MULTIPLE ROLES OF ALIEN PLANTS IN AQUATIC ECOSYSTEMS: FROM PROCESSES TO MODELLING

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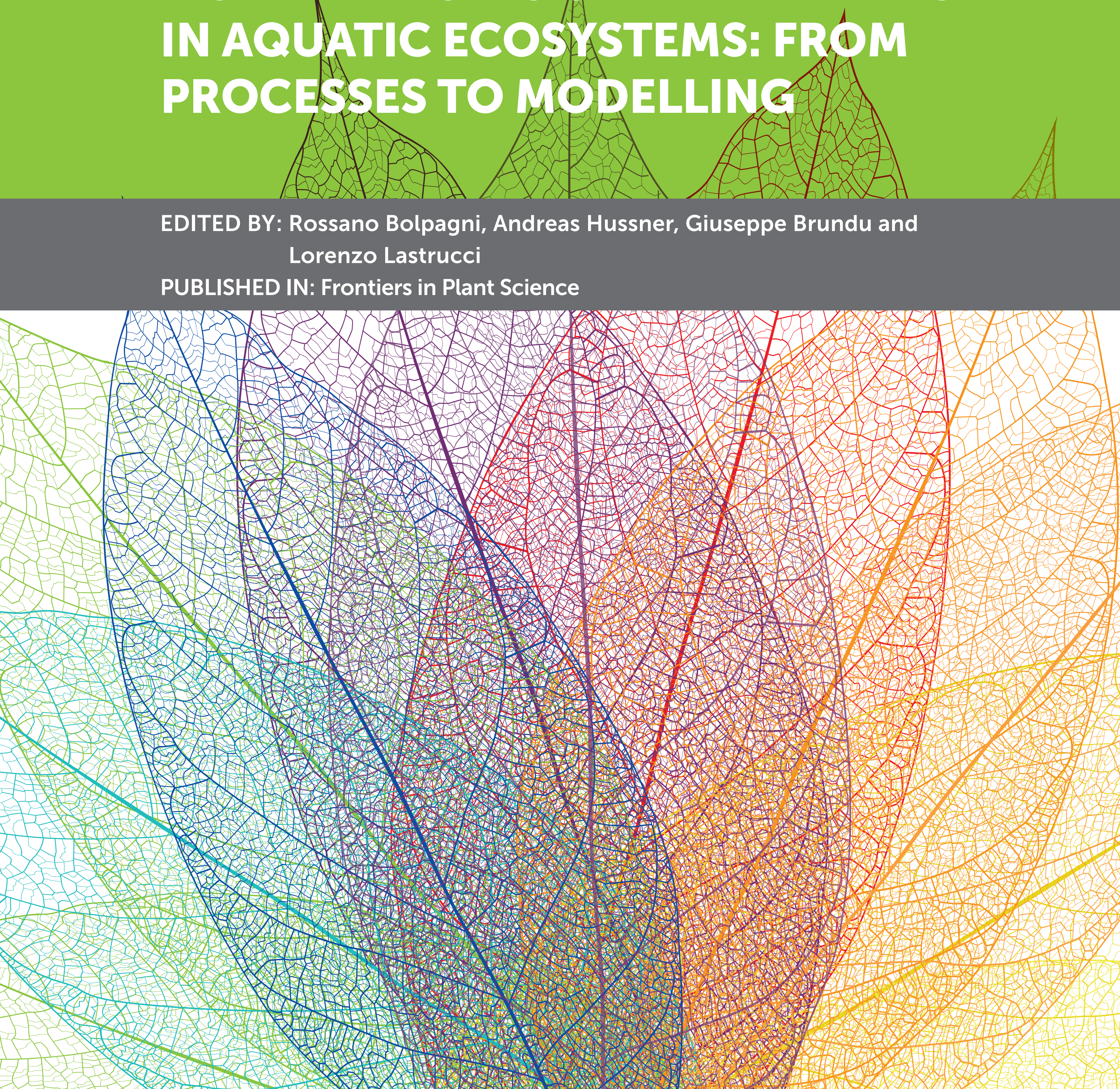

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MULTIPLE ROLES OF ALIEN PLANTS IN AQUATIC ECOSYSTEMS: FROM PROCESSES TO MODELLING

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Editorial: Multiple Roles of Alien Plants in Aquatic Ecosystems: From Processes to Modelling

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Editorial on the Research Topic

Multiple Roles of Alien Plants in Aquatic Ecosystems: From Processes to Modelling

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BACKGROUND

Invasive alien species are one of the most significant contemporary challenges threating biodiversity, ecosystem functioning, and human wellbeing (Rai and Singh, 2020), resulting in major economic and environmental damages and losses (Pimentel et al., 2005). Biological invasions are favored by ecosystems' over-exploitation and climate change, and the progressive accumulation of invasive species strongly weakens the invaded communities by occupying empty phylogenetic and functional spaces or by excluding natives (Dalle Fratte et al., 2019). Among ecosystems, freshwaters seem to be particularly prone to invasions due to their high natural dynamism associated with a global hydrological and trophic alteration due to human activities (Dudgeon, 2019). This urgently calls for a better understanding of the multiple roles played by invasive aquatic alien plants (IAAPs). To this regard, the present Research Topic offers novel perspectives on IAAPs science and on the implications of their establishment.

ENVIRONMENTAL DRIVERS AND FUNCTIONAL TRAITS OF IAAPS

A key question is how IAAPs can progressively replace native counterparts. The invasive success of IAAPs depends in part on the characters of invaded ecosystems, as highlighted by Shen et al. Testing the role of sediments in enhancing the invasibility of aquatic ecosystems, they showed that the soil spatial heterogeneity might have a primary role in promoting the invasive success of *Myriophyllum aquaticum* by regulating morphological traits of individual clonal ramets. Wu and Ding reviewed the role of fundamental drivers such as global change, water pollution, and economic growth in IAAPs introduction and invasions in China, clarifying that "support to aquaculture" and "ecological restoration" are major introduction pathways. Furthermore, IAAPs invasion risk is expected to be boosted by climate change, as well as by increased nutrient availability, and it seems to be under the total control of future economic trends.

Beside the invasibility of the ecosystems, the attributes of IAAPs play a major role for successful invasion. Szabó et al. explored the eco-physiology of two invasive elodeids (*Elodea canadensis* and *E. nuttallii*) as a key to understanding their relative invasiveness. Their investigation of the combined effects of nutrient and light environment demonstrated that relative growth rate, chlorophyll concentration, and efficiency of photosystem II provide *E. nuttalli* the ability to replace congeneric species in the invaded sites. Similarly, Tóth et al. discussed the roles of physiological and ecological traits in explaining the differentiated performances of invasive macrophytes, obtaining evidence on the existence of key adaptive responses of IAAPs. Importantly, *Ludwigia hexapetala*—one of the most invasive IAAPs globally—showed exceptionally high photosynthetic efficiency, resulting in an elevated tolerance to high light intensities. Both papers reinforce the direct implication of plant traits for the invasiveness of IAAPs, suggesting the importance to gain knowledge on this topic. In this respect, Asaeda et al. have investigated habitat preference of *Egeria densa*, focusing on physiological stress proxies. Based on the tissue concentrations of hydrogen peroxide (H_2O_2), it is possible to define critical thresholds for the species survival in terms of flow velocity and light intensity, with relevant implications for designing management solutions to counteract the spread of IAAPs.

IAAPS AND BIOLOGICAL INTERACTIONS

However, beyond the characteristics of habitats and IAAPs, biological interactions—including competition, facilitation, invasional meltdown, and novel weapons—are also crucial for enlightening the IAAPs success. The study by Michelan et al. outlined how the influence of IAAPs on the colonization performance of native species is density dependent, studying the alien *Urochloa arrecta* and the two native *Pontederia cordata* and *Leersia hexandra* performances in terms of biomass and plant length. In their manipulative experiment, they found that if *U. arrecta* density increases (as number of fragments), the biomass and length of native species decrease substantially. However, the responses of the native species to the invader is species-specific, with differentiated biomass allocation behaviours. Wegner et al. investigated the biological mechanisms underlying the boom-bust dynamics of *E. nuttallii*, by modelling the temporal changes in water quality, and abundances of *E. nuttallii* and of another critical invader, the quagga mussel, in a temperate shallow lake. They found that the dynamics of multi-invasion processes can be characterized by different, contrasting phases. After an initial mutual facilitation—as predicted by the invasional meltdown hypothesis—a strong space competition followed. This study offers a new mechanistic explanation of the typical boom-bust dynamics of invasive elodeids.

Likewise, Strange et al. modelled the key interactions involved in the replacing of a floating IAAP (*Pistia stratiotes*) by a submerged one (*E. densa*), evaluating the implications of top-down vs bottom-up processes mediated by biological control efforts and nutrient loading, respectively. Their main message highlighted the risks associated with the adoption of control and eradication actions without planning active recovery interventions, as this lack may create opportunity windows for secondary invasions. Moreover, Thiebaut et al. examined the allelopathic potential of four aquatic plants (including IAAPs) on *L. hexapetala*, monitoring its relative growth rate and physiological traits in response to the exposition to root and leaf leachates of *L. peploides*, *M. aquaticum*, and *Mentha aquatica*, finding complex allelopathic responses. Specifically, *L. hexapetala* exibits a positive autoallelopathy which explains its invasiveness. In addition, they highlighted the risk of occurrence of potential invasional meltdown phenomena, with huge consequences on the local success of *L. hexapetala*, and invasive *Ludwigia* species in general.

IAAPS AS ECOSYSTEM ENGINEERS

IAAPs can deeply alter the functioning of ecosystems. Ribaudo et al. stressed the biogeochemistry implications of *E. densa* and *Lagarosiphon major*, as they are capable to trigger huge stratification events for several key chemical parameters (O_2, C, N), as recurrent hypoxia events. They confirmed that the spread of IAAPs involved a change in the metabolism of ecosystems, resulting in a net nutrient release towards the water column, concurrently with a net increase in carbon emission towards the atmosphere.

PERSPECTIVES ON IAAPS SCIENCE

This Research Topic goes beyond the classical simplistic paradigm on the only negative impacts of IAAPs and increases the awareness on the multiple roles played by IAAPs in aquatic ecosystems. To date, available knowledge is not sufficient for an effective control and management of aquatic invaders (Hussner et al., 2017; Hofstra et al., 2020). Additional global efforts are needed to organize in a comprehensive and cross-cutting framework the processes of introduction, establishment, and spread, as well as the assessment of ecosystem changes and impacts mediated by IAAPs, keeping an eye on genetic and functional aspects (Lastrucci et al., 2018; Molina-Montenegro et al., 2018). In this frame, all the papers collected here will boost scientific interest in these issues, reaffirming the importance of tailored researches to get satisfactory answers to complex issues.

AUTHOR CONTRIBUTIONS

All authors conceived the idea for the special issue. RB coordinated the reviewing and editing of the papers included in the special issue and wrote the first version of the Editorial with major inputs from LL, GB and AH. All authors commented on the Editorial, read, and approved the final version.

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ORIGINAL RESEARCH
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Competitive Effects Hinder the Recolonization of Native Species in Environments Densely Occupied by One Invasive Exotic Species

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The responses of native plants to competition with invasive plants depend mainly on the density of the invasive plants and on the ability of the native plants to compete for resources. In this study, we tested the influence of the invasive exotic *Urochloa arrecta* (Poaceae) on the early colonization of two native species (*Pontederia cordata* and *Leersia hexandra*) of aquatic macrophytes. Our hypotheses were (i) the competitive effects of *U. arrecta* on the native species *P. cordata* and *L. hexandra* are density-dependent and that (ii) these species respond differently to competitive interactions with the invasive species. We conducted the experiments in a greenhouse and in the field, in a tropical reservoir. The biomass of *U. arrecta* (ranging from 206.2 to 447.1 g) was manipulated in the greenhouse in trays with different densities. After the establishment of the invasive species, we added *P. cordata* and *L. hexandra* propagules to each tray. In the field, a propagule of *P. cordata* was planted in 36 sites with different densities of *U. arrecta*. The biomass and length of the natives and the biomass of the invasive species were measured in the greenhouse and in the field experiments. The biomass and length of the native plants decreased with increasing biomass of the exotic species in both experiments, showing that the competition between *U. arrecta* and native species depends on the density of the exotic species. The root:shoot ratio of *L. hexandra* decreased with increasing *U. arrecta* biomass, but the opposite occurred for *P. cordata*. These results indicate that native species exhibit different strategies of biomass allocation when interacting with *U. arrecta*. The strong competitive effects of *U. arrecta* and the different responses of the native species help to explain the reduced diversity of native macrophytes observed in sites colonized by *U. arrecta*. The results also suggest that in a scenario of dominance of exotic species, recolonization by native macrophytes is unlike to occur naturally and without human interventions that reduce the biomass of the exotic species.

Keywords: competition, non-native macrophytes, Poaceae, density-dependent effect, resource competition

INTRODUCTION

In general, only a small fraction of introduced species become successfully established and exhibit population growth to the point of becoming "invasive" (Levine, 2008; Davis, 2009). When they do become invasive, they can reduce the richness and abundance of native species (Madsen et al., 1991; Daehler and Strong, 1994; Roberts et al., 1999; Michelan et al., 2010b). In addition, they can even change the environmental conditions of the invaded sites (Pyšek et al., 2008; Strayer, 2010), causing ecological and economic damage (Richardson and Pyšek, 2008; Carey et al., 2016; Cuassolo et al., 2016). Invasion success depends on multiple factors, including species-specific traits (e.g., growth rate, competitiveness and dispersal ability; Rejmánek, 2011) and the characteristics of the invaded ecosystem (e.g., environmental conditions, disturbances and diversity; Fridley, 2011).

The impacts of invasive species on native species depend largely on the abilities of interacting species to compete for resources (Seabloom et al., 2003; Blindow et al., 2016). Competition is an important biological interaction that influences the structure and development of plant communities (Kiaer et al., 2013; Blindow et al., 2016). Additionally, species respond differently to competition depending on the abiotic conditions and on the density of each population (Gopal and Goel, 1993; Nunes and Camargo, 2017). As a direct result of competition with invasive species, one can predict changes in the structure of invaded communities and a decrease in biodiversity at local and regional scales (Michelan et al., 2010b; Powell et al., 2011, 2013; Amorim et al., 2015).

Competition among plants occurs predominantly by nutrient ("root competition") and/or light acquisition ("above-ground competition"). The roots and shoots of the plants acquire different resources from the environment, and some studies therefore try to separate the effects of the competition of each plant part (Wang et al., 2008; Kiaer et al., 2013; Richter and Gross, 2013). One of the methods to evaluate which organ is most involved in the competition is to use root:shoot biomass ratio (Robinson et al., 2010). High values of this ratio indicate that competition for nutrients and water (by root) is more important, while lower values indicate greater competition for light (Wang et al., 2008; Craine and Dybzinski, 2013; Kiaer et al., 2013; Richter and Gross, 2013; but see Cahill, 2003 for another point of view).

Coexistence between species under natural conditions can be facilitated by several mechanisms, such as disturbances and trade-offs between competitive and dispersal abilities (Grime, 1979; Connell, 1983). However, the competitive effects of invasive species occurring at high densities may be so intense that, at least at fine spatial scales, native species are excluded by competition (Madsen et al., 1991). Yet, little is known about the tolerable limits of the biomass of invasive macrophytes for the recolonization of native macrophyte species. Thus, it is important to evaluate the competitive interactions between native and invasive macrophyte species at different biomasses of the latter. Studies employing this approach would help to identify native species with higher potential for recolonizing environments dominated by invasive species and to identify thresholds of invasive biomass that allow native recolonization and survival.

