

## Research Article

## Impact of non-native invasive plant species cover on phytoplankton and zooplankton communities in temperate ponds

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**Editor's note:**

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

**Abstract**

More than one-third of non-native freshwater organisms are known to cause ecological impacts, but there is still a knowledge gap on how impacts are related to non-native plant abundance. We investigated the relationship between coverage of three aquatic non-native invasive species *Hydrocotyle ranunculoides*, *Ludwigia grandiflora* and *Myriophyllum aquaticum* and phytoplankton and zooplankton abundances. We hypothesized that uninvaded ponds, with a diverse submerged macrophyte community, would have higher plankton biodiversity compared to invaded ponds. In addition, we predicted that invasive plant cover would have a negative impact on plankton biodiversity. We sampled the phytoplankton and zooplankton communities of 18 ponds in Belgium, concurrently with 11 local environmental variables including cover of the invasive species. Our results showed that uninvaded ponds, with a high abundance of submerged vegetation, were associated with indicators of a clear water state and several zooplankton taxa. Variation partitioning showed that both environmental variables and invasive species cover explained zooplankton community variation. We observed a negative relationship between invasive species cover and zooplankton density as expected. In particular, large cladocerans and littoral taxa were absent when the cover of invasive species was high. This is possibly due to low dissolved oxygen concentrations, physical obstruction and a putative allelopathic property of the invasive plants. Invasive species cover was not significantly correlated with phytoplankton biovolume, although some evidence indicated that the biovolume of functional groups (FG) reflected the differences in habitat between invaded and uninvaded ponds. Reynolds functional Y group (*Cryptomonas* spp.) shows a tolerance to low light availability and was the dominant FG in the invaded ponds. Our study provides evidence that it is necessary to include cover of an invasive species to disentangle patterns of impact.

**Key words:** abundance, alien, amphibious macrophytes, freshwater ecosystem, *Hydrocotyle ranunculoides*, *Ludwigia grandiflora*, *Myriophyllum aquaticum*, Reynolds functional groups

**Introduction**

Due to global trade the number of new introduced species has exponentially increased, a trend that is especially evident in aquatic ecosystems. Vilà et al. (2010) showed that freshwater organisms are of parti-

cular concern as more than one-third of the non-native species registered in the DAISIE database are known to cause an ecological impact. Today, more than 400 non-native aquatic and semi-aquatic plant species for aquarium or pond purposes are in trade in Europe (Hussner 2008). As aquatic environments are relatively

homogeneous at a large spatial scale (Santamaría 2002), many aquatic plants can survive and colonise habitats outside their native geographic range (Cook 1985), causing a diverse impact on aquatic biodiversity.

In an aquatic system, complex ecological interactions exist between native macrophytes and different biological communities (Jeppesen et al. 1998). The structure of a macrophyte assemblage plays a large role in determining density and composition in a variety of organismal groups (Declerck et al. 2005; Kuczyńska-Kippen and Nagengast 2006; Bakker et al. 2010; Declerck et al. 2011). In addition, submerged macrophytes control phytoplankton in temperate regions through several mechanisms such as allelopathy (Hilt and Gross 2008), by providing shelter for large zooplankton against predators (Burks et al. 2002) or by lowering nutrient levels (van Donk and van de Bund 2002).

When a non-native plant establishes in a freshwater ecosystem it becomes part of the food web and will compete for light and nutrients with phytoplankton, periphyton and native plants. Moreover, a shift in the primary-production base of a pond will cascade throughout the ecosystem and affect multiple trophic levels. The magnitude of impacts will depend on the identity and density of the species and quality of the habitat prior to invasion (Schultz and Dibble 2012). Invasive macrophytes (defined as non-natives that cause negative environmental or economic impacts) are well known and often cursed ecosystem modifiers in the broadest sense of the word (Strayer 2010). They can create dense beds that limit water movement and light penetration, cause hypoxia or anoxia in the underlying waters, interfere with important human activities that rely on water resources (e.g. recreation and navigation) and outcompete native species (Dandelot 2004; Villamagna and Murphy 2010; Sousa 2011; Hussner 2014). On the other hand, there is some evidence that invasive species can facilitate native species, especially through habitat modification (Rodríguez 2006). Invasive species can provide a unique and complex structure to the freshwater ecosystems in which they establish, thereby increasing diversity and abundance of invertebrates (Thorp et al. 1997; Masifwa et al. 2001; Strayer et al. 2003; Phillips 2008; Nguyen et al. 2015). For submerged invasive species in particular, there is experimental evidence that the replacement of native with non-native plants does not affect invertebrate communities (Grutters et al. 2015).

