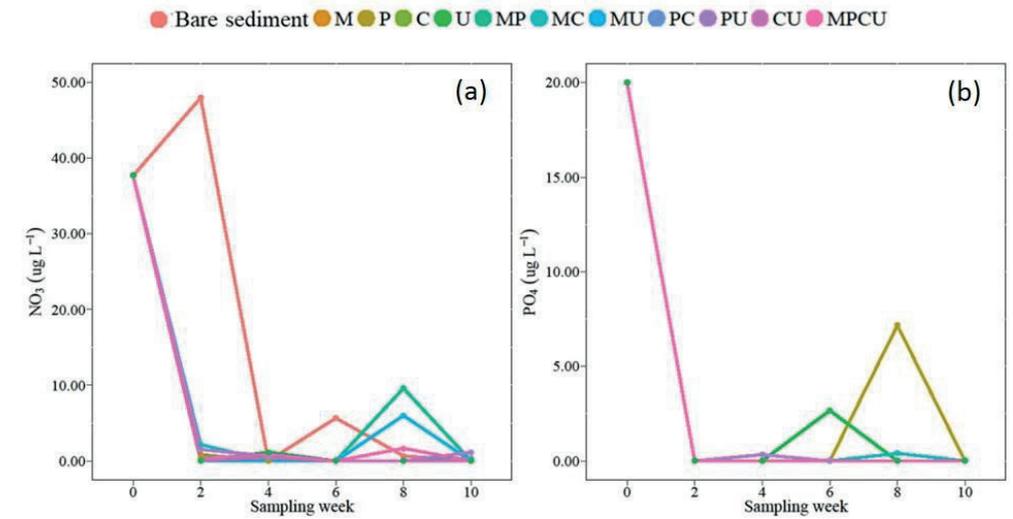
Supplementary Figure 3. Photograph of the monocultures of rooted submerged aquatic macrophytes and *L. major* fragments on top showing the physical barrier imposed by their canopies. (a) *Myriophyllum spicatum* L. and (b) *Potamogeton perfoliatus* L..



Supplementary Figure 4. Pore water (a) nitrate and (b) phosphate concentrations of the treatments over the experiment. Values are means per treatment (n=6). Treatments are abbreviated with: No res – bare sediment, M- *Myriophyllum spicatum* L., P- *Potamogeton perfoliatus* L., C- *Ceratophyllum demersum* L. and U- *Utricularia vulgaris* L. in a full factorial design. Samples were collected every two weeks after the start of the experiment.



Supplementary Figure 5. Water column (a) nitrate and (b) phosphate concentrations in the treatments over the experiment. Values are means per treatment (n=6). Treatments are abbreviated with: No res – bare sediment, M- *Myriophyllum spicatum* L., P- *Potamogeton perfoliatus* L., C- *Ceratophyllum demersum* L. and U- *Utricularia vulgaris* L. in a full factorial design. Samples were collected at the start and every two weeks of the experiment.





Chapter 3

Species identity and diversity effects on invasion resistance of tropical freshwater plant communities

Antonella Petruzzella, Tauany A. da S. S. R. Rodrigues, Casper H. A. van Leeuwen, Francisco de Assis Esteves, Marcos Paulo Figueiredo-Barros and Elisabeth S. Bakker

Scientific Reports (in review)

Abstract

Biotic resistance mediated by native plant diversity has long been hypothesized to reduce the success of invading plant species in terrestrial systems in temperate regions. However, still little is known about the mechanisms driving invasion patterns in other biomes or latitudes. We help to fill this gap by investigating how native plant community presence and diversity, and the presence of native phylogenetically closely related species to an invader, would affect invader *Hydrilla verticillata* establishment success in tropical freshwater submerged plant communities. The presence of a native community suppressed the growth of *H. verticillata*, but did not prevent its colonisation. Invader growth was negatively affected by native plant productivity, but independent of native species richness and phylogenetic relatedness to the invader. Native plant production was not related to native species richness in our study. We show that resistance in tropical aquatic plant communities is mainly driven by the presence and biomass of a native community independent of native species diversity. Our study illustrates that resistance of tropical freshwater plant communities to invasive species contrasts to resistance described for other ecosystems. This emphasizes the need to include understudied systems when predicting patterns of species invasiveness and ecosystem invasibility across biomes.

Introduction

Biological invasions represent a key component of human-induced global changes, and increasingly challenge the conservation and management of freshwater, marine and terrestrial ecosystems across the globe (Simberloff et al. 2013, Gallardo et al. 2016). Although our knowledge on invasion ecology has increased considerably over the last decades, research has been strongly biased towards certain geographical regions and ecosystems (Pyšek et al. 2008, Lowry et al. 2013, Bellard and Jeschke 2016). Most of our understanding about the process, patterns and mechanisms of invasions emerges from studies performed in temperate terrestrial systems, whereas the impact on megadiverse tropical regions – that together harbor the greatest part of the Earth’s biodiversity – remains surprisingly understudied (Rodríguez 2001, Lowry et al. 2013, Bellard and Jeschke 2016, Barlow et al. 2018). Similarly, freshwater systems, which are both hotspots of biodiversity and have been heavily impacted by human activities, are the least studied among ecosystem realms (Dudgeon et al. 2006, Lowry et al. 2013). The lack of information is especially problematic because tropical systems are among the most threatened on earth, and freshwater ecosystems show the strongest biodiversity decline (Sala et al. 2000, Lövei et al. 2012, WWF 2018). Understanding the mechanisms underlying the success or failure of invasions is essential for possible prevention, control or management of invasive species.

Studies have suggested that tropical systems may be less susceptible to invasions than temperate systems (Sax 2001, Freestone et al. 2013). An important rationale for this idea is that the highly species-rich native communities generally found in tropical systems, may resist invasions through biotic processes such as competition (Levine et al. 2004). The biotic resistance hypothesis predicts that, during alien plant invasion, more diverse communities are less susceptible to invasions than

less diverse communities (Elton 1958). At small spatial scales, plant diversity may provide resistance through different niche-based mechanisms. For example, complementarity among multiple species (or functional groups) with non-overlapping resource use strategies could lead to a better use of available resources, leaving less resources available for a potential invader (the complementarity effect) (Naeem et al. 2000, Byun et al. 2013). Higher native diversity could also provide biotic resistance by increasing the probability to have a better competitor or a more productive species present in a more diverse native community, a mechanism known as the sampling effect (Wardle 2001). This hypothesis is largely supported in temperate regions where plant diversity has been experimentally manipulated (Naeem et al. 2000, Hector et al. 2001, Petruzzella et al. 2018), but there is little empirical evidence in tropical freshwater systems (Michelan et al. 2013, Alofs and Jackson 2014).

In line with the biotic resistance hypothesis, stronger resistance to alien species may be expected by phylogenetically more closely related native species. Phylogenetic relatedness of alien species to native species of a community has been used as a metric of niche overlap, and lead to the concept of “ecological similarity” of species (Strauss et al. 2006, Burns and Strauss 2011). Darwin (1859) in *On the origin of species* already proposed that invaders more closely related to native species would be more ecologically similar and therefore have greater niche overlap, leading to greater competition for the same resources (Darwin 1859, Daehler 2001, Burns and Strauss 2011). It is important to highlight that the biotic resistance hypothesis and the ecological similarity concept are not mutually exclusive and can work synergistically (Davies et al. 2011).

The current research bias on biological invasions towards temperate terrestrial ecosystems limits accurate predictions on the establishment and impact of

invasive species on tropical aquatic systems, and therefore also limits the development of the field of invasion ecology in general and our ability to draw robust generalizations across biomes (Pyšek et al. 2008). Here, we address this knowledge gap by presenting the results of an experiment in which we manipulated species diversity and identity of tropical native submerged plant communities, and simulated an invasion by the highly invasive alien submerged rooted plant *Hydrilla verticillata* (L.f.) Royle. *Hydrilla verticillata* is spreading rapidly through tropical and non-tropical areas (Hussner 2012) and has negative impacts on shipping, recreation activities, fisheries, hydropower generation and threatens the ecological integrity of many tropical freshwater ecosystems (Sousa et al. 2009, Sousa 2011). We focused on invader establishment success, here defined as the ability of plant fragments to colonise and grow. We hypothesized that invader establishment success would be (1) greatest in the absence of a native plant community, (2) lower in species-richer plant communities, (3) lowest in the presence of the phylogenetically closely related species *Egeria densa*.

Material and Methods

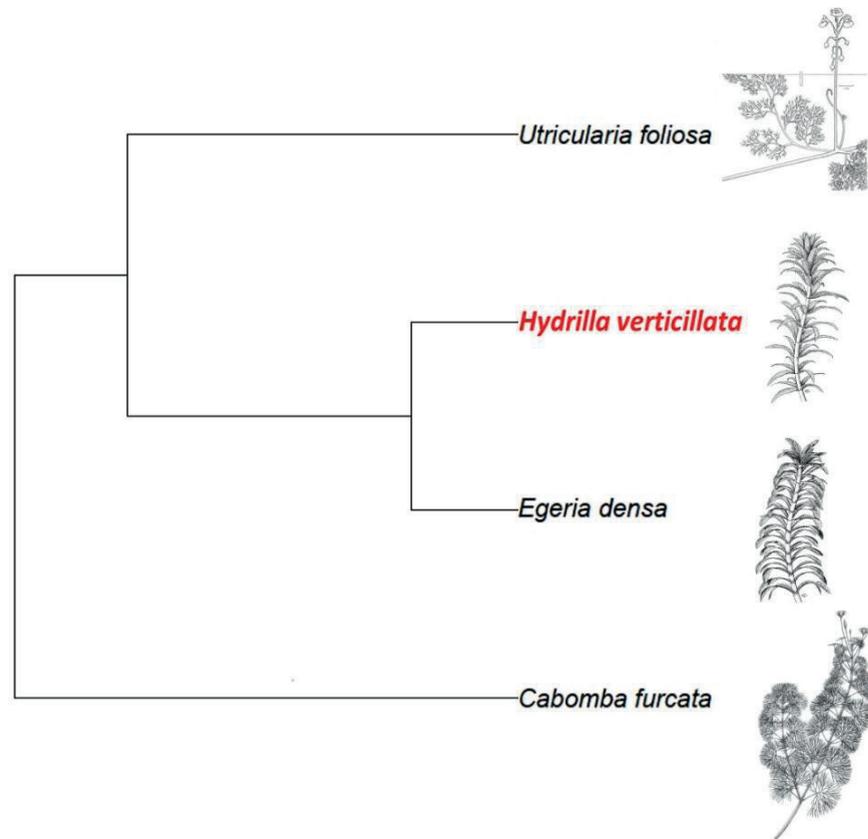
We performed an outdoor mesocosm experiment in the northern region of the State of Rio de Janeiro at Núcleo em Ecologia e Desenvolvimento Sócio-Ambiental de Macaé (NUPEM), Federal University of Rio de Janeiro (UFRJ)- Macaé campus, Brazil (22°19'38.4" S, 41°44'13.41" W). The experiment ran for 10 weeks (71 days) from mid March to the end of May in 2017, during which the mean temperature in this region ranged from 22.7°C to 23.8°C (Instituto Brasileiro de Meteorologia, INMET).

Plant material

We selected the highly invasive rooted submerged aquatic plant *H. verticillata* (Hydrocharitaceae) as invader. *Hydrilla verticillata* is native to Asia and Australia, but globally invades both tropical and temperate inland aquatic ecosystems due to its wide ecological amplitude, high growth rate, vegetative dispersal ability and low nutrient requirements for growth (Cook and Luond 1982, Sousa 2011). It can produce large amounts of vegetative propagules such as turions and fragments, which can float in the water column for days or weeks before settling and rooting (Langeland 1996, Chadwell and Engelhardt 2008). Once *H. verticillata* is established it can rapidly produce large quantities of biomass and displace native macrophyte species (Langeland 1996, Sousa 2011).

To test potential invasion resistance mediated by native plant communities on the establishment success of *H. verticillata* free floating fragments, here defined as the ability of plant fragments to colonise and grow, we established eight different native plant communities using combinations of three submerged macrophyte species: (1) the rooted submerged macrophyte *Cabomba furcata* Schult. & Schult. f. (Cabombaceae), (2) the rooted submerged species *Egeria densa* Planch. (Hydrocharitaceae), phylogenetically closely related to the invader, and (3) the non-rooted submerged species *Utricularia foliosa* L. (Lentibulariaceae) (Fig. 1). All three native species commonly co-occur and are widely distributed in tropical inland freshwater ecosystems of South America (Camargo et al. 2006).

Figure 1. Phylogeny displaying phylogenetic relationships among the four species included in this study based on PhytoPhylo megaphylogeny (Qian and Jin 2016). The invader species *Hydrilla verticillata* is represented in red.



Plant species drawings were modified from Lima et al. (2014) (*C. furcata*), Umetsu et al. (2012) (*H. verticillata* and *E. densa*) and <http://stacyfrank.com> (*U. foliosa*).

The three native plant species also co-occur in the Jurubatiba lagoon, a freshwater coastal lagoon located in the Restinga de Jurubatiba National Park near the university campus (22°17'57.38" S and 41°41'21.27" W). However, because *C. furcata* and *E. densa* densities were low in the lagoon at the time of the experiment

we obtained native plant material from three different locations in Rio de Janeiro State: *C. furcata* from São João River (22°34'54.9" S, 42°18'1.04" W), *E. densa* from Juturnaíba lagoon (22°36'47.9" S, 42°16'30.03" W) and *U. foliosa* from Jurubatiba lagoon. The invader *H. verticillata* was acquired from a commercial plant trader in Rio de Janeiro, Brazil (Flora Aquática). All four plant species were cultivated at the same growth conditions in 310 L water tanks (0.55 m height and 0.75 m diameter, two tanks per species). Each tank was filled with a layer of fertile substrate especially sold for plants in aquaria (7.5 kg/Mega fértil – Box Reef, organic matter content = 16%), a layer of washed sand (30 kg, ≤ 1.0 mm grain size, organic matter content = 0.5%), and water collected from Jurubatiba lagoon (mean \pm SD, $n=6$ water samples, $3.6 \pm 0.4 \mu\text{g L}^{-1}$ P-PO₄; $91.8 \pm 12 \mu\text{g L}^{-1}$ N-NO₃; $0.3 \pm 0.8 \mu\text{g L}^{-1}$ N-NO₂; $19.6 \pm 31.6 \mu\text{g L}^{-1}$ N-NH₄; pH 7.8 ± 0.3). After at least two weeks of cultivation, 183 non-rooted apical fragments without lateral branches were collected randomly from the six cultivation tanks for each of the native species. For *U. foliosa* the entire plant was collected instead of only the apical part. The plant fragments were cut to be 15 cm long and washed in running tap water to remove any material attached. Of the 183 fragments of each native species, 15 were randomly selected, dried to a constant mass at 60°C for at least 48h, and individually weighed for initial biomass measurements. The remaining 168 fragments were used in the experiment.

Experimental set up

We performed a mesocosm experiment with 48 white plastic buckets (20L, 36.7 cm height and 26 cm diameter) filled with a bottom layer of substrate for planted aquaria (Mega fértil – Box Reef, 1 cm/400 g) with a top layer of washed sand (5 cm deep, 4 kg sand per bucket). All mesocosms were placed outdoor in a covered area at NUPEM (Supplementary Figure 1). We filled all mesocosms with water from

Jurubatiba lagoon. Water levels in the mesocosms were maintained constant during the experiment by refilling with lagoon water once a week to compensate for evapotranspiration. The physical and chemical parameters of the water were measured weekly in all mesocosms and the growing conditions were found to be suitable for the plants (mean values at daytime throughout the experiment, mean \pm SD, n=528: water temperature $23.4 \pm 1.6^\circ\text{C}$, dissolved oxygen concentration $8.8 \pm 1.2 \text{ mg L}^{-1}$, pH 7.9 ± 0.8 , n=288: alkalinity $478.1 \pm 126.7 \text{ mEq L}^{-1}$, turbidity $1.8 \pm 4.2 \text{ NTU}$).

To test possible effects of native plant diversity and identity on the invasion potential of *H. verticillata* we established four levels of native plant species richness: zero species (bare sediment), one species (monocultures), and mixtures of two and three species (Table 1). The in total eight treatments were applied in a full factorial design, each replicated six times, using a randomized block design resulting in 48 mesocosms. Initial plant density in the mesocosms was kept constant across treatments at 12 plant fragments per mesocosm, which is equivalent to 240 plants m^{-2} . Thus, mesocosms with two species contained six individuals of each species, whereas those with three species contained four individuals of each species. Such shoot densities are within the range of the shoot densities of submerged macrophyte communities in natural conditions (Li et al. 2015). The plant fragments were planted 5 cm deep in the sediment.

After the native plant community was allowed to establish in the mesocosms (determined by the growth of new shoots of at least one individual of each species, which took two weeks), we introduced two *H. verticillata* fragments per mesocosm. Each fragment consisted of a 15 cm long shoot with apical tip, but no lateral branches, to minimize variation in the size of the initial shoot material. This number of fragments is considered to represent medium propagule pressure (Li et al. 2015). We selected

fragments with an apical tip, because these have higher regeneration and colonization abilities, and higher growth rates than fragments without apical tips (Riis et al. 2009). Invader fragments were not planted in the sediment but were dropped into the mesocosms simulating how alien species arrive in a new area where a native community is already established (Riis and Sand-Jensen 2006). To determine the introduced biomass in dry weight (DW), fifteen *H. verticillata* fragments were randomly selected, dried to a constant mass at 60°C for at least 48h, and individually weighed.

Table 1. Overview of the 8 treatments used in the mesocosm experiment. Bare= Bare sediment, C= *Cabomba furcata*, E= *Egeria densa*, U= *Utricularia foliosa*.

Diversity treatment	Abbreviated community composition	Species	Species richness
1	Bare	Bare sediment	0
2	C	<i>Cabomba furcata</i>	1
3	E	<i>Egeria densa</i>	1
4	U	<i>Utricularia foliosa</i>	1
5	CE	<i>Cabomba furcata</i> + <i>Egeria densa</i>	2
6	CU	<i>Cabomba furcata</i> + <i>Utricularia foliosa</i>	2
7	EU	<i>Egeria densa</i> + <i>Utricularia foliosa</i>	2
8	CEU	<i>Cabomba furcata</i> + <i>Egeria densa</i> + <i>Utricularia foliosa</i>	3

Plant harvest and data collection

We defined invader establishment success in terms of the ability of the fragments to colonize and grow. *H. verticillata* successful colonization was defined as at least one of the introduced fragments having its roots attached in the sediment. At the end of the experiment, i.e. eight weeks (57 days) after the introduction of the invader fragments, we censused the mesocosms to check whether they were successfully colonized or not. *H. verticillata* growth was defined as biomass increase (DW, g). After the census, the invader was harvested, separated in root and shoot aiming to verify biomass allocation, dried to a constant mass at 60° for at least 48h, and weighed. We determined *H. verticillata* total root and total shoot DW by summing the values from both introduced fragments. From these values we calculated the root:shoot ratio and relative growth rate (RGR). The RGR was calculated considering the total biomass of the invader (including roots plus shoots from both fragments) in the mesocosms as follows:

$$\text{RGR} = (\ln W_f - \ln W_i) / t_{\text{days}}$$

where W_f = final DW, W_i = initial DW and t_{days} = time in days. At the end of the experiment, the native plant community biomass was harvested, sorted by species, dried to a constant mass at 60° for at least 48h, and weighed.

Phylogenetic tree construction

In order to show the phylogenetic relatedness among the study species we constructed a phylogenetic tree using the updated megaphylogeny of vascular plants (PhytoPhylo) as a backbone (Qian and Jin 2016). This megaphylogeny was constructed based on seven gene regions (i.e., 18S rDNA, 26S rDNA, ITS, *matK*,

rbcl, *atpB*, and *trnL-F*), which include both slowly and quickly evolving regions. The time scale for the phylogeny was based on 39 fossil calibrations. Both minimum and maximum age constraints were utilized for each fossil calibration. We used S.PhyloMaker package in R developed by Qian and Jin (2016).

Data analyses

We analyzed the colonization success of *H. verticillata* fragments in the experimental mesocosms by comparing the proportion of colonized mesocosms between the bare sediment and planted treatments in a Fisher's exact test. We fitted multiple General Linear Mixed-effects Models (GLMMs) to test how four growth parameters (i.e. root DW, shoot DW, root:shoot ratio and RGR) of the invader depended on (a) the presence of the native community, (b) native species richness (analyzed both including and excluding the bare sediment treatment, i.e. 0 species richness level from the analysis), and (c) the presence of the phylogenetically closely related species *E. densa*, using the *lme* function in the R package *nlme* (Pinheiro et al. 2018). We used Tukey's HSD multiple comparison test to compare means of species identity effect by each single native species. In two separate GLMMs we tested the effects of total native community biomass on total final invader biomass and native species richness on native community biomass. Block (the 6 replicates) was included as random factor in all models. 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. Response variables were log-transformed when necessary. All statistical analyses and graphics were performed in R (R Core Team 2017).

Results

The presence of a native community

The presence of native plants did not decrease invader colonization success, i.e. the ability of the invader having its roots attached in the sediment (Fisher's exact test, odds ratio= 1.869, $p=0.503$). *H. verticillata* had fragments with roots attached to the sediment in 5 out of 6 mesocosms with bare sediment (bare) (83.3% colonised), whereas 38 out of 42 planted treatments, i.e. 90.5% of the mesocosms, were successfully colonised even though a native plant community was present. However, the presence of native plants did suppress *H. verticillata* growth, measured as root biomass (53.5% decrease), shoot biomass (27.5% decrease), root:shoot ratio (42.7% decrease), and RGR (18.9% decrease) (Table 2a).

Effects of native community diversity and biomass on biotic resistance

Native species richness significantly decreased invader root and shoot biomass, root:shoot ratio and RGR (Table 2b; Orange solid line - Fig. 2.a-d). However, when we excluded the bare sediment treatment from our analysis there was no effect of species richness on growth of *H. verticillata* (Table 2b; Black dashed line - Fig. 2.a-d). Native community biomass affected total final invader biomass significantly negatively (Fig. 3), whereas native plant biomass was not related to native species richness ($t = 0.44$, $P = 0.6613$). Total final native community biomass for all treatments is shown in Table 3.

Table 2. Results of General Linear Mixed effect models testing how four different growth parameters of the invader species *Hydrilla verticillata* depended on (a) the presence of a native community, (b) native species richness (data shown in Fig. 2) and (c) the presence of the phylogenetically related species *Egeria densa*. Block is included as a random factor.

Fixed effects	Root DW			Shoot DW			Root:shoot			RGR		
	d.f.	t	P	d.f.	t	P	d.f.	t	P	d.f.	t	P
(a) Presence of native community	41	-4.85	<0.001	41	-3.15	0.003	41	-3.63	<0.001	41	-3.34	0.002
(b) Species richness (including bare sediment)	41	-3.56	<0.001	41	-2.31	0.026	41	-3.07	0.004	41	-2.23	0.031
Species richness (excluding bare sediment)	35	-1.40	0.171	35	-0.48	0.633	35	-1.20	0.238	35	-0.22	0.825
(c) Presence of <i>Egeria densa</i>	41	-2.26	0.029	41	-1.51	0.139	41	-1.66	0.105	41	-1.24	0.221

Significant effects ($p < 0.05$) are in bold. (log) means the response variable was log-transformed. Root DW= Root dry weight, Shoot DW=Shoot dry weight, Root:shoot= Root:shoot ratio, RGR= Relative

Figure 2. Effect of native plant species richness on the invader *Hydrilla verticillata* (a) root biomass (dry weight), (b) shoot biomass (dry weight), (c) root:shoot ratio, (d) relative growth rate (RGR). The orange solid lines are based on GLMMs that include the bare sediment treatments (0 species level) in the analysis, and are all statistically significant (Table 2). Black dashed lines are without including bare sediment (0 species level) in the analyses and are all not significant (Table 2). Each dot represents a replicate.

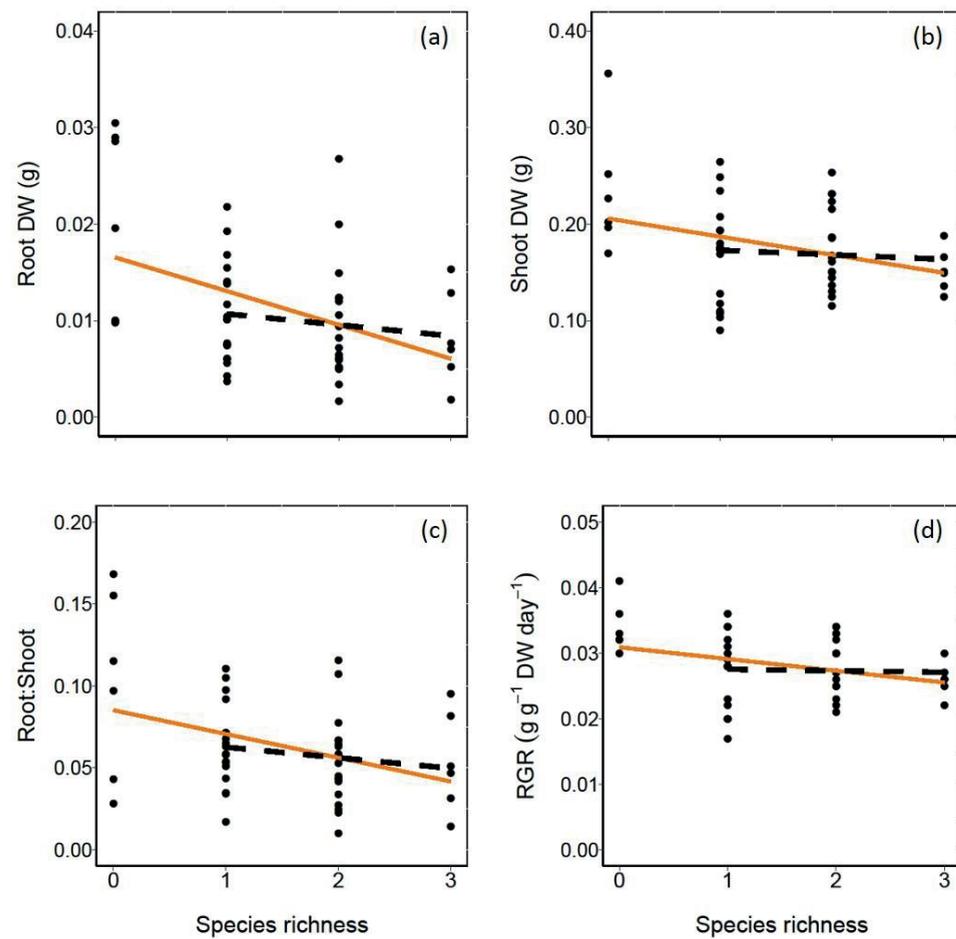
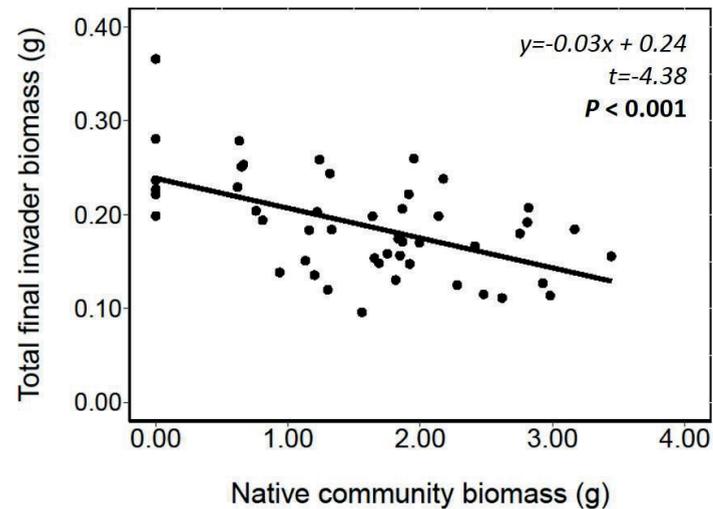


Table 3. Final mean native community biomass dry weight (g) across different treatments. Bare= Bare sediment, C= *Cabomba furcata*, E= *Egeria densa*, U= *Utricularia foliosa*.

Diversity treatment	Abbreviated community composition	Native community biomass (g) Mean \pm SD, n=6
1	Bare	0 \pm 0
2	C	0.69 \pm 0.08
3	E	2.86 \pm 0.19
4	U	1.67 \pm 0.57
5	CE	1.89 \pm 0.18
6	CU	1.26 \pm 0.24
7	EU	2.29 \pm 0.60
8	CEU	1.88 \pm 0.56

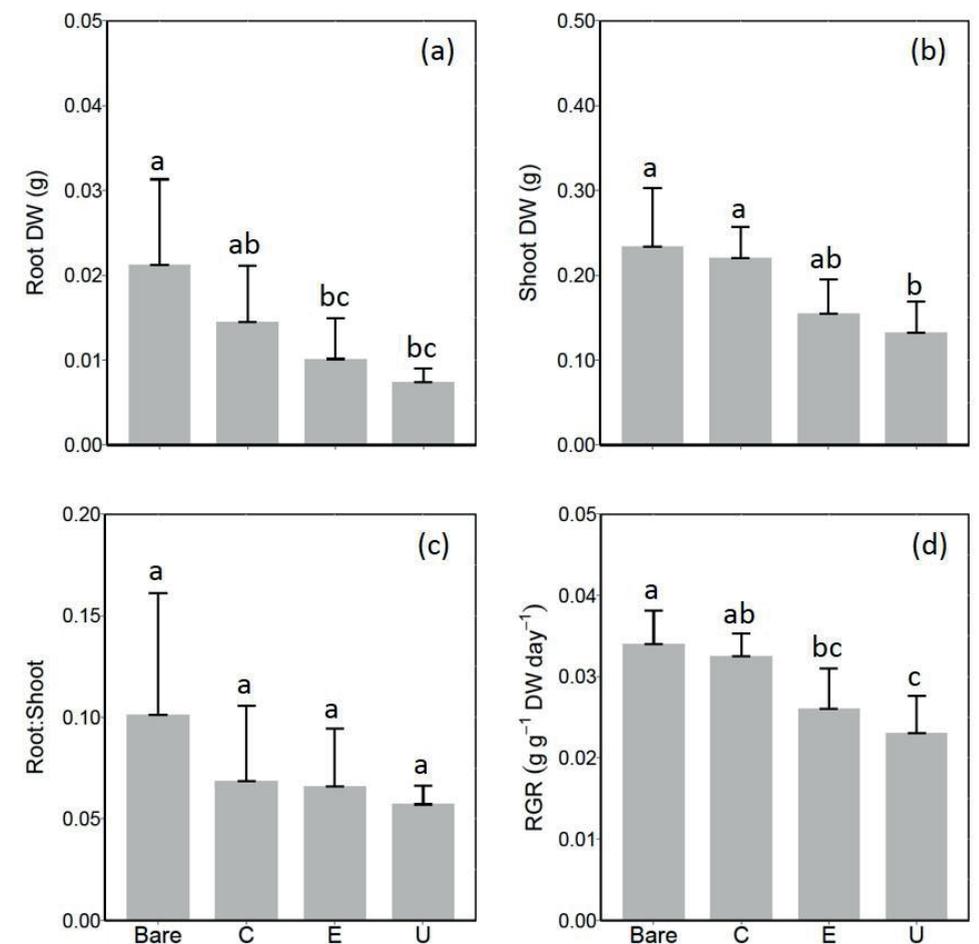
Figure 3. Relationship between the native community biomass and the total final biomass DW (dry weight, g) of the invader *Hydrilla verticillata*.