Many species belonging to the family Poaceae are highly invasive in several aquatic ecosystems (Bunn et al., 1998; Bell et al., 2011; Mugwedi et al., 2015). In general, they have greater competitive effects than species of other groups, such as herbaceous and leguminous plants (Kiaer et al., 2013). This is also the case for *Urochloa arrecta* (Hack. ex T. Durand & Schinz) Morrone & Zuloaga, a species native to Africa, which has colonized tropical and subtropical aquatic ecosystems. In particular, this species is invading and causing ecological impacts in various Brazilian aquatic ecosystems (Pott et al., 2011; Fernandes et al., 2013; Amorim et al., 2015). *U. arrecta* forms large patches, accumulates large amounts of biomass in littoral zones (Michelan et al., 2010b; Fernandes et al., 2013; Amorim et al., 2015), regenerates rapidly after disturbances (Michelan et al., 2010a) and can thrive even in relatively oligotrophic environments with nutrient-poor sandy substrates (Fasoli et al., 2015). However, there is a paucity of experimental studies evaluating the competitive effects of *U. arrecta* on individual macrophyte species.

In this study, we investigated the biomass-dependent effects of *U. arrecta* on the recolonization of two native species of macrophytes (*Pontederia cordata* L. and *Leersia hexandra* Sw.) and tested whether the competitive effects on them differ. First, we developed a greenhouse experiment to test the effects of *U. arrecta* on the biomass and on the root:shoot ratio of the two native species. Then, we repeated the experiment in the field, using *P. cordata* as a focal species, to test the generality of our results obtained in the greenhouse. We tested the hypotheses that (i) the competitive effects of *U. arrecta* on *P. cordata* and *L. hexandra* depend on the invasive biomass and (ii) that native species respond differently to these effects. These hypotheses were postulated because previous studies in the field showed that the native macrophyte biomass decreases in the presence of *U. arrecta* (Michelan et al., 2010b) and that the frequencies of co-occurrence between native macrophytes and *U. arrecta* are species-specific, indicating that native species may respond differently to increasing invasive biomass (Thomaz and Michelan, 2011). We predicted that the effects of *U. arrecta* on *L. hexandra* would be higher than those on *P. cordata* because of the morphological similarity and phylogenetic relationship between the first pair of species. Consequently, they should use resources more similarly, which intensifies competition and reduces the chances of co-existence (Chesson and Kuang, 2008; Rejmánek, 2011). Finally, to place our results in a broader context, we compared our results with those obtained in a recent meta-analysis (Jauni and Ramula, 2015).

We believe that the use of *U. arrecta* as a model plant in our study contributes to a broader view regarding the impacts of exotic plants on native species because this species belongs to the family Poaceae, which is responsible for the greatest ecological impacts among invasive plants (Kiaer et al., 2013). In addition, by conducting experiments in a greenhouse and in the field, we believe that our outcomes can be useful to assess whether the former can be extrapolated to nature, an issue that has been questioned by some investigations that highlight the shortcomings of microcosm experiments (e.g., Wilson and Keddy, 1991).

MATERIALS AND METHODS

We performed two experiments employing an additive design (Gibson et al., 1999) to assess the ability of native species to colonize and grow in sites with different biomasses of *U. arrecta*, one experiment in a greenhouse at the State University of Maringá (Paraná State, Brazil) and the other *in situ* in the Rosana Reservoir (Paraná/São Paulo, Brazil; 22°39′26.19″ S 52°46′52.35″ W; see **Supplementary Figure S1** for photos of the experiment in a greenhouse and *in situ*).

Greenhouse Experiment

Urochloa arrecta (exotic), *P. cordata*, and *L. hexandra* (natives) were used in our greenhouse experiment. The native species were selected based on different levels of co-occurrence with *U. arrecta* (details about this selection in Michelan et al., 2013). *L. hexandra* (Poaceae species often found to co-occur with *U. arrecta*) is a perennial species that can grow vigorously in aquatic ecosystems (Pott and Pott, 2000; Moreira et al., 2011). The Pontederiaceae *P. cordata* has low level of co-occurrence with *U. arrecta* and is a perennial herbaceous species that can also form dense stands (Pott and Pott, 2000). Both species reproduce sexually and asexually (by stems and rhizomes). *P. cordata* rhizomes can survive to fire or dry seasons (Pott and Pott, 2000). The macrophytes were collected in the Rosana Reservoir and taken to the greenhouse.

We used trays (0.30 m × 0.37 m × 0.14 × m) that were filled halfway with sediment and maintained with a 3–5 cm water layer. The water was replaced with tap water whenever necessary. To create a gradient of *U. arrecta* biomass, we added fragments with two nodes each of *U. arrecta* from the apical stems, at densities of 0, 5, 10, 15, 20, 25, and 30 fragments per tray, with five replicates for each treatment, amounting to 35 trays. Trays were randomized inside the greenhouse to offset any undetected environmental variation.

A clear gradient in biomass, a necessary condition to test our hypotheses, was formed by 200 days after planting *U. arrecta* (0–450 g DW m^{-2}). Then, we added one propagule of *P. cordata* and one of *L. hexandra* at the opposite extremes of each tray (separated from each other by ca. 30 cm). These propagules of *P. cordata* and *L. hexandra* were collected in the Rosana Reservoir and brought to the greenhouse, where we removed their leaves and roots in order to allow all plants to start to grow at similar conditions. In addition, we selected propagules with similar weights. The distance between the native species in the microcosms (ca. 30 cm) was assumed to be enough to avoid interaction between them. Although we did not measure the survival rates of the propagules of the native species during the course of the experiment, all propagules survived in our experiment and even those planted in more dense microcosms formed small individuals.

The experiment was completed 3 months after the introduction of the native species. The length of *P. cordata* and the average length of shoots generated by *L. hexandra* were measured with a tape (cm). Afterward, the biomass of each species was washed to remove sediment and was separated into shoots and roots. After drying in an oven (70°C, until constant weight), the dry mass of roots and shoots of each species was measured on a scale with a precision of 0.01 g. We emphasize that the experimental design of this study simulates a situation in which the recolonization by native aquatic macrophyte species occurs after the occupation of an invasive exotic species that is dominant in an ecosystem, a common situation in several Neotropical aquatic environments.

Field Experiment

The field experiment was conducted in one arm of the Rosana Reservoir (between 22°39′19.29″ S; 52°46′58.93″ W–22°40′27.19″ S; 52°47′10.32″ W and 22°39′32.42″ S; 52°46′36.52″ W–22°40′26.18″ S; 52°46′47.43″ W; Brazil), near the sites where the native macrophytes were collected for the greenhouse experiment. We first selected 50 sites (squares of 0.09 m^2–0.3 m × 0.3 m) with different densities of *U. arrecta*. These sites were identified and demarcated with stakes, and a propagule of *P. cordata* was planted in each site. The *P. cordata* propagules were treated the same way as those used in the greenhouse (see details above for the greenhouse experiment). We monitored the field experiment at every week and observed that the propagules of *P. cordata* were consumed by herbivores in 14 sites. Thus, 3 months after the establishment of the native species, only 36 sites were used in this study.

At the end of the experiment (90 days), the individuals of *P. cordata* and the shoots of *U. arrecta* in an area of 0.09 m^2 (0.3 m × 0.3 m) around the native species were collected. For each site, the species were separated and washed, packed and placed in an oven at 70°C until reaching a constant weight. The dry shoot biomass of *U. arrecta* and the shoot and root biomass of *P. cordata* were obtained by using a precision scale with an accuracy of 0.01 g.

Data Analysis

Following Goldberg and Scheiner (2001), we used an analysis of covariance (ANCOVA) for each response variable (i.e., total biomass, length and root:shoot ratio of native plants). In each ANCOVA model, the quantitative and categorical predictor variables were the shoot biomass of *U. arrecta* and the native species identity (*L. hexandra* and *P. cordata*), respectively. The response variables were standardized to the values of these variables expected in the absence of *U. arrecta*. Thus, we expressed them as log response ratios, $lnRR = \ln(Y_u/Y_c)$, where Y_c was the value of a response variable without *U. arrecta* and Y_u was the value of this response variable under the influence of *U. arrecta*. To estimate Y_c, we used the mean values of the response variables in the control experimental units. Thus, *lnRR* is equal to 0.0 in the absence of competitive effects, and the more negative *lnRR* is, the higher the effect of *U. arrecta* in limiting the growth of the natives. As our objective was to analyze the competition between the invasive and native species and to evaluate whether they responded differently to the competition effect in a scenario of exotic dominance, we chose to remove the sites with the absence of the invasive species (but see the **Supplementary Figure S2** for the results based on the entire gradient of *U. arrecta* biomass—with the addition of treatment 0—absence of competition).

In the field experiment, the effect of *U. arrecta* biomass on *P. cordata* biomass was tested using a second-order polynomial regression due to the non-linearity of the data. All statistical analyses were performed in R (R Development Core Team, 2014).

Comparison of the Results With Those Obtained in the Literature

To contrast our results with those obtained in the literature, we first transformed the Pearson correlation coefficient (r) between the total biomass of the invasive species and the total biomass of the native species into Cohen's d using: $d = 2r/\sqrt{1-r^2}$ (Borenstein et al., 2009). The variance of d (V_d) is given by: $V_d = 4V_r/\left(1-r^2\right)^3$, where V_r is the variance of r $\left(V_r = \left(1-r^2\right)^2/n-1\right)$ and n is the sample size. Finally, d was transformed into Hedges' g after multiplying d by a correction factor j (see Eq. 4.22 in Borenstein et al., 2009). The variance of g was estimated by $V_g = j^2 \times V_d$. Second, we compared the values of g estimated in our study with the results of a recent meta-analysis conducted by Jauni and Ramula (2015). This meta-analysis was based on 75 competition studies between exotic and native species. These studies (observational and experimental) evaluated "how exotic plant species influence the fitness components of native plants" (Jauni and Ramula, 2015) and were based on the following response variables: establishment (i.e., germination), growth rate, biomass, reproductive success and survival (or mortality). Fifty-eight exotic species were included, and it is important to note that no study with *U. arrecta* was used in this meta-analysis, ensuring independent results. We focused our comparison on results obtained for biomass, based on studies with 19 exotic species (mainly from the order Poales) and 36 native species (see Figure 1 of Jauni and Ramula, 2015). In general, the approach of quantitatively comparing the results obtained in an experiment with those obtained in a meta-analysis can be considered a type of cumulative meta-analysis (Leimu and Koricheva, 2004).

RESULTS

Greenhouse Experiment

We found that the total biomass of both native species significantly decreased with the increase in the biomass of *U. arrecta*. The slopes of the relationships did not differ significantly (test of parallelism: $F_{1,56} = 3.47$; $P = 0.0676$; **Figure 1A**). However, the coefficient of determination of the model for *L. hexandra* ($R^2 = 0.70$; $P < 0.001$) was substantially higher than that observed for *P. cordata* ($R^2 = 0.51$; $P < 0.001$).

Similar to what we found for biomass, the native species' length decreased significantly with the increase in the biomass of *U. arrecta* (**Figure 1B**). However, the slope of the relationship between the biomass of *U. arrecta* and length of *L. hexandra* ($b = -0.0032 \pm 0.0006$ SE; $R^2 = 0.54$; $P < 0.001$) was significantly steeper (test of parallelism: $F_{1,56} = 5.14$; $P = 0.0272$) than that for *P. cordata* ($b = -0.0017 \pm 0.0003$ SE; $R^2 = 0.48$; $P < 0.001$).

The effects of the *U. arrecta* biomass on the root:shoot ratio clearly differed between the two native species ($F_{1,56} = 60.58$;

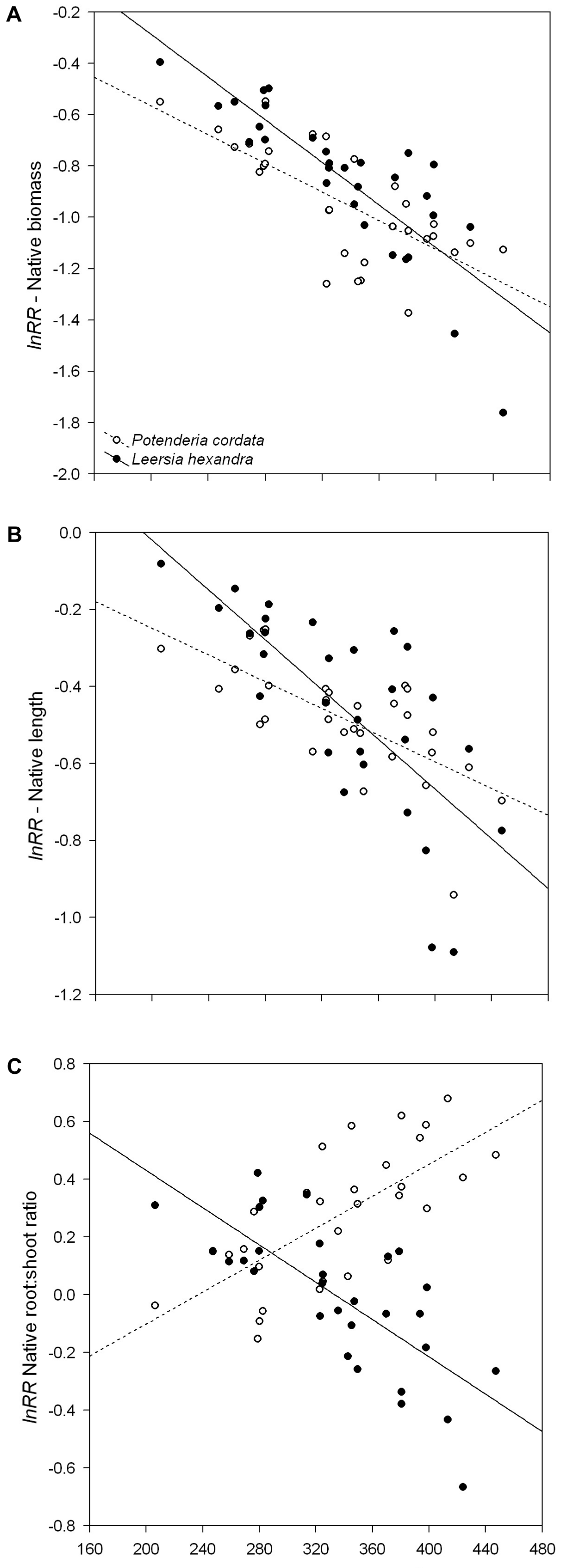

FIGURE 1 | Relationship between the shoot biomass of *U. arrecta* and different traits of two species of native macrophytes. Shown are the results for **(A)** total biomass, **(B)** length, and **(C)** root:shoot ratio.

$P < 0.001$; **Figure 1C**), being positive for *P. cordata* ($b = 0.0028 \pm 0.0005$ SE; $R^2 = 0.49$; $P < 0.001$) and negative for *L. hexandra* ($b = -0.0032 \pm 0.0006$ SE; $R^2 = 0.54$; $P < 0.001$). Despite the opposite effects, the magnitudes of the slopes were similar. These results indicate that *L. hexandra* invests more in shoots, while *P. cordata* invests more in belowground structures (roots), with an increasing degree of competition with *U. arrecta*.

Field Experiment

The results obtained in the field experiment with *P. cordata* followed the pattern found in the greenhouse. The biomass of *U. arrecta* negatively affected the biomass of *P. cordata* ($b = -0.0472 \pm 0.006$ SE; $R^2 = 0.85$; $P < 0.001$; **Figure 2A**). The root:shoot ratio of the biomass of this native species was positively and significantly affected by the biomass of the invasive species ($b = 0.003 \pm 0.0001$ SE; $R^2 = 0.76$; $P < 0.001$; **Figure 2B**).

Comparison of the Results With Those Obtained in the Literature

The effect of *U. arrecta* on native species was substantially larger than the effects reported in the meta-analysis of Jauni and Ramula (2015). The results of this comparison also indicate that the negative effect of *U. arrecta* on *L. hexandra* was greater than that estimated for *P. cordata* in the greenhouse experiment. However, the largest effect size was estimated for *P. cordata* when the experiment was carried out in the field, despite the high overlap between the confidence intervals (**Figure 3**).

DISCUSSION

We found that the competitive effects of *U. arrecta* on *P. cordata* and *L. hexandra* are biomass-dependent, corroborating our first hypothesis. Most importantly, in accordance with our second hypothesis, we showed that the native species responded differently to the competitive interaction with the invader, at least in terms of plant height and root:shoot ratios. In addition, it is likely that the increase in *U. arrecta* biomass increases the competition for nutrient acquisition more in *P. cordata* than in *L. hexandra*, given that the former species invested more in root growth when in greater competitive interaction with *U. arrecta* than the latter. However, although these native species responded differently to the competitive interaction with the invader, *L. hexandra* was not the most negatively affected by *U. arrecta* (as suggested by similar responses in terms of biomass production), which contradicts our prediction in this regard.