In shaping phytoplankton and zooplankton assemblages, both the characteristics of the invader (i.e. plant identity, plant architecture, cover) as well as the environmental setting (i.e. predator intensity, habitat quality, nutrient concentrations) will be

important. Studies that have focused on how invasive species influenced phytoplankton and zooplankton assemblages are scarce. Most studies compared abundance and composition of plankton in patches of the invasive species with areas of open water (Brendonck et al. 2003; Mazzeo et al. 2003; but see Villamagna 2009). Vilà et al. (2011) conducted a meta-analysis on the ecological effects of non-native invasive species and highlighted the knowledge gap of how cover of an invasive species affects native communities.

Non-native invasive *Ludwigia grandiflora* (Michx.) Greuter and Burdet (native to South-America), *Myriophyllum aquaticum* (Velloso) Verdcourt (native to South America) and *Hydrocotyle ranunculoides* L. fil. (native to North and South America) possess a trait that sets them apart from previous invaders. All three species exhibit a rapid stoloniferous perennial growth, including production of floating and emergent stems, which might explain their current and future success as invaders. The three species are recognized as noxious weeds in several European countries (DAISIE 2009). They only recently became invasive and might not have yet completed their spread within Europe (Hussner 2012). Once a water body is invaded, diverse native macrophyte communities are displaced by dense monospecific mats on both the open water and exposed mud i.e. amphibious species (Stiers et al. 2011).

The main objective of this study was to evaluate how these three invasive species affect phytoplankton and zooplankton assemblages. We hypothesized that uninvaded ponds, with a diverse submerged macrophyte community, would have higher plankton biodiversity compared to invaded ponds. In addition, we expected that 1) cover of the invasive species would have a negative impact on plankton biodiversity and 2) invasive species identity would play a role in the observed differences.

## Material and methods

### *Field sampling and sample analysis*

In total 18 ponds (Table S1) were sampled in Belgium during August 2008 to estimate the impact of coverage of the three invasive species on environmental variables, phytoplankton and zooplankton biodiversity. The studied ponds represent a gradient of percent cover of one of the invasive species present (uninvaded ponds,  $n = 6$ ; *H. ranunculoides* invaded ponds,  $n = 3$ ; *L. grandiflora* invaded ponds,  $n = 5$ ; *M. aquaticum* invaded ponds,  $n = 4$ , Table S1). A particular focus was given to sites of high biological value (i.e. nature reserves and Natura 2000 sites).

**Table 1.** Variation partitioning and associated P values of the phytoplankton and zooplankton data matrices into the different components.

Variation	Phytoplankton		Zooplankton	
	Variation explained (%)	P	Variation explained (%)	P
[ENV + EXOT]	35	0.001	50.2	0.001
[ENV]	28.1	0.002	41.9	0.001
[EXOT]	7.2	0.25	17.9	0.03
[ENV   EXOT]	27.8	0.002	32.3	0.002
[EXOT   ENV]	6.9	0.16	8.3	0.05
[ENV ∩ EXOT]	0.3		9.6	
1 – [ENV + EXOT]	65		49.8	

All water bodies are small and shallow ranging from 0.01 to 0.29 ha in surface area with a depth ranging from 0.5 to 4 m.

We visually estimated submerged and invasive macrophyte cover in each pond using a DAFOR scale (Kent and Coker 1992). The following variables were measured in situ: water temperature, pH, dissolved oxygen, conductivity with portable probes and transparency with a Secchi disk. In the uninvaded ponds, phytoplankton and zooplankton samples were randomly taken around and within the submerged macrophyte vegetation. In the invaded ponds samples were taken randomly near the edge and in the interior of the invasive plant mats. Phytoplankton samples were collected with a plastic tube sampler (diameter: 4.5 cm; length: 70 cm) that closes in the lower part. An extension was fixed to the sampler to reach the deeper parts of the ponds when necessary. Five random subsamples from each pond were mixed and 500 ml of this was taken for phytoplankton analysis, 1 L for chemical analyses and 1 L for chlorophyll *a* (Chl *a*) determination. In the laboratory, the following variables were measured: chlorophyll *a* (Chl *a*) and main nutrients (total phosphorus – TP; soluble reactive phosphorus – SRP; dissolved inorganic nitrogen – DIN i.e. NO<sub>x</sub> (NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>) and NH<sub>4</sub><sup>+</sup>) following standard methods (APHA-AWWA-WEF 1995).

Phytoplankton samples were fixed in situ with alkaline lugol, sodium thiosulfate and buffered formaline (Kemp et al. 1993) and stored in the dark. Identification up to genus level and counting were performed with an inverted microscope. For each sample, at least 100 individuals (filament, colony, and single-celled organisms) were enumerated and the results were expressed as biovolume. Phytoplankton biovolume (mm<sup>3</sup> l<sup>-1</sup>) was estimated using the approximations of cell shapes to geometrical forms from the measurements of 30–40 individuals per genus (whenever possible) following Hillebrand et al. (1999) and Vadrucchi et al. (2007). Each genus was classified into functional groups (FG) sensu Reynolds et al.