Limited effect of phylogenetic closely related species

The presence of the phylogenetically most closely related species *E. densa* in the native community significantly reduced invader root production, but shoot biomass, root:shoot ratio and RGR were not affected (Table 2c). Native monocultures generally reduced invader growth, but the effect of *E. densa* monocultures was not stronger than of monocultures of *C. furcata* or *U. foliosa* (Fig. 4a-d). Only *U. foliosa* monocultures significantly decreased *H. verticillata* growth compared to the bare sediment treatment (Fig. 4a, b, d). However, their slightly negative effect did not significantly differ from the other plant species monocultures either (Fig. 4a, b, d) (with the exception of the *C. furcata* treatment in which *H. verticillata* RGR was as large as in the bare sediment treatment). Root:shoot ratios of the invader were similar in all monocultures and the bare sediment treatment.

Figure 4. Effects of single native species on the invader *Hydrilla verticillata* (a) root biomass (dry weight), (b) shoot biomass (dry weight), (c) root:shoot ratio and (d) relative growth rate (RGR). Bare- Bare sediment, C- *Cabomba furcata*, E- *Egeria densa*, U- *Utricularia foliosa*. Different lowercase letters indicate statistically significant differences among treatments (Tukey's HSD post hoc test). Significance levels were determined after Bonferroni's correction for multiple testing ($P < 0.008$). The values are means \pm standard error of the mean (n=6).



Discussion

Competition from native species can decrease the establishment success of alien species (Levine et al. 2004). Evidence for this mechanism originates predominantly from temperate terrestrial ecosystems, despite an increasing pressure of invasive species on megadiverse aquatic tropical systems (Lövei et al. 2012). We experimentally found that communities of native submerged tropical plants can have negative effects on the growth of the highly devastating invading species *H. verticillata*, but that this does not prevent its colonization. Accordingly, we found that native community biomass negatively affected invader growth. Native productivity however, was not related to species richness in the community. Contrary to previous views, our results did not support the prediction that native species diversity provides greater resistance to plant invasions in tropical freshwater. The presence of the most closely related and ecologically similar native species (*E. densa*) also had only a limited effect on *H. verticillata* growth. Our study illustrates that resistance of tropical native freshwater plant communities to invasive species contrasts to resistance described for other ecosystems.

In our first hypothesis we predicted that invader establishment success, defined as the ability of invader fragments to colonize and grow, would be greatest in the absence of a native community. The proposed mechanism was that the presence of native plants should directly reduce resource availability for the invader in terms of nutrients or space, as observed for species in temperate systems (Petruzzella et al. 2018). However, the presence of the native community did not affect *H. verticillata* colonization success (almost 90% of all mesocosms were colonized), despite that the native plants formed dense canopies in our experiment resulting from the uptake of available nutrients. We observed that invader fragments did not need to sink towards the sediment to attach their roots, as observed in other

species of the family Hydrocharitaceae (Petruzzella et al. 2018). Instead, they started to produce long adventitious roots, which are fine and filiform (Umetsu et al. 2012), when they were still floating on the water surface. The roots attached to the sediment after growing in between the shoots of the native plants (Supplementary Figure 2). Fragments of *H. verticillata* take more time to sink than fragments of other species (Wu et al. 2007), but this ability to efficiently produce long roots while trapped in the native vegetation canopy gives them a competitive advantage to rapidly access nutrients in the sediment. Successful colonization by *H. verticillata* is highly dependent on nutrient availability, as in a previous mesocosm experiment invader fragments could not colonize due to nutrient drawdown by the native plants (Chadwell and Engelhardt 2008). Root production may explain why this species is a strong colonizer in the field, as *H. verticillata* fragments were observed to colonize wetlands despite the presence of the native species *Vallisneria americana* Michaux (Chadwell and Engelhardt 2008) and *Potamogeton pectinatus* (L.) Boemer or *Potamogeton gramineus* L. (Spencer and Rejmánek 1989). We therefore partially reject our first hypothesis that the presence of native vegetation would decrease *H. verticillata* colonization, although it is supported regarding the ability of the invader to grow. The presence of a native plant community notably lowered root production of *H. verticillata* (53.5% decrease), so this could on the longer term at least slow down the colonization process. This is found for a tropical invasive Poaceae species *Urochloa arrecta* (Hack. ex T. Durand & Schinz) Morrone & Zuloaga, which growth is limited by the native plant species but not sufficiently to completely repel the invader (Michelan et al. 2013).

Our second hypothesis follows Elton's prediction that species-richer communities are more resistant to invasion than species poorer-communities (Elton 1958), as has been shown in a number of terrestrial and marine communities (Naeem

et al. 2000, Stachowicz et al. 2002, Fargione and Tilman 2005, Marraffini and Geller 2015). However, only few studies have investigated this principle in freshwater systems, and even fewer in tropical systems (Michelan et al. 2013, Alofs and Jackson 2014, Petruzzella et al. 2018). We did not find a relationship between native species richness and invader growth, although this depended on whether we included the bare sediment treatment in the analysis, as a treatment with zero species. If we did, we found a negative relationship between native species richness and growth parameters, whereas if we excluded the bare sediment treatment there was no relationship between native species richness and invader growth. Clearly, this indicates that this pattern is driven by the presence of native plants, but independent of the native community diversity.

Few studies to date have acknowledged the potential that the densities and biomass of native plants may be more important for providing invasion resistance than native species richness *per se* in freshwater ecosystems (Capers et al. 2007, Michelan et al. 2013, Petruzzella et al. 2018, Yu et al. 2018). An underlying mechanism of increased resistance to invader growth with increasing native species richness could be that communities with higher species richness have a higher productivity, and thus provide more resource competition through complementarity and/or sampling effects. Indeed, in our study invader growth was negatively affected by greater biomass production of native species, suggesting an underlying mechanism of increased resource competition. However, in our study, there was no relationship between native species richness and native biomass production, explaining the absence of increased resistance at higher native plant species richness. Therefore, we reject our second hypothesis.

Egeria densa and *H. verticillata* are species of the same family Hydrocharitaceae with similar ecological strategies and growth form (Sousa 2011).

Although there is debate whether close relatives are more likely to have similar niches (Vamosi et al. 2009, Cavender-Bares et al. 2009), phylogenetic relatedness has been used as proxy for missing functional trait information, especially when using understudied tropical species. We expected lower invader establishment success in the presence of the closely related species *E. densa* (hypothesis 3). However, our study provides little support for the prediction that closely related species will compete more strongly than more distantly related species, hence we reject our third hypothesis. Therewith our study is in line with other studies partly supporting and partly rejecting this hypothesis over the last decade (Strauss et al. 2006, Thuiller et al. 2010).

Our general understanding of biological invasions can be greatly advanced by coupling knowledge on invasions in all ecosystems worldwide. Our study highlighted that the ability of tropical native freshwater aquatic plant communities to resist invasive species may differ in some aspects from what is found in most other ecosystems (Levine et al. 2004, Kimbro et al. 2013). These contrasts are important to understand when aiming to generalize patterns of species invasiveness and ecosystem invasibility across biomes. In contrast to most studies performed at small spatial scales in terrestrial ecosystems (Levine et al. 2004), we found biomass to be more important for resistance to invasions than species richness *per se*. This is in line with the few previous studies on biotic resistance in freshwater ecosystems: even though some found a species richness effect on the invader success, most highlighted a crucial role of native species biomass for resistance (Michelan et al. 2013, Petruzzella et al. 2018, Yu et al. 2018). However, native productivity was not driven by native diversity. This suggests a mechanistic difference between aquatic and terrestrial ecosystems that warrants further investigation. Even though we should be cautious about these considerations due to the low number of studies performed, this confirms

the view that successful invasions are very context-dependent (Ricciardi and Atkinson 2004, Ricciardi et al. 2013). A system-specific understanding of community invasibility seems crucial for prediction and management of invasive species.

The intrinsic nature of tropical freshwater ecosystems, together with their importance and demand from a human perspective, may place them at greater risk of invasions than their temperate and terrestrial counterparts. Even though native plant biomass can decrease invader growth, it seems unable to repel establishment. Current intensification of transportation and trade in tropical areas increases the risk of new invader introductions in coming decades (Lövei et al. 2012). Together with great human-induced pressure, particularly overexploitation and habitat degradation and destruction which has been accelerating aquatic vegetation loss (Zhang et al. 2017), highlights the need to further study high susceptibility of tropical ecosystems to biological invasions.

Acknowledgments

This is collaborative research between the Netherlands Institute of Ecology (the Netherlands) and Núcleo em Ecologia e Desenvolvimento Sócio-Ambiental de Macaé (NUPEM/UFRJ, Brazil). We sincerely acknowledge the help of a dedicated field technician João Marcelo Silva de Souza, all colleagues from Laboratório Integrado de Ecologia Aquática (NUPEM), Vagner Leonardo M. Dos Santos, students of Laboratório de Limnologia (UFRJ/RJ), and Nico Helmsing for field and laboratory assistance. We also thank Rosanne Beukeboom for helping to construct the phylogenetic tree included in this paper. AP specially acknowledges the Science without Borders Program and CNPq (Brazilian National Council for Scientific and

Technological Development) through a grant no. 207514/2014-3. We also thank ICMBio for the license permits to collect the plants (ICMBio/SISBIO 57269).

Supplementary material

Supplementary Figure 1. Overview of the experiment (photo taken March 2017)



Supplementary Figure 2. Photograph of *Hydrilla verticillata* fragments producing long adventitious roots, which are fine and filiform, when they were still floating on the surface and attached to the sediment among the shoots of the monoculture of the native submerged species *Cabomba furcata*.



Chapter 4

Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems

Antonella Petruzzella, Bart M. C. Grutters, Sidinei M. Thomaz and
Elisabeth S. Bakker

Aquatic Invasions (2017) 12(3):343-353

doi: 10.3391/ai.2017.12.3.08



Abstract

Invasions of tropical and subtropical aquatic plants threaten biodiversity and cause ecological and economic impacts worldwide. An urgent question is whether native herbivores are able to inhibit the spread of these alien species thus providing biotic resistance. The potential for biotic resistance to these plants depends on plant traits that affect palatability to herbivores, i.e., plant nutritional quality and the presence of secondary metabolites related to anti-herbivory defenses. Studies across latitudinal gradients suggest that aquatic plants from lower latitudes may be less nutritious and better defended than high latitude plants. Therefore, we hypothesized that native herbivores prefer temperate plants over plants from tropical and subtropical regions which would limit the strength of biotic resistance that native temperate herbivores can provide against alien tropical and subtropical plants. Drawing upon the published literature we (1) investigated whether native temperate herbivores reduce the establishment or performance of tropical and subtropical alien plants in the field, and (2) analyzed herbivore consumption of tropical and subtropical versus temperate plants in laboratory feeding trials. In our literature survey, we found only three field studies which in contrast to our hypothesis all demonstrated that the native herbivores (beavers, coots and generalist insect herbivores respectively) significantly reduced the success of invading tropical and subtropical plant species. The analysis of the feeding trials yielded mixed results. Ten out of twelve feeding trials showed that (sub)tropical and temperate plants were consumed in equal amounts by both temperate and tropical generalist ectothermic herbivores. The remaining trials showed a higher consumption rate of temperate plants by a temperate snail and tropical plants by a tropical snail respectively. Although a body of evidence suggests that tropical plants are nutritionally poor and better defended (i.e., less palatable)

compared to temperate plants, we conclude that in the majority of cases, herbivores would eat tropical plants as much as temperate plants. Thus, in agreement with the available field studies, evidence suggests that there is potential for biotic resistance from native generalist herbivores to tropical invasive plants in non-tropical areas.

Key words: Consumption rate, feeding trial, herbivore trait, herbivory, latitudinal gradients, palatability, plant traits

Introduction

Aquatic plant invasions threaten biodiversity and cause ecological and economic impacts worldwide (Vilà et al. 2011, Hussner 2012). Invasive aquatic plants can change community structure and composition, decrease biodiversity of aquatic communities, alter nutrient cycling and the abiotic environment, and have a negative impact on ecosystem services to humans, harming for example, fishing, navigation, recreation activities and the esthetic value of aquatic ecosystems (Charles and Dukes 2007, Michelan et al. 2010, Vilà et al. 2011, Brundu 2015, Thomaz et al. 2015). Tropical and subtropical aquatic plants are among the world's worst invasive species (Rejmánek 2011). Notorious examples include the floating plants *Eichhornia crassipes* (Mart.) Solms, 1883, *Pistia stratiotes* L., 1753 and *Salvinia* spp., and the submerged plants *Egeria densa* Planch., 1849, *Hydrilla verticillata* (L.f.) Royle, 1839, and *Myriophyllum aquaticum* (Vell.) Verdec, 1973 (Hussner 2012, Thomaz et al. 2015).

Although freshwater ecosystems are the most threatened compared to terrestrial and marine ones (WWF 2016), and despite the prevalence of tropical invasive freshwater plant species, most invasion ecology research has been conducted in terrestrial ecosystems (Jeschke et al. 2012b, Lowry et al. 2013). Moreover, the majority of studies focus on temperate species and temperate areas (Lowry et al. 2013, Evangelista et al. 2014). Therefore, the factors that may limit the success of the worst invasive freshwater plant species, which often originate from tropical and subtropical areas and are invasive elsewhere, remain largely unknown (Liu et al. 2006, Coetzee et al. 2011, Brundu 2015).

Tropical and subtropical alien plants have mainly spread due to the aquarium and ornamental trade (Martin and Coetzee 2011, Hussner 2012). Although many of these species are introduced to a new range, only a few become invasive. The failure of some alien species in either establishing or spreading into a new habitat is often attributed to biotic resistance (Elton 1958, Levine et al. 2004). Biotic resistance is mainly determined by ecological interactions in two layers of the food web: competition with native vegetation and consumption by native herbivores. A recent meta-analysis found that in freshwater environments biotic resistance is driven by consumption rather than competition (Alofs and Jackson 2014). However, despite the relevance of herbivory for biotic resistance (Levine et al. 2004, Alofs and Jackson 2014, Wood et al. 2017), few studies have investigated its importance in reducing the abundance of tropical and subtropical alien plant species in the field. Furthermore, most of these studies focused on biological control, i.e., the introduction of alien herbivores to reduce invading plant species (Marko et al. 2005, Tewari and Johnson 2011, Coetzee et al. 2011, Walsh and Maestro 2016). Hence, the potential of native herbivores to provide biotic resistance to tropical and subtropical alien aquatic plants is generally unexplored.

In this study, we assess whether native herbivores can provide biotic resistance to the establishment and growth of tropical and subtropical alien aquatic plants. We consider in particular the case where tropical and subtropical plants invade non-tropical communities in which native temperate herbivores may feed on these plants and thereby prevent their establishment or reduce their performance. Biotic resistance from native herbivores will be even more effective if the herbivores prefer feeding on alien over native plants, since they will both suppress alien plants and give the native plants a competitive advantage (Van Donk and Otte 1996, Parker et al. 2006). The potential for biotic resistance to alien plants depends on plant traits that

affect palatability to herbivores. Here, we consider the two most important traits: plant nutritional quality (often expressed as plant C:N ratios or %N) and the presence of secondary metabolites related to anti-herbivory defenses (Cronin et al. 2002, Dorenbosch and Bakker 2011, Gross and Bakker 2012). Studies across latitudinal gradients suggest that aquatic plants at lower latitudes may be less nutritious and better defended than high latitude plants (Pennings et al. 2001, 2009, Morrison and Hay 2012). Therefore, we hypothesize that herbivores native to temperate regions prefer temperate plants over plants from tropical and subtropical regions which would limit the strength of biotic resistance that temperate native herbivores can provide against tropical and subtropical alien plants.

We draw upon the published literature to compile multiple lines of evidence to estimate the potential for biotic resistance by native temperate herbivores to tropical and subtropical aquatic plant invasions. We (1) investigated whether native temperate herbivores reduce the establishment or performance of tropical and subtropical alien plants in the field and (2) analyzed herbivore consumption of tropical and subtropical versus temperate plants in laboratory feeding trials. We summarize our findings by predicting the scope for biotic resistance to tropical and subtropical macrophyte invasions based on plant and herbivore traits, identifying research gaps and proposing future directions to test the biotic resistance hypothesis.

Material and Methods

Literature searches

Field studies

We searched the literature for field studies that tested biotic resistance by native temperate herbivores to tropical and subtropical alien aquatic plant species. First, we searched for studies in previous meta-analyses (Parker et al. 2006, Alofs and Jackson 2014, Wood et al. 2017). In addition, we carried out a literature search in the ISI Web of Science (1945 – April 2017) using the following combination of keywords ("biotic resistance" OR "biotic resistance hypothesis" OR "diversity-invasibility hypothesis" OR "invasion resistance") AND (plant* OR "aquatic plant*" OR macrophyte* OR producer OR vegetation), which yielded 639 journal articles. From this set, we considered field studies that reported measurements of establishment and performance of tropical and subtropical alien aquatic plant species in the presence and absence of native herbivores.

Feeding trials

We searched the publications on no-choice feeding trials with aquatic herbivores that measured the amount of aquatic plants consumed (e.g., consumption rate: g plants consumed $\text{g}^{-1}(\text{animal weight}) \text{day}^{-1}$; animals feeding: %). We included in our analysis only studies that included both temperate and tropical or subtropical aquatic plant species.

Aquatic plants and documentation of their native distribution

The aquatic plants considered in this study are vascular aquatic photosynthetic organisms that depend on humid environments to survive, including hydrophytes and amphibious plants, but also some helophytes "whose photosynthetically active parts are permanently, or at least for several months each year, submerged or floating on the water surface" (Cook et al. 1974). They colonize a variety of running waters and wetlands, such as swamps and salt marshes, lakes, coastal lagoons and floodplains.

We documented the native latitudinal range of the plants used in the selected feeding trials based on the literature (Stuckey 1974, Orchard 1979, Hussner 2012, Grutters et al. 2017a, Redekop et al. 2018) and online databases (Agricultural Research Service - Germplasm Resources Information Network (ARS-GRIN), eFloras, International Union for Conservation of Nature's Red List of Threatened Species (IUCN Red List), Global Biodiversity Information Facility (GBIF), New Zealand Plant Conservation Network (NZPCN) and United States Department of Agriculture (USDA) – Natural Resources Conservation Service). We classified the latitudinal range of species based on whether their native distribution mainly lies in frost-free (tropical and subtropical, hereafter referred to as "Tropical") or mainly in frost-prone (hereafter referred to as "Temperate") regions (Supplementary Table 1).

Data analyses

We re-analyzed the results of the studies that performed feeding trials to specifically test whether herbivore consumption rates differed on tropical versus temperate plants. For each study, we classified the plant species used according to

Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems | 103

their native distribution. Species whose latitudinal range could not be established owing to their wide native distribution, as well as macro-algae species, were excluded from the analyses. We defined a feeding trial as a separate experiment in which the consumption of multiple temperate and tropical plant species by a single herbivore species was measured. One study could therefore include more than one feeding trial: for example, Parker and Hay (2005) performed three feeding trials, testing consumption rates by three different species of herbivores. Each feeding trial was analyzed separately because the consumption rate measurements were not directly comparable on a single axis due to the different parameters measured in the studies. Thus, for each feeding trial, we tested differences between means of consumption of tropical and temperate plant species groups using a non-parametric Wilcoxon Rank-Sum test. The mean group consumption of tropical versus temperate plant species was calculated by extracting the mean consumption of each plant species from the original papers and averaging these over the number of tropical plant species and the number of temperate plant species used in the respective feeding trial. When the same plant species was measured multiple times in the same feeding trial, we averaged their mean consumption rate to determine a single value per species. The mean consumption rate per plant species could be extracted directly from papers when they were given in Tables, or using DataThief software when they appeared in graphs or figures. Statistical analyses were performed in R version 3.3.2. Data are available in Online Supplementary material Table S3, S4 and S5.

Results

Field studies testing biotic resistance to tropical plants

We found only three studies that addressed the effect of native herbivores on tropical invasive plant species in the field. All three found evidence that native herbivores may contribute to reducing invasion success.

Parker et al. (2007) excluded the native North American beaver (*Castor canadensis* (Kuhl, 1820)) from two wetlands near Atlanta, Georgia, USA (33°54'N, 84°26'W) over 2 years. Total aquatic plant biomass was reduced by 60% through beaver herbivory compared to the exclosures, which also resulted in severely altered plant species composition. Beavers demonstrated feeding selectivity as they reduced the abundance of the South-American alien plant species *M. aquaticum* in particular by nearly 90%. This species was 7.9 fold more abundant in exclosures compared to beaver foraging areas.

Another exclosure study demonstrated that herbivory by American coots (*Fulica americana* (Gmelin, 1789)) had negative impacts on the invasive Eurasian *H. verticillata* in Lake Fairfield, Texas, USA (31°47'N, 96°03'W) during periods of high wetland bird activity (Esler 1989). After six months, *H. verticillata* biomass was over three-fold greater in exclosures compared to plots accessible by coots.

In China, generalist native insect herbivores were found to disproportionately reduce the abundance of the invasive South-American *Alternanthera philoxeroides* (Mart.) Griseb relative to native wetland plant vegetation (Fan et al. 2016). Exclosure studies on islands in Liangzi Lake (30°16'N and 114°34' E) revealed that shoot biomass of *A. philoxeroides* was almost twice as high after five months compared to plots open to native insect herbivores. *Alternanthera philoxeroides* is consumed by

Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems | 105

the native insect *Atractomorpha sinensis* (Bolívar, 1905), the larvae of *Cassida nebulosi* (Linnaeus, 1758), *Spoladea recurvalis* (Fabricius, 1775), *Pieris rapae* (Linnaeus, 1758) and some aphids and spiders (Fan et al. 2016). The higher consumption of *A. philoxeroides* compared to the native plant species was positively correlated with its higher leaf nitrogen concentration.

Feeding trials

We found nine studies that included twelve feeding trials that included both temperate and tropical plants that determined aquatic herbivore consumption rates on aquatic plants. We found that herbivores generally seemed not to consume more temperate than tropical aquatic plants (Fig 1). This was valid across a range of herbivores varying from invertebrates, such as four species of snail (*Pomacea insularum* (d'Orbigny, 1839), *Pomacea canaliculata* (Lamarck, 1822), *Radix swinhoei* (Adams, 1866), *Lymnaea stagnalis* (Linnaeus, 1758)), two species of crayfish (*Procambarus spiculifer* (LeConte, 1856), *Procambarus acutus* (Girard, 1852)), and two species of insects (*Parapoynx stratiotata* (Linnaeus, 1758), *Hygraula nitens* (Butler, 1880)); and one vertebrate species (grass carp *Ctenopharyngodon idella* (Valenciennes, 1844)). These studies were conducted at a variety of latitudes (e.g, from temperate North America to tropical Asia) and with very different selections of plant species. Across all twelve feeding trials presented in Figure 1, in only one was the mean consumption rate on temperate plants significantly higher than that of tropical plants, by the tropical herbivorous snail *P. canaliculata* ($n_{\text{temperate}}=23$, $n_{\text{tropical}}=15$, $W=248$, $P=0.02$, Fig. 1 panel 3). However, another feeding trial showed the opposite pattern in that the consumption rate of the tropical snail *P. insularum* was higher on tropical than on temperate plants ($n_{\text{temperate}}=3$, $n_{\text{tropical}}=8$, $W=8$, $P=0.04$)

(Fig. 1, panel 2). In the other ten feeding trials, there was no difference between temperate and tropical plant consumption rates (Fig. 1 all other panels), regardless whether the herbivores themselves were predominantly tropical or temperate (Table 1).

Figure 1. Summary of no-choice feeding trials showing average consumption rates (mean \pm SE) on aquatic plants from temperate (dark grey bars) and tropical (including subtropical) latitudinal range, here referred to as tropical (light grey bars). Each graph corresponds to one feeding trial (see Table 1 for the number of temperate and tropical plant species included in each feeding trial). Animal symbols indicate the functional group of plant consumers used in the feeding trials, but they do not reflect the individual species. See Table 1 for details on the individual feeding trials. A non-parametric Wilcoxon Rank-Sum test was used to test for differences in consumption between temperate and tropical plant species in each panel. Data are available in Online Supplementary material Table S3, S4 and S5.

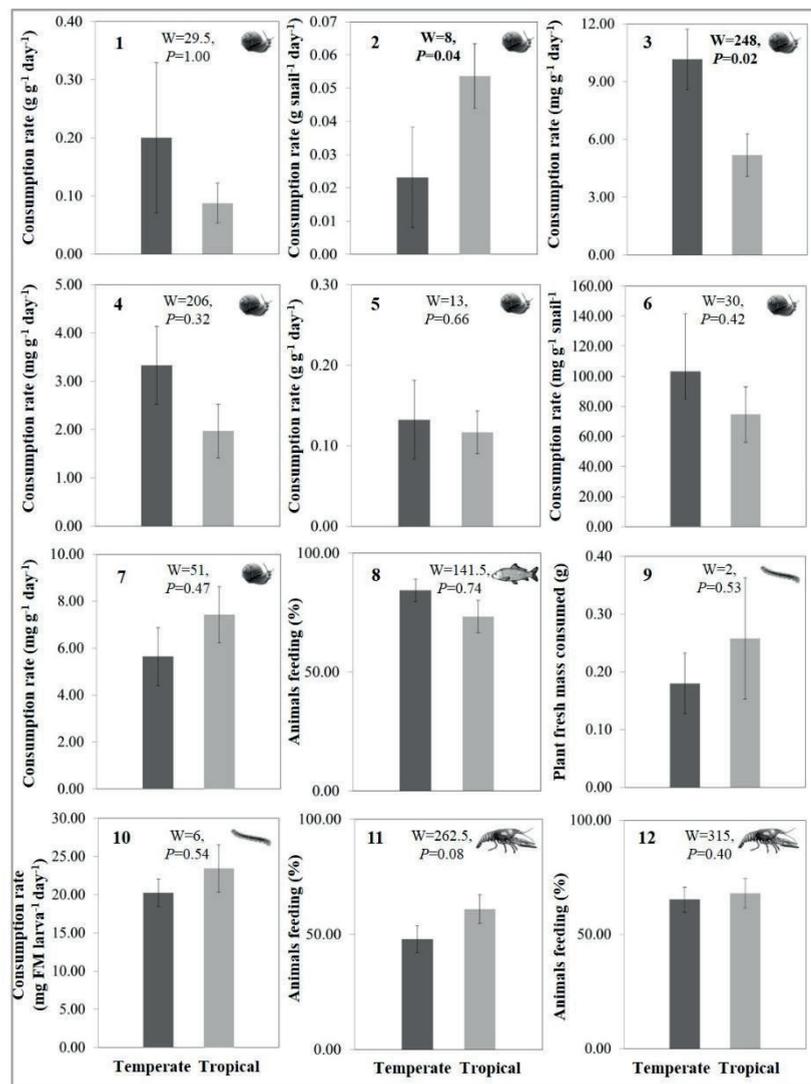


Table 1. Summary of the data presented in Figure 1, indicating the feeding trial studies from which the data were extracted, names of herbivore species, their native distribution and number of temperate and tropical plant species included in the analysis. The native distribution of individual plants species is given in Supplementary Table 1.

Graph number	Feeding trial studies	Herbivore species	Native distribution of herbivore	Number of temperate plant species included	Number of tropical plant species included*
1	Baker et al. (2010)	<i>Pomacea insularum</i>	Tropical and subtropical America	5	12
2	Burlakova et al. (2009)	<i>Pomacea insularum</i>	Tropical and subtropical America	3	8
3	Grutters et al. (2017)	<i>Pomacea caniculata</i>	South America	23	15
4	Grutters et al. (2017)	<i>Lymnaea stagnalis</i>	Europe and Asia	23	15
5	Qiu and Kwong (2009)	<i>Pomacea caniculata</i>	South America	3	7
6	Wong et al. (2010)	<i>Pomacea caniculata</i>	South America	3	15
7	Xiong et al. (2008)	<i>Radix swinhoei</i>	Asia	9	14
8	Parker and Hay (2005)	<i>Ctenopharyngodon idella</i>	Asia	12	22
9	Grutters et al. (2016)	<i>Paraponyx stratiotata</i>	Europe	6	3
10	Redekop et al. (2016)	<i>Hygraula nitens</i>	Australasia	2	4
11	Parker and Hay (2005)	<i>Procambarus spiculifer</i>	North America	28	26
12	Parker and Hay (2005)	<i>Procambarus acutus</i>	North America	28	26

*: including subtropical species (see methods).