The negative effects of *U. arrecta* on native species were even stronger in the field experiment, where the growth of *P. cordata* was nearly suppressed at high densities of *U. arrecta* (see **Figure 2A**). These results, along with those obtained in the greenhouse, indicate that high densities of the invasive species decrease recolonization success by native species, which may explain the pattern of reduced macrophyte diversity with the increase of *U. arrecta* biomass at small spatial scales (Michelan et al., 2010b; Amorim et al., 2015). Our findings agree with studies carried out with other invasive species that showed the importance of density in the establishment success of native species, mainly in controlled experiments (Doyle et al., 2003; Martin and Coetzee, 2014). Thus, in field conditions, the effects of exotic species on the growth of native species are likely to be much stronger than the effects measured in greenhouse experiments. For example, the biomass that *U. arrecta* may attain in the field (approx. 7000 g DW m^{-2}; Carniatto et al., 2013) is much higher than the highest biomass in our experiment (approx. 2000 g DW m^{-2}).

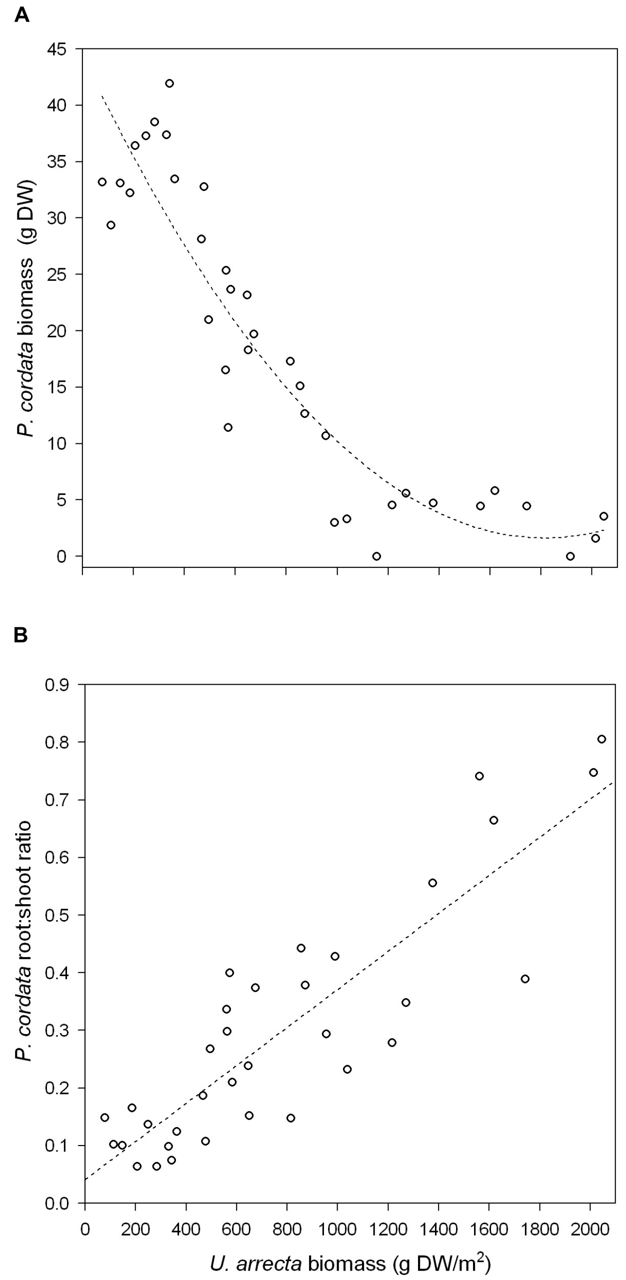

FIGURE 2 | Relationship between the shoot biomass of *U. arrecta* and the biomass **(A)** and root:shoot ratio **(B)** of *Pontederia cordata* (data from the *in situ* experiment).

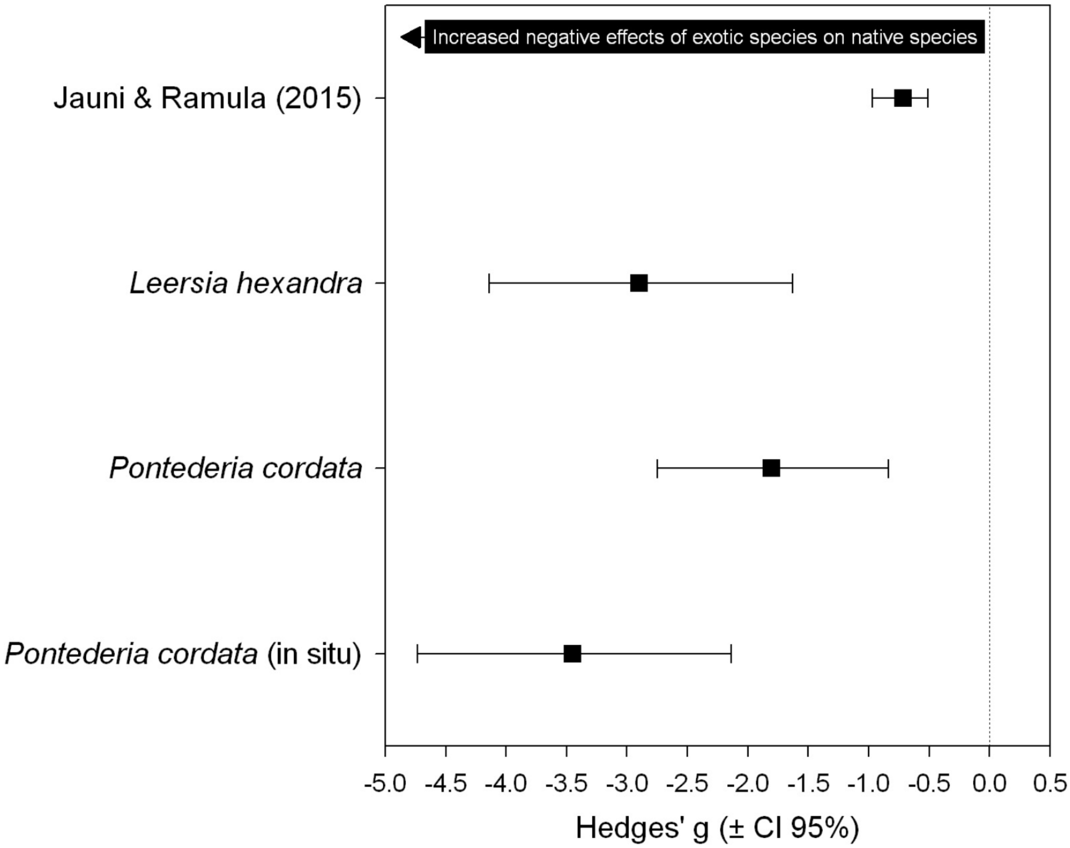

FIGURE 3 | Cumulative effect size estimated by Jauni and Ramula (2015) and effect sizes estimated in this study. These assess the effects of invasive exotic plants on the biomass of native plants.

Macrophytes respond to competition (Burns and Winn, 2006) and other environmental factors, such as increase in water level (Gomaa and AbdElgawad, 2012), by shoot elongation. The elongation (or etiolation) of terrestrial and aquatic plants is, in general, a response to light limitation (e.g., Goldsborough and Kemp, 1988; Paciullo et al., 2008, 2011; Li et al., 2011). However, our results indicate a reduction in the length of native species with increased competition (and shading) by *U. arrecta* (see **Figure 1B**). Thus, our results agree with other studies showing that not all species are able to etiolate in the face of light competition. For example, Burns and Winn (2006) demonstrated that competition reduced the lengths of two grass species. A plausible explanation for the lack of etiolation in face of competition, applicable only to *L. hexandra*, is that the light limitation was offset by the increased investment in the biomass of the shoots, as shown by the results for the root:shoot ratio (see below). By contrast, *P. cordata* has broader leaves than *L. hexandra*, and thus, increased light acquisition may be obtained by increases in leaf area instead of etiolation. These factors may also explain the steeper reduction in length for *L. hexandra* than for *P. cordata* along the invasive biomass gradient.

The native species responded differently to the increase in *U. arrecta* biomass in terms of investment in belowground or aboveground structures (see **Figure 1C**), revealing different strategies to overcome competition with the invasive species. The increase in plant density exacerbates competition, which may occur for space, nutrients and/or light (Witkowski, 1991; Daehler, 2003; Doyle et al., 2003; Davis, 2009). Increased investment in shoots indicates a predominance of competition between aboveground plant structures, while high investment in roots indicates dominance of competition between belowground structures (e.g., Berendse and Möller, 2009; Janeček et al., 2014). Based on this premise, our results indicate that *L. hexandra* growth becomes increasingly limited by light availability over a gradient of *U. arrecta* biomass, while *P. cordata* growth becomes increasingly limited by nutrients and space over the same biomass gradient. The largest investment in shoots by *L. hexandra* at high densities of the invasive probably occurs because Poaceae are, in general, highly sensitive to shading conditions. Allocation to shoot biomass, relative to root biomass, is likely to be a response to light limitation under high competition, as observed for other herbaceous species (Gibson et al., 2004; Awan et al., 2015). On the other hand, increased allocation to roots in *P. cordata* indicates a response to root competition (for other examples, see Bakker and Wilson, 2001; Schiffers et al., 2011; Zhu et al., 2015).

Despite the changes in biomass allocation of the native species over the competition gradient, there was a reduction of approximately 90% of the native species biomass at high biomass of *U. arrecta*, and the reduction was even higher for *P. cordata* in the field. The significant reduction in the growth of native species when exotic species are dominant, as simulated in our experiments, supports the model of preemptive competition, as found in other studies (Grace, 1987; Seabloom and van der Valk, 2003; Moore and Franklin, 2012; Moore et al., 2014). In practical terms, our experimental results indicate that native species have low capacities to recolonize sites dominated by invasive species. In addition, along with results of "invasiveness" experiments (e.g., Xu et al., 2004; Michelan et al., 2013), our results suggest that pre-occupation is key to predicting competition effects. The negative effects on the native species derived from the pre-occupation of the exotic species are likely to be more pronounced in ecosystems subject to anthropogenic impacts, since anthropogenic impacts are more favorable to the success of invasive plants (Daehler, 2003; Havel et al., 2005; Engelhardt, 2011). In summary, we speculate that the natural recolonization by native species in environments dominated by *U. arrecta* is unlikely and that their success can only occur if the invasive species is manipulated, reducing its occupation.

Experiments in greenhouses and controlled conditions are criticized for using small spatial and temporal scales and for not replicating the complexity found in natural environments (Gibson et al., 1999). Experiments like ours could, for example, bias the effects of shoot competition because of limited soil volume, which reduces shoot growth, and because of edge effects, which allow more access to light than would occur in the field (Kiaer et al., 2013). However, the data obtained in the field for *P. cordata* demonstrate that at least the direction and intensity of the competitive effects exerted by *U. arrecta* were similar to those found in the greenhouse. This congruence suggests that the data obtained experimentally in the greenhouse can be extrapolated to field situations, as the results obtained in the latter also indicate the importance of density-dependent effects of an invasive species on native species.

Finally, we believe that the larger effect sizes in our experiment compared to those estimated by Jauni and Ramula (2015) may indicate that *U. arrecta* possesses higher competitive effects than other invasive species. The consistent negative effect of exotic plants on the biomass of native plants, according to Jauni and Ramula (2015), may be explained by considering three mechanisms that are not mutually exclusive. The first mechanism, and probably the most important, is related to competition for light and nutrients, which reduces biomass and may cause a decrease in the reproductive success and survival of native plant species, leading to population decline (Jauni and Ramula, 2015). The other two mechanisms are related to pollinators and survival of recruits, but these mechanisms cannot be used to explain our results because our experiments considered only one plant generation. Interestingly, the results obtained by Jauni and Ramula (2015) suggest that native plants can be established when associated with exotic plants. These authors suggest that the population dynamics of native plants may not be limited by the availability of micro-sites but by competition with exotic species in later stages of the life cycle. Our results corroborate this expectation, since most of the native propagules established even in high biomass of the exotic species, but they attained extremely low growth and did not flower in this condition.

In short, our hypotheses that the effects of competition between *U. arrecta* and native macrophytes are density dependent and that *P. cordata* and *L. hexandra* respond differently to this competitive interaction were corroborated. Our results suggest that in a scenario of dominance of invasive Poaceae, recolonization by native macrophytes is unlike to occur naturally. Our results also indicate that the reduction of the diversity of native macrophytes observed in sites colonized by *U. arrecta* can be explained by the competitive effects of this invasive species. In practical terms, due to the density-dependent competitive effects, when it is not possible to eliminate the invasive species, a strategy to maintain it at low density would be required to allow recolonization by natives and to maintain local biodiversity. Also in this context, we believe that increasing the number of propagules to analyze the capacity of native species to recolonize environments densely colonized by exotic species would be an interesting avenue for further research.

AUTHOR CONTRIBUTIONS

TM and ST conceived the ideas and designed the experiments. TM, ST, and FB conducted the greenhouse and field experiments. TM and LB analyzed and interpreted the data. TM led the writing of the manuscript. ST and LB gave major input into the first draft. All authors contributed critically to the final version of the manuscript and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.01261/full#supplementary-material

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Invasive Aquatic Plants as Ecosystem Engineers in an Oligo-Mesotrophic Shallow Lake

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Exotic hydrophytes are often considered as aquatic weeds, especially when forming dense mats on an originally poorly colonized environment. While management efforts and research are focused on the control and on the impacts of aquatic weeds on biodiversity, their influence on shallow lakes' biogeochemical cycles is still unwell explored. The aim of the present study is to understand whether invasive aquatic plants may affect the biogeochemistry of shallow lakes and act as ecosystem engineers. We performed a multi-year investigation (2013–2015) of dissolved biogeochemical parameters in an oligo-mesotrophic shallow lake of south-west of France (Lacanau Lake), where wind-sheltered bays are colonized by dense mats of exotic *Egeria densa* Planch. and *Lagarosiphon major* (Ridl.) Moss. We collected seasonal samples at densely vegetated and plant-free areas, in order to extrapolate and quantify the role of the presence of invasive plants on the biogeochemistry, at the macrophyte stand scale and at the lake scale. Results revealed that elevated plant biomass triggers oxygen (O_2), dissolved inorganic carbon (DIC) and nitrogen (DIN) stratification, with hypoxia events frequently occurring at the bottom of the water column. Within plants bed, elevated respiration rates generated important amounts of carbon dioxide (CO_2), methane (CH_4) and ammonium (NH_4^+). The balance between benthic nutrients regeneration and fixation into biomass results strictly connected to the seasonal lifecycle of the plants. Indeed, during summer, DIC and DIN regenerated from the sediment are quickly fixed into plant biomass and sustain elevated growth rates. On the opposite, in spring and autumn, bacterial and plant respiration overcome nutrients fixation, resulting in an excess of nutrients in the water and in the increase of carbon emission toward the atmosphere. Our study suggests that aquatic weeds may perform as ecosystem engineers, by negatively affecting local oxygenation and by stimulating nutrients regeneration.

Keywords: carbon emission, methane, hypoxia, water stratification, nutrients regeneration, seasonal, primary production, exotic plants

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INTRODUCTION

Global changes, such as the increase of water temperature, the modifications of lakeshore for anthropic activities and the unintentional introduction of plant fragments may favor the spread of exotic aquatic plants (Gillard et al., 2017; Bertrin et al., 2018). The settlement of invasive species, and the massive developed biomass, has been recently recognized to strongly influence biodiversity (Hussner et al., 2009; Strayer, 2010). However, the effect of invasive species on ecosystem functioning is little known and could be either neutral or positive, by triggering significant changes on the basic processes of the ecosystem (Crooks, 2002; Havel et al., 2015).