(2002) (updated by Padisák et al. 2009) with an indication of their life form and presence in the uninvaded and invaded sites (Table S2).

At the same time, zooplankton samples were collected by taking 6–9 random subsamples of 1 L with the same sampler used for phytoplankton and nutrients. The samples were mixed and filtered through a 64 µm-mesh net, preserved in 5% formaldehyde and stored at 4 °C. For each sample, we counted and identified a minimum of 100 individuals using inverted microscopy. Different levels of identification were used: cladocerans were identified up to genus level, copepods were divided into cyclopods, calanoids and nauplii. Rotifers were counted to give an indication of their abundance but not identified. Based on literature the cladoceran genera were categorized into two groups according to habitat use: planktonic taxa and littoral, predominantly epiphytic taxa (Flossner 2000), and size category: large (large cladocera density, LCD) vs. small (Moss et al. 2003), with an indication of presence in the uninvaded and invaded sites (Table S3). The length of maximally 20 *Daphnia* or *Diaphanosoma* individuals (large cladocera length, LCL) was measured as an indicator of grazing intensity and size-selective predation (Carpenter et al. 2001).

#### Data analysis

To determine compositional similarities in zooplankton and phytoplankton taxa between invaded and uninvaded ponds, we used Bray-Curtis similarity calculated on relative density for zooplankton (based on taxon) and on relative biovolume for phytoplankton (based on genus and FG) using PRIMER v6 (Clarke and Gorley 2006). Relative density/biovolume (percentage contribution of a taxon to the total) was used because differences in phytoplankton biovolume and zooplankton density can exist between ponds with contrasting ecological states (Scheffer 1998). Mann-Whitney U tests, with Bonferroni correction, were used to compare environmental variables between invaded and uninvaded ponds. At

pond level, Spearman rank correlation analyses were performed between invasive species cover and total phytoplankton biovolume and total zooplankton density, respectively. Analyses were performed using STATISTICA 8.0 (Statsoft, inc (2007), Tulsa, OK, USA).

To visualize the variation present in the ponds based on environmental data, a principal component analysis (PCA) was used with phytoplankton and zooplankton taxa as supplementary variables. Phytoplankton biovolume, aggregated to division level, and zooplankton density were Hellinger transformed prior to analyses (Legendre and Gallagher 2001). Normality of the environmental variables was checked visually using histograms and using the K-S test. Based on this analysis Secchi depth (SD), exotic species cover (EC), submerged species cover (SC), conductivity (cond), pH, temperature ( $T^{\circ}$ ) and dissolved oxygen ( $O_2$ ) were not transformed and the remaining variables were  $\log_{10}(x+1)$  transformed. Variation partitioning was used to investigate the relative contribution of environmental variables (ENV) and exotic species cover (EXOT). Only the environmental variables that were significant in a forward selection procedure (Monte Carlo permutation tests,  $n = 999$ ), and thus important in explaining phytoplankton and zooplankton community composition, were included in the model. We used redundancy analysis (RDA) since a preliminary detrended correspondence analysis (DCA) showed that the species respond linearly to gradients (gradient length of 1.493 and  $1.821 < 3$  SD for phytoplankton and zooplankton, respectively). The matrix of environmental variables (ENV) included conductivity ( $p = 0.008$ ) and dissolved  $O_2$  ( $p = 0.023$ ) for the analysis of phytoplankton community composition, and pH ( $p = 0.002$ ) for the analysis of zooplankton community composition. The total variation in the dependent matrices (phytoplankton biovolume and zooplankton density) was broken down into different components: total explained variation [ENV + EXOT]; environmental explained variation [ENV]; exotic species cover explained variation [EXOT]; the variation explained by the environmental factors independent of exotic species cover [ENV | EXOT] and the variation explained by the exotic species cover independent of any environmental variable [EXOT | ENV]. The significance of the different components was tested with Monte Carlo permutations under the reduced model. Two other factors were derived without significance testing: the unexplained variation ( $= 1 - [ENV + EXOT]$ ), and the species variation explained by the sharing of environmental and exotic species cover data ( $= [ENV \cap EXOT]$ ). Analyses were performed using CANOCO for Windows 4.5 (ter Braak and Smilauer 1998).