Discussion

Herbivory is an important biotic process in aquatic ecosystems because it regulates plant abundance and may limit invasion success of aquatic plants (Alofs and Jackson 2014, Bakker et al. 2016, Wood et al. 2017). We found that field studies that tested whether consumption by native herbivores can limit or decrease the establishment and growth of tropical alien aquatic plants are scarce as most studies focus on temperate species (Evangelista et al. 2014, Alofs and Jackson 2014). Even though our very broad search of plant invasion and biotic resistance yielded 639 hits, only 3 provided field tests including native herbivores and tropical aquatic plant species. In contrast to our hypothesis, these studies found that native beavers, coots and insects significantly reduced the success of invading plant species. The analysis of laboratory feeding trials showed that tropical plants were consumed in equal amounts as temperate plants in ten out of twelve trials. In one study, tropical plants were consumed more than temperate plants and in one study herbivores consumed more temperate plants. We conclude that in the majority of cases, herbivores would eat tropical plants as much as temperate plants, and thus may be able to provide biotic resistance to tropical plant invasions, which is also in contrast to our hypothesis.

Plant perspective

Herbivore consumption rate and preference depends on plant palatability which is determined by multiple plant traits and their interactions (Agrawal 2011, Grutters et al. 2017a). The main plant traits that determine aquatic plant palatability are the plant nutritional value and presence of anti-herbivore defenses (Cronin et al. 2002, Sotka et al. 2009). Plants with higher nutritional quality tend to support higher

consumption rates (Wong et al. 2010, Dorenbosch and Bakker 2011, Bakker and Nolet 2014) and better herbivore performance, such as faster growth, longer survival and higher fecundity (Lach et al. 2000, Ho and Pennings 2013, Grutters et al. 2016). Similarly, in the field the higher consumption of the alien *A. philoxeroides* by native insects compared to native plants coincided with its higher leaf nitrogen concentration (Fan et al. 2016).

Plant nutrient concentration and defenses differ systematically along a latitudinal gradient. Tropical aquatic plants are exposed to high temperatures, which directly influences the length of the growing season and the plant's physiology, which usually results in a low nutritional content (Reich and Oleksyn 2004), which may make the plants less attractive for herbivores. Accordingly, Morrison and Hay (2012) found that two crayfish species strongly preferred high latitude over low latitude plants after they removed the plants' structural characteristics by grinding them. Similarly, Moles et al. (2011) showed a significant herbivore preference for plant material from higher latitudes in 27 (59%) of the 48 studies which they analyzed.

Whereas several studies that we analyzed used feeding trials to investigate whether herbivores prefer native or exotic non-native plants (Lach et al. 2000, Xiong et al. 2008, Burlakova et al. 2009, Qiu and Kwong 2009, Baker et al. 2010), only one study was designed to investigate whether herbivores prefer temperate or tropical plants (Grutters et al. 2017a). In this study, both generalist herbivores consumed more temperate than tropical plants, which was positively related to the plant nitrogen content and negatively to the plant total phenolic compounds (Grutters et al. 2017a).

Hence, the general patterns in plant traits suggest lower palatability of tropical plants relative to temperate plants (Table 2). However, overall, we found that herbivores seem not to consume more temperate than tropical aquatic plants in the

feeding trials but consumed them mostly in equal amounts. Given the fact that apart from Grutters et al. (2017a) none of the studies aimed to test the consumption rates on tropical versus temperate plants, variation in the data due to unequal and sometimes small sample sizes for temperate and tropical plants, could possibly explain the lack of consistent patterns. The field studies demonstrated strong consumption of tropical plants by temperate herbivores, but here only one tropical species per study was available to the herbivores. Furthermore, the herbivores were different among the field studies and differed from those used in the feeding trials. The traits of the herbivores may also play a role in their potential to provide biotic resistance.

Table 2. Properties of plants and herbivores and their influence on the potential for biotic resistance by native temperate herbivores to tropical and subtropical plant invasion.

Property	Contribution to potential for biotic resistance at high latitude	Mechanism	References
Plant palatability	Negative	Tropical plants have lower nutritional quality (i.e., low C:N, %N); are better defended, and have lower herbivore consumption rates than temperate plants	Lach et al. (2000); Xiong et al. (2008); Burlakova et al. (2009); Qiu and Kwong (2009); Baker et al. (2010); Morrison and Hay 2012
Herbivore pressure (i.e., herbivore abundance and grazing damage)	Negative	At higher latitudes there are lower herbivory rates resulting in lesser top-down control than at lower latitudes	Pennings et al. (2009); Gonzalez-Bergonzoni et al. (2012)
Herbivore thermoregulation	Negative	Ectothermic herbivores feed less or become inactive in cold environments	Seals et al. (1997); He and Silliman (2016)
Herbivore body size	Positive	Animals in colder environments are larger; larger animals can live on a diet of plant material	Clements et al. (2009); Wood et al. (2012)
Herbivore diet breadth	Negative	Lower amount of plants in the diet of omnivores at higher latitudes	Jeppesen et al. (2010); Gonzalez-Bergonzoni et al. (2012)

Herbivore perspective

Latitudinal pattern of herbivory

Biotic interactions, such as predation, competition and herbivory are widely assumed to be more intense and specialized in tropical regions due to the lack of freezing winters and the favorable and predictable climate (Dobzhansky 1950, Schemske et al. 2009). Studies in a wide range of ecosystems have supported that herbivore consumption pressure increases toward the equator (Coley and Aide 1991, Coley and Barone 1996, Bolser and Hay 1996, Pennings et al. 2007, 2009), even though others argue that it needs further investigation (Moles and Ollerton 2016). Despite the debate, the majority of studies suggest that herbivore grazing pressure is less in temperate than tropical areas, which would reduce the potential for temperate herbivores to provide sufficient biotic resistance to invasions of tropical plants (Table 2).

Herbivore traits

Herbivore consumption rate of aquatic plants is generally determined by the density of herbivores and their traits including taxonomic identity, body size and diet breadth (Bakker et al. 2016, Wood et al. 2017).

Higher herbivore density leads to a stronger reduction of plant biomass (Kelkar et al. 2013, Wood et al. 2017). Herbivore taxa differ in their effect on plant abundance: echinoderms, mollusks and fish have a relatively large impact compared to insects and birds (Wood et al. 2017). A recent meta-analysis on consumer control of vegetation in coastal wetlands suggested that animal thermoregulation also plays a role: top-down control by ectothermic rather than endothermic herbivores

increases with increasing annual mean temperature, resulting in weaker top-down control at higher latitudes (He and Silliman 2016). Ectothermic herbivores feed less or become inactive in cold environments reducing overall grazing pressure by the herbivores (Seals et al. 1997, Vejříková et al. 2016). As a result, the potential for biotic resistance from herbivores may be lower at higher latitudes (Table 2), unless endotherms become more important at higher latitudes.

Most aquatic plant consumers are omnivorous animals and their body size has been shown to be a strong predictor of the degree of plant consumption (Bakker et al. 2016). In water birds, the bigger the animals, the more plant biomass is included in the diet, relative to animal prey. The smallest water birds include a lot of animal prey in their diet, the larger ones eat animal prey and seeds and the largest consume mostly plants, with a lot of green leaf material (Wood et al. 2012). Similarly, in fish, larger fish consume more plant material. This can be seen both in fish ontogeny, where only the older, and thus larger, fish consume plant material, as well as between species, where especially in cold water conditions, only the large fish species can live of a diet of plant material (Clements et al. 2009). The positive relationship between body size and the ability to live of green plant material can be explained by the length of the digestive tract. Larger animals have larger digestive tracts, allowing them more time to break down plant cell walls and extract their nutrients (Demment and Van Soest 1985). As animals tend to be bigger at higher latitudes, this would favor their impact on aquatic plants and their potential for providing biotic resistance (Table 2).

Specialist herbivores are rare in aquatic systems (Lodge et al. 1998, Shurin et al. 2006) hence introduced aquatic plant species are less likely to benefit from natural enemy release (Keane and Crawley 2002, Xiong et al. 2008). Instead, with most aquatic herbivores being generalists, these may provide biotic resistance due to less

selective feeding, thus consuming most plant species, including alien plants (Table 2). As generalist herbivores can consume significant amounts of aquatic plants their potential to provide biotic resistance can be high (Cyr and Pace 1993, Bakker et al. 2016, Wood et al. 2017). This is in line with the field studies that we analyzed, where generalist herbivores reduced the abundance of alien tropical plants.

As most aquatic plant consumers are omnivorous, to predict grazing pressure across latitude it is very important to consider the distribution of feeding modes across latitude. At lower latitudes, there is higher richness and abundance of herbivorous and omnivorous fish, whereas carnivorous fish are more abundant towards the poles (Jeppesen et al. 2010, González-Bergonzoni et al. 2012). These patterns are both observed at the level of fish communities as well as within a single species, such as the marine *Girella nigricans* (Ayres, 1860), which includes more plants in its diet at lower compared to higher latitudes (Behrens and Lafferty 2012). This distribution of feeding modes suggests lower plant consumption at higher latitudes, reducing the potential for biotic resistance (Table 2).

Predicting the scope for biotic resistance to tropical plant invasions

Macro-ecological latitudinal patterns suggest that tropical plants have lower nutrient concentrations and higher defenses and are less palatable compared to temperate plants (Table 2). Furthermore, herbivore abundance is lower at higher latitudes, more animals are carnivorous and ectotherms in general eat less at colder temperatures, all resulting in lower grazing pressure on plants at higher latitudes. Together this suggests that temperate herbivores have less potential to provide biotic

resistance to tropical plants, as they consume limited amounts of plants and would not select for these plant species. Only the generally larger body size of temperate plant consumers would favor their potential to provide biotic resistance.

Whereas these macro-ecological patterns are well-established, interestingly, our analysis of empirical data does not support the general idea that the potential for biotic resistance by native temperate herbivores would be low. Instead, we found that native herbivores can provide strong biotic resistance to tropical plants, particularly in the field studies. Also, in the feeding trials herbivores were rather unselective, and thus would have a potential to provide biotic resistance or at least to inhibit the spread of alien tropical plants to a certain extent.

Knowledge gaps and future research

Our current understanding of biotic resistance by native herbivores to tropical invasive aquatic plants is constrained by a lack of empirical studies. Current gaps in this regard are the lack of appropriate experimental designs to compare herbivory on tropical versus temperate macrophytes. Furthermore, most studies are exclusively temperate. A solution is to design experiments including plant species from both latitudes in a balanced design, i.e. with equal amounts of temperate and tropical plant species to allow statistical testing of the results. Furthermore, the lack of field studies strongly limits our ability to judge the quantitative strength of herbivore impact on tropical plant invasions in aquatic systems and hence their potential as a natural defense system. Plant-species interactions are always context dependent. In this respect, the role of endotherm herbivores should be further investigated: in temperate areas especially, endotherms such as water birds and mammals, particularly large species, may have the strongest potential to provide biotic

Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems | 117

resistance. Moreover, to reach firm conclusions on the role of herbivory on invasiveness of macrophytes, experiments should be conducted in both their native and introduced ranges. For example, to conclude that invasion success is explained by enemy release, it is necessary to show that the growth of macrophytes in the absence of herbivores is higher in their invasive than in their native range (see Prior et al. 2015). In this case, experiments should measure plant traits related to invasion success for the same plant species, in the native and invasive range, and in the presence and absence of herbivores (Prior et al. 2015). This could be achieved by international collaborations employing similar protocols in different regions, such as described for feeding trials (Elger and Barrat-Segretain 2002, Burlakova et al. 2009). Experiments design to investigate the joint effects of herbivory and abiotic conditions on invasiveness of macrophytes is recommended, since these factors interact (e.g. Coetzee and Hill 2012). In summary, future studies on the role of native herbivores providing biotic resistance to plant invasions are urgently needed. Our study provides the relevant parameters to measure as well as hypotheses that can easily be tested in feeding trials or field studies.

Acknowledgements

AP acknowledges the Science Without Borders Programme and CNPq (Brazilian National Council for Scientific and Technological Development) for her scholarship. BMCG and ESB acknowledge the Netherlands Organization for Scientific Research (NWO) for financial support through Biodiversity works grant 841.11.011. This is publication 6359 of the Netherlands Institute of Ecology (NIOO-KNAW). SMT is especially thankful to the Brazilian National Council for Scientific and Technological Development (CNPq) for providing continuous funding through a Research Productivity Grant. We are also thankful to three anonymous reviewers and the editor Rob S. E. W. Leuven for constructive suggestions that helped to significantly improve this manuscript.

Supplementary material

Table 1. Metadata of the plant species used in the no-choice feeding trial studies included in our analysis. Information regarding the native range was obtained in literature and online databases (see Source). The latitudinal range was determined based on whether the plant native distribution mainly lies in frost-free (subtropical and tropical, together referred to as “Tropical”) or in frost-prone regions (referred to as “Temperate”). The ‘X’ indicates which plant species was used in each of the feeding trial studies.

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Acorus calamus</i> L.	Asia	Both	ARS-GRIN									X
<i>Ageratum conyzoides</i> L.	C-, S- America	Tropical	ARS-GRIN								X	
<i>Alisma plantago-aquatica</i> L.	Holarctic	Temperate	Grunters et al. (2017)			X						

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Alisma subcordatum</i> Raf.	N- America	Temperate	USDA					X				
<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	S- America	Tropical	Hussner (2012)	X	X			X			X	X
<i>Alternanthera sessilis</i> (L.) R. Br. ex DC.	Asia	Tropical	ARS-GRIN									X
<i>Amaranthus gangeticus</i> L.	Asia	Tropical	ARS-GRIN						X		X	
<i>Apium graveolens</i> L.	Europe, Asia, N- Africa	Temperate	ARS-GRIN						X			
<i>Azolla caroliniana</i> Willd.	N-, C-, S- America	Tropical	Hussner (2012)					X				
<i>Azolla filiculoides</i> Lam.	N-, C-, S- America	Tropical	Grunters et al. (2017)				X					

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Bacopa caroliniana</i> (Walter) B. L. Rob.	N- America	Tropical	USDA					X				
<i>Bacopa monnieri</i> (L.) Pennell	N-, C-, S- America, Asia, Africa	Tropical	ARS-GRIN								X	
<i>Brasenia schreberi</i> J. F. Gmel.	N-, C- America, Asia, Africa	Both	ARS-GRIN					X				X
<i>Cabomba caroliniana</i> A. Gray	S- America	Tropical	Grunters et al. (2017)			X	X					X
<i>Callitriche platycarpa</i> Kutz.	Europe, Asia	Temperate	Grunters et al. (2017)				X					

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Canna glauca</i> L.	N-, C-, S- America	Tropical	ARS-GRIN		X							
<i>Carex lurida</i> Wahlenb.	N-, S- America	Temperate	ARS-GRIN					X				
<i>Carex</i> sp.	-	-	-					X				
<i>Centella erecta</i> (L. f.) Fernald	N- America	Tropical	USDA					X				
<i>Ceratophyllum demersum</i> L.	Cosmopolitan	Both	Grunters et al. (2017)	X	X	X	X	X		X		X
<i>Chara contraria</i> A. Braun ex Kutz.	-	-	-			X						
<i>Chara</i> sp.	-	-	-	X				X				
<i>Colocasia esculenta</i> (L.) Schott	Australasia	Tropical	ARS-GRIN	X	X			X	X		X	

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Commelina diffusa</i> Burm. f.	N-, C-, S- America, Asia, Africa	Tropical	ARS-GRIN USDA								X	
<i>Commelina virginica</i> L.	N- America	Temperate	ARS-GRIN					X				
<i>Crassula helmsii</i> A. Berger	Australia	Tropical	Grunters et al. (2017)			X						
<i>Echinodorus cordifolius</i> (L.) Griseb.	N-, S- America	Both	ARS-GRIN					X				
<i>Egeria densa</i> Planch.	S- America	Tropical	Grunters et al. (2017)	X			X	X		X	X	X
<i>Egeria najas</i> Planch.	S- America	Tropical	Grunters et al. (2017)			X						

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Eichhornia crassipes</i> (Mart.) Solms	S- America	Tropical	Grunters et al. (2017)	X	X		X	X	X		X	X
<i>Eleocharis baldwinii</i> (Torr.) Chapm.	N- America	Tropical	ARS-GRIN					X				
<i>Eleocharis obtusa</i> (Willd.) Schult.	N- America	Temperate	USDA					X				
<i>Eleocharis quadrangulata</i> (Michx.) Roem. & Schult.	N- America	Temperate	USDA					X				
<i>Elodea canadensis</i> Michx.	N- America	Temperate	Grunters et al. (2017)				X			X		X
<i>Elodea nuttallii</i> (Planch.) H. St. John	N- America	Temperate	Grunters et al. (2017)			X						

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Eryngium aquaticum</i> L.	N- America	Temperate	ARS-GRIN					X				
<i>Gratiola virginiana</i> L.	N- America	Temperate	USDA					X				
<i>Hottonia palustris</i> L.	Worldwide	Temperate	Grunters et al. (2017)			X						
<i>Hydrilla verticillata</i> (L. f.) Royle	Asia	Tropical	Hussner (2012)	X				X		X		X
<i>Hydrocharis dubia</i> (Blume) Backer	Asia	Tropical	IUCN Red List									X
<i>Hydrocharis morsus-ranae</i> L.	Europe, Asia	Temperate	Grunters et al. (2017)			X						
<i>Hydrocleys nymphoides</i> (Humb. & Bonpl. ex Willd.)	C-, S- America	Tropical	ARS-GRIN									X

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Hydrocotyle ranunculoides</i> L. f.	N-, C-, S- America	Tropical	Grunters et al. (2017)				X	X				
<i>Hydrocotyle sibthorpioides</i> Lam.	Asia, Africa	Tropical	ARS-GRIN								X	
<i>Hydrocotyle umbellata</i> L.	N-, C-, S- America	Tropical	ARS-GRIN	X				X				
<i>Hydrocotyle vulgaris</i> L.	Europe, Asia	Temperate	Grunters et al. (2017)				X					
<i>Hydrolea quadrivalvis</i> Walter	N- America	Temperate	USDA					X				
<i>Hymenocallis lirioides</i> (Raf.) Shinners	N- America	Tropical	USDA		X							
<i>Ipomoea aquatica</i> Forssk.	Asia, Africa, Australia	Tropical	ARS-GRIN						X		X	

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Juncus acuminatus</i> Michx.	N-, C- America	Temperate	ARS- GRIN GBIF					X				
<i>Juncus effusus</i> L.	Worldwide	Both	ARS- GRIN					X				
<i>Lagarosiphon major</i> (Ridley) Moss	South Africa	Tropical	Grunters et al. (2017)			X				X		
<i>Landoltia punctata</i> (G. Mey.) D.H. Les & D.J. Crawford	Worldwide	Tropical	ARS- GRIN					X				
<i>Leersia hexandra</i> Sw.	Pantropic	Tropical	ARS- GRIN					X				
<i>Leersia oryzoides</i> (L.) Sw.	N- America, Europe, Asia	Temperate	ARS- GRIN					X				

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Lemna minor</i> L.	N- America, Europe, Asia, Africa	Both	ARS- GRIN Hussner (2012)					X			X	
<i>Limnobium spongia</i> (Bosc) Rich. ex Steud.	N- America	Temperate	ARS- GRIN	X				X				
<i>Limnophila sessiliflora</i> Blume	Asia	Tropical	ARS- GRIN					X				
<i>Ludwigia adscendens</i> (L.) H. Hara	Asia	Tropical	eFloras						X		X	
<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	S- America	Tropical	Grunters et al. (2017)				X					

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Gruiters et al. (2016)	Gruiters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Ludwigia hexapetala</i> (Hook. & Arn.) Zardini et al.	C-, S-America	Tropical	GBIF					X				
<i>Ludwigia palustris</i> (L.) Elliott	N-, C-, S-America, Europe, Asia, Africa	Both	ARS-GRIN GBIF					X				
<i>Ludwigia peploides</i> (Kunth) P. H. Raven	S-America	Tropical	Gruiters et al. (2017)			X						
<i>Luziola fluitans</i> (Michx.) Terrell & H. Rob.	N-, C-America	Tropical	ARS-GRIN					X				
<i>Lysichiton americanus</i> Hultén & H. St. John	W N-America	Temperate	Gruiters et al. (2017)				X					

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Gruiters et al. (2016)	Gruiters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Menyanthes trifoliata</i> L.	Holarctic	Temperate	Gruiters et al. (2017)				X					
<i>Mimulus guttatus</i> DC.	W N-America	Temperate	Gruiters et al. (2017)				X					
<i>Monochoria vaginalis</i> (Burm. f.) C. Presl ex Kunth	Asia	Tropical	ARS-GRIN									X
<i>Murdannia keisak</i> (Hassk.) Hand.-Maz.	Asia	Tropical	Hussner (2012)					X				
<i>Murdannia nudiflora</i> (L.) Brenan	Asia	Tropical	ARS-GRIN						X			
<i>Myriophyllum aquaticum</i> (Vell.) Verdc.	S-America	Tropical	Gruiters et al. (2017)	X		X	X	X	X		X	X

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Myriophyllum heterophyllum</i> Michx.	SW N- America	Tropical	Grunters et al. (2017)	X		X	X	X				
<i>Myriophyllum pinnatum</i> (Walter) Britton, Sterns & Poggenb.	N- America	Temperate	USDA					X				
<i>Myriophyllum spicatum</i> L.	Europe, Asia	Temperate	Grunters et al. (2017)			X	X	X				X
<i>Myriophyllum triphyllum</i> orchard	New Zealand	Temperate	Orchard (1979) Redekop et al. (2016)							X		
<i>Myriophyllum verticillatum</i> L.	N- America, Europe, Asia	Temperate	ARS- GRIN		X							

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Najas guadalupensis</i> (Spreng.) Magnus	N-, C-, S- America, Asia	Both	ARS- GRIN Hussner (2012)	X								
<i>Najas minor</i> All.	Europe, Asia, N- Africa	Temperate	ARS- GRIN					X				X
<i>Nasturtium officinale</i> W. T. Aiton	Europe, Asia, Africa	Temperate	ARS- GRIN						X		X	
<i>Nelumbo lutea</i> Willd.	N-, C- America	Both	ARS- GRIN					X				
<i>Nelumbo nucifera</i> Gaertn.	Asia, Africa	Tropical	ARS- GRIN Hussner (2012)								X	
<i>Nuphar lutea</i> (L.) Sm.	Europe, Asia	Temperate	Grunters et al. (2017)				X					
<i>Nymphaea alba</i> L.	Europe, Asia	Temperate	Grunters et al. (2017)				X				X	X

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Nymphaea odorata</i> Aiton	N-, C- America	Temperate	ARS-GRIN	X				X				
<i>Nymphoides aquatica</i> (J.F. Gmel.) Kuntze	N- America	Tropical	ARS-GRIN				X					
<i>Nymphoides indica</i> (L.) Kuntze	C-, S- America, Africa, Asia, Australasia	Tropical	ARS-GRIN									X
<i>Nymphoides peltata</i> (S.G. Gmel.) Kuntze = <i>N. peltatum</i>	Europe, Asia	Temperate	Grunters et al. (2017)				X					X
<i>Ottelia alismoides</i> (L.) Pers.	Asia, Australia	Tropical	Hussner (2012)									X
<i>Panicum hemitomon</i> Schult.	N-, S- America	Tropical	ARS-GRIN		X			X				

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Panicum repens</i> L.	Asia, Africa, Mediterranean region	Tropical	ARS-GRIN	X				X				
<i>Paspalum distichum</i> L.	N-, C-, S- America	Both	ARS-GRIN					X				
<i>Paspalum repens</i> P. J. Bergius	N-, S- America	Both	ARS-GRIN GBIF IUCN Red List					X				
<i>Peltandra virginica</i> (L.) Schott	N- America	Temperate	ARS-GRIN					X				
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Cosmopolitan	Both	ARS-GRIN GBIF								X	
<i>Pistia stratiotes</i> L.	S- America	Tropical	Grunters et al. (2017)	X			X	X			X	

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Pluchea camphorata</i> (L.) DC.	N- America	Temperate	eFloras GBIF USDA					X				
<i>Polygonum amphibium</i> L.	N- America, Europe, Asia, Africa	Temperate	ARS- GRIN					X				
<i>Polygonum barbatum</i> L.	Asia	Tropical	eFloras								X	
<i>Polygonum densiflorum</i> Meisn.	N-, C-, S- America, Asia, Africa	Tropical	ARS- GRIN USDA					X				
<i>Polygonum hydropiper</i> L.	Europe, Asia, Australia	Temperate	ARS- GRIN						X			
<i>Polygonum hydropiperoides</i> Michx.	N-, C-, S- America	Both	ARS- GRIN					X				

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Polygonum pennsylvanicum</i> L.	N- America	Temperate	ARS- GRIN					X				
<i>Polygonum sagittatum</i> L.	N- America, Asia	Temperate	ARS- GRIN					X				
<i>Polygonum setaceum</i> Baldw.	N- America	Temperate	USDA					X				
<i>Pontederia cordata</i> L. = <i>P. lanceolata</i>	N-, S- America	Both	Grunters et al. (2017)	X	X		X	X				
<i>Potamogeton crispus</i> L.	Europe, Asia, Australia	Temperate	ARS- GRIN									X
<i>Potamogeton diversifolius</i> Raf.	N- America	Temperate	eFloras USDA					X				

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Potamogeton illinoensis</i> Morong	N-, C- America	Temperate	ARS-GRIN	X				X				
<i>Potamogeton lucens</i> L.	Europe, Asia	Temperate	Grunters et al. (2017)		X	X						
<i>Potamogeton maackianus</i> A. Bennett	Asia	Temperate	eFloras									X
<i>Potamogeton malaiianus</i> Miq. = <i>P. nodosus</i>	Worldwide	Both	ARS-GRIN					X				X
<i>Potamogeton natans</i> L.	Holarctic	Temperate	Grunters et al. (2017)				X					
<i>Potamogeton ochreateus</i> Raoul	Australia, New Zealand, SE Asia	Tropical	NZPCN Redekop et al. (2016)							X		

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Potamogeton perfoliatus</i> L.	Holarctic, Australia	Temperate	Grunters et al. (2017)				X					
<i>Potamogeton pusillus</i> L.	Worldwide	Temperate	Grunters et al. (2017)			X	X	X				
<i>Ranunculus circinatus</i> Sibth.	Europe	Temperate	Grunters et al. (2017)			X	X					
<i>Ranunculus sceleratus</i> L.	N- America, Europe, Asia	Temperate	ARS-GRIN								X	
<i>Ruppia maritima</i> L.	Cosmopolitan	Both	ARS-GRIN		X							
<i>Sagittaria graminea</i> Michx.	N- America	Temperate	ARS-GRIN		X							
<i>Sagittaria kurziana</i> Gluck	SE N- America	Tropical	ARS-GRIN	X								

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Sagittaria lancifolia</i> L.	N-, C+, S- America	Tropical	ARS-GRIN	X	X							
<i>Sagittaria latifolia</i> Willd.	N- America	Temperate	ARS-GRIN Hussner (2012)	X			X	X				
<i>Sagittaria sagittifolia</i> L. = <i>S. trifolia</i>	Europe, Asia	Temperate	Grunters et al. (2017)			X						X
<i>Salix nigra</i> Marshall	N- America	Temperate	ARS-GRIN					X				
<i>Salvinia minima</i> Baker	C-, S- America	Tropical	ARS-GRIN					X				
<i>Salvinia molesta</i> Mitchell	S- America	Tropical	Grunters et al. (2017)				X					
<i>Saururus cernuus</i> L.	N- America	Temperate	ARS-GRIN					X				

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Schoenoplectus californicus</i> (C.A. Mey.) Palla	N-, C+, S- America	Tropical	ARS-GRIN		X							
<i>Scirpus maritimus</i> L.	N-, S- America, Europe, Asia, Africa	Both	ARS-GRIN		X							
<i>Spartina alterniflora</i> Loisel.	N-, C+, S- America	Both	ARS-GRIN		X							
<i>Spirodela polyrhiza</i> (L.) Schleid.	N-, C- America, Europe, Asia	Both	ARS-GRIN					X				
<i>Spirogyra</i> sp.	-	-	-					X				
<i>Stratiotes aloides</i> L.	Europe	Temperate	Grunters et al. (2017)				X					

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Thalia dealbata</i> Fraser ex Roscoe	N- America	Temperate	ARS- GRIN eFloras		X							
<i>Trapa natans</i> L. = <i>T. Bispinosa</i>	Europe, Asia	Temperate	Grunters et al. (2017)			X						X
<i>Triadenum walteri</i> (J.G. Gmel.) Gleason	N- America	Temperate	ARS- GRIN				X					
<i>Typha latifolia</i> L.	N-, C-, S- America, Europe, Asia, Africa	Temperate	ARS- GRIN GBIF	X	X							
<i>Utricularia aurea</i> Loureiro	Asia, Australia	Tropical	IUCN Red List									X

Plant species	Native range	Latitudinal range	Source	Baker et al. (2010)	Burlakova et al. (2009)	Grunters et al. (2016)	Grunters et al. (2017)	Parker and Hay (2005)	Qiu and Kwong (2009)	Redekop et al. (2016)	Wong et al. (2010)	Xiong et al. (2008)
<i>Utricularia biflora</i> L. = <i>U. gibba</i>	N-, C- America, Asia	Both	GBIF Hussner (2012)					X				
<i>Utricularia vulgaris</i> L.	Europe, Asia	Temperate	Grunters et al. (2017)				X					
<i>Vallisneria americana</i> Michx.	N-, C- America	Both	ARS- GRIN eFloras	X				X				
<i>Vallisneria natans</i> (Lour.) H. Hara	Asia	Both	GBIF IUCN Red List								X	
<i>Vallisneria spiralis</i> L.	North Africa, Asia, Europe	Tropical	Grunters et al. (2017)				X					X
<i>Wolffia gladiata</i> (Hegel.) Hegelm.	N- America	Tropical	ARS- GRIN eFloras					X				

Sources: **ARS-GRIN** (Agricultural Research Service - Germplasm Resources Information Network). <https://npgsweb.ars-grin.gov> / **eFloras**. <http://www.efloras.org> **GBIF** (Global Biodiversity Information Facility). <http://www.gbif.org> / **Grutters BMC**, Roijendijk YOA, Verberk WCEP, Bakker ES (2017) Plant traits and plant biogeography control the biotic resistance provided by generalist herbivores. *Functional Ecology*. Online. doi: 10.1111/1365-2435.12835 / **Hussner A (2012)** Alien aquatic plant species in European countries. *Weed Research* 52:297-306. doi: 10.1111/j.1365-3180.2012.00926.x / **IUCN Red List** (International Union for Conservation of Nature's Red List of Threatened Species). <http://www.iucnredlist.org> / **NZPCN** (New Zealand Plant Conservation Network). <http://www.nzpcn.org.nz> / **Orchard AE (1979)** *Myriophyllum* (Haloragaceae) in Australasia. I. New Zealand: A revision of the genus and a synopsis of the family. *Brunonia* 2:247-287 / **Redekop P**, Gross EM, Nuttens A, Hofstra DE, Clayton JS, Hussner A (2016) *Hygraula nitens*, the only native aquatic caterpillar in New Zealand, prefers feeding on an alien submerged plant. *Hydrobiologia*. doi: 10.1007/s10750-016-2709-7 / **Stuckey RL (1974)** The introduction and distribution of *Nymphoides peltatum* (Menyanthaceae) in North America. *Bartonia* 42:14-23 / **USDA** (United States Department of Agriculture) – Natural Resources Conservation Service. <http://plants.usda.gov>



Chapter 5

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

Antonella Petruzzella, Casper H. A. van Leeuwen, Ellen van Donk and Elisabeth S. Bakker

Journal of Ecology (in review)

Abstract

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 tropical plant invasions, understand the underlying mechanisms and search for potential interactive effects of competition and herbivory on invader performance (i.e. growth). We mimicked natural temperate mesotrophic and eutrophic freshwater lakes in mesocosms, by growing three native submerged species individually (*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 tropical highly invasive alien species *Egeria densa*. We found that competition by native plant species consistently and significantly reduced invader performance at the highest level of native plant competition, regardless of native species identity. Herbivory had no negative effect on invader performance. Snails fed mainly on the available filamentous algae, which are commonly found in meso- and eutrophic systems, instead of on the plants. However, we found a significant positive interaction between competition and herbivory, which depended on the native plant species involved. In the treatment with *C. demersum* the snails indirectly facilitated the invasion of *E. densa* by enhancing its performance. Our study highlights the important role of indirect interactions to understand the potential of biotic resistance in natural ecosystems.