The presence of invasive macrophytes can strongly modify aquatic local conditions, and particularly the water temperature, the sediment chemistry and the nutrients cycling of the colonized area, especially in the case of rootless or floating-leaved hydrophytes (Urban et al., 2006; Pierobon et al., 2010; Andersen et al., 2017; Vilas et al., 2017). Indeed, the massive plant coverage at the air–water interface is recognized to generate thermal and chemical stratification, even within very shallow waters (Andersen et al., 2017). While submerged rooted macrophytes at moderate biomass are known to increase sedimentary redox potential thanks to radial oxygen loss (Racchetti et al., 2010; Ribaudo et al., 2011), extremely dense submerged canopies may lead to decreased redox potentials and increased benthic nutrients fluxes, as a result of limited water mixing (Boros et al., 2011). As in the case of floating hydrophytes, the oxygen consumption from mineralization of plant detritus may favor the production of anaerobic end-products and nutrients regeneration such as methane and ammonium (Bianchini et al., 2008; Pierobon et al., 2010; Oliveira-Junior et al., 2018).

Benthic nutrients release from densely vegetated sediments is favored by particles trapping by submerged shoots and sediment accretion, and may therefore constitute a functional advantage for plant development (Madsen et al., 2001). Macrophytes will use nutrients regenerated from the sediment for their growth and deplete them, especially in nutrients-poor contexts (Bini et al., 2010). In oligotrophic systems, characterized by a low productivity, the settlement of fast growing primary producers can thus accelerate nutrients cycling and boost organic matter degradation, especially in summer, in correspondence with the maximum growth rates and elevated temperatures. On the opposite, during the senescence of the plants in autumn, respiration processes will be prevailing over nutrients fixation and regenerate nutrients toward the water column (Bowes et al., 1979; Pierobon et al., 2010; Ribaudo et al., 2011, 2012).

The balance between nutrients regeneration from sediments and uptake by plants is a key concept for investigating the net effect of the presence of large macrophytes stands within nutrient-poor shallow lake (Bowes et al., 1979). Indeed, at the lake scale, vegetated littoral zones are recognized as hotspots of primary production that take advantage from watershed nutrients incomes, while nutrients and organic matter outputs to the pelagic zone depend on water currents and wind velocity (Wetzel, 1992). Abundant plant biomass can self-sustain thanks to organic matter accretion and nutrients regeneration even when the input from the watershed is low (Marion and Paillisson, 2003).

In this study, several sampling campaigns were carried out in aquatic weed dense meadows of a shallow oligo-mesotrophic lake, with the aim of understanding whether two invasive aquatic plants can act as ecosystem engineers in a nutrient-poor system (Crooks, 2002). More precisely, we hypothesized that dense invasive macrophytes stands will (i) induce thermal and nutrients stratification in the water column, and (ii) contribute to the regeneration of nutrients from the sediments according to a lifecycle seasonal pattern. To test those hypotheses, we worked at two different spatial scales: (1) at the vegetated stand scale for understanding the role of the two invasive hydrophytes in enhancing nutrients regeneration and (2) at the lake scale, to contextualize the role of massive stands in shallow lakes concerning nutrients and carbon budgets.

MATERIALS AND METHODS

Study Area

Lacanau Lake is one of the oligo-mesotrophic shallow lakes of the French Atlantic Lakes chain, located between the Gironde and the Adour estuaries in South-West of France, together with Carcans-Hourtin, Cazaux-Sanguinet, and Parentis-Biscarrosse lakes (Cellamare et al., 2012; Moreira et al., 2015). French Atlantic Lakes are *Lobelia* shallow lakes, known for being colonized by a few macrophyte species, which are typically distributed along the first meter of the water column. Macrophyte community is here mainly composed by isoetids (*Lobelia dortmanna* L., *Littorella uniflora* (L.) Asch. and *Isoetes boryana* Durieu) together with some species of charophytes (Bertrin et al., 2018). This community is recognized to reduce carbon benthic fluxes and to contribute to sediment oxygenation through radial oxygen loss. Their slow metabolism and low growth rates do not affect biochemical cycles on the short term nor water stratification (Ribaudo et al., 2017). Within those lakes, the nutrients budget is mainly driven by benthic fluxes and input from the small watershed and rainfalls (Buquet et al., 2017).

In French Atlantic Lakes, the strong wind and the oligotrophic conditions do not allow the settlement of large canopy-forming hydrophytes, which typically require still waters, nutrients availability and organic-rich sediments. Nevertheless, since about 40 years, large submerged stands of two caulescent aquatic plants [*Egeria densa* Planch and *Lagarosiphon major* (Ridl.) Moss] have been found in some areas of those lakes. *E. densa* and *L. major* are two non-native hydrophytes belonging to the Hydrocharitaceae family, characterized by long erected stems with alternate or opposed whorled leaves. They preferentially settle and develop in sheltered creeks and ports of the lake, between −0.5 and −3.5 m; sparse shoots could be present until 6 m deep. Within French Atlantic Lakes, those hydrophytes develop a total biomass up to 4000 g_{DW} m^{-2} (Bertrin et al., 2017). They present elevated growth rates, giving them a selective advantage over other hydrophytes species, notably thanks to the presence of adventitious roots allowing vegetative multiplication and large dispersion capacities (Haramoto and Ikusima, 1988). According to available past reports (François, 1948), the two species have not replaced other hydrophytes in French Atlantic Lakes. Indeed, *Myriophyllum* spp. were present in 1940's with sparse shoots, but never developed such biomass and meadow extent.

Lacanau Lake's surface is 16.2 km^2 and the mean depth is 2.6 m, Secchi disk is 3.5 m (Moreira et al., 2015). Within the lake, *E. densa* and *L. major* form dense stands, with total biomass >50 g_{DW} m^{-2} occupying 1.19 km^2 (about 7% of the lake surface, according to Bertrin et al., 2017). The substrate on which plants develop is composed of a sandy substrate covered by a thick layer of labile organic matter-rich sediment presenting 35 ± 21% ($n = 59$) as loss of ignition (Bertrin et al., 2017).

Seasonal Vertical Stratification

In dense vegetated stands, water sampling was performed at 15 sites between June 2013 and November 2015 (**Figure 1** and **Appendix 1**). For each site, measurements were carried out in duplicates, twice during the day (in the morning, at around 11 a.m. and in the early afternoon, before 3 p.m.) and repeated during the growing seasons in this temperate region of France: spring (March to June), summer (June to September), and autumn (September to November). Water samplings were performed from a boat using a silicone pipe connected to a peristaltic pump. One pipe's extremity was inserted into the plants stand deep to about 40 cm above the sediment; the other end was connected to a syringe on the boat. Samples within the plants (*Vegetated – Bottom* samples) were collected at depths ranging from 100 to 330 cm, with an average of 249 ± 72 cm. Samples taken just below the water column surface (*Vegetated – Surface* samples) were collected directly from the boat without the use of the pump. During each sampling cycle, the vegetated stand height was systematically measured using a graduated pole.

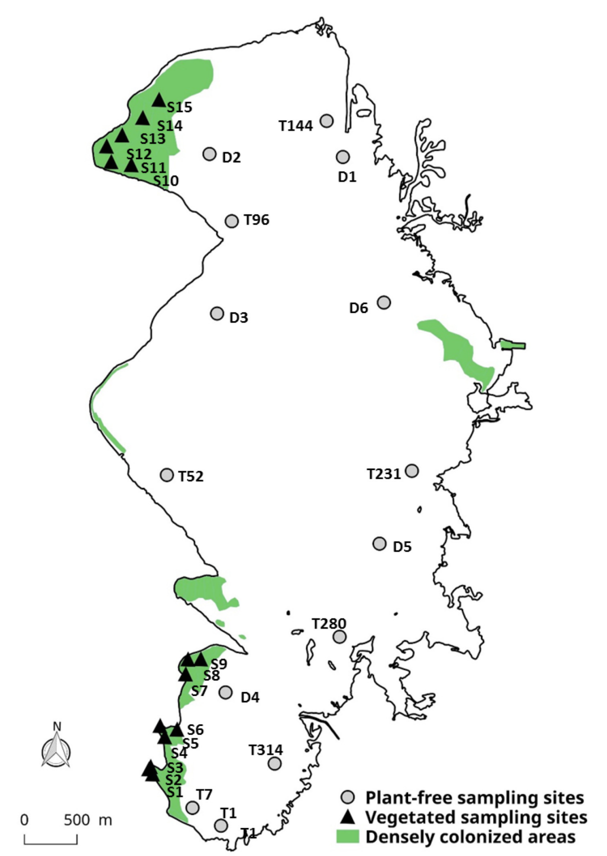

FIGURE 1 | Location of the sampling stations within Lacanau Lake; the correspondence to the sampling sites is reported in **Appendix 1**. Distribution map of *E. densa* and *L. major* densely colonized areas (>50 g_{DW} m^{-2}) refers to 2014 and is taken from Bertrin et al. (2017).

In unvegetated areas, water sampling was carried out during the day ($n = 1$–6, collected between 10 a.m. and 5 p.m.) between May 2013 and November 2015, at 14 sites where aquatic weeds were totally absent, within parallel studies (Buquet et al., 2017; Ribaudo et al., 2017; Jamoneau, unpublished; **Figure 1** and **Appendix 1**). Samples were collected at the top of the water column (hereafter *Plant-free – Surface* samples) and, where the depth allowed it, samples were also collected at −3 m by using a 2L Niskin bottle, hereafter referred as *Plant-free – Bottom* samples (Buquet et al., 2017).

For each sampling, an aliquot was immediately transferred to a beaker, in which water temperature and pH were measured with a YSI Multiple Probe (model 556). Separated aliquots were sampled in borosilicate vials and then analyzed in the laboratory for dissolved oxygen (O_2 – by Winkler method), alkalinity (TAlk – by titration with HCl 0.1 M), carbon dioxide [CO_2 – by retrocalculation starting from TAlk and *in situ* pH, validated by measures of dissolved inorganic carbon, DIC, to verify that organic alkalinity was negligible (Abril et al., 2015)] and methane (CH_4 – by headspace GC-FID method) analyses, following the methods reported in Ribaudo et al. (2017). A separate aliquot was filtered (Whatman GF/F filters) and transferred to a 50 ml plastic vial for subsequent dissolved inorganic nitrogen (NO_3^- and NH_4^+) analyses by ionic chromatography (METROHM 881 – compact). An aliquot of 50 ml was filtered by GF/F filters and transferred to a borosilicate glass flask for measurement of dissolved organic carbon (DOC) by COTmeter. An aliquot of 500 ml was transferred to a PE-HD flask for total phosphorus (TP) measurement by spectrophotometric assay after NF acid mineralization T90-023, and total nitrogen (TN) after selenium mineralization NF EN 25663. TP and TN were measured only at vegetated sites and at the surface of some of plant-free sites (see **Appendix 1**).

For vegetated stands, we calculated excess dissolved inorganic carbon (eDIC, μM), as the difference between the *in situ* DIC and a theoretical DIC at atmospheric equilibrium (for $CO_2 = 400$ ppmv), according to Abril et al. (2000). The apparent oxygen utilization (AOU, μM) was calculated for vegetated stands according to Dinauer and Mucci (2017), as the deviation of oxygen from an O_2 concentration in equilibrium with the atmosphere.

Seasonal Nutrients and Carbon Budget

Together with seasonal samplings, plant harvesting was carried out for biomass measurements. An additional winter sample was

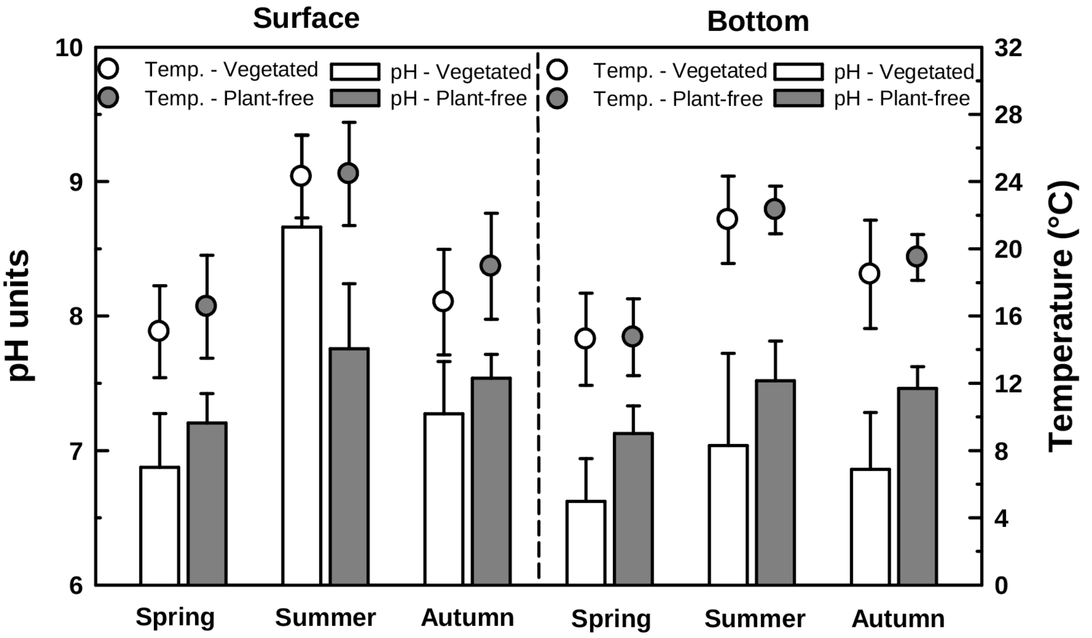

FIGURE 2 | Water pH (bars, left scale) and temperature (points, right scale) variations measured along different seasons in vegetated stands and plant-free areas at the surface and at the bottom of the water column. Error bars represent standard deviation.

taken in February 2014 ($n = 7$), in order to obtain a winter value for growth rate calculations. Samples were always collected by the same operator to minimize the sources of error (Johnson and Newman, 2011), using a telescopic rake (ground sampling area = 0.28 m^2). The plants were kept cold, transported fresh to the laboratory in opaque bags and transferred to water-filled containers until the moment of measurement, in order to facilitate their handling. In laboratory, the dry weight (g_{DW}) was determined after 72 h at 70°C and expressed as total biomass (below + aboveground biomass, g_{DW} m^{-2}). Number of shoots was counted for obtaining a shoot density (shoots m^{-2}).

The gross growth rate (GGR, expressed as g_{DW} $g_{DW}{}^{-1}d^{-1}$) was calculated as follows:

$$\mathrm{GGR} = \mathrm{NGR} + \mathrm{abs(DR)}$$

where NGR is the net growth rate (g_{DW} $g_{DW}{}^{-1}d^{-1}$), measured at different temperatures for *Egeria* spp. by Haramoto and Ikusima (1988) and Tavechio and Thomaz (2003) corresponding to a value of 0.020, 0.050, 0.030, and 0.005 g_{DW} $g_{DW}{}^{-1}d^{-1}$, for spring, summer, autumn, and winter, respectively. DR is the biomass decay rate (g_{DW} $g_{DW}{}^{-1}d^{-1}$), measured at different seasons for *Egeria* spp. by Carvalho et al. (2005), Carrillo et al. (2006), and Suzuki et al. (2015), corresponding to a value of 0.016, 0.045, 0.036, and 0.014 g_{DW} $g_{DW}{}^{-1}d^{-1}$, for spring, summer, autumn, and winter, respectively.

The GGR, NGR, and DR obtained for each sampling site and season were multiplied by the biomass measured in the same site and season, in order to obtain a daily gross primary production (GPP, g_{DW} $m^{-2}d^{-1}$), a daily net primary production (NPP, g_{DW} $m^{-2}d^{-1}$) and a daily decomposition (DD, g_{DW} $m^{-2}d^{-1}$). Each seasonal value (averaged from $n = 15$ sites) was then multiplied by 90 days (corresponding to one season of 3 months) and then summed in order to obtain an annual estimation. Further, weight production/decomposition was converted into nutrients uptake/loss rates, by using an average content of 0.360 g C $g_{DW}{}^{-1}$, 0.015 g N $g_{DW}{}^{-1}$ and 0.003 g P $g_{DW}{}^{-1}$ in the plant tissue (Carvalho et al., 2005; Yarrow et al., 2009; Suzuki et al., 2015). In this way, we obtained an estimation of the amount of carbon and nutrients fixed/loss annually into/from biomass at the local and at the lake scale by considering a total surface covered by plants of 1.19 km^2.