## Results

### *Environmental variables and vegetation*

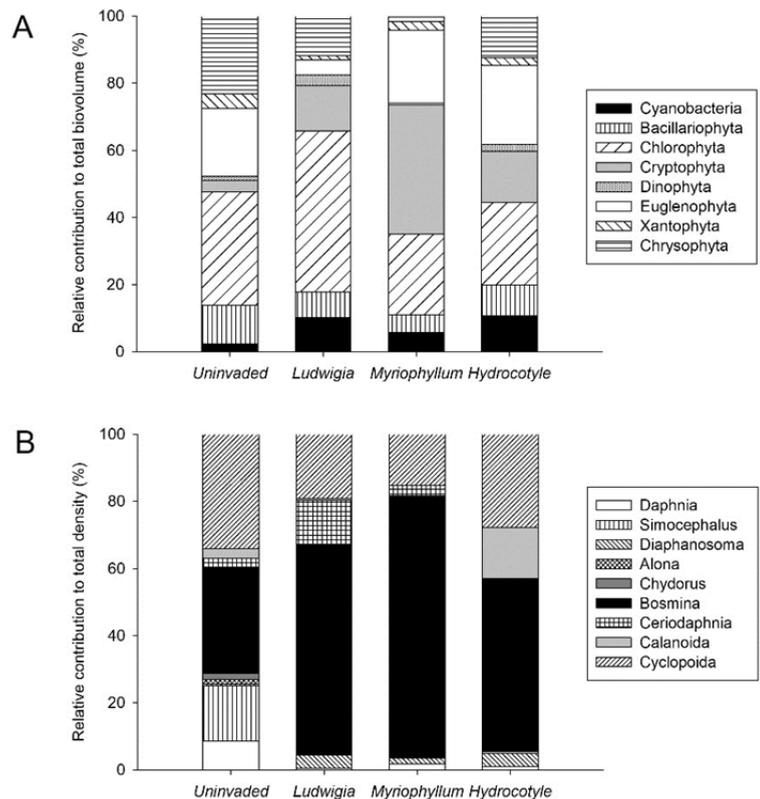
The nutrient gradient ranged from oligotrophic to eutrophic conditions in uninvaded ponds and from mesotrophic to hypereutrophic in invaded ponds (Table S4). No significant differences were observed between invaded and uninvaded ponds in nutrient concentrations (TP, SRP and DIN, Mann-Whitney U-test, all P values  $> 0.05$ ). Although rank values for chl *a*, temperature, conductivity and pH tended to be higher for invaded ponds, and vice versa for water transparency and dissolved  $O_2$ , the differences were not significant (Mann-Whitney U-test, all P values  $> 0.05$ ). However, dissolved  $O_2$  reached low values ( $< 5 \text{ mg l}^{-1}$ ) only in invaded ponds (Table S4).

All uninvaded ponds harboured submerged vegetation (Table S4) with half of the ponds having more than 75% coverage. Recorded species were *Ceratophyllum demersum*, *Utricularia vulgaris*, *Callitriche truncata*, *Elodea nuttallii*, *Hottonia palustris* and *Myriophyllum spicatum*. In only 33% of the invaded ponds, submerged vegetation was present with a maximum cover of  $< 50\%$  (Table S4). Two species were recorded: *Ceratophyllum demersum* and *Utricularia vulgaris*.

### *Plankton communities associated with the non-native invasive species*

A total of 81 phytoplankton genera were identified, corresponding principally to Chlorophyta, Cryptophyta and Euglenophyta. The sorting rendered 23 FG though for 13 genera we could not assign an FG (Table S2). Table S4 shows the dominant FG in the uninvaded and invaded ponds ( $\sim 15\%$  of total biovolume). We found 65 genera in uninvaded ponds, followed by 56 in *M. aquaticum* invaded ponds, 54 in *L. grandiflora* invaded ponds, and 47 in *H. ranunculoides* invaded ponds. Considering FG, uninvaded ponds, *L. grandiflora* invaded ponds, and *M. aquaticum* invaded ponds had 20 FG and *H. ranunculoides* invaded ponds had 18 FG.

The field data showed a considerable overlap in the composition of phytoplankton communities between the invaded and uninvaded ponds (Figure 1A). Similarity ranged from 63–70% based on FG and from 51–60% based on genera. All invaded ponds, irrespective of species, shared one FG which was group Y (13, 23 and 16% in *L. grandiflora*, *M. aquaticum* and *H. ranunculoides* invaded ponds, respectively). Species of group Y are typically Cryptomonads and show a tolerance to low light availability. Uninvaded ponds were dominated by group  $W_s$  (21%), which inhabit humic environments



**Figure 1.** Composition of (A) phytoplankton and (B) zooplankton in the uninvaded ( $n = 6$ ), *L. grandiflora* ( $n = 5$ ), *M. aquaticum* ( $n = 4$ ) and *H. ranunculoides* ( $n = 3$ ) invaded ponds.

and typical representatives are *Synura* spp. Another abundant FG was W2 (18%) for uninvaded ponds, with bottom dwelling *Trachelomonas* spp. as typical representatives. Group F (33%) was abundant for *L. grandiflora* invaded ponds with colonial chlorophytes as representatives, which show a tolerance to low nutrient level. In *M. aquaticum* invaded ponds, group X2 (18%) was also relatively abundant and this group is represented by small flagellates in meso-eutrophic systems. In *H. ranunculoides* invaded ponds, group W1 (18%) was abundant, which are representatives of small organic ponds dominated by Euglenoids.