Keywords: Alien invasive species, aquatic macrophytes, biological invasion, competition, *Egeria densa*, freshwater ecosystems, herbivory, plant-herbivore interactions

Introduction

Alien species are increasingly intentionally and unintentionally introduced into new environments due to the intensification of trade, transport and tourism (Meyerson and Mooney 2013). However, only few of these introduced species truly establish, become invasive and cause negative impacts (Williamson and Fitter 1996, Meyerson and Mooney 2007, Caley et al. 2008). This can partly be explained by the concept of biotic resistance (Elton 1958, Levine et al. 2004), which states that interactions with native species can reduce the establishment or spread of alien species. While the potential of biotic resistance to alien species invasions has been extensively investigated in terrestrial ecosystems, far fewer studies explored this mechanism in freshwater systems (Alofs and Jackson 2014). Freshwater and terrestrial ecosystems differ in many ways including primary producer traits such as growth rate and nutritional quality, strength of trophic interactions and disturbance regimes (Shurin et al. 2006, Bakker et al. 2016). Also, 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). These factors may influence the strength of biotic resistance, therefore, it is unclear how research results for one ecosystem will translate to another ecosystem. The lack of studies on biotic resistance in freshwater systems (e.g. Alofs and Jackson 2014) is particularly surprising because invasive species are a significant threat, have strong negative impacts and challenge the conservation of freshwater systems (Dudgeon et al. 2006, Gallardo et al. 2016). A better understanding of the processes that may limit the establishment success of alien species in freshwater ecosystems is urgently needed.

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 effects on the performance of invading plant species in freshwater ecosystems (Petruzzella et al. 2018), grasslands (Corbin and D'Antonio 2004, te Beest et al. 2018), forests (Fine 2002), saltmarshes (Amsberry et al. 2014) and marine systems (Britton-Simmons 2006). However, less is known about the potential of herbivores to provide biotic resistance in freshwater ecosystems. A recent meta-analysis suggested that biotic resistance in freshwater is mostly driven by consumption (herbivory and predation) and less by competition – but also concluded that available information and comparative studies are still very scarce (Alofs and Jackson 2014).

Whereas herbivory was until recently considered to have a minimum impact on freshwater aquatic plants, an increasing body of evidence shows that herbivores have large impacts on plants, and even greater in aquatic systems than reported for terrestrial plants (Bakker et al. 2016). Herbivores can reduce significant amounts of plant biomass, increase nutrient turnover rates and affect plant species composition and distribution (Bakker et al. 2016, Wood et al. 2017). Invasive alien plants can be released from top-down control because native herbivores generally selectively feed on native plants, a concept known as the enemy release hypothesis (Keane and Crawley 2002). However, this is more likely to occur with specialist, and not generalist, enemies. This distinction is important because within freshwater ecosystems, specialist herbivores are rare (Lodge et al. 1998, Shurin et al. 2006). Generalist herbivores, usually vertebrates and large invertebrates, often have larger impacts on plant communities than specialists, in particular insects (Parker et al. 2006, Bakker et al. 2016, Wood et al. 2017). Generalists can therefore provide biotic resistance through

removal of a lot of plant biomass, provided that this includes the alien plants. However, their feeding preferences are more difficult to predict compared to specialist herbivores. Feeding trials have demonstrated that freshwater generalist herbivores can prefer native plants (Xiong et al. 2008) or alien plants (Parker and Hay 2005, Morrison and Hay 2011) or have no preference for either (Grutters et al. 2017a). A further limitation in our understanding of the potential of biotic resistance by herbivory in freshwater 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 interactions, in mesocosms or the field are scarce (White et al. 2006, Alofs and Jackson 2014, Petruzzella et al. 2017).

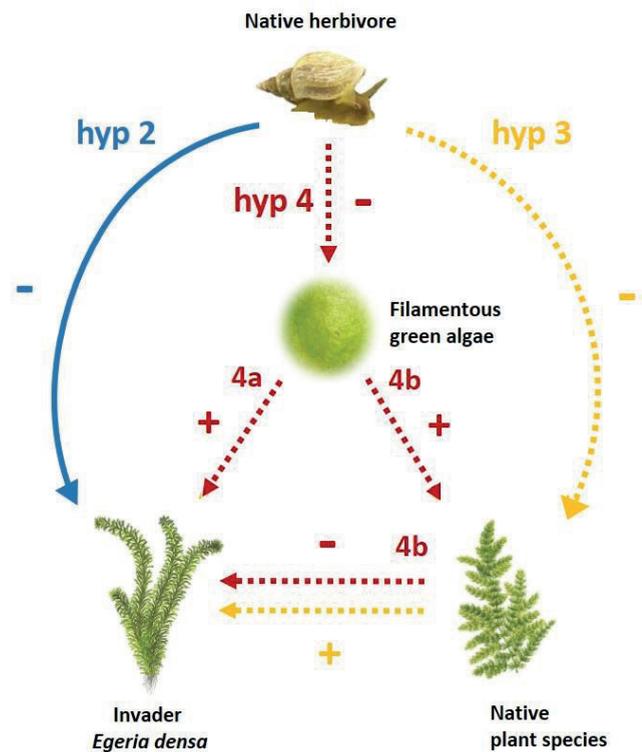
In freshwater ecosystems, several generalist herbivores can also feed on algae instead of plant matter (Elger et al. 2007). Filamentous green algae often grow on aquatic plants in mesotrophic and eutrophic conditions; their removal can promote the growth of plants by reducing the negative effects of shading and nutrient competition which would have an indirect effect on either the invading (Fig. 1 – Red dashed arrow (4a)), native (Fig. 1 – Red dashed arrow (4b)) or both native and invading plant species (No overall effect) (Brönmark 1985, 1989, Bakker et al. 2013, Hidding et al. 2016). Furthermore, herbivores can also indirectly affect alien plant establishment through alteration of resource availability, for example by increasing nutrient recycling (White et al. 2006). Complex interactions between aquatic plants, epiphytes and aquatic herbivores, particularly snails, have been described (Brönmark 1985, 1989, Bakker et al. 2013, Hidding et al. 2016). To take this real-world complexity into account, more studies are needed to investigate the direct and indirect effects of herbivores on biotic resistance, its interactions with plant-plant competition, and whether they are able to provide biotic resistance in understudied freshwater ecosystems. To our knowledge, only studies in the context of biological control – i.e.

using alien herbivores – have explored the interaction between these mechanisms in submerged freshwater plants (Van et al. 1998, Doyle et al. 2007), whereas the role of indirect effects influencing invasion success has rarely been considered (White et al. 2006).

Here, our aims were to determine whether temperate native aquatic plants and herbivores can provide biotic resistance to tropical 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 individually three native submerged species (*Ceratophyllum demersum* L., *Myriophyllum spicatum* L. and *Potamogeton perfoliatus* L.) at three competition levels (no, low and high) without and with the native aquatic generalist snail *Lymnaea stagnalis* L., and simulated an early stage of establishment of the tropical highly invasive alien species *Egeria densa* Planchon. We used three different plant species as competitors to assess the role of competitor identity and palatability on these interactions. 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) in the absence of herbivores native plants provide biotic resistance: increasing native plant density reduces *E. densa* performance; (2) In the presence of herbivores, herbivores have a direct effect by feeding on the invader thereby providing biotic resistance through reducing *E. densa* performance (Fig. 1 – Blue solid arrow). Alternatively, (3) herbivores have an indirect interactive effect on biotic resistance if they prefer to feed on native plants, reducing the competitive effect of native plants on *E. densa* and therefore increasing its performance (antagonistic effect) (Fig. 1 – Yellow dashed arrows). (4) Herbivores have an indirect effect on biotic resistance when they feed on filamentous algae (Fig. 1 – Red dashed arrows), which may provide relative benefits to either (a) the invader, and

directly stimulate its growth, (b) the native plant species, and indirectly inhibit invader growth via competition (c) both the invader and native plants, which may have no overall effect.

Figure 1. Schematic representation of the direct (solid line) and indirect effects (dashed lines) of the native herbivore on invader *Egeria densa* performance. The sign of the interaction (+/-) shows whether biomass gain or loss accrues in the direction of the arrow. Generalist herbivores have a direct effect on providing biotic resistance by feeding on the invader reducing *E. densa* performance (hypothesis 2 – Blue solid arrow). Herbivores have an indirect interactive effect on biotic resistance feeding on native plants, reducing the competitive effect of native plants on *E. densa* and therefore increasing its performance (hypothesis 3 – Yellow dashed arrows). Herbivores have an indirect effect on biotic resistance feeding on filamentous algae (hypothesis 4 – Red dashed arrows) which may provide relative benefits to either (a) the invader, and directly stimulate its growth, (b) the native plant species, and indirectly inhibit invader growth via competition (c) both the invader and native plants, which may have no overall effect (not included in the figure).



Material and methods

Aquatic plants

The Brazilian waterweed *E. densa* (Hydrocharitaceae) is a rooted, submerged, freshwater perennial plant native to South America. It is a popular aquarium plant in Europe and worldwide; 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 developed 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. The species already colonized 12 European countries and is listed on 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). 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 (Thiébaud et al. 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 et al. 1999).

The three common native submerged species that we used in the experiments are widely distributed in Northwestern Europe and co-occur in temperate shallow lakes (Van De Haterd and Ter Heerd 2007). *Ceratophyllum demersum* (Ceratophyllaceae) 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 h light/dark cycle at a temperature of $21 \pm 3^\circ\text{C}$ during the day and $16 \pm 3^\circ\text{C}$ during the night (Supplementary Figure 1). The tanks were filled with a 3.4 kg bottom layer of artificial plant pond sediment (Plant soil Moerings – Velda, organic matter=34.31%), 44.9 kg 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 ($52^\circ12'55.2''$ N, $5^\circ02'25.7''$ E). Lake Terra Nova is a shallow peat lake located in the center of the Netherlands where all three native plant species used in the experiment co-occur (Van De Haterd and Ter Heerdt 2007). The lake is characterized by high nutrient concentrations in the water (water used in the experiment: mean \pm SD, $n=6$ water samples, 0.14 ± 0.05 mg L⁻¹ P-PO₄; 0.55 ± 0.46 mg L⁻¹ N-NO₃). The plants were cultivated under the following conditions: water temperature $22.3\pm0.8^\circ\text{C}$, dissolved oxygen 12.5 ± 1.3 mg L⁻¹, conductivity 263 ± 28 $\mu\text{S cm}^{-1}$, pH 9.8 ± 0.3 and alkalinity 2.37 ± 0.52 mEq L⁻¹. Plants were pre-cultivated for at least 20 days before the start of the experiment.

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 m⁻² 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 (Elger and Barrat-Segretain 2002, 2004, Bakker et al. 2013, Grutters et al. 2017a, Zhang et al. 2018a).

Adult snails were collected from a pond located at the Netherlands Institute of Ecology (NIOO-KNAW, $51^\circ59'16.8''$ N, $5^\circ40'24.7''$ E, Wageningen, the Netherlands). They were acclimated to laboratory conditions for at least two weeks in 15 L buckets filled with groundwater at 20°C and constant aeration and exposed to a 16:8h day:night cycle, before being experimentally used. The snails were fed butterhead lettuce (*Lactuca sativa* L.) six 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. 2017a).

Experimental design and set up

A greenhouse experiment was established at the Netherlands Institute of Ecology (NIOO-KNAW) ($51^\circ59'15.3''$ N and $5^\circ40'14.8''$ E) during the summer of 2018 (July - October). The experiment was set up as a full factorial randomized block design, with $3 \times 3 \times 2$ 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 (Supplementary Figure 1). The greenhouse controlled conditions consisted of a 16/8 h light/dark cycle at a mean temperature of $21 \pm 3^\circ\text{C}$ during the day and $16 \pm 3^\circ\text{C}$ during the night.

Direct and indirect effects of native plants and herbivores on biotic resistance to alien aquatic plant invasions | 159

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 (mean±SD, $n=1166$, water temperature $23.3\pm 1.0^\circ\text{C}$, dissolved oxygen $12.9\pm 1.9\text{ mg L}^{-1}$, conductivity $283\pm 30\ \mu\text{s cm}^{-1}$, pH 9.7 ± 0.7 and alkalinity $2.12\pm 0.47\text{ mEq L}^{-1}$).

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 h, 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 fragments (no competition, invader growing alone), 1:2 (low competition) and 6:2 (high competition), corresponding to $37.04\text{ plants m}^{-2}$ (low competition) and $222.22\text{ plants m}^{-2}$ (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.

The native plants were left to establish for two weeks (24-July to 06-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 (07-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 et al. 2009). To determine the introduced biomass in DW, 15 *E. densa* shoots were randomly selected, dried to a constant mass at 60°C for at least 48h, and individually weighed. *Egeria densa* was allowed to root for two 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 $30\pm 1\text{ mm}$, wet weight $2.19\pm 0.27\text{ g}$, mean±SD, $n=108$) and starved the snails for 48 h before adding them to standardize their appetite as is common practice in feeding trials (following Grutters et al. 2017a).

Harvest and data collection

At the end of the experiment (after 8 weeks, on 8-October), we removed the herbivores and harvested the filamentous algae and alien and native plants. We harvested the filamentous algae biomass present on the plants and in the water column (Supplementary Figure 2). 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 48h, 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 48h, and weighed to determine DW.

Feeding trials

To determine plant palatability for the snails, we performed 24h no-choice feeding trials following established protocols (Elger and Barrat-Segretain 2002, 2004, Grutters et al. 2017a). 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, mean \pm 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 μ S/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 h prior to the trial to standardize their appetite. All cups were covered with a mesh 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 16:8h day:night cycle (Supplementary Figure 3). All snails were removed from their respective cup after 24

h 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 h. The dry weights of plant fragments remaining in each cup were determined as described previously (see *Harvest and data collection*).

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

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

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.

Data analyses

For each of the three native plant species, we fitted a General Linear Mixed-effects Model (GLMM) to test the effects of competition (factor with 3 levels, controls without native plants present as intercept), herbivory (factor with 2 levels, treatment without snails present as intercept) and their interactions on the invader performance (i.e. growth). We also fitted a GLMM to test whether native species biomass, RCR and filamentous green algae differed between treatments. Block (the 6 replicates) was included as random factor in all models. 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. To determine which terms of interest we should retain in the best models, we performed model selection based on the Akaike Information Criterion (AICc

indicated for small sample sizes (Burnham et al. 2011)) on models fitted with Maximum Likelihood estimations. We used model averaging over the best models within $<2.0 \Delta AICc$ of the top model if needed (details on model selections can be found in supporting information Table S1) (Burnham et al. 2011). All models were fitted using Maximum Likelihood estimations in the *lme* function in the R package *nlme* (Pinheiro et al. 2018).

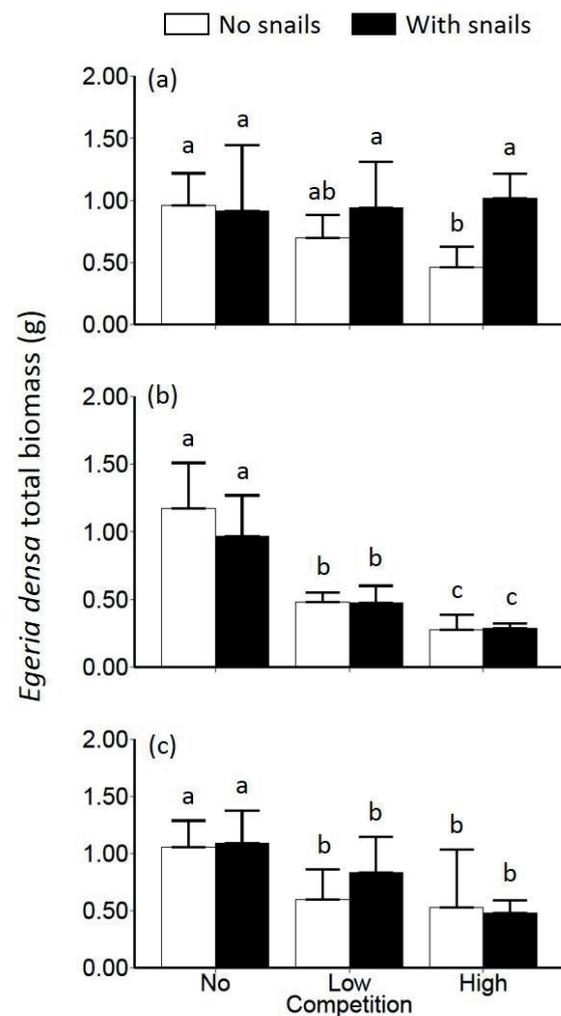
Results

Competition by native plant species consistently and significantly reduced invader performance at the highest native plant densities, regardless of native species identity (Table 1). Without herbivory, the native species *C. demersum* suppressed *E. densa* total biomass (52% decrease) at high levels of competition compared to the no competition treatment (invader growing alone in the absence of competitors). At the low competition level *E. densa* biomass was intermediate showing a 27% decrease which was not significantly different from either no competition and high competition (Fig. 2a – white bars). Low and high densities of *M. spicatum* proportionally decreased *E. densa* performance with 59% and 76%, respectively (Fig. 2b). *Potamogeton perfoliatus* significantly decreased invader performance by 44% and 50% at low and high levels of competition, respectively, whereas there was no significant difference between these levels (Fig. 2c).

Table 1. Effects of competition by native plant species, the presence of herbivores and their interaction on the performance of the invader *Egeria densa* (measured as total biomass accumulation after 8 weeks). Parameter estimates and their significance are given for terms that remained in final General Linear Mixed-effects Models (GLMMs) after model selection based on AICc-values (Supplementary Table 1). Effects of the explanatory variables competition (intercept: 'No competition'), herbivory (intercept: 'No snails') and their interaction are shown for three models with *Ceratophyllum demersum*, *Myriophyllum spicatum* or *Potamogeton perfoliatus* as the native species, respectively.

Native plant species	Response variable	Explanatory variable (s)	Estimate	SE	z-value	t-value	p-value
<i>C. demersum</i>	Total invader biomass	Intercept	0.782	0.150	5.116	-	<0.001
		Low competition (LC)	-0.264	0.160	1.579	-	0.114
		High competition (HC)	-0.500	0.1593	2.984	-	0.003
		Herbivory (H)	0.162	0.182	0.872	-	0.383
		LC x H	0.289	0.225	1.218	-	0.223
		HC x H	0.599	0.225	2.527	-	0.011
<i>M. spicatum</i>	Total invader biomass	Intercept	1.082	0.060	17.277	-	<0.001
		Low competition (LC)	-0.593	0.078	7.268	-	<0.001
		High competition (HC)	-0.790	0.078	9.685	-	<0.001
<i>P. perfoliatus</i>	Total invader biomass	Intercept	1.078	0.096	-	11.221	<0.001
		Low competition (LC)	-0.366	0.117	-	-3.112	0.005
		High competition (HC)	-0.574	0.117	-	-4.884	<0.001

Figure 2. Effect of the competition levels of native plant species (a) *Ceratophyllum demersum*, (b) *Myriophyllum spicatum* and (c) *Potamogeton perfoliatus* on the performance of the invader *Egeria densa* (total biomass, g) without (white bars) and with (black bars) the generalist herbivore *Lymnaea stagnalis*. Bars represent mean values \pm CI (95% confidence intervals). Different lowercase letters indicate statistically significant differences between treatments after contrast analysis at a significance level of $p < 0.05$.



Herbivory had no effect on invader performance, i.e. the snails did not significantly feed on *E. densa* as we also observed (pers. obs.) (Table 1). In the absence of native plant species, the snails did not affect *E. densa* total biomass (Fig. 2a-c). However, with native plant competitors present we found a significant positive interaction between competition and herbivory on invader performance for one of the three native plant species, *C. demersum* (Table 1). The total biomass of *E. densa* was significantly larger when grown with than without snails at high competition levels of *C. demersum* (Fig. 2a). Herbivory did not significantly interact with competition for the other two native species (Fig. 2b-c).

The snails did not significantly negatively affect any of the native species even though we observed grazing activity in *P. perfoliatus* (pers. obs.) (Fig. 3). *Ceratophyllum demersum* even produced significantly more biomass at high competition levels in the presence of snails (Fig. 3a). The generalist herbivore consumed all the plant species, but the average plant palatability did not differ among the invading plant species and the three natives (Fig. 4). Snails were predominantly feeding on the filamentous green algae that appeared in the aquaria, and significantly reduced their biomass to almost zero in each of the native plant species treatments (Fig. 5).

Figure 3. Native biomass production (g DW) of (a) *Ceratophyllum demersum*, (b) *Myriophyllum spicatum* and (c) *Potamogeton perfoliatus* at low and high competition levels without (white bars) and with (black bars) the generalist herbivore *Lymnaea stagnalis*. Bars represent mean values \pm CI (95% confidence intervals). Different lowercase letters indicate statistically significant differences between treatments after contrast analysis at a significance level of $p < 0.05$.

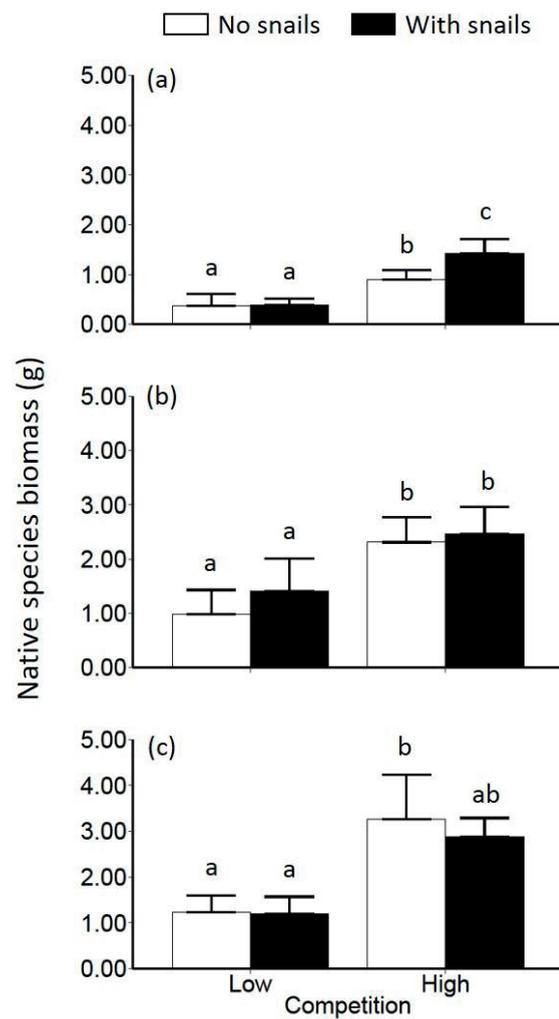


Figure 4. Relative consumption rate (RCR, DW) by the generalist herbivore *Lymnaea stagnalis*. Bars represent mean values \pm CI (95% confidence intervals), $n=12$. Different lowercase letters indicate statistically significant differences between plant species after contrast analysis at a significance level of $p < 0.05$. P= *Potamogeton perfoliatus*, M= *Myriophyllum spicatum*, E= *Egeria densa* and C= *Ceratophyllum demersum*.

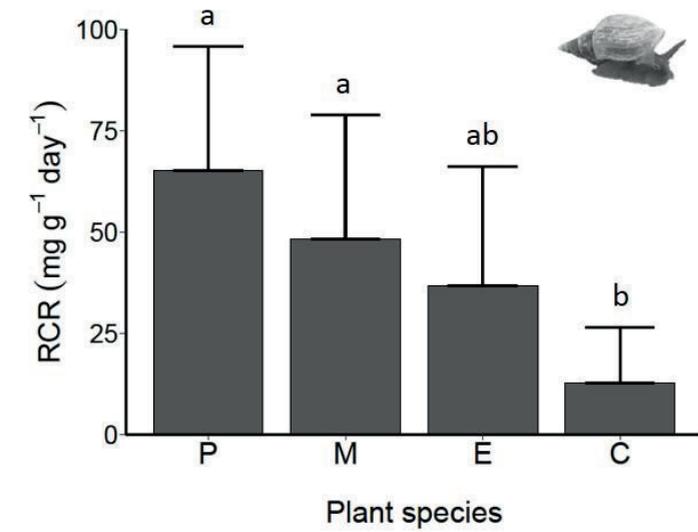
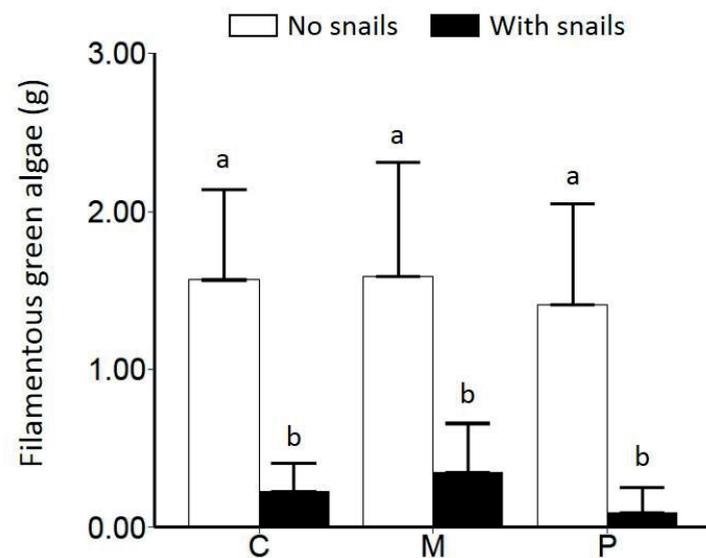


Figure 5. Biomass of filamentous green algae (dry weight per aquarium, g) at the end of the experiment for each of native species C= *Ceratophyllum demersum*, M= *Myriophyllum spicatum* and P= *Potamogeton perfoliatus* without (white bars) and with (black bars) the generalist herbivore *Lymnaea stagnalis*. Bars represent mean values \pm CI (95% confidence intervals), $n=18$ (M, C), $n=16$ (P). Different lowercase letters indicate statistically significant differences between plant species after contrast analysis at a significance level of $p < 0.05$.



Discussion

Our experiment showed clear and negative effects of competition by native plant species on invader performance, regardless of native species identity. Herbivory had no net negative effect on invader performance. In contrast, we found a significant positive interaction between competition by native species *C. demersum* and

herbivory. The presence of snails at high levels of competition by *C. demersum* indirectly facilitated the invasion by *E. densa*. Below we discuss our findings and the mechanisms that may underlie the observed invader facilitation.

In our first hypothesis we expected that in the absence of the herbivore increasing native plant density reduces *E. densa* performance. Indeed, overall competition by native plants reduced invader performance – thus confirming our first hypothesis that also in freshwater systems, native plants provide biotic resistance to alien plants. The degree to which native plants reduced the invader biomass varied among the three native species competitors. 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* 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 reducing the performance of *E. densa* proportionally with increasing plant density as we expected. *Potamogeton perfoliatus* plants also reduced *E. densa* performance but less than *M. spicatum* and low and high planting densities did not differ in their suppression of the invader. Both rooted submerged native species are from the same functional group as the invader, which also rooted, hence their spatial resource use overlaps, which increases the competitive strength of both native species in reducing invader species success (Petruzzella et al. 2018). We found that *C. demersum* only provided biotic resistance at high densities. This species is fully floating and uses nutrients from the water column. Therefore, it does not compete with the invader for nutrients and may be less efficient in suppressing invader growth. *Ceratophyllum demersum* has been observed to displace other aquatic plant species by shading due to closed dense

canopy formation (Wells et al. 1997, Stiers et al. 2011). However, at low densities *C. demersum* may provide low resistance to recently established alien rooted plant propagules (Stiers et al. 2011). 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.