Starting from the surface gas concentrations and local wind speed, CO_2 and CH_4 diffusive fluxes at the water–air interface were calculated, following the two-layer model proposed by Liss and Slater (1974). Diffusive fluxes at the water–air interface (F) were calculated as follows:

$$F = k \times (C_{\mathrm{meas}} - C_{\mathrm{eq}})$$

where C_{meas} is the gas concentration measured in the surface sample expressed as mg C L^{-1}, C_{eq} is the gas concentration in surface sample in equilibrium with the atmosphere (calculated in function of the temperature from Henry's law – Sander, 1999) and k is the gas transfer velocity constant (cm h^{-1}). Gas transfer velocity varies in function of turbulence at the water–air interface, which is mostly generated by winds in lakes (Repo et al., 2007; Cole et al., 2010). Gas transfer velocity was calculated using the average wind speed of 3.5 m s^{-1} measured over

TABLE 1 | Summarized results of the three-way ANOVA on physicochemical parameters (plant presence, season, and sampling depth as fixed factors; sampling site as random factor). For TN and TP only, a two-way ANOVA was performed (season and sampling depth as fixed factors; sampling site as random factor).

	pH		**Temperature**		**O_2**		**CO_2**		**CH_4**	
Source	***df*, residuals**	***p***	***df*, residuals**	***p***	***df*, residuals**	***p***	***df*, residuals**	***p***	***df*, residuals**	***p***
Plant presence	1, 31	0.0596	1, 31	0.0018	1, 31	<0.001	1, 28	<0.001	1, 26	0.0847
Sampling depth	1, 305	<0.001	1, 305	0.6804	1, 478	<0.001	1, 437	<0.001	1, 471	<0.001
Season	1, 305	<0.001	1, 305	<0.001	1, 478	<0.001	1, 437	<0.001	1, 471	0.0550
Plant × Depth	1, 305	<0.001	1, 305	0.4911	1, 478	<0.001	1, 437	<0.001	1, 471	0.0130
Season × Depth	1, 305	<0.001	1, 305	<0.001	1, 478	0.1675	1, 437	0.9790	1, 471	0.3187
Plant × Season	1, 305	<0.001	1, 305	0.1870	1, 478	<0.001	1, 437	0.0312	1, 471	0.6093
Plant × Season × Depth	1, 305	<0.001	1, 305	0.2377	1, 478	0.9595	1, 437	0.9122	1, 471	0.7920

	NH_4^+		**NO_3^-**		**DOC**		**TN**		**TP**	
	***df*, residuals**	***p***	***df*, residuals**	***p***	***df*, residuals**	***p***	***df*, residuals**	***p***	***df*, residuals**	***p***
Plant presence	1, 31	0.4659	1, 31	0.0082	1, 26	<0.001	1,12	–	1, 12	–
Sampling depth	1, 538	<0.001	1, 519	0.0052	1, 216	0.0667	1, 56	0.0497	1, 56	0.4002
Season	1, 538	<0.001	1, 519	<0.001	1, 216	0.0045	1, 56	<0.001	1, 56	0.0141
Plant × Depth	1, 538	0.0046	1, 519	0.9777	1, 216	0.1326	1, 56	–	1, 56	–
Season × Depth	1, 538	0.0287	1, 519	<0.001	1, 216	0.9738	1, 56	0.4862	1, 56	0.6519
Plant × Season	1, 538	0.0220	1, 519	<0.001	1, 216	<0.001	1, 56	–	1, 56	–
Plant × Season × Depth	1, 538	0.0871	1, 519	0.1509	1, 216	0.8363	1, 56	–	1, 56	–

the lake at all seasons (Bertrin et al., 2017) and the equation of Crusius and Wanninkhof (2003). Fluxes were calculated for CO_2 and CH_4 and then summed for obtaining a total carbon flux: F was then expressed as mg C $m^{-2}h^{-1}$. Calculated fluxes were thus diffusive and did not account for ebullition processes; in our study, CO_2 and CH_4 diffusive fluxes merely served to compare the dynamics of C with and without plants. Hourly fluxes were averaged and upscaled for each sampling season (90 days each), and then summed to obtain a budget for the growing season of the plants (March to November). Finally, fluxes calculated for vegetated stands were upscaled to the lake's surface covered by invasive plants (1.19 km^2), while fluxes calculated in

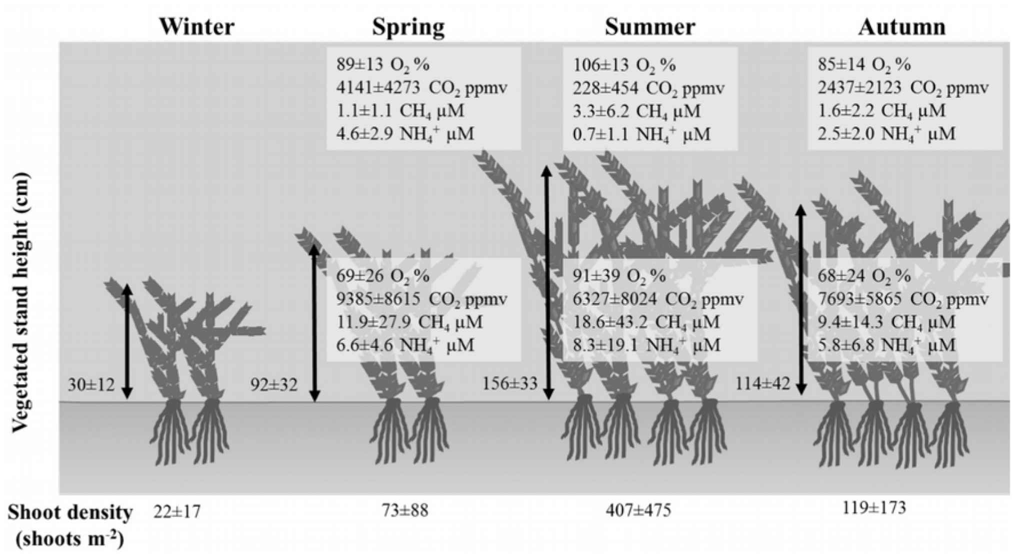

FIGURE 3 | Seasonal variation of some physicochemical parameters in function of plant density and space occupation by invasive aquatic plants. Mean ± SD are reported.

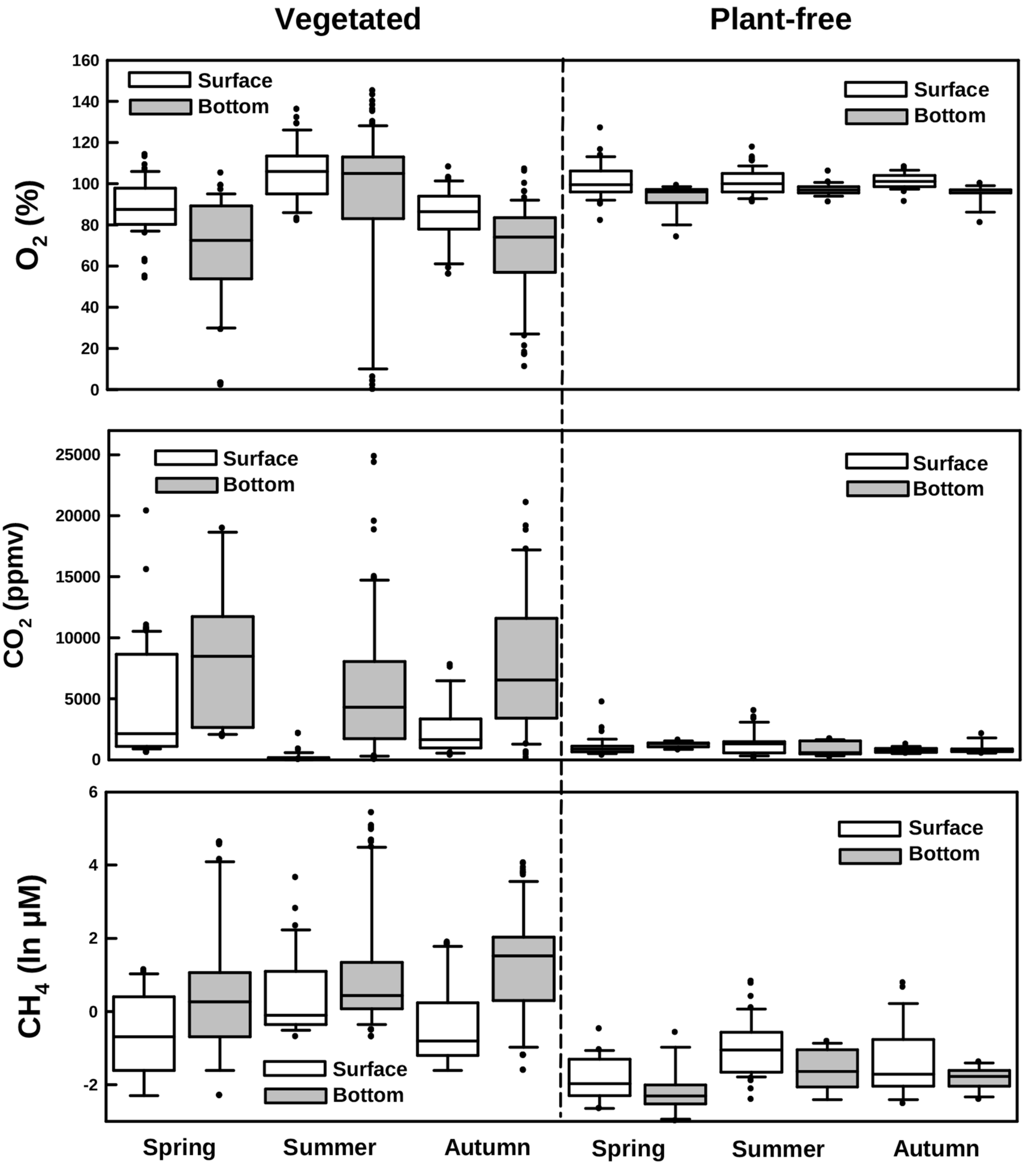

FIGURE 4 | Boxplots of dissolved oxygen, carbon dioxide, and methane measured along different seasons in vegetated and plant-free areas at the surface and the bottom of the water column.

plant-free areas were upscaled to the unvegetated lake's surface (15.01 km^2).

Statistical Analyses

We tested the influence of the presence/absence of dense vegetated stands on the biogeochemistry of the water column by a three-way ANOVA. The presence/absence of vegetated stands (*Plant presence*, two levels: vegetated vs. plant-free areas), season (*Season,* three levels: spring vs. summer vs. autumn) and sampling depth (*Depth*, two levels: surface vs. bottom) were considered as fixed factors, while sampling site (*Site*, 29 levels) was considered as a random factor. When checking

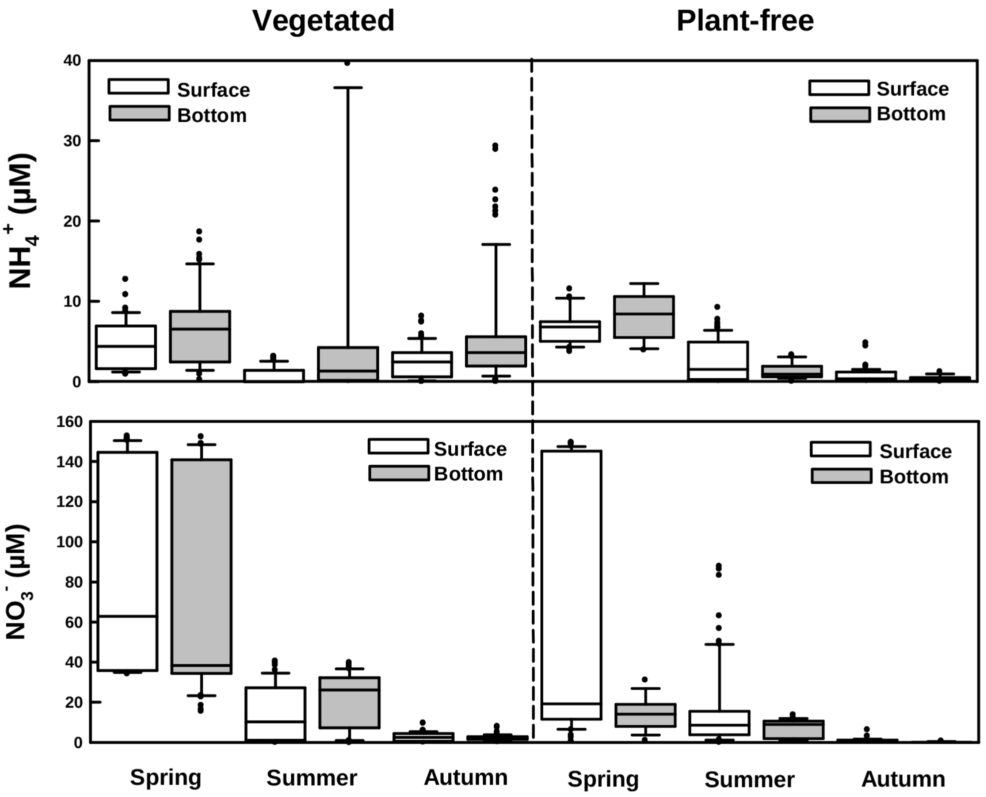

FIGURE 5 | Boxplots of ammonium and nitrate measured along different seasons in vegetated and plant-free areas at the surface and the bottom of the water column.

for analysis of variance assumptions, we found that almost every physicochemical parameter was not normally distributed (Shapiro–Wilk test for normality assumption). Nevertheless, considered the width of the dataset and that homogeneity of variances was always attained (Levene's test for homoscedasticity assumption), we decided not to apply any data transformation. *Post hoc* analyses were performed by Tukey's Honestly Significant Difference (HSD) test.

In order to test the correlation between physicochemical parameters, linear regressions and Pearson correlation coefficients were performed. Statistical analyses were performed with R Program (R – Development Core Team 2018). Mean values are reported with their standard deviation.

RESULTS

Seasonal Vertical Stratification

Water temperature measured in vegetated stands was significantly lower than that measured in plant-free areas irrespective of the season (annual mean 18.7 ± 4.4 and 20.0 ± 4.3°C, for vegetated and plant-free areas, respectively). At both vegetated and plant-free areas, water temperature varied seasonally (summer higher than spring and autumn; HSD test, $p < 0.001$) (**Figure 2** and **Table 1**). The vegetated stands were thermally stratified in summer (surface warmer than bottom; HSD test, $p < 0.001$), contrary to plant-free sites, which were never stratified. pH differences between vegetated and plant-free sites depended upon the season and the depth (**Figure 2**). At plant-free areas, differences between surface and bottom were significant only during summer (HSD test, $p < 0.001$), unlike vegetated stands, where the water column was stratified all year around.