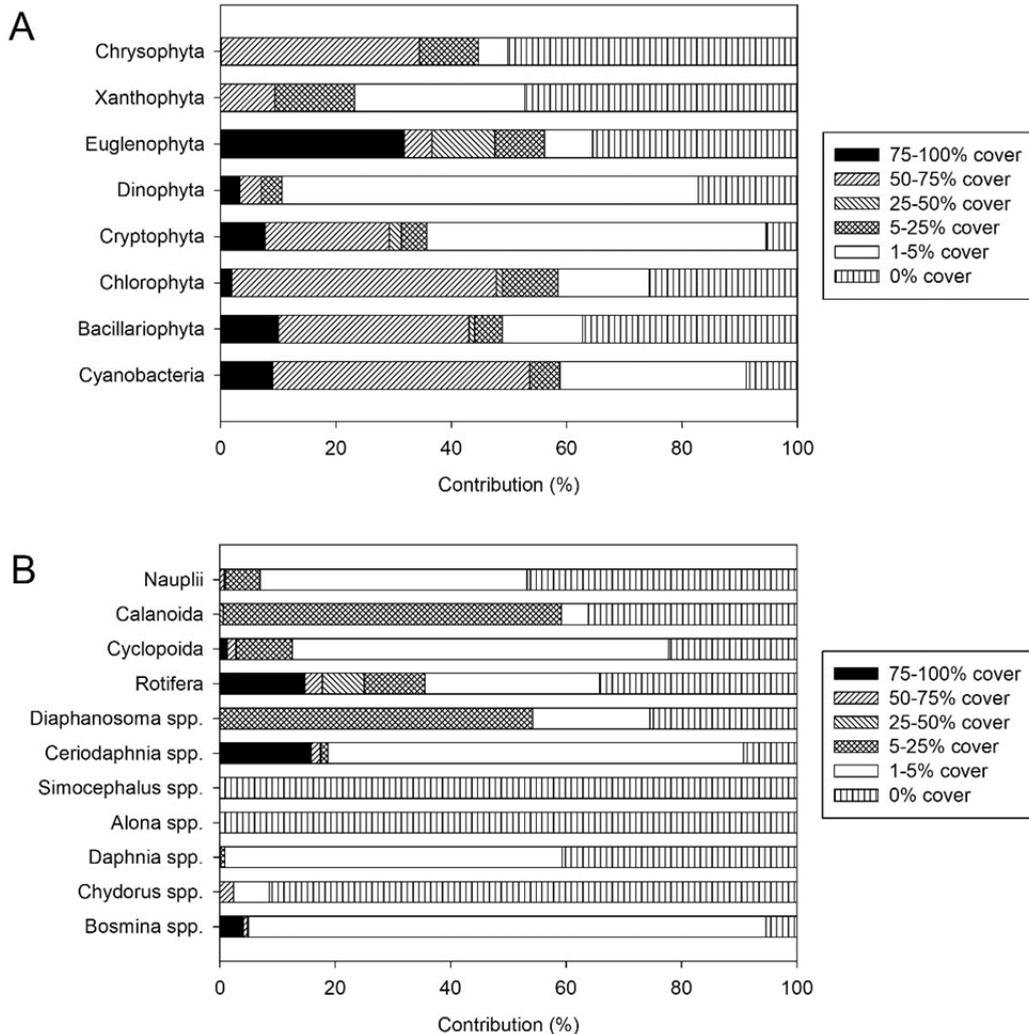
Zooplankton composition varied markedly between invaded and uninvaded ponds (range 32–69% similarity, Figure 1B). The community in uninvaded ponds was dominated by cyclopoid copepods and *Bosmina* spp. (34 and 31% respectively). The invaded ponds, irrespective of species, were dominated by *Bosmina* spp. (> 52%). We observed 3 littoral and 4 pelagic cladocera genera (Table S3). Uninvaded ponds hosted all cladocera genera, followed by *L. grandiflora* and *H. ranunculoides* invaded ponds (both 5 genera) and *M. aquaticum* invaded ponds (4 genera). Two littoral genera, *Simocephalus* spp. and

*Alona* spp. were never observed in association with the invasive plant mats (Figure 2B).

#### *Relationship between plankton and cover of the non-native invasive species*

Total phytoplankton biovolume showed no association with invasive species cover. However, phytoplankton biovolume seemed lower at very dense cover (75–100%) compared to low cover (1–5%), except for Euglenophyta (Figure 2A).