There is potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems (Petruzzella et al. 2017). We hypothesized that herbivores could provide biotic resistance by directly consuming the invader. Most studies that test this hypothesis rely on feeding trials comparing the consumption rate of native and alien plants by herbivores (e.g. Parker and Hay 2005, Grutters et al. 2017a). In our experiment, the feeding trials indicated that the herbivores consumed all plant species, whereas the consumption rate of the invader was not different from the native species. This corresponded with the mesocosm experiment, where we did not find any significant effect of herbivory alone on *E. densa* performance among the native species treatments. Thus, we 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). This contradiction may be due to differences in the densities or identities of either the herbivore or plants among studies, or may be related to often-ignored indirect effects that clearly played a role in our study.

Our third hypothesis stated that the herbivore could indirectly facilitate the invader when it feeds on the native plants. We found a significant positive interaction between competition and herbivory: we observed that the invader *E. densa* increased its performance at high levels of competition by native species *C. demersum* in the

presence of the native generalist snails. This effect is in line with our hypothesis. However, in the feeding trial the consumption rate of the snails of *C. demersum* was lower than the consumption of the other native plants and similar to the consumption rate of the invader, which does not suggest preferential feeding on *C. demersum*. Furthermore, snails also promoted the growth of *C. demersum*, hence, whereas the pattern that we observed is in line with our third hypothesis, the mechanism cannot be preferential feeding on the native plant species. Therefore, we reject our third hypothesis.

Our experimental system was mimicking natural situations found in temperate mesotrophic and eutrophic freshwater lakes and as a result we observed the growth of filamentous green algae, as can be found in the field. Our fourth hypothesis was that herbivores have an indirect effect on biotic resistance with either negative, neutral or positive effects on the invader. This indirect effect would apply when the herbivores feed on filamentous algae and recycle nutrients, thus affecting the plant competition. Indeed, in our mesocosms the generalist snails were feeding on the filamentous algae covering the plants, but hardly on the plants themselves. The presence of invertebrate herbivores can not only reduce aquatic plant abundance, but can also promote their growth in freshwater ecosystems (Brönmark 1989) as in particular snails can decrease the effects of shading and increase nutrient recycling rates by reducing filamentous algae (Brönmark 1985, Bakker et al. 2013). We also observed a positive effect of snails on the biomass of *C. demersum* at the high planting densities treatment, indicating facilitation of *C. demersum* by the snails. As *C. demersum* is a non-rooted species it can directly benefit from the increase of nutrient availability in the water column through recycling of the nutrients from the grazed filamentous algae biomass. Positive effects of snails on the abundance of *C. demersum* have been shown in previous experimental (Brönmark 1985, Underwood

1991, Pinowska 2002) as well as field studies (Underwood et al. 1992). In contrast, we did not find such an effect in the rooted species, which is probably due to their resource uptake, which is mostly from the sediment, not from the water column.

The highest experimental density of *C. demersum* significantly reduced the invader biomass, thus providing biotic resistance in the absence of snails. Therefore, if biomass of *C. demersum* in the presence of snails increased due to facilitation, one would expect an even stronger biotic resistance to the invader. Surprisingly, the opposite was true: at the highest planted density of *C. demersum* the invader biomass was significantly 2-fold higher in the presence of snails than when snails were absent. Therefore, the snail presence facilitated *C. demersum* and indirectly also the invader. In fact, in the presence of snails, the invader biomass in the *C. demersum* treatments was the same, regardless of the abundance or even the presence of *C. demersum* plants. This suggests that the non-rooted species *C. demersum* has a limited effect on *E. densa* performance. Neither the presence of filamentous algae alone nor low densities of *C. demersum* with (no snails) and without (with snails) filamentous algae affected invader performance (Fig. 2a). Therefore, the observed biotic resistance provided by high densities of *C. demersum* was possibly the result of the combined effect of its own biomass production associated with that of the filamentous algae.

Although snails exhibited the same feeding pattern – i.e. feeding on the filamentous algae – in the other native plant species treatments (Fig. 5), we did not observe indirect facilitation on *E. densa* performance. For *M. spicatum*, for example, allelopathy enables this species to reduce the filamentous algae on its leaves, even when the algae are abundant in the immediate vicinity (Gross et al. 1996, Gross 2001). Thus, snails would have a very limited effect because this species is a greater competitor *per se*. As expected for our fourth hypothesis herbivore feeding

preference for filamentous algae determined the direction and strength of its effect on the performance of the invader *E. densa*, but this was a species-specific response.

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 et al. 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).

Implications

Both competition and herbivory have been shown to decrease alien plant performance in terrestrial and marine systems (Levine et al. 2004, Kimbro et al. 2013). 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 and Jackson 2014). Our experiment provided important insights into the mechanisms and their interactive effects on 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 tropical 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 depended on the native plant species involved. Our study highlights the

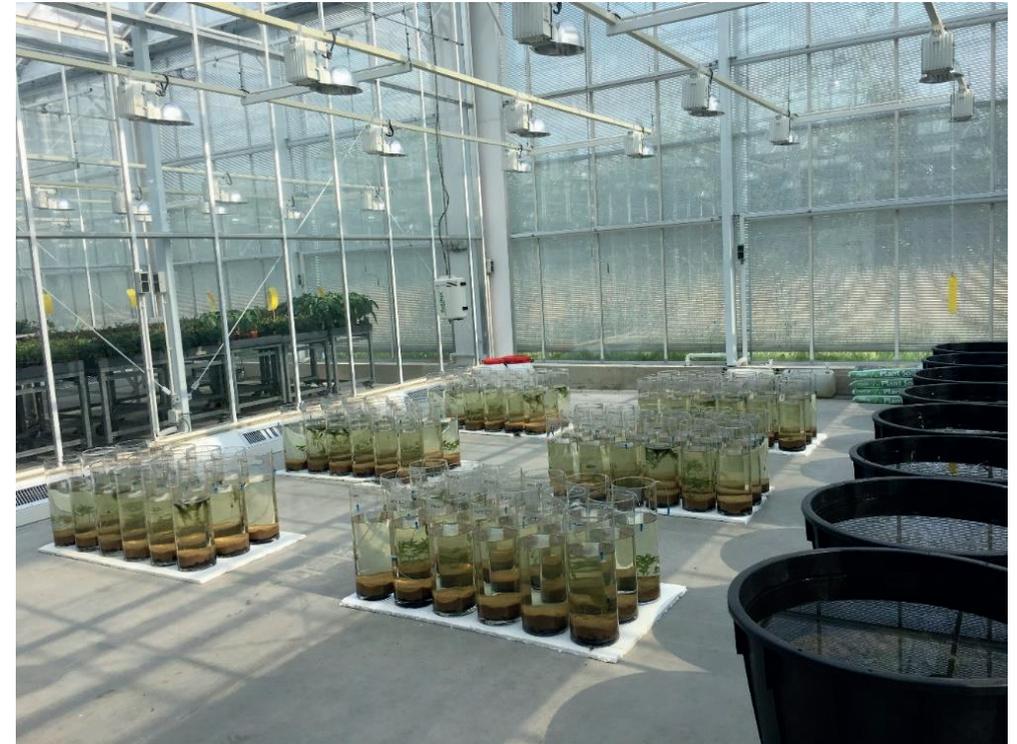
important role of indirect interactions to understand the potential of biotic resistance in real ecosystems. These results do not only apply to freshwater ecosystems, but are generally valid. Important indirect interactions initiated by herbivores that may affect biotic resistance to invading plants including physical processes, such as bioturbation and chemical processes, such as nutrient recycling, deserve further investigation.

Acknowledgements

For help for setting up the experiment, sampling and data measurements we especially thank the technicians Dennis Waasdorp, Erik Reichman, Nico Helmsing and Suzanne Wiezer of the Aquatic Ecology department of Netherlands Institute of Ecology (NIOO-KNAW). A special thanks to all colleagues from the department and outside of the department especially Dr. Cameron Hudson. Without them this work would not have been possible. AP specially acknowledges the Science without Borders Program and CNPq (Brazilian National Council for Scientific and Technological Development) through a grant no. 207514/2014-3. The authors do not have a conflict of interest to declare.

Supplementary material

Supplementary Figure 1. Overview of the experiment (photo taken July 2018).



Supplementary Figure 2. Photography showing two experimental aquaria, one in the front with the presence of the generalist snail *Lymnaea stagnalis* (With snails) and the other in the back in the absence of the snail (No snails). Grazing of *L. stagnalis* keeps the front aquarium free of filamentous algae.



Supplementary Figure 3. Photo of the feeding trial set-up.



Table S1. Model selection for the response variables. Model selection procedure based on AICc-values of models fitted using Maximum Likelihood estimation. Indicated are: the native plant species, the response variable, the possible terms of interest in the model, AICc values of all models, the $\Delta AICc$ value relative to the model with the lowest AICc value, and Akaike weights of all models. "+" indicates the variable that was included in the model. We applied model averaging over the best models within $<2.0 \Delta AICc$ of the top model (greyed models) if needed.

Native species	Response variable (s)	Competition (Comp)	Herbivory (Herb)	Comp x Herb	AICc	$\Delta AICc$	Weight
<i>C. demersum</i>	Total invader biomass	+	+		26.8	0.00	0.518
		+	+	+	28.6	1.72	0.219
		+		+	29.6	2.75	0.131
		+			30.0	3.16	0.107
		+			32.9	6.07	0.025
<i>M. spicatum</i>	Total invader biomass	+			-8.1	0.00	0.659
		+	+		-6.5	1.60	0.296
		+	+	+	-2.7	5.37	0.045
					39.7	47.78	0.000
		+			41.9	50.03	0.000
		+			20.7	0.00	0.736
		+	+		22.9	2.24	0.240
		+	+	+	27.6	6.94	0.023
<i>P. perfoliatus</i>	Total invader biomass				33.7	13.02	0.001
		+			35.4	14.69	0.000



Chapter 6

Synthesis

Biotic resistance to alien freshwater plant species

Alien freshwater plant species are increasingly crossing geographical barriers – mainly due to the ornamental and aquarium trade – which introduces them into many tropical and temperate freshwater ecosystems (Padilla and Williams 2004a, Martin and Coetzee 2011, Peres et al. 2018). Invasive aquatic plant species are among the major threats to freshwater biodiversity, strongly affecting the structure and functioning of these ecosystems (Dudgeon et al. 2006, Strayer 2010). However, only a small portion of all introduced plant species becomes invasive, because most of them fail to establish or integrate into native communities at low densities. This ‘resistance’ by the local ecosystems can partly be attributed to biotic filters, i.e. biotic resistance.

Biotic resistance is the ability of native species to reduce the success (colonization, survival, growth and reproduction) of alien species via biotic mechanisms such as competition, herbivory, predation or disease (Levine et al. 2004). Although the patterns and the underlying mechanisms of establishment success of alien plant species have been widely debated in terrestrial and marine systems (Levine et al. 2004, Kimbro et al. 2013), few experimental studies have explored this topic in freshwater ecosystems (Alofs and Jackson 2014). Similarly, most experimental studies to date have focused on temperate ecosystems, while invasions cause problems in both temperate and tropical regions. This makes tropical regions surprisingly understudied. Our current lack of information is especially problematic if we aim to draw robust generalizations regarding the factors and mechanisms underlying establishment success of alien species across ecosystems and geographic regions (Pyšek et al. 2008).

The major aim of this thesis was to determine whether tropical and temperate native freshwater species communities can provide biotic resistance to alien plant invasions, and to understand the underlying mechanisms in freshwater ecosystems. In this synthesis, I discuss the main findings in the context of invasion and community ecology theory, their implications for conservation and restoration, and suggest potential future research avenues.

Can native communities provide biotic resistance to alien plant invasions in freshwater ecosystems?

In this thesis I distinguished two stages during the establishment success of alien submerged plants. I tested the ability of propagules to colonize, which I defined as invaders having their roots attached to the sediment after introduction of plant fragments into the water. This simulates how alien species commonly arrive in new areas with already established native communities (Figure 1). Additionally, I tested the ability of the invader to grow, defined as accumulating biomass (performance) once they successfully colonized (Figure 1). Biotic resistance to both colonization and growth has been tested throughout this thesis for freshwater ecosystems, for which I summarize the evidence in Table 1.

Figure 1. Schematic figure showing where in the invasion process I investigated biotic resistance to the establishment of several alien submerged species and how I define establishment success in this thesis. Establishment success of alien submerged plants is defined as the ability of alien species to colonize and grow. Figure modified from Levine et al. 2004.

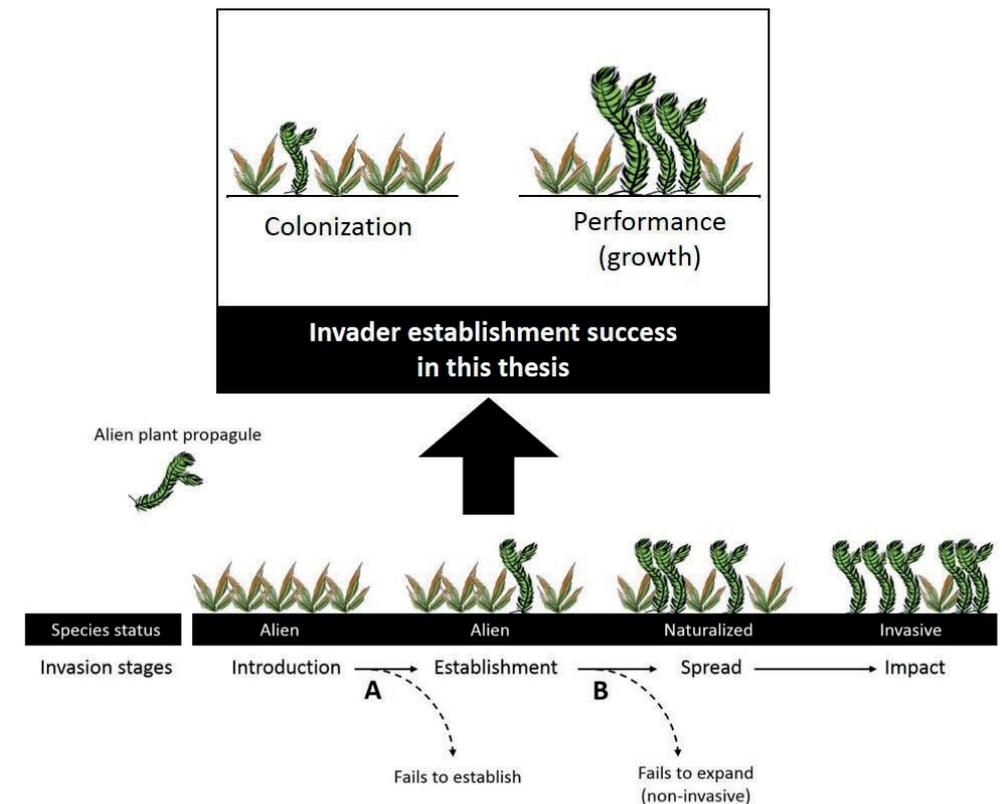


Table 1. Summary of the evidence for biotic resistance to alien submerged plants in freshwater ecosystems provided in this thesis.

Mechanisms	Evidence for biotic resistance	References	
Native vegetation (competition)	Presence-absence	Physical barrier imposed by the temperate native vegetation reduced the colonization success of alien plant <i>Lagarosiphon major</i> propagules	Chapter 2
		Physical barrier imposed by the tropical native vegetation did not reduce the colonization success of alien plant <i>Hydrilla verticillata</i>	Chapter 3
		Tropical native biomass reduced <i>Hydrilla verticillata</i> performance	Chapter 3
	Species diversity	Biomass of higher-diversity native communities overyielded due to the presence of rooted submerged species (sampling effect), which reduced <i>Lagarosiphon major</i> performance	Chapter 2
		No species diversity effect was found on <i>Hydrilla verticillata</i> performance	Chapter 3
	Limiting similarity (functional group)	Presence of a similar functional group (rooted submerged) to the invading plant <i>Lagarosiphon major</i> further reduced its performance	Chapter 2
Presence of a native species from the same functional group of the invading species <i>Egeria densa</i> further reduced its performance		Chapter 5	
Limiting similarity (phylogenetic relatedness)	Phylogenetic closely related native species to invader <i>Hydrilla verticillata</i> did not reduce its performance	Chapter 3	
Native herbivore (herbivory)	Selective feeding on native or alien plants	Herbivores reduced the success of alien species in field studies	Chapter 4
		Consumption rates of tropical and temperate plants in feeding trials did not differ	Chapter 4
		No direct effect of herbivory on <i>Egeria densa</i> performance	Chapter 5
	Indirect effects	Removing filamentous algae facilitates invader <i>Egeria densa</i> increasing its performance	Chapter 5

I investigated the colonization success of submerged invading propagules in **Chapters 2 and 3**. I found that the presence of a temperate native plant community strongly decreased the ability of the invader *Lagarosiphon major* to colonize (only 7.6% of the experimental mesocosms with a native plant community were colonized, **Chapter 2**). However, in **Chapter 3**, the invader *Hydrilla verticillata* colonized 90.5% of the mesocosms with tropical native plant species. This difference seems surprising because both tropical and temperate native species used in the experiments are known to produce dense canopies in natural systems which have the potential to act as a physical barrier to the colonization of these propagules. A likely explanation for their contrast is the difference in colonization strategies between the two invading species. Propagules of both invading species *L. major* and *H. verticillata* are able to grow roots while floating in the water. *Lagarosiphon major* grows thick adventitious roots, but the plant fragments soon need to sink towards the sediment to attach themselves. In contrast, floating fragments of *H. verticillata* are able to produce long adventitious roots, which are fine and filiform, and can grow in between the shoots of native plants while the plant fragment itself is still floating while being trapped in the vegetation canopy. This ability of *H. verticillata* gives the species a competitive advantage to rapidly access nutrients in the sediment. This shows how attributes, i.e. traits, of alien species can interact with the potential of native communities to provide biotic resistance.

After an invader colonized an area, further establishment involves growth (Fig. 1). I investigated biotic resistance to growth of invading species in three of my chapters (**Chapter 2, 3 and 5**). In **Chapter 2**, I found that the growth of *L. major* significantly decreased with increasing temperate native plant species richness. The native biomass of higher-diversity communities overyielded due to the presence of rooted submerged species – from a functional group similar to the invader – which

further lowered the growth of the invader. In **Chapter 3**, I found that tropical native plant biomass significantly reduced *H. verticillata* growth, but in contrast to my results in **Chapter 2** this effect was not related to native species richness or limiting similarity through phylogenetic relationships. **Chapter 5** showed that the growth of the tropical invader *Egeria densa* decreased due to competition with native temperate plant species, but also revealed that the identity of the native plant species determined the strength of this biotic resistance, similar to the findings in **Chapter 2**. Overall, this suggests that the strength of biotic resistance is importantly determined by the identity of both the invading and native species.

Besides experimental evidence, my literature survey on field observations in **Chapter 4** additionally showed that herbivores can strongly reduce the performance of alien species in the field. Published feeding trials indicated that herbivores feed rather unselective, and thereby ingest tropical alien plants as much as temperate plants. Although both experimental and field evidence indicate that there is potential for biotic resistance from herbivores, I did not find direct effects of herbivores on *E. densa* growth in **Chapter 5** (indirect effects of herbivory will be discussed in more detailed later).

Which mechanisms determine biotic resistance to alien aquatic plant invasions?

This thesis' findings have shown that there is biotic resistance to alien submerged plants in freshwater ecosystems. Colonization and growth of alien plants can be reduced by biotic interactions with native species. However, my experimental results varied depending on the biotic mechanism that was explored. In this section I

discuss in more detail which mechanisms I identified as important for the observed biotic resistance to the establishment success of alien submerged species.

The role of species diversity

Biotic resistance mediated by native plant diversity has long been hypothesized to reduce the success of invading plant species, known as the biotic resistance hypothesis (Elton 1958). The rationale behind this idea is that high-diversity communities have multiple species (or functional groups) that complement each other in resource use, and thereby reduce resources for potential invaders. This mechanism is known as the complementarity effect (Fargione and Tilman 2005). High-diversity communities may also be more likely to contain particular species (or functional groups) that are better competitors or more productive in the community. The presence of such productive, competing species can also provide resistance to invading species, a mechanism known as sampling effect (Wardle 2001).

In **Chapter 2**, temperate native species diversity negatively affected the establishment success of the invader *L. major*. By disentangling the two potential mechanisms of diversity, I could demonstrate that the observed diversity effect was not explained by species richness *per se* but was mainly due to a sampling effect. The most productive species (or functional group), in this case the rooted submerged species, were more likely to be included in more diverse communities and had greatest species-specific impacts on native biomass. In **Chapter 3** I manipulated tropical native species diversity, however, I found no effects of native species richness on invader performance. Although I did not find support for the biotic resistance hypothesis i.e. species diversity effect, biotic resistance was observed. There was a negative correlation between native species biomass and invader total biomass. This

implies that an increase in native biomass – irrespective of species richness – can also provide biotic resistance by itself.

Unlike studies conducted mostly in terrestrial systems, which often show a strong relationship between species (or functional) diversity and primary productivity, as observed in e.g. grasslands (Craven et al. 2016), forests (Zhang et al. 2012) and a variety of other systems (Cardinale et al. 2012), I found in **Chapter 3** that species diversity did not promote native biomass production. Contrary to what I found in **Chapter 2** that different species had different growth and the most productive ones dominated at higher diversity communities, the tropical submerged species did not differ much in biomass production among each other at the end of the experiment. Since communities dominated by different species show differences in plant growth (Bakker et al. 2010), likely the lack of dominance in our tropical plant communities – which have a completely different set of species compared to the temperate communities – explains the differences observed. This lack of a positive diversity-productivity relationship was observed in other studies with submerged plant species (Engelhardt and Ritchie 2001, Zhang et al. 2018a). A possible explanation for the general result of positive plant diversity-productivity positive relationships in terrestrial systems and inconsistent results for freshwater systems is the lack of complementarity effects among similar growth forms as submerged plants which adapted to growing under water (Zhang et al. 2018a, Riis et al. 2018).

Submerged plant communities often have a lower number of species compared to grasslands systems (Engelhardt and Ritchie 2001, Hector et al. 2001). Higher species richness commonly generates a stronger complementarity effect, resulting in a more complete resource use such as observed among grasses, legumes and forbs (Tilman et al. 1996, Weisser et al. 2017). Compared to these terrestrial

systems there are fewer submerged plant species and submerged plant species are more similar in their resource use, thus complementarity could be weaker among submerged species (Zhang et al. 2018a, Riis et al. 2018). Therefore, based on my findings, I conclude that species diversity is an important attribute of the native communities to reduce invasibility since the sampling effect is a valid mechanism by which diversity effects may express themselves. Also, native biomass plays a crucial role in reducing invasibility and this high biomass can come from the dominance of more productive species but also from the native community as a whole, as shown in this thesis.

Limiting similarity

The principal of limiting similarity suggests that species must be functionally different to coexist (MacArthur and Levins 1967). It is based on the idea that functionally different species should exploit resources differently, i.e. occupy different niches. Ecological similarities (or niche overlaps) among species can be estimated based on functional groups (trait similarity) or on phylogenetic relationships (under the assumption that closely related species share similar traits) (Strauss et al. 2006, Burns and Strauss 2011, Byun et al. 2013). The concept of functional (or phylogenetic) similarity has been applied in attempts to understand successful invasions from two different points of view: which alien species are more likely to become invasive, and which native communities are less susceptible to invasions? Based on limiting similarity theory, functional groups (or species) that are most similar (or phylogenetically closely related) to an invader will provide the greatest invasion resistance due to niche overlap (Darwin 1859, Hooper and Dukes 2010).

Besides exploring the mechanisms of taxonomic diversity (species richness) on biotic resistance (**Chapter 2 and 3**), I also explored whether the identities of functional groups (**Chapter 2**) or species (**Chapter 3**) in the native community would affect establishment success of the introduced species. I found that the performance of *L. major* depended on which functional groups were present in the native community (**Chapter 2**). *Lagarosiphon major*, as a rooted submerged plant, faced greater resistance by the native rooted plants than by the non-rooted species (**Chapter 2**). In **Chapter 5**, I also showed that competition was stronger between the alien and native species from the same functional group, although we did not test limiting similarity directly. The native non-rooted species *C. demersum* had little effect on the early establishment phase of the invasion by alien *E. densa*. Similar results have been found for several studies in terrestrial ecosystems (Naeem et al. 2000, Fargione et al. 2003, Fargione and Tilman 2005, Pokorny et al. 2005, Byun et al. 2013). For example, overall more functionally diverse serpentine grassland communities were less invaded because of the presence of functional groups similar to invading species (Hooper and Dukes 2010).

However, I found little support that phylogenetically closely related species necessarily provide greater resistance (**Chapter 3**). The tropical native submerged species *E. densa* had little effect on invader *H. verticillata* growth, although both are from the same family (Hydrocharitaceae). Based on my findings in these two chapters, I conclude that functional similarity is a better predictor of biotic resistance than phylogenetic relationships *per se*.

The role of native herbivores

Herbivory on aquatic plants was considered to have minimum impact on aquatic ecosystems for a long time (e.g. Wetzel 1983, Polunin 1984). However, a large body of evidence now demonstrates that herbivores can have large impacts on plants in aquatic systems, likely even greater than reported for terrestrial plants (Bakker et al. 2016). Herbivores can reduce significant amounts of plant biomass, increase nutrient turnover rates and affect plant species composition and distributions in aquatic systems (Bakker et al. 2016, Wood et al. 2017). This offers potential for herbivores to negatively or positively affect plant invasion outcomes.

Our current understanding of biotic resistance by native herbivores to alien freshwater plants is constrained by a lack of studies, particularly for (sub)tropical regions. In **Chapter 4** I reviewed the literature searching for empirical and theoretical evidence on potential biotic resistance from herbivores in tropical and subtropical alien aquatic plant species, because tropical aquatic invasive species are among the world's worst invasive species (Rejmánek 2011). Macro-ecological latitudinal patterns suggest that tropical aquatic plants have low palatability, are better defended and face less top-down control, which suggests that tropical invasive species may face little biotic resistance from herbivores, at higher latitudes (Pennings et al. 2009, Qiu and Kwong 2009, Morrison and Hay 2012). However, empirical evidence showed that temperate herbivores (beavers, coots and insects) significantly reduced the success of tropical invading species. This may be explained by my analysis of feeding trials that many herbivores in aquatic ecosystems forage rather unselectively (**Chapter 4**). Therefore, I conclude there is potential for biotic resistance from herbivores to alien tropical plants invading temperate regions. However, as biotic resistance research advances, it has become clear that this phenomenon is much more complex than

expected. Native species are able to interact with aliens in multiple ways that either decrease or increase the probability of alien establishment and spread.

In **Chapter 5** I empirically addressed biotic resistance by herbivory myself. I tested whether temperate herbivores can indeed provide biotic resistance to an alien tropical freshwater plant species, and included potential interactive effects of herbivory with competition by native plant species. It is important to consider that herbivores can have direct effects on invader establishment by foraging on them, but can also have indirect effects that are often neglected in invasion biology (White et al. 2006). Invader success may be indirectly influenced if herbivores alter native plant species abundances, nutrient cycling, physical disturbance rates. For example, the combined effect of herbivory and small-scale disturbances, such as fish which shred plants, contribute to reduce invasive *H. verticillata* growth (Ribas et al. 2017). However, these disturbances can play an ambiguous role at other stages of the invasion process due to the increased production and the release of vegetative propagules by herbivore activity (Ribas et al. 2017). Likewise, in **Chapter 5** I observed indirect effects of herbivores on biotic resistance, in this case, herbivores were facilitating *E. densa* invasion. At high levels of plant competition with the native species *Ceratophyllum demersum*, the generalist snail was mainly feeding on the available filamentous algae, which are often found in the meso- to eutrophic system that we mimicked, instead of on the plants. This indirectly facilitated the success of the invader *E. densa* by enhancing its performance. **Chapters 4 and 5** illustrate the need of further understanding of the role of herbivores, including their indirect interactions, on biotic resistance in natural ecosystems.

Implications for conservation and restoration

Freshwater ecosystems are heavily impacted by human activities suffering from eutrophication, habitat modification, degradation and destruction, pollution and climate change (Dudgeon et al. 2006). Considering the threat that invasive alien species pose to diversity, functioning and management of these systems, there is a growing interest in ecological restoration². Constraining invasions already during their establishment phase should be a priority of restoration because invasive species are difficult to eradicate once established (reviewed by Kettenring and Adams 2011). This could efficiently mitigate loss of biodiversity, ecosystem functions and ecosystem services. Researchers have seen this as an opportunity to apply the concepts of ecological resistance and reassemble communities more resistant to invasions (Funk et al. 2008, Byun et al. 2018). Here I will discuss how the concepts related to biotic resistance that I addressed in this thesis can be used to strengthen resistance of freshwater plant communities to the establishment of alien species, and improve restoration practices.

An essential component of restoration is to reassemble communities starting from bare sediment due to intense degradation or following the removal of invasive species. Ecosystem restoration projects are particularly prone to invasion, as the restoration process results in disturbance and increased resource availability opening opportunities for invaders (D'Antonio and Meyerson 2002). Thus, the optimal invasive species management programme should focus on conserving or restoring the native aquatic plant community (Hussner et al. 2017). Reintroducing native plant species can

² Ecological restoration is 'the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed' (SER 2004).

actively help to fill these empty available niches ensuring a high rate of biomass accumulation by the native plant community to serve as a natural barrier to the colonization and spread of alien species according to **Chapter 2 and 3**. For example, active management to stimulate a rapid development of a dense canopy in a wetland reduced the establishment of the highly invasive reed canary grass (*Phalaris arundinacea* L.) (Lindig-Cisneros and Zedler 2002). Upon restoration, the sites that were more vulnerable to *P. arundinacea* invasion were the ones that had sparse or no vegetation (Lindig-Cisneros and Zedler 2002). Here, higher species diversity provided more complex canopies reducing the *P. arundinacea* recruitment (Lindig-Cisneros and Zedler 2002).