Dissolved oxygen and carbon dioxide significantly varied according to the sampling depth and the season at vegetated sites (**Table 1**; HSD test, $p < 0.001$), whereas in plant-free areas values were constant along the year and homogenous in the water column (**Figures 3**, **4**; HSD test, $p < 0.001$). At plant-free sites, O_2 averaged 97 ± 17% and CO_2 averaged 945 ± 65 ppmv. At vegetated sites, CO_2 was generally much above 400 ppmv and presented significantly higher values than plant-free sites (**Figures 3**, **4**; HSD test, $p < 0.001$). The bottom of the vegetated stands was generally undersaturated: often hypoxic (<50%) and frequently below <20%. Average O_2 values measured at the surface of vegetated stands were <100% in spring and autumn, while in summer they were >100%. In this season, CO_2 values measured at the surface of vegetated stands averaged 228 ± 39 ppmv and reached very low values (down to 8 ppmv). At vegetated stands, DIC mean values were of 0.8 ± 0.4, 0.6 ± 0.4, and 0.8 ± 0.3 mM (surface+bottom pooled data) for spring, summer, and autumn, respectively. At plant-free areas, DIC mean values were of 0.4 ± 0.3,

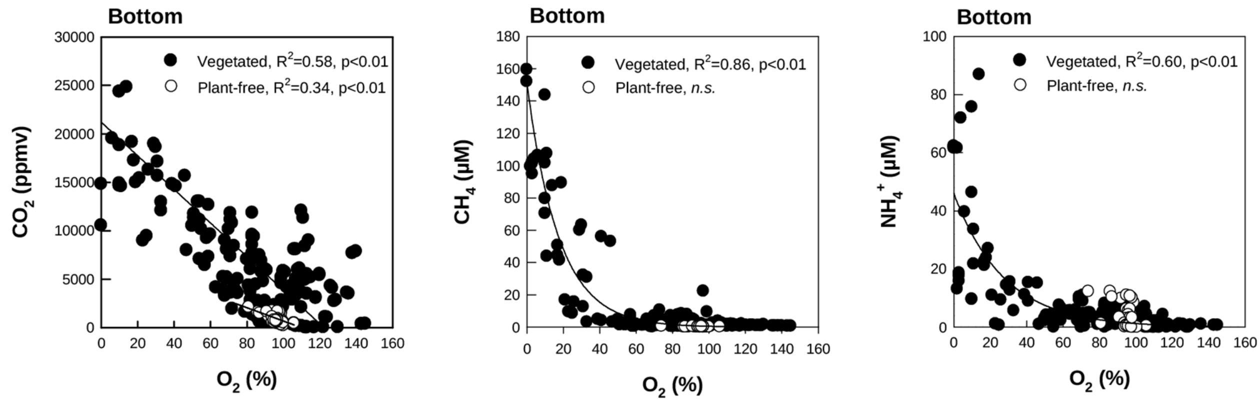

FIGURE 6 | Scatter plots of O_2 and CO_2 (Left), CH_4 (Middle), and NH_4^+ (Right) measured at the bottom of the vegetated and plant-free areas (seasonal data pooled). Regressions are calculated through a linear model for CO_2, and through an exponential model for CH_4 and NH_4^+. *n.s.* indicates a not significant relationship.

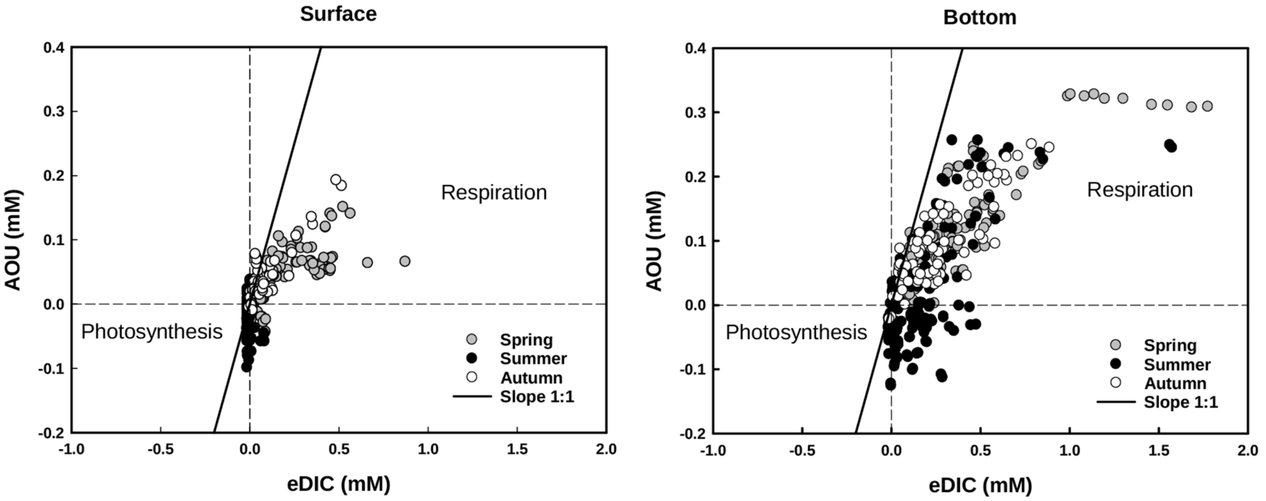

FIGURE 7 | Apparent oxygen utilization (AOU) and excess of dissolved inorganic carbon (eDIC) plotted according different seasons at the surface and at the bottom of the vegetated stands. Respiration and remineralization processes are reflected in positive values of AOU and eDIC, whereas the effects of photosynthesis are reflected in negative values. The graphical representation takes inspiration on Dinauer and Mucci (2017).

0.5 ± 0.1, and 0.4 ± 0.1 mM for spring, summer, and autumn, respectively.

CH_4 values did not vary seasonally at vegetated neither at plant-free areas (**Figures 3**, **4** and **Table 1**); at vegetated stands, the water column was significantly stratified for CH_4, with mean values of 1.92 ± 0.91 µM (from 0.05 to 38.7 µM) and 13.71 ± 31.97 µM (from 0.05 to 227 µM), measured at the surface and at the bottom, respectively (HSD test, $p < 0.001$). At the bottom of vegetated stands, values were significantly higher values than at the bottom of plant-free sites (HSD test, $p < 0.001$), where CH_4 averaged 0.20 ± 0.06 µM and 0.19 ± 0.05 µM, for the surface and the bottom, respectively. Overall, values at plant-free sites were comprised between 0.05 and 0.67 µM.

Dissolved inorganic nitrogen varied seasonally, with NO_3^- declining from spring to autumn, at both vegetated and plant-free areas. Values were significantly higher at vegetated sites; nevertheless, no stratification of the water column was detected (**Figures 3**, **5** and **Table 1**). At vegetated stands, NO_3^- values averaged 78.1 ± 51.1, 19.5 ± 14.0, and 2.5 ± 1.7 µM (surface + bottom pooled data) for spring, summer, and autumn, respectively. At plant-free sites, NO_3^- values averaged 20.0 ± 17.8, 16.4 ± 20.0, and 0.8 ± 1.2 µM (surface + bottom pooled data) for spring, summer, and autumn, respectively. NH_4^+ values varied seasonally at both vegetated and plant-free sites, with a marked stratification in the vegetated water column (**Figures 3**, **5** and **Table 1**). Here, surface values were comprised between 0.01 and 12.7 µM (mean 2.7 ± 0.6 µM), while bottom values were comprised between 0.01 and 86.9 µM (mean 7.0 ± 4.3 µM). At plant-free sites, values ranged from 0.1 to 11.5 µM (mean 3.4 ± 0.5 µM) and from 0.1 to 12.0 µM (mean 3.2 ± 0.4 µM), at the surface and at the bottom, respectively.

TABLE 2 | Dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP) measured along different seasons in vegetated stands and plant-free areas at the surface and at the bottom of the water column (mean ± SD).

		Vegetated		**Plant-free**	
		Surface	**Bottom**	**Surface**	**Bottom**
DOC (mg L^{-1})	Spring	12.3 ± 0.3	12.4 ± 0.5	14.2 ± 1.6	14.9 ± 0.9
	Summer	13.2 ± 0.4	13.1 ± 0.4	13.9 ± 0.9	14.4 ± 0.9
	Autumn	13.5 ± 0.5	13.6 ± 0.7	13.4 ± 0.8	13.8 ± 1.2
TN (mg L^{-1})	Spring	0.7 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	–
	Summer	0.5 ± 0.1	0.7 ± 0.3	0.6 ± 0.1	–
	Autumn	1.1 ± 0.1	1.3 ± 0.5	1.2 ± 0.4	–
TP (mg L^{-1})	Spring	0.06 ± 0.07	0.06 ± 0.06	0.02 ± 0.01	–
	Summer	0.06 ± 0.04	0.07 ± 0.03	0.02 ± 0.01	–
	Autumn	0.08 ± 0.09	0.12 ± 0.06	0.06 ± 0.04	–

In general, CO_2, CH_4, and NH_4^+ concentrations at the bottom layer of the water column were inversely dependent on O_2 values for vegetated stands (**Figure 6**), whereas their relationship was never significant for values measured at the surface. In vegetated stands, AOU at the surface ranged from −0.10 (summer) to 0.20 mM (autumn), with mean values comprised between −0.01 ± 0.03 and 0.5 ± 0.5 mM, measured in summer and autumn, respectively. At the bottom, values were comprised between −0.13 (summer) and 0.33 mM (spring), with mean values ranging from 0.02 ± 0.09 to 0.11 ± 0.09 mM, measured in summer and spring, respectively. eDIC at the surface ranged from −0.01 (summer and spring) to 0.52 mM (autumn), with mean values comprised between 0.00 ± 0.02 and 0.17 ± 0.17 mM, measured in summer and spring, respectively. At the bottom, eDIC values ranged between −0.01 (summer and autumn) to 1.78 mM (spring), with mean values ranging from 0.19 ± 0.23 and 0.38 ± 0.35 mM, measured in summer and spring, respectively. The relationship between AOU and eDIC in vegetated stands (**Figure 7**), evidenced a prevalence of respiration processes at the bottom all year round, while photosynthesis was prevailing during summer at the surface of colonized areas.

For DOC, TN, and TP, values varied seasonally, with no significant differences between surface and bottom values (**Tables 1**, **2**). At vegetated stands, DOC values were significantly lower than those measured at plant-free sites at all seasons except in autumn (HSD test, $p < 0.001$).

Seasonal Nutrients and Carbon Budget

In vegetated stands, the height of the vegetation and the relative proportion of occupation in the water column varied according to the season (one-way ANOVA, $p < 0.001$, $F_{2,22}$), with relatively short stems in winter and spring, when plants occupied about 44% of the water depth (**Figure 3**), which turned longer and thicker in summer and autumn (56 and 52% of the water depth). In some cases, the vegetated stands occupied 80% of the water column height; shoot density varied between 22 ± 17 to 407 ± 475 shoots m^{-2}, measured in winter and summer, respectively.

Vegetated stands were mainly constituted by *Egeria densa* (at least 80% of the biomass at each station), whereas *Lagarosiphon major* occurred only in few stations with a much lower biomass. Biomass values, as well as GPP, DD, and NPP, varied seasonally at all stations, with a marked increase in summer and autumn compared to spring and winter (**Table 3A**). Biomass ranged from 46 to 1339 g_{DW} m^{-2}, measured in winter and summer, respectively. When transforming GPP, DD, and NPP in nutrients uptake, loss, and fixation, respectively, we can estimate that dense vegetated stands fix a positive amount of nutrients, on an annual scale (**Table 3B**). When upscaling those values to the vegetated areas within the lake, on an annual basis, we can estimate that vegetated stands fix 2319 ± 1196 tons C $year^{-1}$, 97 ± 50 tons N $year^{-1}$ and 19 ± 10 tons P $year^{-1}$ during their growth.

Coherently with concentrations measured at the surface of the water column, diffusive carbon fluxes calculated at the water–air interface followed a seasonal pattern (**Figure 8**). At vegetated stands, the highest values were recorded in spring (2.3 ± 2.4 mg C $m^{-2}d^{-1}$) and the lowest in summer (0.2 ± 0.8 mg C $m^{-2}d^{-1}$); at plant-free sites, the highest value was recorded in spring (0.6 ± 0.7 mg C $m^{-2}d^{-1}$) and the lowest in autumn (0.2 ± 0.1 mg C $m^{-2}d^{-1}$). During 1 year, at the scale of the growing season of the plants (March to November) we can estimate that vegetated stands release 332 ± 417 mg C m^{-2}, while plant-free areas release 108 ± 94 mg C m^{-2}. At both vegetated and plant-free sites, the major contribution to diffusive fluxes carbon is given by CO_2 and, only in a minor part, by CH_4. When upscaling to the lake scale, we can estimate that dense vegetated stands emit 396 ± 489 kg C per growing season, whereas plant-free areas emit, in the same period, an estimated amount of 1622 ± 1403 kg C.

DISCUSSION

Biogeochemical Functioning in Invasive Macrophyte Stands

The first evidence of our study is that the presence of a massive biomass development of invasive plants generates the stratification of the water column. The thermal and chemical stratification of the water column has been reported in a few studies concerning other submerged aquatic vegetation (Mazzeo et al., 2003; Andersen et al., 2017). Vilas et al. (2017) found that water stratification due to extensive colonization of *Potamogeton crispus* started when plants occupied more than 50% of the water column, which is a value that is overpassed in most of our samplings. Nevertheless, the variability measured within our dataset could be given by episodic wind events disturbance on sediment resuspension and water mixing. Conversely, water stagnation and related extreme values can be attributable to prolonged and extremely calm weather conditions (Søndergaard et al., 1992; Gale et al., 2006). Water stratification/mixing is also dependent on the biomass degree and stand ages of the sites, which can present a variable quantity of organic matter buildup in the sediment and thus influence the degree of respiration and consumption of oxygen (Boros et al., 2011).

The stratification of dissolved oxygen has important cascade effects on the local biogeochemistry, and in particular on

TABLE 3 | (A) Total biomass (measured), gross primary production, decomposition, and net primary production (estimated) for vegetated stands of *E. densa* and *L. major* at different seasons. **(B)** Nutrients mobilization (uptake, calculated from GPP; loss, calculated from DD; fixation, calculated from NPP) within dense mats of *E. densa* and *L. major* at stand and lake scales.

(A)	**Total biomass** g_{DW} m^{-2}	**GPP** g_{DW} $m^{-2}d^{-1}$	**DD** g_{DW} $m^{-2}d^{-1}$	**NPP** g_{DW} $m^{-2}d^{-1}$
Spring	319 ± 245	11.7 ± 5.2	−5.2 ± 2.3	6.5 ± 2.9
Summer	668 ± 414	76.5 ± 38.7	−32.8 ± 16.6	36.4 ± 18.4
Autumn	567 ± 537	36.9 ± 26.2	−20.1 ± 14.3	16.8 ± 11.9
Winter	87 ± 50	1.6 ± 1.0	−1.2 ± 0.7	0.4 ± 0.3

(B)		**Carbon** g C $m^{-2}year^{-1}$	**Nitrogen** g N $m^{-2}year^{-1}$	**Phosphorus** g P $m^{-2}year^{-1}$
Stand scale	*Uptake*	4105 ± 2125	171 ± 89	34 ± 18
	Loss	−1920 ± 1007	−80 ± 42	−16 ± 8
	Fixation	1949 ± 1005	81 ± 42	16 ± 8
		tons C $year^{-1}$	**tons N $year^{-1}$**	**tons P $year^{-1}$**
Lake scale	*Uptake*	4885 ± 2528	204 ± 105	41 ± 21
	Loss	−2285 ± 1198	−95 ± 50	−19 ± 10
	Fixation	2319 ± 1196	97 ± 50	19 ± 10

the dynamics of carbon and nitrogen. In the dark bottom of the water column, under a thick layer of stems, plants respiration is not compensated by an equivalent oxygen release (Tavechio and Thomaz, 2003). The elevated heterotrophic respiration rates, filled up by the availability of labile dead biomass, generate here important amounts of DIC. This carbon is diffused to the water column surface, where it can be emitted toward the atmosphere as CO_2. This process appears to be connected to the seasonal variations in net primary production and biomass decomposition of the plants. Indeed, unlike the plant-free areas, carbon emissions from vegetated stands are accentuated in spring and autumn, and much lower

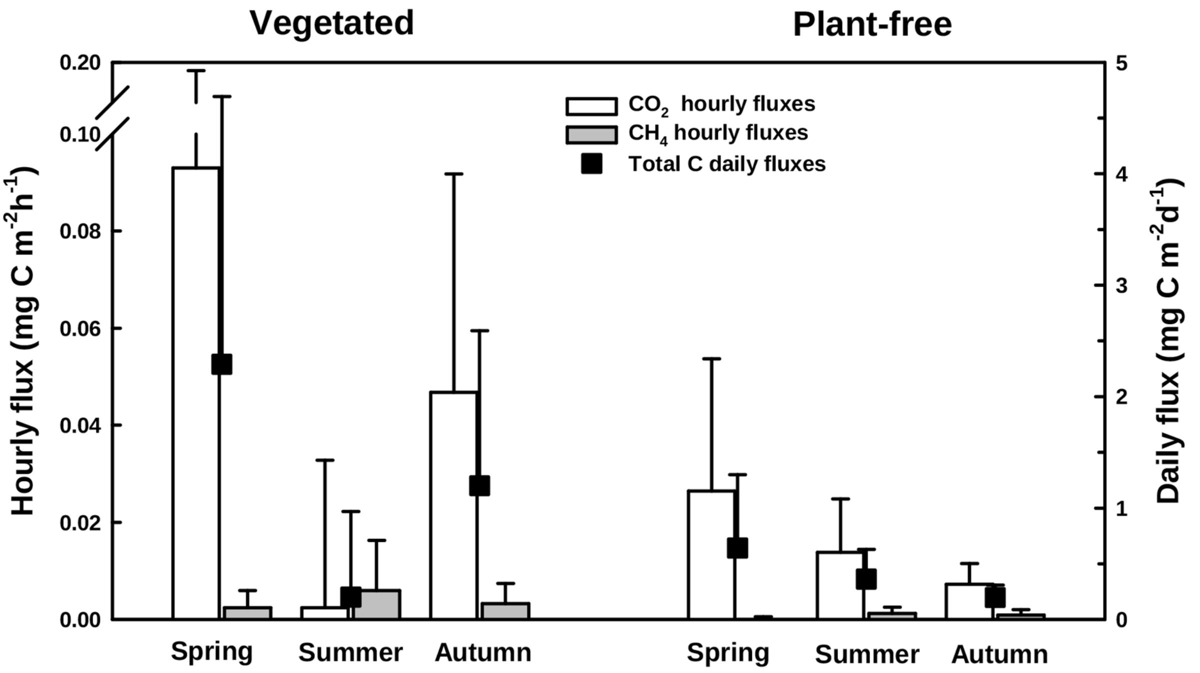

FIGURE 8 | Hourly and total diffusive carbon fluxes (from CO_2 and CH_4) calculated from concentrations measured at the surface of the water column of vegetated and plant-free areas.

in summer. Coherently, the negative eDIC values measured in summer in the euphotic layer above the canopy indicate that this carbon is likely re-utilized by photosynthesis and only partially diffused toward the surface and then toward the atmosphere. In summer, the CO_2 undersaturation measured at the surface indicates indeed a complete sequestration of CO_2 in the water column. This is typical of freshwater systems where the biogeochemical functioning is seasonally determined by the ecophysiology of dense macrophyte mats (Bowes et al., 1979; Wang et al., 2006; Pierobon et al., 2010).