Total zooplankton density was negatively correlated with invasive species cover among invaded ponds ( $n = 12$ ,  $r_s = -0.66$ ,  $P = 0.02$ , Figure S1A) but not over all ponds. Zooplankton density was highest when the invasive species was absent or present at low coverage (1–5%). Additionally, a strong negative relationship between invasive species cover and large cladocera density among all ponds ( $r_s = -0.73$ ,  $P < 0.001$ ) and among invaded ponds only ( $r_s = -0.88$ ,  $P < 0.001$ ) was detected (Figure S1B). No large cladocera species could be detected when the cover of invasive species was > 50%. Littoral zooplankton (only *Chydorus* spp.) was present as long as the cover of invasive species was < 25% (Figure 2B).



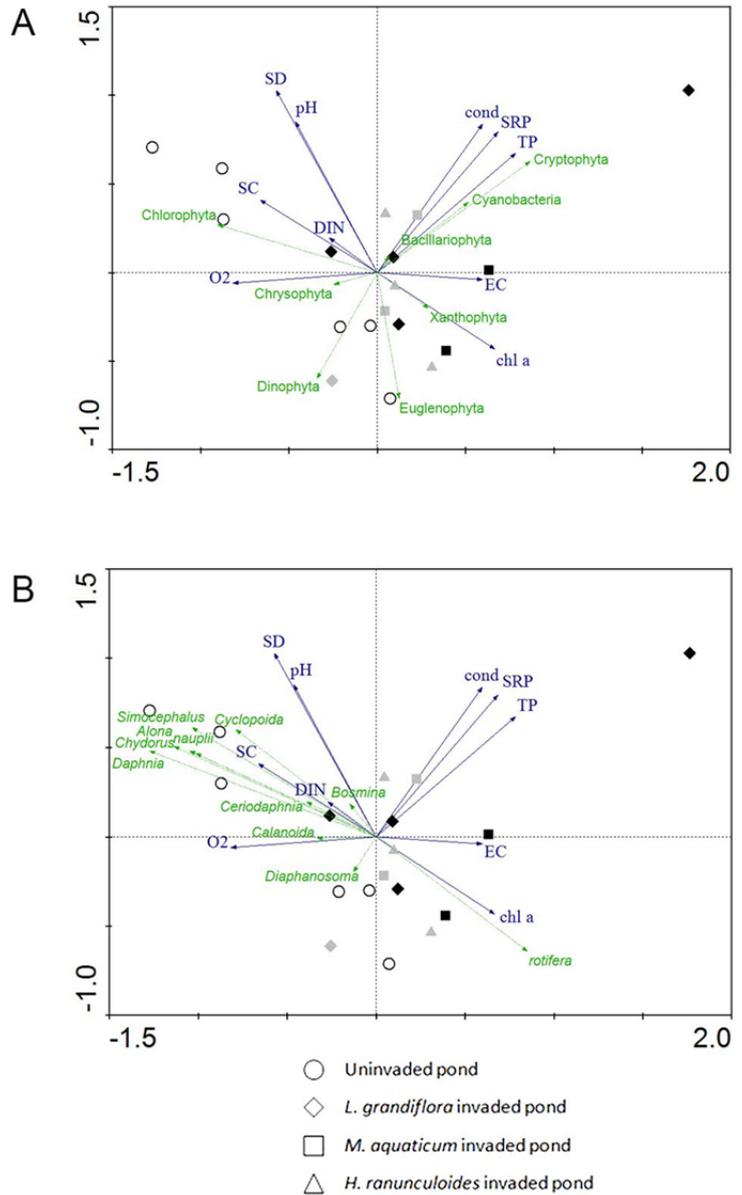
**Figure 2.** Relative abundance of (A) phytoplankton divisions in terms of biovolume ( $\text{mm}^3 \text{ l}^{-1}$ ), and (B) zooplankton taxa in terms of density ( $\text{ind l}^{-1}$ ), associated with invasive species cover.

### Plankton – environmental responses

The first ordination axis of the PCA, based on environmental variables and phytoplankton and zooplankton communities, explained respectively 32.9% and 39.0% of the total variation. This axis mainly separated uninvasive ponds with a high submerged plant cover, high dissolved  $\text{O}_2$  concentration, high pH and high Secchi depth from more turbid ponds with higher nutrient concentrations and exotic species cover (Figure 3A, 3B). Phytoplankton taxa associated with these uninvasive ponds were Chlorophyta, while there was no clear association of phytoplankton taxa with the other ponds (Figure 3A). All uninvasive ponds were associated with higher abundance of large cladocera (*Simocephalus* spp.,

*Diaphanosoma* spp. and *Daphnia* spp.), littoral zooplankton (*Alona* spp., *Chydorus* spp.), cyclopoida and nauplii (Figure 3B). The second ordination axis, which explained respectively 25.8% and 18.8% of the total variation, separated ponds associated with Euglenophyta and Dinophyta and to a lesser extent Rotifera from the other ponds.