Another factor which is important to be considered during ecosystem restoration is the selection of the species. I have shown in this thesis that biotic resistance strength is determined by the identity of both the invading and native species. Selecting native species with highly competitive attributes which can produce high biomass such as presented by rooted submerged species are good candidates for restoration practices (**Chapters 2 and 5**). Thus, the selection and combination of plants is crucial as just randomly adding species may enhance diversity but not increase resistance (**Chapter 3**). Furthermore, **Chapter 2** addressed the principals of similarity in terms of functional groups. Establishing native species functionally similar to potential invaders may be successful in limiting alien plant invasions (Fargione et al. 2003, Funk et al. 2008). However, to apply the limiting similarity concept to restoration it must be assumed that the potential invaders are known.

Unfortunately, biotic resistance enhancement is not often taken into account when measures are planned to restore submerged vegetation (Hilt et al. 2006), although some efforts have been made for emergent wetland vegetation and the

results are mixed (Kettenring and Adams 2011, Byun et al. 2018). In conclusion, increasing our knowledge on how biotic resistance may affect establishment success of alien species has the potential to improve ecosystem restoration efforts in multiple ways helping to select species, planting diversity and densities.

Future research avenues

Current understanding of biotic resistance to alien plants in freshwater ecosystems is constrained by a lack of studies examining this aspect. The importance and limitations of biotic resistance to the establishment of alien plant species in these systems have been highlighted in this thesis. However, this thesis also showed that biotic resistance is a complex result of species interactions. Native biotic interactions can additionally change over a gradient of abiotic conditions, disturbance and habitat type (Milbau et al. 2009, Bulleri et al. 2016). Thus, I fully encourage that the results of this thesis should be scaled-up to field conditions to expand the realism, relevance and predictive ability. A powerful approach would be to combine controlled mesocosm experiments to study the underlying mechanisms of biotic resistance, with field studies that can unravel the biotic processes in natural settings.

Many megadiverse developing countries are located in the tropics and are currently undergoing intensive development (Lövei et al. 2012). It seems that the growing threat from alien species invasions has not yet received the necessary attention in these rapidly developing areas. The lack of studies in the tropics is alarming. This knowledge gap is especially problematic as it limits the development of the field of invasion ecology, limiting robust generalizations across biomes and the power to predict alien species invasions and their impacts. I encourage more studies

to be performed in tropical areas around the globe. This could be achieved by international collaborations between wealthy and developing countries.

Another point that is important to address is, that indirect effects of herbivores are important in influencing the invasion outcomes as I have shown in **Chapter 5**. I suggest that these more complex interactions deserve further investigation and should be considered in future studies. Designing experiments that are able to investigate both direct and indirect effects will give insight in the importance of indirect effects on invasions.

Closing remarks

The aim of this thesis was to answer two main questions: (I) can native communities provide biotic resistance to alien plant invasions in freshwater ecosystems? And (II) which mechanisms are underlying biotic resistance to aquatic plant invasions?

I have shown that native communities provide biotic resistance to alien plant invasions in freshwater ecosystems. Based on my findings, I conclude that plant competition is an effective mechanism to provide biotic resistance. Native biomass plays a crucial role in reducing invasibility of these systems and this high biomass can come from the dominance of more productive species but also from the native community as a whole. Even though I did not experimentally find direct effects of herbivores in providing biotic resistance, I showed that they can have indirect effects on invaders, indirectly facilitating their establishment. This finding highlights the complexity of species interactions in freshwater ecosystems.

The context-dependent nature of interactions between alien and native species in freshwater ecosystems make generalizations difficult across terrestrial and marine ecosystems. This thesis illustrates the importance of continuously jointly studying invasions and loss of native biodiversity, including the novel interactions among native and invasive species in freshwater ecosystems. Furthermore, my work emphasizes the importance of conserving and restoring our native aquatic plant communities, because preserving native species increases the robustness of our aquatic ecosystems to species invasions.



Chapter 7

References



A

- Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence. *Functional Ecology* 25:420–432.
- Alofs, K. M., and D. A. Jackson. 2014. Meta-analysis suggests biotic resistance in freshwater environments is driven by consumption rather than competition. *Ecology* 95:3259–3270.
- Aloo, P., W. Ojwang, R. Omondi, J. M. Njiru, and D. Oyugi. 2013. A review of the impacts of invasive aquatic weeds on the bio-diversity of some tropical water bodies with special reference to Lake Victoria (Kenya). *Biodiversity Journal* 4:471–482.
- Amsberry, L., M. A. Baker, P. J. Ewanchuk, and M. D. Bertness. 2014. Clonal integration and the expansion of *Phragmites australis*. *Ecological Applications* 10:1110–1118.
- Arenas, F., I. Sánchez, S. J. Hawkins, S. R. Jenkins, I. Sanchez, S. J. Hawkins, and S. R. Jenkins. 2006. The invasibility of marine algal assemblages: Role of functional diversity and identity. *Ecology* 87:2851–2861.

B

- Baker, P., F. Zimmanck, and S. M. Baker. 2010. Feeding rates of an introduced freshwater gastropod *Pomacea insularum* on native and nonindigenous aquatic plants in Florida. *Journal of Molluscan Studies* 76:138–143.
- Bakker, E. S., I. Dobrescu, D. Straile, and M. Holmgren. 2013. Testing the stress gradient hypothesis in herbivore communities: facilitation peaks at intermediate

- nutrient levels. *Ecology* 94:1776–1784.
- Bakker, E. S., E. Van Donk, S. A. J. Declerck, N. R. Helmsing, B. Hidding, and B. A. Nolet. 2010. Effect of macrophyte community composition and nutrient enrichment on plant biomass and algal blooms. *Basic and Applied Ecology* 11:432–439.
- Bakker, E. S., and B. A. Nolet. 2014. Experimental evidence for enhanced top-down control of freshwater macrophytes with nutrient enrichment. *Oecologia* 176:825–836.
- Bakker, E. S., K. A. Wood, J. F. Pagès, G. F. F. (Ciska) Veen, M. J. A. Christianen, L. Santamaría, B. A. Nolet, and S. Hilt. 2016. Herbivory on freshwater and marine macrophytes: a review and perspective. *Aquatic Botany* 135:18–36.
- Balestri, E., F. Vallerini, V. Menicagli, S. Barnaba, and C. Lardicci. 2018. Biotic resistance and vegetative propagule pressure co-regulate the invasion success of a marine clonal macrophyte. *Scientific Reports* 8:16621.
- Barlow, J., F. França, T. A. Gardner, C. C. Hicks, G. D. Lennox, E. Berenguer, L. Castello, E. P. Economo, J. Ferreira, B. Guénard, C. Gontijo Leal, V. Isaac, A. C. Lees, C. L. Parr, S. K. Wilson, P. J. Young, and N. A. J. Graham. 2018. The future of hyperdiverse tropical ecosystems. *Nature* 559:517–526.
- Bates, D., M. Mächler, E. Zurich, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using *lme4*. *Journal of Statistical Software* 67:1–48.
- te Beest, M., N. J. Mpandza, and H. Olf. 2018. Biotic resistance affects growth and reproduction, but not survival of a high-impact woody invader in African savannas. *Journal of Vegetation Science* 29:532–540.
- Behrens, M. D., and K. D. Lafferty. 2012. Geographic variation in the diet of Opaleye (*Girella nigricans*) with respect to temperature and habitat. *PLoS ONE* 7:e45901.
- Bellard, C., and J. M. Jeschke. 2016. A spatial mismatch between invader impacts and research publications. *Conservation Biology* 30:230–232.
- Bini, L. M., S. M. Thomaz, K. J. Murphy, and A. F. M. Camargo. 1999. Aquatic macrophyte distribution in relation to water and sediment conditions in the Itaipu Reservoir, Brazil. *Hydrobiologia* 415:147–154.
- Bolser, R. C., and M. E. Hay. 1996. Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. *Ecology* 77:2269–2286.
- Britton-Simmons, K. H. 2006. Functional group diversity, resource preemption and the genesis of invasion resistance in a community of marine algae. *Oikos* 113:395–401.
- Brönmark, C. 1985. Interactions between macrophytes, epiphytes and herbivores - An experimental approach. *Oikos* 45:26–30.
- Brönmark, C. 1989. Interactions between epiphytes, macrophytes and freshwater snails: A review. *Journal of Molluscan Studies* 55:299–311.
- Brönmark, C. 1990. How do herbivorous freshwater snails affect macrophytes?--A comment. *Ecology* 71:1212–1215.
- Brown, C. S., and K. J. Rice. 2010. Effects of belowground resource use complementarity on invasion of constructed grassland plant communities. *Biological Invasions* 12:1319–1334.
- Brundu, G. 2015. Plant invaders in European and Mediterranean inland waters: profiles, distribution, and threats. *Hydrobiologia* 746:61–79.

Bulleri, F., L. Benedetti-Cecchi, A. Jaklin, and L. Iveša. 2016. Linking disturbance and resistance to invasion via changes in biodiversity: a conceptual model and an experimental test on rocky reefs. *Ecology and Evolution* 6:2010–2021.

Burlakova, L. E., A. Y. Karatayev, D. K. Padilla, L. D. Cartwright, and D. N. Hollas. 2009. Wetland restoration and invasive species: Apple snail (*Pomacea insularum*) feeding on native and invasive aquatic plants. *Restoration Ecology* 17:433–440.

Burnham, K. P., D. R. Anderson, and K. P. Huyvaert. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology* 65:23–35.

Burns, J. H., and S. Y. Strauss. 2011. More closely related species are more ecologically similar in an experimental test. *Proceedings of the National Academy of Sciences* 108:5302–5307.

Butchart, S. H. M., M. Walpole, B. Collen, A. Van Strien, J. P. W. Scharlemann, R. E. A. Almond, J. E. M. Baillie, B. Bomhard, C. Brown, J. Bruno, K. E. Carpenter, G. M. Carr, J. Chanson, A. M. Chenery, J. Csirke, N. C. Davidson, F. Dentener, M. Foster, A. Galli, J. N. Galloway, P. Genovesi, R. D. Gregory, M. Hockings, V. Kapos, J. F. Lamarque, F. Leverington, J. Loh, M. A. McGeoch, L. McRae, A. Minasyan, M. H. Morcillo, T. E. E. Oldfield, D. Pauly, S. Quader, C. Revenga, J. R. Sauer, B. Skolnik, D. Spear, D. Stanwell-Smith, S. N. Stuart, A. Symes, M. Tierney, T. D. Tyrrell, J. C. Vié, and R. Watson. 2010. Global biodiversity: Indicators of recent declines. *Science* 328:1164–1168.

Byun, C., S. de Blois, and J. Brisson. 2013. Plant functional group identity and diversity determine biotic resistance to invasion by an exotic grass. *Journal of Ecology* 101:128–139.

Byun, C., S. de Blois, and J. Brisson. 2018. Management of invasive plants through ecological resistance. *Biological Invasions* 20:13–27.

C

Caley, P., R. H. Groves, and R. Barker. 2008. Estimating the invasion success of introduced plants. *Diversity and Distributions* 14:196–203.

Camargo, A. F. M., M. M. Pezzato, G. G. Henry-Silva, and A. M. Assumpção. 2006. Primary production of *Utricularia foliosa* L., *Egeria densa* Planchon and *Cabomba furcata* Schult & Schult.f from rivers of the coastal plain of the State of São Paulo, Brazil. *Hydrobiologia* 570:35–39.

Capers, R. S., R. Selsky, G. J. Bugbee, and J. C. White. 2007. Aquatic plant community invasibility and scale-dependent patterns in native and invasive species richness. *Ecology* 88:3135–3143.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. MacE, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. *Nature* 486:59–67.

Carniatio, N., S. M. Thomaz, E. R. Cunha, R. Fugi, and R. R. Ota. 2013. Effects of an invasive alien Poaceae on aquatic macrophytes and fish communities in a neotropical reservoir. *Biotropica* 45:747–754.

Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. *Ecology Letters* 12:693–715.

CBD. 2002. Decision VI/23 of the Conference of the Parties to the Convention on Biological Diversity.

- Chadwell, T. B., and K. A. M. Engelhardt. 2008. Effects of pre-existing submersed vegetation and propagule pressure on the invasion success of *Hydrilla verticillata*. *Journal of Applied Ecology* 45:515–523.
- Charles, H., and J. S. Dukes. 2007. Impacts of Invasive Species on Ecosystem Services. Pages 217–237 in W. Nentwig, editor. *Biological Invasions*. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Christianen, M. J. A., F. O. H. Smulders, M. S. Engel, M. I. Nava, S. Willis, A. O. Debrot, P. J. Palsbøll, J. A. Vonk, and L. E. Becking. 2019. Megaherbivores may impact expansion of invasive seagrass in the Caribbean. *Journal of Ecology* 107:45–57.
- Clements, K. D., D. Raubenheimer, and J. H. Choat. 2009. Nutritional ecology of marine herbivorous fishes: ten years on. *Functional Ecology* 23:79–92.
- Coetzee, J. A., and M. P. Hill. 2012. The role of eutrophication in the biological control of water hyacinth, *Eichhornia crassipes*, in South Africa. *BioControl* 57:247–261.
- Coetzee, J. A., M. P. Hill, M. J. Byrne, and A. Bownes. 2011. A review of the biological control programmes on *Eichhornia crassipes* (C.Mart.) Solms (Pontederiaceae), *Salvinia molesta* D.S.Mitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides*. *African Entomology* 19:451–468.
- Coley, P. D., and T. M. Aide. 1991. Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. Pages 25–49 in P. W. Price, T. M. Lewinsohn, G. W. Fernandes, and W. W. Benson, editors. *Plant-Animal interactions: evolutionary ecology in tropical and temperate regions*. Wiley, New York, USA.
- Coley, P. D., and J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* 27:305–335.
- Cook, C. D. K., B. J. Gut, E. Martyn Rix, J. Schneller, and M. Seitz. 1974. Water plants of the world - A manual for the identification of the genera of freshwater macrophytes. Junk.
- Cook, C. D. K., and R. Luond. 1982. A revision of the genus *Hydrilla* (Hydrocharitaceae). *Aquatic Botany* 13:485–504.
- Corbin, J. D., and C. M. D'Antonio. 2004. Competition between native perennial and exotic annual grasses: implications for an historical invasion. *Ecology* 85:1273–1283.
- Craven, D., F. Isbell, P. Manning, J. Connolly, H. Bruelheide, A. Ebeling, C. Roscher, J. van Ruijven, A. Weigelt, B. Wilsey, C. Beierkuhnlein, E. de Luca, J. N. Griffin, Y. Hautier, A. Hector, A. Jentsch, J. Kreyling, V. Lanta, M. Loreau, S. T. Meyer, A. S. Mori, S. Naeem, C. Palmberg, H. Wayne Polley, P. B. Reich, B. Schmid, A. Siebenkäs, E. Seabloom, M. P. Thakur, D. Tilman, A. Vogel, and N. Eisenhauer. 2016. Plant diversity effects on grassland productivity are robust to both nutrient enrichment and drought. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371.
- Cronin, G., D. M. Lodge, M. E. Hay, M. Miller, A. M. Hill, T. Horvath, R. C. Bolser, N. Lindquist, and M. Wahl. 2002. Crayfish feeding preferences for freshwater macrophytes: the influence of plant structure and chemistry. *Journal of Crustacean Biology* 22:708–718.
- Cyr, H., and M. L. Pace. 1993. Magnitude and patterns of herbivory in aquatic ecosystem. *Nature* 361:148–150.

D

- D'Antonio, C., and L. A. Meyerson. 2002. Exotic plant species as problems and solutions in ecological restoration: a synthesis. *Restoration Ecology* 10:703–713.
- Daehler, C. C. 2001. Darwin's naturalization hypothesis revisited. *The American Naturalist* 158:324–330.
- Darwin, C. R. 1859. *The Origin of Species*. John Murray, London.
- Davies, K. F., J. Cavender-Bares, and N. Deacon. 2011. Native communities determine the identity of exotic invaders even at scales at which communities are unsaturated. *Diversity and Distributions* 17:35–42.
- Davis, M. A., J. P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: A general theory of invasibility. *Journal of Ecology* 88:528–534.
- Demment, M. W., and P. J. Van Soest. 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *The American Naturalist* 125:641–672.
- Denny, P. 1972. Sites of nutrient absorption in aquatic macrophytes. *Journal of Ecology* 60:819–829.
- Dickinson, M. B., and T. E. Miller. 1998. Competition among small, free-floating, aquatic plants. *American Midland Naturalist* 140:55–67.
- Diez, J. M., J. J. Sullivan, P. E. Hulme, G. Edwards, and R. P. Duncan. 2008. Darwin's naturalization conundrum: dissecting taxonomic patterns of species invasions. *Ecology Letters* 11:674–681.
- Dobzhansky, T. 1950. Evolution in the tropics. *American Scientist*:209–221.

Van Donk, E., and A. Otte. 1996. Effects of grazing by fish and waterfowl on the biomass and species composition of submerged macrophytes. Pages 285–290 *in* J. M. Caffrey, P. R. F. Barrett, K. J. Murphy, and P. M. Wade, editors. *Management and Ecology of Freshwater Plants*. Springer Netherlands, Dordrecht.

Dorenbosch, M., and E. S. Bakker. 2011. Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. *Freshwater Biology* 56:1783–1797.

Doyle, R., M. Grodowitz, M. Smart, and C. Owens. 2007. Separate and interactive effects of competition and herbivory on the growth, expansion, and tuber formation of *Hydrilla verticillata*. *Biological Control* 41:327–338.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81:163–182.

E

Early, R., B. A. Bradley, J. S. Dukes, J. J. Lawler, J. D. Olden, D. M. Blumenthal, P. Gonzalez, E. D. Grosholz, I. Ibañez, L. P. Miller, C. J. B. Sorte, and A. J. Tatem. 2016. Global threats from invasive alien species in the twenty-first century and national response capacities. *Nature Communications* 7:12485.

Elger, A., and M.-H. Barrat-Segretain. 2002. Use of the pond snail *Lymnaea stagnalis* (L.) in laboratory experiments for evaluating macrophyte palatability. *Fundamental and Applied Limnology* 153:669–683.

- Elger, A., and M. H. Barrat-Segretain. 2004. Plant palatability can be inferred from a single-date feeding trial. *Functional Ecology* 18:483–488.
- Elger, A., T. De Boer, and M. E. Hanley. 2007. Invertebrate herbivory during the regeneration phase: experiments with a freshwater angiosperm. *Journal of Ecology* 95:106–114.
- Elger, A., N. J. Willby, and M. Cabello-Martinez. 2009. Invertebrate grazing during the regenerative phase affects the ultimate structure of macrophyte communities. *Freshwater Biology* 54:1246–1255.
- Elton, C. S. 1958. *The Ecology of Invasions by Animals and Plants*. Springer US, Boston, MA.
- Engelhardt, K. A. M., and M. E. Ritchie. 2001. Effects of macrophyte species richness on wetland ecosystem functioning and services. *Nature* 411:687–689.
- Engelhardt, K. A. M., and M. E. Ritchie. 2002. The effect of aquatic plant species richness on wetland ecosystem processes. *Ecology* 83:2911–2924.
- Esler, D. 1989. An assessment of American coot herbivory of *hydrilla*. *The Journal of Wildlife Management* 53:1147–1149.
- Evangelista, H. B. A., S. M. Thomaz, and C. A. Umetsu. 2014. An analysis of publications on invasive macrophytes in aquatic ecosystems. *Aquatic Invasions* 9:521–528.

F

- Fan, S., H. Yu, X. Dong, L. Wang, X. Chen, D. Yu, and C. Liu. 2016. Invasive plant *Alternanthera philoxeroides* suffers more severe herbivory pressure than native

competitors in recipient communities. *Scientific Reports* 6:36542.

- Fargione, J., C. S. Brown, and D. Tilman. 2003. Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences* 100:8916–8920.
- Fargione, J. E., and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementarity effects. *Ecology Letters* 8:604–611.
- Feijoó, C., M. E. García, F. Momo, and J. Toja. 2002. Nutrient absorption by the submerged macrophyte *Egeria densa* Planch.: effect of ammonium and phosphorus availability in the water column on growth and nutrient uptake. *Limnetica* 21:96–104.
- Fine, P. V. A. 2002. The invasibility of tropical forests by exotic plants. *Journal of Tropical Ecology* 18:687–705.
- Fleming, J. P., and E. D. Dibble. 2015. Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia* 746:23–37.
- Fletcher, D., D. MacKenzie, and E. Villouta. 2005. Modelling skewed data with many zeros: a simple approach combining ordinary and logistic regression. *Environmental and Ecological Statistics* 12:45–54.
- Frankow-Lindberg, B. E. 2012. Grassland plant species diversity decreases invasion by increasing resource use. *Oecologia* 169:793–802.
- Frankow-Lindberg, B. E., C. Brophy, R. P. Collins, and J. Connolly. 2009. Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. *Annals of Botany* 103:913–921.
- Freestone, A. L., G. M. Ruiz, and M. E. Torchin. 2013. Stronger biotic resistance in

tropics relative to temperate zone: effects of predation on marine invasion dynamics. *Ecology* 94:1370–1377.

Friday, L. E. 1992. Measuring investment in carnivory: seasonal and individual variation in trap number and biomass in *Utricularia vulgaris* L. *New Phytologist* 121:439–445.

Fridley, J. D., J. J. Stachowicz, S. Naeem, D. F. Sax, E. W. Seabloom, M. D. Smith, T. J. Stohlgren, D. Tilman, and B. Von Holle. 2007. The invasion paradox: reconciling pattern and process in species invasions. *Ecology* 88:3–17.

Funk, J. L., E. E. Cleland, K. N. Suding, and E. S. Zavaleta. 2008. Restoration through reassembly: plant traits and invasion resistance. *Trends in Ecology & Evolution* 23:695–703.

G

Gallardo, B., M. Clavero, M. I. Sanchez, and M. Vila. 2016. Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology* 22:151–163.

González-Bergonzoni, I., M. Meerhoff, T. A. Davidson, F. Teixeira-de Mello, A. Baattrup-Pedersen, and E. Jeppesen. 2012. Meta-analysis shows a consistent and strong latitudinal pattern in fish omnivory across ecosystems. *Ecosystems* 15:492–503.

Gross, E. 2001. Seasonal and spatial dynamics of allelochemicals in the submersed macrophyte *Myriophyllum spicatum* L. *Internationale Vereinigung für Theoretische und Angewandte Limnologie Verhandlungen* 27:2116–2119.

Gross, E. M., and E. S. Bakker. 2012. The role of plant secondary metabolites in freshwater macrophyte-herbivore interactions: limited or unexplored chemical

defenses. Pages 154–169 in G. R. Iason, M. Dicke, and S. E. Hartley, editors. *The Integrative Role of Plant Secondary Metabolites in Ecological Systems*. Cambridge University Press.

Gross, E. M., H. Meyer, and G. Schilling. 1996. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. *Phytochemistry* 41:133–138.

Grutters, B. M. C., E. M. Gross, and E. S. Bakker. 2016. Insect herbivory on native and exotic aquatic plants: phosphorus and nitrogen drive insect growth and nutrient release. *Hydrobiologia* 778:209–220.

Grutters, B. M. C., Y. O. A. Rojendijk, W. C. E. P. Verberk, and E. S. Bakker. 2017a. Plant traits and plant biogeography control the biotic resistance provided by generalist herbivores. *Functional Ecology* 31:1184–1192.

Grutters, B. M. C., B. Saccomanno, E. M. Gross, D. B. Van de Waal, E. van Donk, and E. S. Bakker. 2017b. Growth strategy, phylogeny and stoichiometry determine the allelopathic potential of native and non-native plants. *Oikos* 126:1770–1779.

H

Van De Haterd, R. J. W., and G. N. J. Ter Heerdt. 2007. Potential for the development of submerged macrophytes in eutrophicated shallow peaty lakes after restoration measures. Pages 277–290 *Hydrobiologia*.

He, Q., and B. R. Silliman. 2016. Consumer control as a common driver of coastal vegetation worldwide. *Ecological Monographs* 86:278–294.

Hector, A., K. Dobson, A. Minns, E. Bazeley-White, and J. H. Lawton. 2001. Community diversity and invasion resistance: an experimental test in a grassland ecosystem

and a review of comparable studies. *Ecological Research* 16:819–831.

Hidding, B., E. S. Bakker, M. J. M. Hootsmans, and S. Hilt. 2016. Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. *Oikos* 125:1489–1495.

Hilt, S., and E. M. Gross. 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology* 9:422–432.

Hilt, S., E. M. Gross, M. Hupfer, H. Morscheid, J. Mählmann, A. Melzer, J. Poltz, S. Sandrock, E. M. Scharf, S. Schneider, and K. van de Weyer. 2006. Restoration of submerged vegetation in shallow eutrophic lakes - A guideline and state of the art in Germany. *Limnologica* 36:155–171.

Ho, C. K., and S. C. Pennings. 2013. Preference and performance in plant-herbivore interactions across latitude - a study in U.S. Atlantic salt marshes. *PLoS ONE* 8:e59829.

Hooper, D. U., and J. S. Dukes. 2010. Functional composition controls invasion success in a California serpentine grassland. *Journal of Ecology* 98:764–777.

Houston, W. A., and L. J. Duivenvoorden. 2002. Replacement of littoral native vegetation with the ponded pasture grass *Hymenachne amplexicaulis*: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia. *Marine and Freshwater Research* 53:1235–1244.

Hulme, P. E. 2007. Biological invasions in Europe: drivers, pressures, states, impacts and responses. *Issues in Environmental Science and Technology* 25:56–80.

Hussner, A. 2012. Alien aquatic plant species in European countries. *Weed Research* 52:297–306.

Hussner, A., I. Stiers, M. J. J. M. Verhofstad, E. S. Bakker, B. M. C. Grutters, J. Haury, J. L. C. H. van Valkenburg, G. Brundu, J. Newman, J. S. Clayton, L. W. J. Anderson, and D. Hofstra. 2017. Management and control methods of invasive alien freshwater aquatic plants: a review. *Aquatic Botany* 136:112–137.

I

Immers, A. K., E. S. Bakker, E. Van Donk, G. N. J. Ter Heerdt, J. J. M. Geurts, and S. A. J. Declerck. 2015. Fighting internal phosphorus loading: an evaluation of the large scale application of gradual Fe-addition to a shallow peat lake. *Ecological Engineering* 83:78–89.

Irfanullah, H. M., and B. Moss. 2004. Factors influencing the return of submerged plants to a clear-water, shallow temperate lake. *Aquatic Botany* 80:177–191.

J

Jeppesen, E., M. Meerhoff, K. Holmgren, I. González-Bergonzoni, F. Teixeira-de Mello, S. A. J. Declerck, L. De Meester, M. Søndergaard, T. L. Lauridsen, R. Bjerring, J. M. Conde-Porcuna, N. Mazzeo, C. Iglesias, M. Reizenstein, H. J. Malmquist, Z. Liu, D. Balayla, and X. Lazzaro. 2010. Impacts of climate warming on lake fish community structure and potential effects on ecosystem function. *Hydrobiologia* 646:73–90.

Jeschke, J., L. Gómez Aparicio, S. Haider, T. Heger, C. Lortie, P. Pyšek, and D. Strayer. 2012a. Support for major hypotheses in invasion biology is uneven and declining. *NeoBiota* 14:1–20.

Jeschke, J. M., L. G. Aparicio, S. Haider, T. Heger, C. J. Lortie, P. Pyšek, and D. L. Strayer. 2012b. Taxonomic bias and lack of cross-taxonomic studies in invasion biology. *Frontiers in Ecology and the Environment* 10:349–350.

Jeschke, J. M., S. Debille, and C. J. Lortie. 2018. Biotic resistance and island susceptibility hypotheses. Pages 60–70 *in* J. M. Jeschke and T. Heger, editors. *Invasion biology: hypotheses and evidence*. CABI, Wallingford.

K

Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17:164–170.

Kelkar, N., R. Arthur, N. Marbà, and T. Alcoverro. 2013. Greener pastures? High-density feeding aggregations of green turtles precipitate species shifts in seagrass meadows. *Journal of Ecology* 101:1158–1168.

Keller, R. P., J. Geist, J. M. Jeschke, and L. Kühn. 2011. Invasive species in Europe: Ecology, status, and policy. *Environmental Sciences Europe* 23:23.

Keller, R. P., A. Masoodi, and R. T. Shackleton. 2018. The impact of invasive aquatic plants on ecosystem services and human well-being in Wular Lake, India. *Regional Environmental Change* 18:847–857.

Kennedy, T. A., S. Naeem, K. M. Howe, J. M. H. Knops, D. Tilman, and P. Reich. 2002. Biodiversity as a barrier to ecological invasion. *Nature* 417:636–638.

Kettenring, K. M., and C. R. Adams. 2011. Lessons learned from invasive plant control experiments: a systematic review and meta-analysis.

Kettunen, M., P. Genovesi, S. Gollasch, S. Pagad, U. Starfinger, P. ten Brink, and C.

Shine. 2009. Technical support to EU strategy on invasive species (IAS) — Assessment of the impacts of IAS in Europe and the EU (final report for the European Commission, Institute for European Environmental Policy (IEEP). Brussels, Belgium.

Kimbro, D. L., B. S. Cheng, and E. D. Grosholz. 2013. Biotic resistance in marine environments. *Ecology Letters* 16:821–833.

Kolar, C. S., and D. M. Lodge. 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16:199–204.

L

Lach, L., D. K. Britton, R. J. Rundell, and R. H. Cowie. 2000. Food preference and reproductive plasticity in an invasive freshwater snail. *Biological Invasions* 2:279–288.