The quantity of DIC present in the whole water column is just satisfactory to support the daily primary production of the plants. Indeed, in summer, plants present a NPP of 36.4 ± 18.4 g_{DW} $m^{-2}d^{-1}$, which corresponds to a fixation of 13.1 ± 6.6 g C $m^{-2}d^{-1}$. If we consider the mean value of 0.6 ± 0.4 mM DIC measured in summer for the whole water column, for a fixed volume of water having a base of 1 m^2 and an average height of 2.8 ± 0.4 m, we can estimate that the water column contains about 20 g C. This quantity is likely entirely consumed in a day by the plants in summer, as the carbon loop constituted by respiration-uptake is very fast and relates of an efficient coupling between bacterial nutrients regeneration and assimilation by plant shoots. Thus, the bottom part of the water column acts as a source of carbon, whereas the canopy of plants at the surface acts as a sink. In spring and autumn, the lower NPP do not allow the sequestration of the whole DIC generated from the organic matter degradation. This approximation is confirmed by the eDIC values, indicating the prevalence of respiration processes in the bottom of the vegetated water column all over the year, and in spring and autumn also at the surface.

The oxygen consumption and the settlement of hypoxic/anoxic conditions favor the production and buildup of methane and ammonium. Methanogenic bacteria develop thanks to the availability of dissolved organic matter (DOM) derived from the decomposition of decaying biomass (Zhang et al., 2018). A part of this methane is possibly consumed by aerobic methanotrophy within the canopy of the plants near the surface (Yoshida et al., 2014). However, a large amount of CH_4 is diffused toward the surface of the water column, that contributing to carbon emissions from the vegetated stands. Nevertheless, calculated carbon diffusive fluxes are much lower than those measured in other systems colonized by floating-leaved invasive plants (Pierobon et al., 2010; Ribaudo et al., 2012; Oliveira-Junior et al., 2018). That confirms that, despite the elevated concentrations measured at the bottom of the water column, the CH_4 oxidation and the CO_2 uptake within submerged-leaved plants can significantly reduce the net effect on carbon emissions. Under hypoxic and anoxic conditions, the nitrification process is limited (Seitzinger, 1988), so that NH_4^+ accumulates in the lower layer of water column. As in the case of CO_2, during summer, NH_4^+ can be efficiently taken up by dense vegetation. Indeed, a mean value of 6 ± 13 μM NH_4^+ measured for the whole water column, corresponds to a quantity of about 0.22 g N for a water column of 2.8 ± 0.4 m high. This quantity is completely depleted by primary production, as the corresponding NPP in summer is of 36.4 ± 18.4 g_{DW} $m^{-2}d^{-1}$, and thus is equivalent to a fixation of 0.5 ± 0.3 g N $m^{-2}d^{-1}$. Inversely, in spring and autumn, the dissolved nitrogen regenerated from decomposition cannot be wholly exhausted by fixation into biomass.

Biogeochemical Processes From Local to Lake Scale

Invasive species are known to colonize new areas thanks to peculiar ecophysiological adaptations, such as fast growth rates and phenotypic plasticity, and to the availability of resources and space (Riis et al., 2010; Gillard et al., 2017). As pristine conditions, the lake investigated in this study is characterized by low concentrations of phosphorus and nitrogen (Cellamare et al., 2012): furthermore, the total nutrients discharge from the watershed has been reduced during the past decades thanks to the management of rural activities (Buquet et al., 2017). Nevertheless, the extended photoperiod and mild temperatures characterizing the region, coupled to the availability of space and the absence of other canopy-forming hydrophytes, constitute a very favorable unsaturated ecological niche for aquatic weeds to spread. The settlement of invasive hydrophytes in originally oligo-mesotrophic systems is reported elsewhere (Nagasaka, 2004; Mjelde et al., 2012; Bolpagni et al., 2015), that contrasting with the general tendency of the establishment of invasive plants in meso-eutrophic freshwaters (Kelly and Hawes, 2005; Hussner et al., 2009). Our study shows that the presence of such a massive area of primary production and biomass decomposition affects the carbon budget of the whole lake. Indeed, if we extend the carbon diffusive fluxes calculated for vegetated and plant-free areas to their correspondent surface area (1.19 and 15.01 km^2, respectively), it results that, during their vegetative growth, plant stands release three fold more C per surface area than plant-free sites. Although they cover only 7% of the lake area, plant stands contribute to 20% of the lake carbon emissions and constitute a hotspot of carbon release (Wang et al., 2006). On the opposite, in Lacanau Lake, dense beds formed by aquatic weeds could be responsible for the storage of a part of the nutrients incoming the lake from the catchment area (Reddy et al., 1987; Søndergaard et al., 2003). During the year 2014, the total mass balance of the lake (including inputs from the watershed and the unvegetated sediments) results in a net storage of 67 tons N $year^{-1}$ and 0.16 tons P $year^{-1}$ within the lake (Buquet et al., 2017). We here estimate that invasive vegetated stands require more than 200 tons N $year^{-1}$ and 40 tons P $year^{-1}$ for their gross primary production, but this quantity is not available in the lake, neither coming from the watershed, neither originating from benthic fluxes in unvegetated areas (Buquet et al., 2017). Concomitantly, the fast renewal (NPP) and decay (DD) of biomass result in the almost total reutilization of the nutrients regenerated from the sediment, and in a small part of nutrients stocked in the sediment, through sediment accretion. In correspondence with vegetated stands, we observe indeed an accumulation of low-density, highly organic fluffy sediments

(Bertrin et al., 2017). The predominance of recycled sedimentary N and P is well known in marine systems for seagrasses growth (Bartoli et al., 1996; Deborde et al., 2008). Carvalho et al. (2005) suggest that due to the rapid decomposition of *Egeria* spp. at high temperatures, a very small exportation of nutrients is expected from its stands to distant regions of the lake. Effectively, DOC measured in vegetated stands being lower than those of plant-free areas, we can expect that a process of priming effect boosts benthic bacterial communities, fueled by the continuous supply of fresh labile plant material, and rapidly degrading organic matter to inorganic compounds (Findlay et al., 1986; Marion and Paillisson, 2003). Bini et al. (2010) also commented that, in dense *Egeria* spp. vegetated stands, the nutrients regeneration is ephemeral, temporally limited to the vegetative growth of the plants, and spatially restricted to the areas of the lake that are colonized. Our results show that effectively, in summer, this could be the case. Nevertheless, the seasonal investigation performed on water chemistry shows an excess of nutrients during the seasons of slow plant growth. Especially in spring, when nutrients regeneration is elevated and not fully compensated by fixation into biomass, the nutrients export from vegetated to plant-free areas could be possible thanks to a low shoot density allowing the water circulation during strong wind events (Losee and Wetzel, 1993; James and Barko, 1994; Bertrin et al., 2017). In addition, according to the elevated concentrations of TN and TP measured in autumn, fresh organic matter deposition, reduced light and dynamic redox conditions in plant stands would make these areas potential temporary sources of available P for phytoplankton in plant-free areas (Buquet et al., 2017). Unfortunately, the sampling strategy adopted in this study was not designed for, from a spatial and a temporal point of view, detecting any diffusive gradient of nutrients between the vegetated stands and unvegetated areas. Moreover, investigations focusing on the sediments underlying the plants are needed to complete the current knowledge.

CONCLUSION

In this study, we show that, once settled, *Egeria densa* and *Lagarosiphon major* are able to act as ecological engineers and modify the sites they colonize, by keeping a not-limiting nutrients level along the year, thanks to the formation of dense stands where a constant production of labile organic matter stimulates the microbial loop. We here show that in vegetated areas of the lake, the whole nutrients level is generally higher than in the rest of the lake, that seasonal fluctuations of oxygen and carbon are intensified and that an impact on the whole-lake ecosystem functioning is possible.

AUTHOR CONTRIBUTIONS

CR, VB, GA, PA, and JT-R conceived and designed the research project. CR, GJ, AJ, and DB collected the field data. All the co-authors commented on and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.01781/full#supplementary-material

APPENDIX 1 | Dates and hours of sampling at different sites of the Lacanau Lake. The complete physicochemical database is reported.

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Aspects of Invasiveness of *Ludwigia* and *Nelumbo* in Shallow Temperate Fluvial Lakes

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The relationship between invasive plant functional traits and their invasiveness is still the subject of scientific investigation, and the backgrounds of transition from non-native to invasive species in ecosystems are therefore poorly understood. Furthermore, our current knowledge on species invasiveness is heavily biased toward terrestrial species; we know much less about the influence of allochthonous plant traits on their invasiveness in aquatic ecosystems. In this paper, we present the results of a study on physiological and ecological traits of two introduced and three native macrophyte species in the Mantua lakes system (northern Italy). We compared their photophysiology, pigment content, leaf reflectance, and phenology in order to assess how the invasive *Nelumbo nucifera* and *Ludwigia hexapetala* perform compared to native species, *Nuphar lutea*, *Nymphaea alba*, and *Trapa natans*. We found *L. hexapetala* to have higher photosynthetic efficiency and to tolerate higher light intensities than *N. nucifera* and the native species especially at extreme weather conditions (prolonged exposure to high light and higher temperatures). Chlorophyll a and b, and carotenoids content of both allochthonous species were substantially higher than those of native plants, suggesting adaptive response to the ecosystem of Mantua lakes system. Higher variability of recorded data in invasive species was also observed. These observations suggest advanced photosynthetic efficiency of the invasive species, especially *L. hexapetala*, resulting in faster growth rates and higher productivity. This was supported by the evaluation of seasonal dynamics mapped from satellite remote sensing data. This study provides empirical evidence for the relationship between specific plant physiological traits and invasiveness of aquatic plant species, highlighting the importance of trait studies in predicting ecosystem-level impacts of invasive plant species.

Keywords: macrophytes, photophysiology, leaf reflectance, leaf pigments, phenology, *Trapa*, *Nuphar*, *Nymphaea*

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INTRODUCTION

Introduced species pose an imminent threat to biodiversity, species composition and structure, as well as general functioning of their new ecosystems (Vitousek, 1990; D'Antonio and Kark, 2002; Vilà et al., 2011). This is especially the case in aquatic environments and for aquatic plants (Gallardo et al., 2016). Invasive species are responsible for displacing large numbers of native species throughout the world (Musil, 1993; Meyer and Florence, 1996; Holmes and Cowling, 1997).

Although the introduction of species to new environments can result from natural processes, the vast majority in recent decades has occurred as either a direct (i.e., purposeful transport) or indirect (i.e., without human awareness) consequence of the anthropogenic collapse of biogeographic barriers (McKinney and Lockwood, 1999). In addition, anthropogenic pressure on ecosystems (e.g., altered land-use patterns, changes in climate regimes, increase in atmospheric CO_2), and especially on aquatic ecosystems, foster favorable conditions for the establishment of introduced species (Dukes and Mooney, 1999; Hussner et al., 2014; Fasoli et al., 2018).

Multiple differences between native and alien species have been observed, and can be summarized as being associated with either more efficient resource use by the invasive plants (Vitousek, 1986; Baruch and Goldstein, 1999), or with invasive species' lack of natural enemies in their new habitats (Callaway and Aschehoug, 2000; Mitchell and Power, 2003). In general, from one side the invasive plants could outperform their native counterparts due to their higher phenotypic variability or phenological plasticity, i.e., the introduced plants are able to better acclimate and find unoccupied spatial and temporal niches where they can prevail (Pyšek and Richardson, 2007; Davidson et al., 2011). The higher phenological plasticity of an alien plant, reflecting into its seasonal dynamics, could affect its invasive success, since the possibility of extending its lifecycle beyond the lifecycles of native species can determine its ability to capture and utilize resources (Wolkovich and Cleland, 2011). From the other side, species could be successful in their new environment in the absence of enemies, pathogens or predators, from their former habitats, consequently freeing vital resources for production and development of the introduced species (Keane and Crawley, 2002; Colautti et al., 2004). Despite the advantages the introduced species have, this does not always correspond to biomass dominance; indeed, the background processes of how these advantages are translated into better performance in their new environments are still not fully understood (Davis et al., 2001; Funk and Vitousek, 2007). Moreover, in invasive plants sometimes even the certain disadvantages (for example in C assimilation) are converted into higher production and fitness (Hussner and Jahns, 2015). Even though invasive plants seem to have higher plasticity and enhanced functional traits in many instances (Pyšek and Richardson, 2007; Davidson et al., 2011), scientific literature still has to reach consensus as to whether the carbon acquisition and distribution of native and invasive plants differ across groups and ecosystems (Daehler, 2003; Van Kleunen et al., 2010; Leffler et al., 2014; Hussner and Jahns, 2015).

Within this context, macrophytes (i.e., aquatic plants) emerge as particularly interesting targets, for several reasons. Macrophytes tend to display higher diversity in temperate areas (Alahuhta et al., 2017), where human impacts are stronger, to show highly cosmopolitan features (Zhou and Zhou, 2009), and to occupy the extremes of the global spectrum of vegetation forms (Díaz et al., 2016). As such, macrophytes are more sensitive to ecosystem degradation and invasion processes, but also provide insight into the functional adaptation potential of plants to environmental conditions different from those of their native range.

The main aim of this study was to investigate and compare selected traits (physiological, spectral, and phenological) of two invasive floating-emergent aquatic plants and native macrophyte species found in a shallow temperate freshwater system. Toward this, we hypothesized that: (i) photophysiological performance (i.e., chlorophyll fluorescence, related to C acquisition) of invasive species is superior to that of native plants; (ii) leaf pigment pools of invasive plants are different in size and composition from native ones; (iii) photophysiological performance and pigment content show higher plasticity in invasive than in native species; (iv) invasive macrophytes can effectively exploit temporal niches left unoccupied by the seasonal dynamics of native species; and that (v) leaf spectroscopy can provide information about physiological traits of both invasive and native macrophytes.