Variation partitioning showed that the selected environmental variables explained a significant proportion of the total phytoplankton (27.8%,  $p = 0.002$ ) and zooplankton (32.3%,  $p = 0.002$ ) variation, independently of exotic species coverage (Table 1). The exotic species cover component significantly explained 17.9% ( $p = 0.03$ ) of the variation in the zooplankton community component (marginally significant after correction for environment,  $p = 0.05$ ).



**Figure 3.** Triplot of the first two axes of a PCA on the different ponds and their relationship with local environmental variables with (A) phytoplankton taxa as supplementary variables explaining 58.7% of the total variation and (B) zooplankton taxa as supplementary variables explaining 57.8% of the total variation. Ponds are shown as symbols and are classified according to invasive species cover: grey = low cover (< 50%); black = high cover (> 50%). Phytoplankton and zooplankton taxa (green) and environmental variables (blue) are shown as arrows with abbreviations as previously defined.

**Discussion**

The field evidence confirmed the structuring role of both environmental factors and non-native invasive species cover for zooplankton, but not phytoplankton, communities. For both communities, environmental variables explained more variation in the composition than non-native invasive species cover alone. This suggests that the environment is the dominant factor in shaping phytoplankton and zooplankton communities. Uninvaded ponds with submerged vegetation were associated with several zooplankton taxa,

higher dissolved O<sub>2</sub> concentration, high Secchi depth and lower chl *a* concentration, all factors associated with a clear water state. The other ponds showed a broad range of nutrient concentrations and were not clearly associated with certain phytoplankton or zooplankton taxa. True separation of pure environmental factors and invasive species cover in invaded systems seems impossible as the three invasive species can be ecosystem engineers (Jones et al. 1994). Here, ecosystem engineering refers to the capability of invasive species to affect native species through abiotic resource modification. The three species form dense monospecific mats, half a meter thick,

that limit water movement and light penetration; and low dissolved O<sub>2</sub> concentrations were observed under the invasive mats (Dandelot 2004; Kuehne et al. 2016 and this study). Several studies have shown that aquatic invasive plants can affect water quality with possible impacts on fish, invertebrate and plankton communities (Villamagna 2009; Strayer 2010; Schultz and Dibble 2012; Kuehne et al. 2016).

There was no relationship between invasive species cover and phytoplankton biovolume or, according to variation partitioning, on the phytoplankton community. The uninvaded ponds did not seem to have their own typical taxa; they were rather similar to those occurring in the invaded ponds but with a lower abundance in the latter. Some FGs were dominant both in invaded and uninvaded ponds, such as group J (which was comprised of different taxa) and group W2 (only *Trachelomonas* spp.), which are common in small, shallow mesotrophic and eutrophic ponds and lakes (Reynolds 2006). However, phytoplankton composition and associated life history strategies also reflected the difference in habitat between invaded and uninvaded ponds. The members of group Ws (*Synura* spp.), which was the dominant FG in uninvaded ponds, prevailed in macrophyte-dominated ponds but only when oxygen concentrations were sufficiently high (Paształeniec and Poniewozik 2013). Chrysophytes, which were quite abundant in uninvaded ponds (23% of total biovolume), show in general a high biomass under good water oxygenation. Some taxon specific responses to invasive species cover and/or environment were also observed. Group Y (*Cryptomonas* spp.) was dominant in the invaded ponds and associated with all invasive species. This pattern agrees with their ecology as they are mixotrophic phytoplankton, which can use phagotrophy or osmotrophy to survive in periods of low light availability (Reynolds 2006). Likewise, certain Cyanobacteria genera, *Chroococcus* spp., *Merismopedia* spp. (group Lo), *Oscillatoria* spp. (group MP) and *Synechococcus* spp. (Z) only occurred in invaded ponds. Most of these blue-green algae are shade adapted and have a high tolerance to a low oxygen level. Similar patterns were found in other studies that investigated the influence of free-floating plants on phytoplankton assemblages (de Tezanos Pinto et al. 2007; Paształeniec and Poniewozik 2013). A high free-floating species cover favoured mixotrophic and heterotrophic species as well as Cyanobacteria with a tolerance to low light and oxygen availability. In the highly invaded ponds (> 75% cover) phytoplankton biovolume for the different taxa was in general low. A decrease of phytoplankton biomass is frequently observed inside vegetation beds (van Donk and van de Bund 2002;

Mangas-Ramirez and Elias-Gutierrez 2004; Greenfield et al. 2007) due to competition for resources (e.g. nutrients and light) and possibly enhanced by allelopathy. There is evidence that the three invasive species negatively affect one or more plant or algae species through the release of chemical compounds (Della Greca et al. 1994; Cheng et al. 2008; Dandelot et al. 2008).