Lambertini, M., J. Leape, J. Marton-Lefèvre, R. A. Mittermeier, M. Rose, J. G. Robinson, S. N. Stuart, B. Waldman, and P. Genovesi. 2011, July 22. Invasives: a major conservation threat. *Science* 333:404–405.

Langeland, K. A. 1996. *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae), “The perfect aquatic weed.” *Castanea* 61:293–304.

Levine, J. M. 2000. Species diversity and biological invasions: relating local process to community pattern. *Science* 288:1998–2000.

Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters* 7:975–989.

Li, H.-L., Y.-Y. Wang, Q. Zhang, P. Wang, M.-X. Zhang, and F.-H. Yu. 2015. Vegetative

- propagule pressure and water depth affect biomass and evenness of submerged macrophyte communities. *Plos One* 10.
- Lima, C. T. de, F. de A. R. dos Santos, and A. M. Giuliatti. 2014. Morphological strategies of *Cabomba* (Cabombaceae), a genus of aquatic plants. *Acta Botanica Brasilica* 28:327–338.
- Lindig-Cisneros, R., and J. B. Zedler. 2002. *Phalaris arundinacea* seedling establishment: effects of canopy complexity in fen, mesocosm, and restoration experiments. *Canadian Journal of Botany* 80:617–624.
- Liu, J., M. Dong, S. L. Miao, Z. Y. Li, M. H. Song, and R. Q. Wang. 2006. Invasive alien plants in China: role of clonality and geographical origin. *Biological Invasions* 8:1461–1470.
- Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* 20:223–228.
- Lodge, D. M., G. Cronin, E. van Donk, and A. J. Froelich. 1998. Impact of herbivory on plant standing crop: comparisons among biomes, between vascular and nonvascular plants, and among freshwater herbivore taxa. Pages 149–174 *in* E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christoffersen, editors. *The Structuring Role of Submerged Macrophytes in Lakes*. Springer, New York, NY.
- Lonsdale, W. M., W. General, and S. Abstracts. 1999. Global patterns of plant invasions and the concept of invasibility. *Ecology* 80:1522–1536.
- Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76.
- Lövei, G. L., T. M. Lewinsohn, and the biological I. in M. R. Network. 2012. Megadiverse developing countries face huge risks from invasives. *Trends in Ecology and Evolution* 27:3–4.
- Lowry, E., E. J. Rollinson, A. J. Laybourn, T. E. Scott, M. E. Aiello-Lammens, S. M. Gray, J. Mickley, and J. Gurevitch. 2013. Biological invasions: a field synopsis, systematic review, and database of the literature. *Trends in Ecology and Evolution* 3:182–196.
- M**
- MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist* 101:377–385.
- MacDougall, A. S., B. Gilbert, and J. M. Levine. 2009. Plant invasions and the niche. *Journal of Ecology* 97:609–615.
- Madsen, T. V., and N. Cedergreen. 2002. Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology* 47:283–291.
- Marko, M. D., E. M. Gross, R. M. Newman, and F. K. Gleason. 2008. Chemical profile of the North American native *Myriophyllum sibiricum* compared to the invasive *M. spicatum*. *Aquatic Botany* 88:57–65.
- Marko, M. D., R. M. Newman, and F. K. Gleason. 2005. Chemically mediated host-plant selection by the milfoil weevil: A freshwater insect-plant interaction. *Journal of Chemical Ecology* 31:2857–2876.
- Maron, J. L., and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society B: Biological Sciences* 273:2575–2584.

- Maron, J. L., and M. Vilà. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* 95:361–373.
- Marruffini, M. L., and J. B. Geller. 2015. Species richness and interacting factors control invasibility of a marine community. *Proceedings of the Royal Society B: Biological Sciences* 282:20150439.
- Martin, G. D., and J. A. Coetzee. 2011. Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa. *Water SA* 37:371–380.
- McCreary, N. J. 1991. Competition as a mechanism of submersed macrophyte community structure. *Aquatic Botany* 41:177–193.
- Meyerson, L. A., and H. A. Mooney. 2007. Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment* 5:199–208.
- Meyerson, L. A., and H. A. Mooney. 2013. Invasive globalization alien species in an era of globalization. *Frontiers in Ecology and the Environment* 5:199–208.
- Michelan, T. S., S. M. Thomaz, and L. M. Bini. 2013. Native macrophyte density and richness affect the invasiveness of a tropical poaceae species. *Plos One* 8.
- Michelan, T. S., S. M. Thomaz, R. P. Mormul, and P. Carvalho. 2010. Effects of an exotic invasive macrophyte (tropical signalgrass) on native plant community composition, species richness and functional diversity. *Freshwater Biology* 55:1315–1326.
- Milbau, A., J. C. Stout, B. J. Graae, and I. Nijs. 2009. A hierarchical framework for integrating invasibility experiments incorporating different factors and spatial scales. *Biological Invasions* 11:941–950.
- Milligan, H., S. Deinet, L. Mcrae, and R. Freeman. 2014. Protecting species: status and trends of the Earth's protected areas. UK.
- Mitchell, C. E., A. A. Agrawal, J. D. Bever, G. S. Gilbert, R. A. Hufbauer, J. N. Klironomos, J. L. Maron, W. F. Morris, I. M. Parker, A. G. Power, E. W. Seabloom, M. E. Torchin, and D. P. Vázquez. 2006, June 1. Biotic interactions and plant invasions. John Wiley & Sons, Ltd (10.1111).
- Moles, A. T., S. P. Bonser, A. G. B. Poore, I. R. Wallis, and W. J. Foley. 2011. Assessing the evidence for latitudinal gradients in plant defence and herbivory. *Functional Ecology* 25:380–388.
- Moles, A. T., and J. Ollerton. 2016. Is the notion that species interactions are stronger and more specialized in the tropics a zombie idea?
- Morrison, W. E., and M. E. Hay. 2011. Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS ONE* 6:e17227.
- Morrison, W. E., and M. E. Hay. 2012. Are lower-latitude plants better defended? Palatability of freshwater macrophytes. *Ecology* 93:65–74.
- Mwangi, P. N., M. Schmitz, C. Scherber, C. Roscher, J. Schumacher, M. Scherer-Lorenzen, W. W. Weisser, and B. Schmid. 2007. Niche pre-emption increases with species richness in experimental plant communities. *Journal of Ecology* 95:65–78.

N

- Naeem, S., J. M. H. Knops, D. Tilman, K. M. Howe, T. Kennedy, and S. Gale. 2000. Plant diversity increases resistance to invasion in the absence of covarying extrinsic

factors. *Oikos* 91:97–108.

O

Olden, J. D., L. Comte, and X. Giam. 2018. The Homogocene: a research prospectus for the study of biotic homogenisation. *NeoBiota* 37:23–36.

Olden, J. D., N. L. R. Poff, M. R. Douglas, M. E. Douglas, and K. D. Fausch. 2004. Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology and Evolution* 19:18–24.

Orchard, A. 1979. *Myriophyllum* (Haloragaceae) in Australasia. 1. New Zealand: A revision of the genus and a synopsis of the family. *Brunonia* 2:247.

P

Padilla, D. K., and S. L. Williams. 2004a. Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems.

Padilla, D. K., and S. L. Williams. 2004b. Beyond ballast water: aquarium and species in aquatic ecosystems. *Frontiers in Ecology and the Environment* 2:131–138.

Parker, J. D., D. E. Burkepille, and M. E. Hayt. 2006. Opposing effects of native and exotic herbivores on plant invasions. *Science* 311:1459–1461.

Parker, J. D., C. C. Caudill, and M. E. Hay. 2007. Beaver herbivory on aquatic plants. *Oecologia* 151:616–625.

Parker, J. D., and M. E. Hay. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters* 8:959–967.

Peng, S., N. L. Kinlock, J. Gurevitch, and S. Peng. 2019. Correlation of native and exotic

species richness: a global meta-analysis finds no invasion paradox across scales. *Ecology* 100:e02552.

Pennings, S. C., C.-K. Ho, C. S. Salgado, K. Wieski, N. Dave, A. E. Kunza, and E. L. Wason. 2009. Latitudinal variation in herbivore pressure in Atlantic Coast salt marshes. *Ecology* 90:183–195.

Pennings, S. C., E. L. Siska, and M. D. Bertness. 2001. Latitudinal differences in plant palatability in Atlantic Coast salt marshes. *Ecology* 82:1344–1359.

Pennings, S. C., M. Zimmer, N. Dias, M. Sprung, N. Davé, C. K. Ho, A. Kunza, C. McFarlin, M. Mews, A. Pfauder, and C. Salgado. 2007. Latitudinal variation in plant-herbivore interactions in European salt marshes. *Oikos* 116:543–549.

Peres, C. K., R. W. Lambrecht, D. A. Tavares, and W. A. Chiba de Castro. 2018. Alien Express: the threat of aquarium e-commerce introducing invasive aquatic plants in Brazil. *Perspectives in Ecology and Conservation*.

Petruzzella, A., B. M. C. Grutters, S. M. Thomaz, and E. S. Bakker. 2017. Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems. *Aquatic Invasions* 12:343–353.

Petruzzella, A., J. Manschot, C. H. A. van Leeuwen, B. M. C. Grutters, and E. S. Bakker. 2018. Mechanisms of invasion resistance of aquatic plant communities. *Frontiers in Plant Science* 9.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino, and T. Tsomondo. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment* 84:1–20.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52:273–288.

Pinheiro, J., D. M. Bates, S. DebRoy, D. Sarkar, and R. C. Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models.

Pinowska, A. 2002. Effects of snail grazing and nutrient release on growth of the macrophytes *Ceratophyllum demersum* and *Elodea canadensis* and the filamentous green alga *Cladophora* sp. *Hydrobiologia* 479:83–94.

Pokorny, M. L., R. L. Sheley, C. A. Zabinski, R. E. Engel, T. J. Svejcar, and J. J. Borkowski. 2005. Plant functional group diversity as a mechanism for invasion resistance. *Restoration Ecology* 13:448–459.

Polunin, N. V. C. 1984. The decomposition of emergent macrophytes in fresh water. *Advances in Ecological Research* 14:115–166.

Price, J. N., and M. Pärtel. 2013. Can limiting similarity increase invasion resistance? A meta-analysis of experimental studies. *Oikos* 122:649–656.

Prior, K. M., T. H. Q. Powell, A. L. Joseph, and J. J. Hellmann. 2015. Insights from community ecology into the role of enemy release in causing invasion success: the importance of native enemy effects. *Biological Invasions* 17:1283–1297.

Pyšek, P., D. M. Richardson, J. Pergl, V. Jarošík, Z. Sixtová, and E. Weber. 2008. Geographical and taxonomic biases in invasion ecology. *Trends in Ecology and Evolution* 23:237–244.

Pyšek, P., D. M. Richardson, M. Rejmánek, G. L. Webster, M. Williamson, and J. Kirschner. 2004. Alien plants in checklists and floras: towards a better

communication between taxonomists and ecologists. *Taxon* 53:131–143.

Q

Qian, H., and Y. Jin. 2016. An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *Journal of Plant Ecology* 9:233–239.

Qiu, J. W., and K. L. Kwong. 2009. Effects of macrophytes on feeding and life-history traits of the invasive apple snail *Pomacea canaliculata*. *Freshwater Biology* 54:1720–1730.

R

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Redekop, P., E. M. Gross, A. Nuttens, D. E. Hofstra, J. S. Clayton, and A. Hussner. 2018. *Hygraula nitens*, the only native aquatic caterpillar in New Zealand, prefers feeding on an alien submerged plant. *Hydrobiologia* 812:13–25.

Reich, P. B., and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences* 101:11001–11006.

Rejmánek, M. 2011. Invasiveness. Pages 379–385 in D. Simberloff and M. Rejmánek, editors. *Encyclopedia of Biological Invasions*. University of California Press, Berkeley.

Ribas, L. G. D., E. R. Cunha, J. R. S. Vitule, R. P. Mormul, S. M. Thomaz, and A. A. Padial. 2017. Biotic resistance by snails and fish to an exotic invasive aquatic plant.

Freshwater Biology 62:1266–1275.

Ricciardi, A., and S. K. Atkinson. 2004. Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems. *Ecology Letters* 7:781–784.

Ricciardi, A., M. F. Hoopes, M. P. Marchetti, and J. L. Lockwood. 2013. Progress toward understanding the ecological impacts of non-native species. *Ecological Monographs* 83:263–282.

Richardson, D. M., and P. Pyšek. 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography* 30:409–431.

Richardson, D. M., P. Pyšek, M. Rejmánek, M. G. Barbour, F. Dane Panetta, and C. J. West. 2000. Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* 6:93–107.

Riis, T., T. V. Madsen, and R. S. H. H. Sennels. 2009. Regeneration, colonisation and growth rates of allofragments in four common stream plants. *Aquatic Botany* 90:209–212.

Riis, T., A. Olesen, S. M. Jensen, A. B. Alnoee, A. Baattrup-Pedersen, T. L. Lauridsen, and B. K. Sorrell. 2018. Submerged freshwater plant communities do not show species complementarity effect in wetland mesocosms. *Biology Letters* 14:20180635.

Riis, T., and K. Sand-Jensen. 2006. Dispersal of plant fragments in small streams. *Freshwater Biology* 51:274–286.

Rodríguez, J. P. 2001. Exotic species introductions into South America: an underestimated threat? *Biodiversity and Conservation* 10:1983–1996.

Rosenzweig, M. L. 2001. The four questions: What does the introduction of exotic species do to diversity? *Evolutionary Ecology Research* 3:361–367.

S

Sala, O. E., F. S. Chapin III, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774.

Santamaría, L. 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologica* 23:137–154.

Sax, D. F. 2001. Latitudinal gradients and geographic ranges of exotic species: implications for biogeography. *Journal of Biogeography* 28:139–150.

Schemske, D. W., G. G. Mittelbach, H. V. Cornell, J. M. Sobel, and K. Roy. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics* 40:245–269.

Seals, C., A. G. Eversole, J. R. Tomasso, and B. R. Petrosky. 1997. Effects of temperature on feeding activity of the white river crayfish *Procambarus acutus acutus*. *Journal of the World Aquaculture Society* 28:133–141.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, M. Arianoutsou, S. Bacher, B. Blasius, G. Brundu, C. Capinha, L. Celesti-Grapow, W. Dawson, S. Dullinger, N. Fuentes, H. Jäger, J. Kartesz, M. Kenis, H. Kreft, I. Kühn, B. Lenzner, A. Liebhold, A. Mosena, D. Moser,

- M. Nishino, D. Pearman, J. Pergl, W. Rabitsch, J. Rojas-Sandoval, A. Roques, S. Rorke, S. Rossinelli, H. E. Roy, R. Scalera, S. Schindler, K. Štajerová, B. Tokarska-Guzik, M. Van Kleunen, K. Walker, P. Weigelt, T. Yamanaka, and F. Essl. 2017. No saturation in the accumulation of alien species worldwide. *Nature Communications* 8:14435.
- SER. 2004. The SER International Primer on Ecological Restoration.
- Shea, K., and P. Chesson. 2002. Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17:170–176.
- Shurin, J. B., D. S. Gruner, and H. Hillebrand. 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proceedings of the Royal Society B-Biological Sciences* 273:1–9.
- Simberloff, D., J. Martin, P. Genovesi, V. Maris, D. A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, P. Pysek, R. Sousa, E. Tabacchi, M. Vila, and I. 2013. Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* 28.
- Sotka, E. E., J. Forbey, M. Horn, A. G. B. Poore, D. Raubenheimer, and K. E. Whalen. 2009. The emerging role of pharmacology in understanding consumer-prey interactions in marine and freshwater systems. *Integrative and Comparative Biology* 49:291–313.
- Sousa, W. T. Z. 2011. *Hydrilla verticillata* (Hydrocharitaceae), a recent invader threatening Brazil's freshwater environments: a review of the extent of the problem. *Hydrobiologia* 669:1–20.
- Sousa, W. T. Z., S. M. Thomaz, K. J. Murphy, M. J. Silveira, and R. P. Mormul. 2009. Environmental predictors of the occurrence of exotic *Hydrilla verticillata* (L.f.) Royle and native *Egeria najas* Planch. in a sub-tropical river floodplain: The Upper River Paraná, Brazil. *Hydrobiologia* 632:65–78.
- Spencer, D. F., and M. Rejmánek. 1989. Propagule type influences competition between two submersed aquatic macrophytes. *Oecologia* 81:132–137.
- Stachowicz, J. J., J. F. Bruno, and J. E. Duffy. 2007. Understanding the effects of marine biodiversity on communities and ecosystems. *Annual Review of Ecology, Evolution, and Systematics* 38:739–766.
- Stachowicz, J. J., H. Fried, R. W. Osman, and R. B. B. Whitlatch. 2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. *Ecology* 83:2575–2590.
- Stiers, I., J. Njambuya, and L. Triest. 2011. Competitive abilities of invasive *Lagarosiphon major* and native *Ceratophyllum demersum* in monocultures and mixed cultures in relation to experimental sediment dredging. *Aquatic Botany* 95:161–166.
- Stiers, I., and L. Triest. 2017. Impact of non-native invasive plant species cover on phytoplankton and zooplankton communities in temperate ponds. *Aquatic Invasions* 12:385–395.
- Stohlgren, T. J., D. T. Barnett, and J. T. Kartesz. 2003. The rich get richer: patterns of plant invasions in the United States. *Frontiers in Ecology and the Environment* 1:11.
- Stohlgren, T. J., D. Binkley, G. W. Chong, M. A. Kalkhan, L. D. Schell, K. A. Bull, Y. Otsuki, G. Newman, M. Bashkin, and Y. Son. 1999. Exotic plant species invade hot spots of native plant diversity. *Ecological Monographs* 69:25–46.

Strauss, S. Y., C. O. Webb, and N. Salamin. 2006. Exotic taxa less related to native species are more invasive. *Proceedings of the National Academy of Sciences of the United States of America* 103:5841–5845.

Strayer, D. L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55:152–174.

Stuckey, R. L. 1974. The introduction and distribution of *Nymphoides peltatum* (Menyanthaceae) in North America. *Bartonia*:14–23.

T

Tewari, S., and S. J. Johnson. 2011. Impact of two herbivores, *Samea multiplicalis* (Lepidoptera: Crambidae) and *Cyrtobagous salviniae* (Coleoptera: Curculionidae), on *Salvinia minima* in south Louisiana. *Journal of Aquatic Plant Management* 49:36–43.

Theoharides, K. A., and J. S. Dukes. 2007. Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist* 176:256–273.

Thiébaud, G., M. Gillard, and C. Deleu. 2016. Growth, regeneration and colonisation of *Egeria densa* fragments: the effect of autumn temperature increases. *Aquatic Ecology* 50:175–185.

Thomaz, S. M., R. P. Mormul, and T. S. Michelan. 2015. Propagule pressure, invasibility of freshwater ecosystems by macrophytes and their ecological impacts: a review of tropical freshwater ecosystems. *Hydrobiologia* 746:39–59.

Thuiller, W., L. Gallien, I. Boulangeat, F. de Bello, T. Münkemüller, C. Roquet, and S. Lavergne. 2010. Resolving Darwin's naturalization conundrum: a quest for

evidence. *Diversity and Distributions* 16:461–475.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379:718–720.

Tomasetto, F., R. P. Duncan, and P. E. Hulme. 2013. Environmental gradients shift the direction of the relationship between native and alien plant species richness. *Diversity and Distributions* 19:49–59.

U

Umetsu, C. A., H. B. A. Evangelista, and S. M. Thomaz. 2012. The colonization, regeneration, and growth rates of macrophytes from fragments: a comparison between exotic and native submerged aquatic species. *Aquatic Ecology* 46:443–449.

Underwood, G. J. C. 1991. Growth enhancement of the macrophyte *Ceratophyllum demersum* in the presence of the snail *Planorbis planorbis*: the effect of grazing and chemical conditioning. *Freshwater Biology* 26:325–334.

Underwood, G. J. C., J. D. Thomas, and J. H. Baker. 1992. An experimental investigation of interactions in snail-macrophyte-epiphyte systems. *Oecologia* 91:587–595.

Urban, R. A., J. E. Titus, and W. X. Zhu. 2006. An invasive macrophyte alters sediment chemistry due to suppression of a native isoetid. *Oecologia* 148:455–463.

V

Vamosi, S. M., S. B. Heard, J. C. Vamosi, and C. O. Webb. 2009. Emerging patterns in the comparative analysis of phylogenetic community structure. *Molecular*

Ecology 18:572–592.

Van, T. K., G. S. Wheeler, and T. D. Center. 1998. Competitive interactions between *Hydrilla* (*Hydrilla verticillata*) and *Vallisneria* (*Vallisneria americana*) as influenced by insect herbivory. *Biological Control* 11:185–192.

Vejříková, I., L. Vejřík, J. Syväranta, M. Kiljunen, M. Čech, P. Blabolil, M. Vašek, Z. Sajdlová, S. H. T. Chung, M. Šmejkal, J. Frouzová, and J. Peterka. 2016. Distribution of herbivorous fish is frozen by low temperature. *Scientific Reports* 6:39600.

Verhofstad, M. J. J. M., M. M. Alirangues Núñez, E. P. Reichman, E. van Donk, L. P. M. Lamers, and E. S. Bakker. 2017. Mass development of monospecific submerged macrophyte vegetation after the restoration of shallow lakes: roles of light, sediment nutrient levels, and propagule density. *Aquatic Botany* 141:29–38.

Vilà, M., C. Basnou, P. Pyšek, M. Josefsson, P. Genovesi, S. Gollasch, W. Nentwig, S. Olenin, A. Roques, D. Roy, P. E. Hulme, P. Andriopoulos, M. Arianoutsou, I. Bazos, I. Kokkoris, A. Yannitsaros, A. Zikos, S. Augustin, P. O. Cochard, C. Lopez-Vaamonde, D. Sauvard, A. Yart, S. Bacher, F. Bretagnolle, J. Gasquez, F. Chiron, S. Kark, S. Shirley, P. Clergeau, C. Cocquempot, A. Coeur d'Acier, F. Dorkeld, A. Migeon, M. Navajas, M. David, P. Delipetrou, K. Georghiou, M. L. Desprez-Loustau, V. Didziulis, F. Essl, B. S. Galil, M. Hejda, V. Jarosik, J. Pergl, I. Perglová, I. Kühn, M. Winter, P. W. Kühn, A. Marcer, J. Pino, M. McLoughlin, D. Minchin, V. E. Panov, M. Pascal, K. Paboljsaj, R. Scalera, O. Sedláček, P. Zagatti, M. Vila, C. Basnou, P. Pyšek, M. Josefsson, P. Genovesi, S. Gollasch, W. Nentwig, S. Olenin, A. Roques, D. Roy, P. E. Hulme, P. Andriopoulos, M. Arianoutsou, S. Augustin, S. Bacher, I. Bazos, F. Bretagnolle, F. Chiron, P. Clergeau, P. O. Cochard, C. Cocquempot, A. Coeur d'Acier, M. David, P. Delipetrou, M. L.

Desprez-Loustau, V. Didziulis, F. Dorkeld, F. Essl, B. S. Galil, J. Gasquez, K. Georghiou, M. Hejda, V. Jarosik, S. Kark, I. Kokkoris, I. Kuhn, P. W. Lambdon, C. Lopez-Vaamonde, A. Marcer, A. Migeon, M. McLoughlin, D. Minchin, M. Navajas, V. E. Panov, M. Pascal, J. Pergl, I. Perglova, J. Pino, K. Paboljsaj, W. Rabitsch, J. Y. Rasplus, D. Sauvard, R. Scalera, O. Sedlacek, S. Shirley, M. Winter, A. Yannitsaros, A. Yart, P. Zagatti, A. Zikos, and D. Partners. 2010. How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment* 8:135–144.

Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarošík, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pyšek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14:702–708.

W

Wakeman, R. W., and D. H. Les. 1994. Interspecific competition between *Potamogeton amplifolius* and *Myriophyllum spicatum*. *Lake and Reservoir Management* 9:125–129.

Walsh, G. C., and M. Maestro. 2016. Impact of introduced native herbivores on a *Pistia stratiotes* infestation close to the Paraná Delta in Argentina. *Biocontrol Science and Technology* 26:35–46.

Wardle, D. A. 2001. Experimental demonstration that plant diversity reduces invasibility - evidence of a biological mechanism or a consequence of sampling effect? *Oikos* 95:161–170.

Weisser, W. W., C. Roscher, S. T. Meyer, A. Ebeling, G. Luo, E. Allan, H. Beßler, R. L. Barnard, N. Buchmann, F. Buscot, C. Engels, C. Fischer, M. Fischer, A. Gessler, G. Gleixner, S. Halle, A. Hildebrandt, H. Hillebrand, H. de Kroon, M. Lange, S. Leimer, X. Le Roux, A. Milcu, L. Mommer, P. A. Niklaus, Y. Oelmann, R. Proulx, J. Roy, C. Scherber, M. Scherer-Lorenzen, S. Scheu, T. Tschardtke, M. Wachendorf, C. Wagg, A. Weigelt, W. Wilcke, C. Wirth, E.-D. Schulze, B. Schmid, and N. Eisenhauer. 2017. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: patterns, mechanisms, and open questions. *Basic and Applied Ecology* 23:1–73.

Wells, R. D. S., M. D. de Winton, and J. S. Clayton. 1997. Successive macrophyte invasions within the submerged flora of Lake Tarawera, Central North Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 31:449–459.

Wetzel, R. G. 1983. *Limnology*. 2nd edition. Saunders College Publishing, Philadelphia.

White, E. M., J. C. Wilson, and A. R. Clarke. 2006. Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions* 12:443–455.

Williamson, M., and A. Fitter. 1996. The varying success of invaders. *Ecology* 77:1661–1666.

Winter, M., O. Schweiger, S. Klotz, W. Nentwig, P. Andriopoulos, M. Arianoutsou, C. Basnou, P. Delipetrou, V. Didziulis, M. Hejda, P. E. Hulme, P. W. Lambdon, J. Pergl, P. Pysek, D. B. Roy, and I. Kuhn. 2009. Plant extinctions and introductions lead to phylogenetic and taxonomic homogenization of the European flora. *Proceedings of the National Academy of Sciences* 106:21721–21725.

Wong, P. K., Y. Liang, N. Y. Liu, and J. W. Qiu. 2010. Palatability of macrophytes to the invasive freshwater snail *Pomacea canaliculata*: differential effects of multiple plant traits. *Freshwater Biology* 55:2023–2031.

Wood, K. A., M. T. O'Hare, C. McDonald, K. R. Searle, F. Daunt, and R. A. Stillman. 2017. Herbivore regulation of plant abundance in aquatic ecosystems. *Biological Reviews* 92:1128–1141.

Wood, K. A., R. A. Stillman, R. T. Clarke, F. Daunt, and M. T. O'Hare. 2012. The impact of waterfowl herbivory on plant standing crop: a meta-analysis. *Hydrobiologia* 686:157–167.

Wootton, K. L. 2017. Omnivory and stability in freshwater habitats: does theory match reality? *Freshwater Biology* 62:821–832.

Wu, Z., J. Zuo, J. Ma, J. Wu, S. Cheng, and W. Liang. 2007. Establishing submersed macrophytes via sinking and colonization of shoot fragments clipped off manually. *Wuhan University Journal of Natural Sciences* 12:553–557.

WWF. 2016. *Living Planet Report*. Gland, Switzerland.

WWF. 2018. *Living Planet Report - 2018: Aiming Higher*. Gland, Switzerland.

X

Xiong, W., D. Yu, Q. Wang, C. Liu, and L. Wang. 2008. A snail prefers native over exotic freshwater plants: implications for the enemy release hypotheses. *Freshwater Biology* 53:2256–2263.

Xu, K., W. Ye, H. Cao, X. Deng, Q. Yang, and Y. Zhang. 2004. The role of diversity and functional traits of species in community invasibility. *Bull. Acad. Sin* 45:149–157.

Y

- Yannelli, F. A., C. Koch, J. M. Jeschke, and J. Kollmann. 2017. Limiting similarity and Darwin's naturalization hypothesis: understanding the drivers of biotic resistance against invasive plant species. *Oecologia* 183:775–784.
- Yarrow, M., V. H. Marín, M. Finlayson, A. Tironi, L. E. Delgado, and F. Fischer. 2009. The ecology of *Egeria densa* planchon (liliopsida: Alismatales): a wetland ecosystem engineer.
- Young, S. L., J. N. Barney, G. B. Kyser, T. S. Jones, and J. M. DiTomaso. 2009. Functionally similar species confer greater resistance to invasion: Implications for grassland restoration. *Restoration Ecology* 17:884–892.
- Yu, H. H., L. G. Wang, C. H. Liu, and S. F. Fan. 2018. Coverage of native plants is key factor influencing the invasibility of freshwater ecosystems by exotic plants in China. *Frontiers in Plant Science* 9.

Z

- Zhang, Q., Y.-P. Liu, F.-L. Luo, B.-C. Dong, and F.-H. Yu. 2018a. Does species richness affect the growth and water quality of submerged macrophyte assemblages? *Aquatic Botany*.
- Zhang, Y., H. Y. H. Chen, and P. B. Reich. 2012. Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. *Journal of Ecology* 100:742–749.
- Zhang, Y., E. Jeppesen, X. Liu, B. Qin, K. Shi, Y. Zhou, S. M. Thomaz, and J. Deng. 2017. Global loss of aquatic vegetation in lakes. *Earth-Science Reviews* 173:259–

265.

- Zhang, Y., H. Meng, Y. Wang, and Q. He. 2018b. Herbivory enhances the resistance of mangrove forest to cordgrass invasion. *Ecology* 99:1382–1390.
- Zheng, Y.-L., J. H. Burns, Z.-Y. Liao, Y. Li, J. Yang, Y. Chen, J. Zhang, and Y. Zheng. 2018. Species composition, functional and phylogenetic distances correlate with success of invasive *Chromolaena odorata* in an experimental test. *Ecology Letters* 21:1211–1220.
- Zhu, D. H., P. Wang, W. Z. Zhang, Y. Yuan, B. Li, and J. Wang. 2015. Sampling and complementarity effects of plant diversity on resource use increases the invasion resistance of communities. *PLoS One* 10.