Consistent with our hypothesis, the cover of invasive species was negatively correlated with zooplankton density. Large cladocerans and littoral taxa in particular were absent when the cover of invasive species was high. Zooplankton distribution is influenced by the presence of aquatic plant beds and various environmental factors such as light intensity, temperature, dissolved oxygen and pH. In addition, food quantity and quality (Burks et al. 2002) and allelopathy (van Donk and van de Bund 2002) are also important aspects. The phytoplankton species associated with the invasive species might be less edible for zooplankton. *Daphnia* species prefer edible chlorophyta (Ebert 2005), an algae group that was associated with submerged plant cover in uninvaded ponds (mainly unicellular small green algae e.g. *Monoraphidium* spp. and *Chlamydomonas* spp.). This is enhanced by the absence of macrophyte-associated species such as *Simocephalus* spp. and *Alona* spp. around the edges or in the dense invasive mats, while these zooplankton species were present in uninvaded ponds. Less edible colonial chlorophytes and cyanobacteria were also more abundant in the invaded ponds. Lower food quality because of shading of phytoplankton, low oxygen concentration under the dense mats, in addition to potential allelopathic properties might explain the aversion of large cladocerans and littoral taxa towards the invasive mats. Furthermore, especially for large cladocera, the dense structure of the root system may hamper movement, a physical cause already suggested for invasive *E. crassipes* and *Pistia stratiotes* by Meerhoff et al. (2006).

Macrophyte architecture, often confounded with macrophyte biomass, can have an influence on zooplankton communities (Kuczyńska-Kippen and Nagengast 2006), although the evidence is equivocal. We observed some differences in the composition of zooplankton communities between invaded and uninvaded ponds but overall invasive species identity had a minor influence. The invasive macrophytes considered have a similar physical structure, at least of the submerged stems in full summer (the time of our study). The three species form a multitude of long creeping or floating stems (up to 4–5 m), which result in dense interwoven mats, usually without submerged leaves, of sometimes half a meter thick

in full summer. In contrast, the physical structure of the submerged vegetation present in the uninvaded ponds is considerably more complex as most plants have finely dissected leaves. Other studies that focus on how dense mats of invasive species shape zooplankton communities are scarce. Villamagna (2009) found that density and taxonomic richness of water column invertebrates were generally higher in association with *E. crassipes*, but that mean percent cover of *E. crassipes* affected the magnitude of differences among habitats and vegetation types; however, in this study comparisons were made between zones with *E. crassipes* and plant-free zones. Meerhoff et al. (2003, 2007) found that submerged plants hosted significantly more cladocerans than dense free-floating mats of *E. crassipes*, even when the latter supported lower densities of fish.

It is also well documented that the density and composition of zooplankton predators cause shifts in the density and community composition of zooplankton species (Jeppesen et al. 1997; Burks et al. 2002). Our study did not quantify fish density but there are indications of high predation pressure in all ponds. First, there is a general low abundance of large-bodied *Daphnia* spp. and their length (LCL) was similar in invaded and uninvaded ponds. Second, most of the ponds harboured fish as they are either fishing ponds, spawning grounds or garden ponds stocked with fish. The possibility also exists that the invasive species affect potential predators but this is largely unknown, although Legrand (2002) reported that *L. grandiflora* populations in France constituted a barrier for fish movement.

A healthy, diverse community of submerged macrophytes is a key-element in freshwater ecosystems. The presence of submerged vegetation is likely to limit high phytoplankton biomass, promoting a clear water state (Scheffer et al. 1993; Jeppesen et al. 1998). In shallow lakes, submerged plants might provide a refuge for zooplankton from fish predation (Burks et al. 2002). In short, our results confirmed that uninvaded ponds, with a high abundance of submerged vegetation, were associated with low chl *a*, high dissolved oxygen concentration, high Secchi depth and a diverse zooplankton community. In contrast, ponds with a high cover of the invasive species seemed to create an adverse environment for large cladocera and littoral taxa. Invasive species cover did not have a significant influence on the phytoplankton community, although some evidence was found that differences in functional group composition occurred between invaded and uninvaded ponds. We showed that it is important to include cover of an invasive species as a necessary element to disentangle patterns of impact.

## Acknowledgements

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** Main characteristics of the studied ponds.

**Table S2.** List of phytoplankton genera encountered during the field study with an indication of the Reynolds functional group, life form\* and presence in invaded and uninvaded sites.

**Table S3.** List of cladoceran genera encountered during the field study with an indication of the habitat use (pelagic or littoral), size-category (large (> 0.5 mm) or small) and presence in invaded and uninvaded sites.

**Table S4.** Range of the main morphological, biological and physico-chemical characteristics of the studied ponds.

**Figure S1.** Relationship between invasive species cover and total zooplankton density among invaded ponds and large cladocera density among all ponds.

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