Chapter 8



Summary /

Nederlandse samenvatting /

Sumário em Português /

Acknowledgements/Agradecimentos /

About the author



/ Summary

Biological invasions represent a key component of human-induced global changes. With the intensification of trade, transport and tourism, the number of introductions of alien species has significantly increased over the last centuries. Especially introductions of alien plants into aquatic ecosystems – mainly via the aquarium and ornamental trade – are among the major threats to global freshwater biodiversity. Invasive aquatic plants can have a wide range of impacts on freshwater ecosystems by altering the structure and functioning of these systems. This commonly results in loss of ecosystem services and annual losses of billions of Euros globally.

Although the impacts of invasive alien species can be alarming, in fact only a small proportion of all introduced species establishes and becomes problematic. Most introduced species fail to establish or have their population size constrained, and never become invasive. Because alien species are important drivers of global biodiversity loss, there is an increasing interest in understanding why some alien plant species establish as invaders and others fail, and why some habitats are more susceptible to invasions than others. However, these questions remain surprisingly understudied in freshwater ecosystems, especially for tropical regions. The lack of information is especially problematic because tropical systems are among the most threatened on Earth, and freshwater ecosystems show the strongest biodiversity decline.

The failure of some alien species in either establishing or spreading into new areas is often attributed to biotic resistance. Biotic resistance is the ability of native species to reduce the success (establishment and performance) of alien species via species interactions such as competition, herbivory, predation or disease. Native aquatic communities may resist invasions through a diversity of niche-based

mechanisms and interactions with other trophic levels. The major aim of this thesis was to determine whether tropical and temperate native freshwater species communities can provide biotic resistance to alien plant invasions and to understand the underlying mechanisms in freshwater ecosystems. I studied this by using a combination of mesocosm experiments and published evidence, in a model system consisting of tropical and temperate submerged plant species and an aquatic generalist herbivore. Throughout the chapters of this thesis I aim to improve our understanding of the biotic mechanisms underlying the establishment success of alien plant species in tropical and temperate freshwater aquatic ecosystems.

In the first two chapters of this thesis I tested the biotic resistance hypothesis, which predicts that more diverse native communities provide more resistance to invasions than less diverse communities (Elton 1958). I manipulated native plant species richness gradients in temperate (**Chapter 2**) and tropical (**Chapter 3**) freshwater ecosystems and experimentally assessed whether native plant diversity can reduce the colonization and performance (i.e. growth) of alien submerged plant species. By increasing plant diversity, also the chance that a similar functional group (**Chapter 2**) or a phylogenetically closely related species to the invader (**Chapter 3**) was present in the native community increased. In **Chapter 2** I describe how temperate native species diversity negatively affected the establishment success of the invader *Lagarosiphon major*. Higher native species diversity resulted in lower establishment success of introduced fragments of the invading species, probably because richer native communities contained species that were functionally similar to the invader (i.e. also rooted). In contrast, in **Chapter 3** I found no effects of tropical native plant diversity on the establishment success of the invader *Hydrilla verticillata*. In this experiment plant biomass of the tropical native community lowered invader success, but species diversity was not important. This suggests that phylogenetically

closely related species do not necessarily provide greater biotic resistance to invaders. Combining my findings in these two chapters, I suggest that increases in native plant diversity as well as biomass productivity can be mechanisms that provide biotic resistance. Greatest biotic resistance can be expected from functionally similar native plant species, with functional similarity being a better predictor of biotic resistance than phylogenetic relationships *per se*.

In **Chapter 4 and 5**, I added another trophic level to my approach by including herbivory by native herbivores as an additional mechanism of biotic resistance. In **Chapter 4**, I used a literature survey – including both field studies and laboratory feeding trials – to assess whether native temperate herbivores can provide resistance to the establishment success of tropical and subtropical alien aquatic plants. I found that even though macro-ecological latitudinal patterns suggest that temperate herbivores provide less biotic resistance to tropical plants, as they consume limited amount of plants and would not prefer to feed on tropical plant species, analysis of the empirical data did not support this general idea. I therefore conclude that native herbivores can provide strong biotic resistance to tropical plants, particularly in field studies. Most herbivores foraged rather unselective in feeding trials, and ingested tropical plants as much as temperate plants.

Because I found biotic resistance via herbivory as well as via competition with native plants, I further explored their potential interactions in **Chapter 5**. In this chapter, I tested how herbivory and competition by native plants interactively affected alien species growth after colonization. Mimicking natural conditions found in temperate mesotrophic and eutrophic freshwater lakes, I grew three native submerged species individually at three competition levels with and without the native aquatic generalist herbivore snail *Lymnaea stagnalis*. I subsequently simulated an

early stage of establishment of the tropical highly invasive alien species *Egeria densa*. I found that competition by native plant species consistently and significantly reduced invader performance at the highest level of native plant competition, regardless of native species identity. Herbivory had no negative effect on invader performance. However, we found a significant positive interaction between competition and herbivory for one native plant species. The herbivorous snail was mainly feeding on the available filamentous algae, which are often found in this kind of system, instead of on the plants. This indirectly facilitated the invasion of *E. densa* by enhancing its performance. **Chapters 4 and 5** illustrate the need of further understanding of the role of herbivores, including their indirect interactions, on biotic resistance in natural ecosystems.

Finally, in **Chapter 6**, I synthesize my findings by gathering all the evidence that my thesis provides for the importance of biotic resistance to alien plant invasions in freshwater ecosystems. The strength of biotic resistance in freshwater ecosystems is importantly determined by the identity of both the invading and native species. Native plant biomass plays a crucial role in providing biotic resistance in these systems, but also species diversity and functional group similarity are important. This thesis illustrates the importance of jointly studying invasions and loss of native biodiversity, including the novel interactions among native and invasive species. Furthermore, my work emphasizes the importance of conserving and restoring our native aquatic plant communities, because preserving native species increases the robustness of our aquatic ecosystems to species invasions.

/ Nederlandse samenvatting

De verspreiding van uitheemse plant- en diersoorten over de hele wereld is één van de belangrijkste effecten van de mens op natuurlijke systemen. Het aantal biologische invasies neemt de laatste eeuwen steeds sneller toe door intensivering van handel, transport en toerisme. Met name introducties van uitheemse waterplanten in zoetwater ecosystemen – voornamelijk via de aquarium- of sierhandel – vormen een grote bedreiging voor de wereldwijde aquatische biodiversiteit. Geïntroduceerde waterplanten die invasief worden kunnen grote gevolgen hebben voor de structuur en het functioneren van zoetwater ecosystemen. Invasieve soorten tasten daarbij ook de ecosysteem diensten aan die deze ecosystemen leveren, wat jaarlijks miljarden euros kost.

Hoewel de gevolgen van invasieve soorten desastreus kunnen zijn, lijkt slechts een klein deel van alle door de mens geïntroduceerde soorten zich ook daadwerkelijk te vestigen en op termijn tot problemen te leiden. De meeste soorten kunnen zich na introductie uiteindelijk niet vestigen, of blijven alleen lokaal aanwezig en worden niet invasief. Omdat uitheemse soorten een belangrijke oorzaak zijn van wereldwijd verlies van biodiversiteit, wordt het steeds belangrijker om te begrijpen waarom sommige uitheemse planten soorten zich vestigen en invasief worden, terwijl anderen daar niet in slagen, en waarom sommige gebieden vatbaarder zijn voor invasies dan andere. Hierover is nog weinig bekend in zoetwatersystemen, met name in de tropen. Dit is een urgent probleem, aangezien wereldwijd de biodiversiteit het sterkst achteruitgaat in zoetwater ecosystemen, en tropische ecosystemen tot de meest bedreigde op aarde behoren.

Het feit dat sommige uitheemse soorten er niet in slagen zich te vestigen of nieuwe gebieden te koloniseren wordt vaak toegeschreven aan “biologische resistentie”. Biologische resistentie is het vermogen van inheemse soorten om het succes (vestiging en groei) van uitheemse soorten te verminderen via interacties tussen soorten zoals competitie, begrazing, predatie of ziektes. Inheemse zoetwater gemeenschappen kunnen resistentie tegen invasies bieden via mechanismen die gebaseerd zijn op overlap in niches, of via interacties met andere trofische niveaus. Het voornaamste doel van dit proefschrift was om te bepalen of tropische en gematigde inheemse zoetwater gemeenschappen resistentie kunnen bieden tegen invasies van uitheemse planten, en om de onderliggende mechanismen in zoetwater ecosystemen te begrijpen. Ik heb dit bestudeerd door een combinatie van experimenten in mesocosms en analyses van gepubliceerde artikelen te gebruiken, in een modelsysteem van ondergedoken tropische en gematigde waterplanten en een algemene aquatische herbivoor. Met dit proefschrift wil ik ons begrip over het succes van uitheemse zoetwaterplanten in tropische en gematigde zones vergroten en de onderliggende biologische mechanismen verder ontrafelen.

In de eerste twee hoofdstukken van dit proefschrift heb ik de biologische resistentie hypothese getest. Deze hypothese stelt dat inheemse plantengemeenschappen met een hogere diversiteit meer resistentie tegen uitheemse soorten zullen bieden dan gemeenschappen met een lagere inheemse diversiteit (Elton 1958). Ik testte deze hypothese experimenteel door het creëren van een gradiënt aan diversiteit van inheemse waterplanten in gematigde (**Hoofdstuk 2**) en tropische (**Hoofdstuk 3**) zoetwater systemen, en door te onderzoeken hoe deze gradiënten de kolonisatie en prestatie (gemeten als groei) van een ondergedoken uitheemse waterplant beïnvloedden. Door het verhogen van de inheemse plantendiversiteit nam ook de kans toe dat inheemse planten van dezelfde

functionele groep (**Hoofdstuk 2**) of fylogenetisch nauw verwante soorten (**Hoofdstuk 3**) aanwezig waren. In **Hoofdstuk 2** beschrijf ik hoe inheemse plantensoorten uit de gematigde zone het vestigingssucces van de invasieve soort *Lagarosiphon major* negatief beïnvloedden. Toename van inheemse diversiteit verlaagde de vestigingskans van plantfragmenten van deze invasieve soort, waarschijnlijk omdat de meer diverse planten gemeenschappen soorten bevatten die een vergelijkbare niche innemen als de invasieve soort (i.e. ook uit de groep van wortelende waterplanten). In **Hoofdstuk 3** vond ik daarentegen geen effecten van de diversiteit van inheemse tropische waterplanten op het vestigingssucces van de invasieve soort *Hydrilla verticillata*. Het succes van *H. verticillata* was in dit experiment lager bij een toename aan totale biomassa van de inheemse planten, maar de diversiteit van de inheemse vegetatie was hierbij niet belangrijk. Dit suggereert dat de aanwezigheid van fylogenetisch nauw verwante soorten geen garantie biedt op een hoge biologische resistentie. Zowel een toename van inheemse plantendiversiteit als een toename van de totale biomassa van de inheemse vegetatie kunnen mechanismen zijn die zorgen voor biologische resistentie. De meeste resistentie is te verwachten van functioneel vergelijkbare soorten, waarbij functionele overlap een betere voorspeller van resistentie is dan fylogenetische verwantschap.

In **Hoofdstuk 4 en 5** heb ik nog een trofisch niveau aan mijn studies toegevoegd, door begrazing door inheemse herbivoren als een extra mechanisme voor biologische resistentie te bestuderen. Door middel van een literatuur studie (**Hoofdstuk 4**) – waarin ik gebruik maakte van zowel publicaties met veldgegevens als experimentele laboratorium studies – beoordeelde ik of inheemse herbivoren in gematigde klimaatzones biologische resistentie kunnen bieden tegen vestiging van tropische en subtropische uitheemse waterplanten. Macro-ecologische patronen suggereren dat herbivoren uit de gematigde zone minder biologische resistentie

zouden bieden tegen tropische planten aangezien ze weinig planten eten en geen voorkeur hebben voor tropische planten. Dit idee werd echter niet ondersteund door de analyse van de beschikbare gegevens. Ik concludeer daarom dat inheemse herbivoren wel degelijk biologische resistentie tegen tropische planten kunnen bieden, wat met name duidelijk wordt uit veldstudies. Uit de laboratorium studies blijkt dat de meeste aquatische herbivoren aselectief foerageren, waarbij ze evenveel tropische planten eten als planten uit de gematigde zone.

Omdat ik biologische resistentie via zowel plant-plant competitie als via begrazing heb gevonden, heb ik ook een mogelijk interactie tussen deze twee vormen van resistentie onderzocht. In **Hoofdstuk 5** heb ik getest of de interactie tussen begrazing en competitie de groei van uitheemse planten na hun vestiging kan beïnvloeden. Ik simuleerde daarvoor de natuurlijke omstandigheden van mesotrofe en eutrofe zoetwatermeren, en liet daar drie inheemse ondergedoken waterplanten in groeien. Voor elke soort creëerde ik monocultures met drie dichtheden (competitieniveaus), en voegde daar wel of niet de inheemse plantenetende zoetwaterslak *Lymnaea stagnalis* aan toe. In deze opzet simuleerde ik een vroeg stadium van de vestiging van de zeer invasieve tropische waterplant *Egeria densa*. Competitie door de inheemse soorten bij de hoogste dichtheid remde de groei van de invasieve soort in alle gevallen, onafhankelijk van de inheemse soortendiversiteit. Begrazing had geen negatief effect op de groei van de invasieve soort. Wel vonden we bij één van de inheemse plantensoorten een significante positieve interactie tussen competitie en begrazing. Dit kwam hoogstwaarschijnlijk doordat de plantenetende slak met name op draadalgen foerageerde (draadalgen komen vaak in dit soort systemen voor) in plaats van op de waterplanten. Dit had indirect positieve gevolgen voor de prestaties (groei) van *E. densa* en faciliteerde daarbij de invasie. **Hoofdstukken 4 en 5** illustreren daarmee het belang van onderzoek naar de rol

van plantenetende dieren op biologische resistentie in natuurlijke systemen, inclusief eventuele interacties met inheemse planten.

In **Hoofdstuk 6** bediscussieer ik tot slot al mijn bevindingen, door al het bewijs uit mijn proefschrift voor het belang van biologische resistentie in zoetwatersystemen samen te voegen. Biologische resistentie wordt sterk bepaald door de identiteit van de uitheemse en inheemse plantensoorten. De biomassa van inheemse soorten speelt daarbij een belangrijke rol voor biologische resistentie, maar ook soorten diversiteit en functionele gelijkenis kunnen belangrijk zijn. Dit proefschrift illustreert daarmee het belang van onderzoek aan het huidige verlies aan biodiversiteit in combinatie met onderzoek naar biologische invasies, inclusief de nieuwe interacties tussen inheemse en invasieve soorten die daarbij ontstaan. Dit proefschrift benadrukt het belang van het behoud en herstel van onze inheemse waterplanten gemeenschappen, omdat dit tot robuuste zoetwater ecosystemen leidt die minder vatbaar zijn voor biologische invasies.

/ Sumário em Português

Invasões biológicas representam uma componente chave das mudanças globais induzidas pelo homem. Com a intensificação do comércio, transporte e turismo, o número de introduções de espécies alienígenas (também chamadas de exóticas) aumentaram significativamente nos últimos séculos. Introduções de plantas alienígenas em ecossistemas aquáticos — principalmente através da aquariofilia e do comércio de plantas ornamentais — estão entre as principais ameaças à biodiversidade global de água doce. Plantas aquáticas invasoras podem ter uma ampla gama de impactos nos ecossistemas de água doce, alterando a sua estrutura e funcionamento, o que resulta em perda de serviços ecossistêmicos e perdas anuais de bilhões de euros globalmente.

Embora os impactos das espécies alienígenas invasoras possam ser alarmantes, apenas uma pequena proporção de todas as espécies introduzidas se estabelece e se torna problemática. A maioria destas não conseguem estabelecer ou tem os seus tamanhos populacionais restringidos, nunca se tornando invasoras. Como espécies alienígenas são importantes impulsionadores da perda de biodiversidade global, há um interesse crescente em entender o porquê algumas espécies se estabelecem como invasoras e outras falham, como também o porquê alguns *habitats* são mais suscetíveis às invasões do que outros. Entretanto, essas questões permanecem surpreendentemente pouco estudadas em ecossistemas de água doce, especialmente em regiões tropicais. A falta de informação é especialmente problemática considerando que estes sistemas tropicais estão entre os mais ameaçados da Terra, e os ecossistemas de água doce mostram o declínio mais forte da biodiversidade.

O fracasso de algumas espécies alienígenas em estabelecer ou se espalhar em novas áreas é frequentemente atribuído à resistência biótica. A resistência biótica é a capacidade de espécies nativas de reduzir o sucesso (estabelecimento e desempenho) de espécies alienígenas via interações entre espécies como competição, herbivoria, predação ou doença. As comunidades aquáticas nativas podem resistir às invasões através de uma diversidade de mecanismos baseados em nicho e interações com outros níveis tróficos. O principal objetivo desta tese foi determinar se comunidades de espécies de água doce nativas tropicais e temperadas podem fornecer resistência biótica às invasões de plantas alienígenas e entender os mecanismos subjacentes em ecossistemas de água doce. Estudei isso usando uma combinação de experimentos em mesocosmos e evidências publicadas, em um sistema modelo que consistia em espécies de plantas submersas tropicais e temperadas e um herbívoro generalista aquático. Ao longo dos capítulos desta tese pretendo melhorar o nosso entendimento sobre os mecanismos bióticos subjacentes ao sucesso de estabelecimento de espécies de plantas alienígenas em ecossistemas aquáticos de água doce, tropicais e temperados.

Nos dois primeiros capítulos desta tese, testei a hipótese da resistência biótica, que prediz que comunidades nativas mais diversas fornecem mais resistência às invasões do que comunidades menos diversas (Elton, 1958). Manipulei gradientes de riqueza em espécies de plantas nativas em ecossistemas de água doce temperado (**Capítulo 2**) e tropical (**Capítulo 3**) e avaliei experimentalmente se a diversidade de plantas nativas pode reduzir a colonização e o desempenho (ou seja, crescimento) de espécies de plantas alienígenas submersas. Ao aumentar a diversidade de plantas, também aumentou a chance de que um grupo funcional similar (**Capítulo 2**) ou uma espécie filogeneticamente relacionada ao invasor (**Capítulo 3**) estivesse presente na comunidade nativa. No **Capítulo 2** descrevo como a diversidade de

espécies nativas temperadas afetou negativamente o sucesso de estabelecimento do invasor *Lagarosiphon major*. A maior diversidade de espécies nativas resultou em menor sucesso de estabelecimento dos fragmentos introduzidos da espécie invasora, provavelmente porque as comunidades nativas mais ricas continham espécies que eram funcionalmente semelhantes ao invasor (ou seja, também enraizadas). Em contraste, no **Capítulo 3** não encontrei nenhum efeito da diversidade de plantas nativas tropicais no sucesso de estabelecimento da invasora *Hydrilla verticillata*. Neste experimento, a biomassa de plantas da comunidade nativa tropical diminuiu o sucesso do invasor, mas a diversidade de espécies não foi importante. Isso também sugere que espécies filogeneticamente relacionadas ao invasor não fornecem necessariamente maior resistência biótica. Combinando as minhas descobertas nesses dois capítulos, sugiro que o aumento na diversidade de plantas nativas e na produção de biomassa podem ser mecanismos que fornecem resistência biótica. A maior resistência biótica pode ser esperada a partir de espécies de plantas nativas funcionalmente semelhantes ao invasor, sendo similaridade funcional um melhor preditor de resistência biótica do que as relações filogenéticas *per se*.

Nos **Capítulos 4 e 5**, adicionei outro nível trófico à minha abordagem, incluindo herbivoria por herbívoros nativos como um mecanismo adicional de resistência biótica. No **Capítulo 4**, usei uma pesquisa bibliográfica — incluindo estudos de campo e ensaios de alimentação em laboratório — para avaliar se herbívoros temperados nativos podem fornecer resistência ao sucesso de estabelecimento de plantas aquáticas alienígenas tropicais e subtropicais. Descobri que, embora padrões latitudinais macroecológicos sugiram que herbívoros temperados forneçam menor resistência biótica às plantas tropicais, uma vez que consomem uma quantidade limitada e não preferem se alimentar dessas espécies, a análise dos dados empíricos não corroborou essa ideia. Concluo, portanto, que

herbívoros nativos podem fornecer forte resistência biótica às plantas tropicais, particularmente em estudos de campo. A maioria dos herbívoros se alimentou não seletivamente nos ensaios de alimentação, e ingeriu plantas tropicais tanto quanto plantas temperadas.

Como encontrei resistência biótica através de herbivoria e também por competição com plantas nativas, explorei ainda mais as suas potenciais interações no **Capítulo 5**. Neste capítulo, testei como a herbivoria e a competição por plantas nativas afetavam interativamente o crescimento de espécies alienígenas depois da colonização. Imitando as condições naturais encontradas em lagos de água doce mesotróficos e eutróficos temperados, cresci três espécies nativas submersas individualmente em três níveis de competição com e sem o caramujo herbívoro generalista nativo *Lymnaea stagnalis*. Subsequentemente, simulei um estágio inicial de estabelecimento da espécie alienígena tropical altamente invasora *Egeria densa*. Descobri que a competição por espécies de plantas nativas reduz consistente e significativamente o desempenho do invasor no mais alto nível de competição de plantas nativas, independentemente da identidade das plantas nativas. Herbivoria não teve efeito negativo no desempenho do invasor. No entanto, encontramos uma interação positiva significativa entre a competição e a herbivoria para uma espécie de planta nativa. O caramujo herbívoro estava se alimentando principalmente das algas filamentosas disponíveis, que são frequentemente encontradas neste sistema, em vez das plantas. Isso indiretamente facilitou a invasão de *E. densa*, melhorando o seu desempenho. Os **Capítulos 4 e 5** ilustram a necessidade de maior compreensão do papel dos herbívoros, considerando interações indiretas, na resistência biótica em ecossistemas naturais.

Finalmente, no **Capítulo 6**, sintetizo as minhas descobertas reunindo todas as evidências que minha tese fornece sobre a importância da resistência biótica às

invasões de plantas alienígenas em ecossistemas de água doce. A força da resistência biótica nos ecossistemas de água doce é determinada pela identidade das espécies invasoras e nativas. A biomassa de plantas nativas desempenha um papel crucial no fornecimento de resistência biótica nestes sistemas, mas a diversidade de espécies e similaridade de grupos funcionais também são importantes. Esta tese ilustra a importância de se estudar conjuntamente invasões e perda de biodiversidade nativa, incluindo as novas interações entre espécies nativas e invasoras. Além disso, meu trabalho enfatiza a importância de conservar e restaurar nossas comunidades nativas de plantas aquáticas, uma vez que a preservação de espécies nativas aumenta a robustez de nossos ecossistemas aquáticos às invasões de espécies.

/ Acknowledgements/Agradecimentos

WHAT A JOURNEY!!!

This PhD journey was guided and supported by so many wonderful people that I found difficult to write this part. It would not have been possible to reach this far without you all. All my respect and gratitude!!! I leave here a personal message to each of you.

QUE JORNADA!!!

Esse doutorado foi guiado e apoiado por tantas pessoas maravilhosas que eu achei difícil escrever essa parte. Não teria sido possível chegar até aqui sem todos vocês. Todo meu respeito e gratidão!!! Deixo aqui minha mensagem pessoal para cada um de vocês.

/ About the author

Antonella Petruzzella was born on 11 of March 1989 in Macaé, a city located 190 km from Rio de Janeiro, Brazil. As a young girl, she was always very curious, communicative, and with a strong sense of adventure. She wanted to see and explore the world and its nature. Her first serious thought about what she wanted to be when growing up was journalism. However, this thought was rapidly replaced when she started having biology classes at school (Escola Alfa). She fell in love with biology because of a science teacher called Vera Lucia Cruz Coutinho (Verinha). When she was 19 years old she left home and went to live in Rio de Janeiro city to go to university. She did her bachelor in Biology in one of the best universities of the country, Federal University of Rio de Janeiro (UFRJ). These were the golden years! During already her first year of studies, she saw an advertisement for an internship about the restoration of Amazonian lakes. It matched perfectly to what she has imagined doing for life and soon she started an internship at the Laboratório de Limnologia - UFRJ (Limnology lab). After 6 beautiful years studying her bachelor and master's degree, under the supervision of Prof. Dr. Francisco de Assis Esteves (Chico), she became an aquatic ecologist. During her masters she investigated the influence of herbivore damage on methane emissions from emergent aquatic macrophytes. Although she was working on carbon cycle, aquatic plants and herbivores ecology caught her interested more than methane. During that period, she had her first contact with Dr. Liesbeth Bakker work who is passionate for any kind of herbivores. In 2015, she got her master's degree and also a personal Brazilian grant to do her full PhD supervised by Dr. Bakker at the Netherlands Institute of Ecology (NIOO-KNAW). This thesis is part of the result of 4 years of lots of work but also fun. She is still full of ideas about how to improve our understanding of the determinants of invasion success but also full of dreams.



Publications

Zhang P, Grutters BMC, van Leeuwen CHA, Xu J, **Petruzzella A**, van den Berg RF, Bakker ES (2019) Effects of rising temperature on the growth, stoichiometry, and palatability of aquatic plants. *Frontiers in Plant Science* 9:1947

Petruzzella A, Manschot J, van Leeuwen CHA, Grutters BMC, Bakker ES (2018) Mechanisms of invasion resistance of aquatic plant communities. *Frontiers in Plant Science* 9:134

Petruzzella A, Grutters BMC, Thomaz SM, Bakker ES (2017) Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems. *Aquatic Invasions* 12(3): 343-353

Petruzzella A, Guariento RD, Gripp AR, Marinho CC, Figueiredo-Barros MP, Esteves FA (2015) Herbivore damage increases methane emission from emergent aquatic macrophytes. *Aquatic Botany* 127: 6-11

Petruzzella A, Marinho CC, Sanches LF, Minello M, Esteves FA (2013) Magnitude and variability of methane production and concentration in tropical coastal lagoons sediments. *Acta Limnologica Brasiliensia* 25(3): 341-351

Gripp AR, Marinho CC, Sanches LF, **Petruzzella A**, Esteves FA (2013) The role played by aquatic macrophytes regarding CO₂ balance in a tropical coastal lagoon (Cabiúnas Lagoon, Macaé, RJ). *Acta Limnologica Brasiliensia* 25(3): 291-301

Contact

E-mail: antonellabio@gmail.com

Researchgate: https://www.researchgate.net/profile/Antonella_Petruzzella

ORCID-iD: orcid.org/0000-0002-3111-3302

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (6 ECTS)

- Potential for biotic resistance from herbivores to tropical and subtropical plant invasions in aquatic ecosystems

Writing of Project proposal (4.5 ECTS)

- Biotic resistance strength against invasive aquatic macrophytes in tropical and temperate wetlands

Postgraduated courses (4.5 ECTS)

- Aquatic Ecology – Robustness of aquatic ecosystems in the face of global change (2018)
- Introduction to Zero Inflated Models with R (2018)
- Meta-analysis (2016)
- Advanced Statistics course Design of Experiments (2016)
- Introduction to R for statistical analysis (2016)

Laboratory training and working visits (4.5 ECTS)

- Species diversity and invasion resistance in tropical freshwater ecosystems (NUPEM/UFRJ – Federal University of Rio de Janeiro, Brazil) (2017)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Aquatic Botany – Plant invasion and soil methane emissions (2016)/Factors limiting *Azolla* productivity (2018)

- Acta Limnologica Brasiliensia (ALB) – Temporary succession of macrophytes in temporary environments (2017)

Competence Strengthening / Skills courses (3.8 ECTS)

- Research data management (workshop) (2016)
- Writing for academic publication (2017)
- Reviewing a scientific paper (2017)
- Research Integrity (2017)

PE&RC Weekend, PE&RC Day, and other PE&RC events (2.1 ECTS)

- PE&RC Weekend for first years (2015)
- PE&RC Day (November 5) (2015)
- PE&RC Day (October 20) (2016)
- PE&RC Weekend for midterm (2017)

Discussion groups / local seminars or scientific meetings (7.5 ECTS)

- NIOO monthly PhD Seminar (2016-2018)
- Growing Peat: New Opportunities and Challenges for the Restoration of Peatlands – CWE symposium (Broek in Waterland) (2015)
- Half a century of fundamental and applied wetland ecology: from acidification to climate change – CWE symposium (Nijmegen) (2015)
- Netherlands Annual Ecology Meeting (NAEM) – NERN and NecoV (Lunteren) (2016)
- The good, the bad or a bit of both? The role of exotic species in aquatic ecosystems – CWE symposium (Wageningen) (2016)
- Rewilding with large herbivores: challenges and opportunities for science and practice – NecoV (Wageningen) (2016)
- Future of aquatic carbon: impacts, feedbacks and mitigation – CWE symposium (Wageningen) (2017)

- Seed dispersal by vertebrates in forests and wetlands: unifying concepts in tropical and temperate systems – NIOO Mini-symposium (Wageningen) (2019)

International symposia, workshops and conferences (9 ECTS)

- 14th International Symposium on Aquatic Plants (Edinburgh, Scotland), poster (2015)
- XXXIII SIL 2016 (Turin, Italy), oral presentation (2016)
- 15th International Symposium on Aquatic Plants (Queenstown, New Zealand), oral presentation (2018)
- ASLO 2018 Summer Meeting (Victoria, BC, Canada), oral presentation (2018)

Lecturing / Supervision of practical's / tutorials (1.5 ECTS)

- Aquatic Ecology bachelor course – Aquatic macrophytes practical (Utrecht University/NIOO) (2016, 2018)

Supervision of MSc student(s) (3 ECTS)

- Species diversity and invasion resistance in temperate freshwater ecosystems
- Species diversity and invasion resistance in tropical freshwater ecosystems

“Reconhece a queda e não desanima

Levanta, sacode a poeira

E dá a volta por cima”

Samba ‘Volta por cima’ written by Paulo Vanzolini