MATERIALS AND METHODS

Study Sites

The Mantua lakes system (Laghi di Mantova), consisting of the Superior, Middle, and Inferior lakes, are three shallow (mean depth: 3.5 m) fluvial lakes adjacent to the city of Mantua, Italy (**Figure 1**). The lakes are surrounded by urban areas, both residential and industrial, and the upstream basin is characterized by intensive agriculture. The Mantua lakes system is therefore exposed to diffuse sources of nutrients (N and P) and other pollutants. As a result, the lakes system is characterized by high turbidity (Secchi disk depth < 1 m in summer), high trophic level (Chl-a up to 200 mg m^{-3}), and the coexistence of phytoplankton and macrophytes, comprising emergent, submerged, floating-leaved and free-floating species (Pinardi et al., 2011; Bresciani et al., 2013; Bolpagni et al., 2014; Villa et al., 2015).

The sacred lotus (*Nelumbo nucifera* Gaertn. Nelumbonaceae), an allochthonous species originating from Southeast Asia, colonizes Superior Lake, forming two large macrophyte islands. The plant is rooted in the sediment and can grow in waters up to 3 m deep. The large, shield-shaped leaves ascend from the rhizomes on waxy, prickly stems and can rise 1–2 m above the water level. Various parts of the plant can be used for different purposes, including food, silk production, and medicine, as well as for esthetical reasons, mainly due to its large, beautiful flowers. *Nelumbo* was originally introduced to the Mantua lakes system in the 1920s, with its rhizomes proposed as an alternative food source. In the subsequent decades, it has been mainly been used for floral collection, and has spread throughout the lakes over the past five decades.

The water primrose [*Ludwigia hexapetala* (Hook. and Arn.) Zardini, H. Y. Gu and P. H. Raven Onagraceae], native to South America, is the other allochthonous species that has spread in the lakes over the past decade, first colonizing calm water bays in the Middle Lake and more recently the riparian zones of Superior and Inferior lakes. In its native range, *Ludwigia* can be found in wet grasslands and wetlands, whereas in areas where it has been introduced it is able to invade lakes, slow fluvial plumes, and wetlands, and is one of the most invasive plant genera in Europe

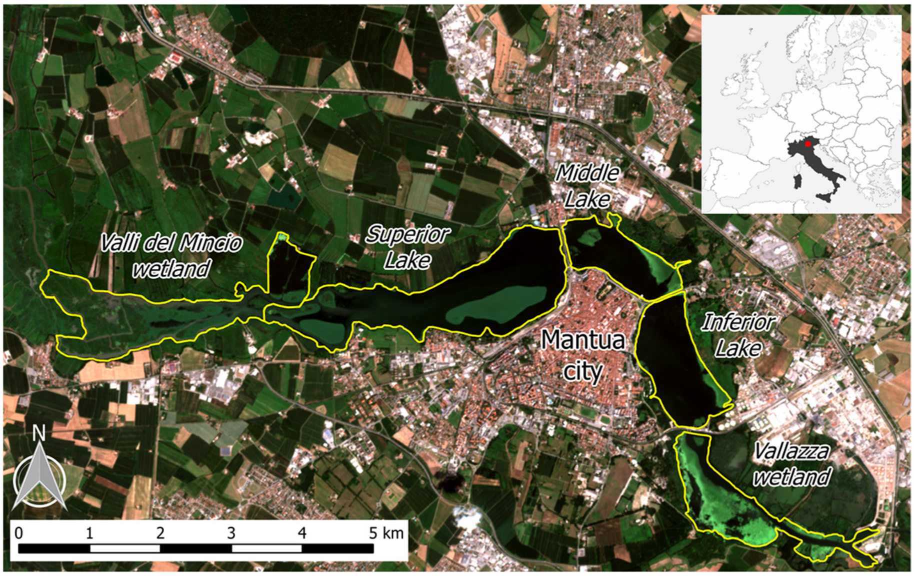

FIGURE 1 | The Mantua lakes system, with the location of the study area in Italy (upper right box), and the different subsystems (lakes and wetlands) indicated. Background image is a Sentinel-2A satellite-acquired true color composite from 28 July 2016.

(EPPO, 2004a,b). *Ludwigia* has large (2–5 cm), yellow flowers, and 8–10 cm long, spear-shaped, pilose leaves. The stem of the plant grows from 2 to 3 meter-deep water to the water surface, and can emerge above the water level.

Water chestnut (*Trapa natans* L. Trapanaceae) is the most widespread native species, present in all three lakes and dominant in Middle and Inferior lakes. Nymphaeids, mainly *Nuphar lutea* (L.) Sm *Nymphaea alba* L., are present in the upstream portion of Superior Lake, as well as in small patches in Middle and Inferior lakes. Submersed species (e.g., *Ceratophyllum demersum* L., *Vallisneria spiralis* L., and *Najas major All.*), predominantly *C. demersum*, are especially present in Superior Lake.

The lakes system is part of the protected Mincio Regional Park, which manages water and macrophytes. The system is characterized by high nutrient and organic matter loads and low water flow rates, resulting in its tendency toward infilling and heightened risk of hypoxia (Pinardi et al., 2011; Bolpagni et al., 2014). For this reason, since 2004, *N. nucifera* and *T. natans* macrophyte stands are controlled by cutting and occasionally harvesting (Pinardi et al., 2011; Villa et al., 2017).

We gathered limnological seasonal data (6 dates per year, ranging from February to November) and meteorological daily data (air temperature, solar radiation, precipitation, and wind velocity), collected in Mantua lakes system by the Environmental Protection Agency of Lombardy region (ARPA Lombardia) during 2016 and 2017. No significant differences in limnological parameters (pH, water temperature, dissolved O_2, total N, and total P) across the three lakes were observed for the two seasons (data not shown).

Photophysiological Measurements

Chlorophyll fluorescence parameters were measured using a chlorophyll fluorometer (PAM-2500, Heinz Walz GmbH, Germany) between 9 and 15 h. Light response curves, i.e., the electron transport rate (ETR) of the photosystem II (PSII), as a function of photosynthetically active radiation (PAR) were measured from mature, healthy-looking leaves after a dark-adapting period of 20 min. After dark adaptation, emitted initial fluorescence yield (F_o) and maximal fluorescence yield (F_m) resulting from a pulse of a saturated light (630 nm, intensity 3000 μmol m^{-2} s^{-1}) were determined. From these, the photochemical PSII efficiency (F_v/F_m), coefficient of photochemical quenching (qP), and coefficient of non-photochemical quenching (qN) were calculated. The measured leaves were exposed to 11 actinic lights (630 nm) for a duration of 15 s, with an intensities between 5 and 787 μmol m^{-2} s^{-1}, and the ETR values were measured after each illumination step with a new pulse of saturated (3000 μmol m^{-2} s^{-1}) light. Exponentially saturating curves (Eilers and Peeters, 1988) were fit to the light response data, and the maximum ETR (ETR_{max}), theoretical saturation light intensity (I_k), and maximum quantum yield for whole chain electron transport (α) were retrieved (Genty et al., 1989).

Pigment Analysis

Leaf discs of 0.6 cm in diameter were collected from intact leaves close to where chlorophyll fluorescence was measured, and stored in aluminum foil in sub 0 temperature until their transfer to a −20°C freezer. Frozen leaf discs samples were homogenized in liquid N_2, then extracted in 80% acetone. The extracts were centrifuged and the supernatant collected and stored at −20°C until analyses. The absorbance spectra (400–750 nm) of extracts were measured using a spectrophotometer (Shimadzu UV-2401PC, dual-beam), and pigment concentrations, i.e., chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), and total carotenoids (Car) were calculated using empirical formulae (Wellburn, 1994) and reported as fresh weight concentrations ($\mu g \, g_{fw}{}^{-1}$).

Spectroradiometric Measurements

Leaf reflectance of the same leaves sampled for photophysiological measurements was measured over the visible to shortwave infrared spectral range (350–2500 nm) using a portable SR-3500 Full Range spectroradiometer (Spectral Evolution, Lawrence, United States). Following a 20-min dark adaptation period, leaf-reflected radiance under near-steady state conditions (60 s after leaf de-shadowing) was measured using a Reflectance Contact Probe (Spectral Evolution, Lawrence, United States). The probe was equipped with a light source (5-watt tungsten halogen bulb) and spectra were calibrated to leaf reflectance using measurements of a 99% Spectralon panel (Labsphere, North Sutton, United States). During measurements, macrophyte leaves were placed over a flat neoprene plate (absorbance > 95%) in order to minimize background reflection of light transmitted through the leaves. This approach was chosen because it is easier and faster under challenging field conditions (e.g., boat-based surveys) compared with using an integrating sphere to fully determine reflectance and transmittance, and only introduces minimal distortion in leaf reflectance measurements (Sims and Gamon, 2002; Potůčková et al., 2016).

Leaf reflectance spectra were used to calculate a set of spectral indices (SIs), belonging to three different groups: (1) SIs sensitive to photosynthetic pigments (Gitelson et al., 2001, 2003; Sims and Gamon, 2002); (2) SIs connected to radiation use efficiency (RUE) and the state of the xanthophyll cycle pigments (Gamon et al., 1992; Wu et al., 2010; Garrity et al., 2011; Hernández-Clemente et al., 2011); and (3) SIs correlated to macrophyte, *Phragmites australis* (common reed), physiological parameters (Stratoulias et al., 2015). The SIs tested in this study, with their formulas and relevant references are provided in **Supplementary Table S1**.

In order to focus on the most relevant leaf spectral features, the collinearity of all SIs tested was assessed by computing the correlation coefficient (Pearson's r) of each SI pair (**Supplementary Figure S1**). Only the SIs carrying the most information were retained for further analysis and discussion, by excluding the ones with $r > 0.9$.

Summary of *in situ* Measurements

Leaf samples were measured in the Mantua lakes system in late July, 2016 – photophysiological (n = 51) and spectroradiometric measurements (n = 45) – late May, 2017 – photophysiological (n = 67), pigments (n = 45) and spectroradiometric measurements (n = 42) – and late July, 2017 – photophysiological (n = 71), pigment (n = 71) and spectroradiometric measurements (n = 68) – covering different years and growing stages. For each period, slightly different sets of sampling sites were visited, due to logistic and technical constraints. A summary of sampled sites and number of samples collected is provided in **Supplementary Table S2**. Leaves were collected from plants located within 2–3 m of the water edge of homogenous, dense, intact macrophyte stands. For *in situ* measurements, from each plant the youngest, mature, intact leaf was chosen. At each sampling site, 3 to 12 plants per species were selected (**Supplementary Table S2**). Following dark adaptation, whole leaves (for *Ludwigia* and *Trapa*) or leaf parts (for *Nelumbo*, *Nuphar,* and *Nymphaea*) were removed from the plants and subjected to measurements. Chlorophyll fluorescence measurements were performed on the dark-adapted part of the leaves, with leaf spectroradiometric reflectance recorded in its close vicinity. In order to avoid possible bias due to light environment differences, only leaves exposed directly to sunlight were measured.

Seasonal Dynamics

Additional information highlighting key macrophyte phenological characteristics were extracted from macrophyte leaf area index (LAI) seasonal dynamics maps. Time series of LAI for floating and emergent macrophytes in the Mantua lakes system were derived from medium resolution satellite data (Landsat 8, SPOT5, and Sentinel-2) for the 2015 growing season (Villa et al., 2018). From these time series, seasonal dynamics metrics were computed, namely: the day of the start of season (SoS), the day of the peak of season (PoS), the day of the end of season (EoS), the duration of the growing season (Length), the maximum LAI value (LAI_{max}), the rate of increase of LAI during the early growth (Growth rate), and the rate of decrease of LAI during the senescence (Senescence rate).

We calculated the mean and standard deviation scores of these seasonality metrics of all pixels dominated by each macrophyte species considered in this work, as well as the area covered by each species and the corresponding percentage of total macrophyte cover. *Nymphaea* pixels are grouped together with *Nuphar* pixels, because they only cover a very small area (< 1 ha).

Statistical Analyses

Graphing, curve fitting, and statistics were performed in Past (Hammer et al., 2001) and various packages in R v.3.4.4, i.e., stats 3.5.2, ggplot2 3.1.0, psych 1.8.12 (R Development Core Team, 2012). Due to non-normality of sample distributions, differences among species in terms of investigated parameters were tested using Kruskal-Wallis One Way Analysis of Variance on Ranks, followed by pairwise multiple comparison procedures (Dunn's Method). For each species, Mann–Whitney U test was used for testing the differences of investigated parameters between July 2016 and July 2017, as well as between May 2017 and July 2017 conditions. Only relevant information, i.e., rejecting the hypothesis of equal distribution among compared samples at confidence level higher than 5% ($P < 0.05$), is reported in the following section, describing the main results.

RESULTS

Photophysiological Measurements

Clear differences in the photophysiological properties of the studied plants and in the photosynthetic activity of *Ludwigia* were observed (**Supplementary Figure S2**; **Figures 2B,C**). Not only did the extent of ETR_{max} and I_k differ significantly for

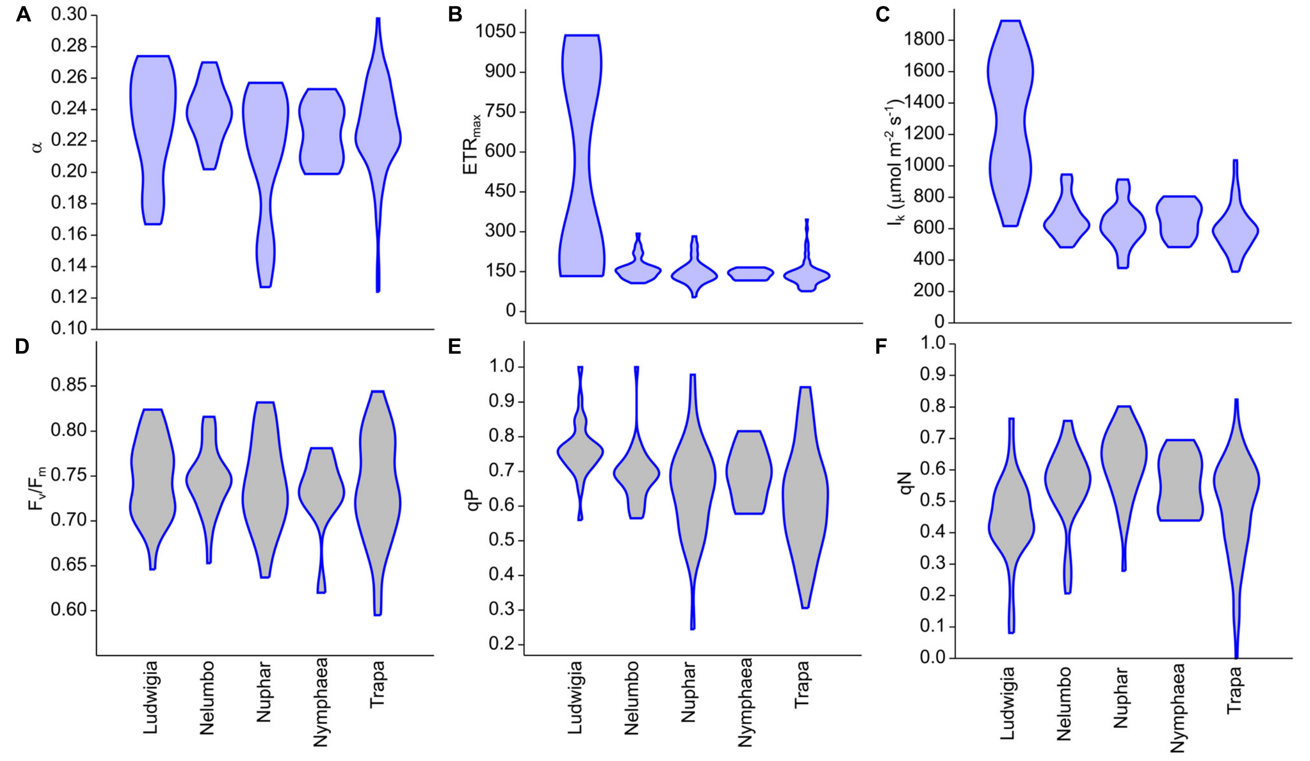

FIGURE 2 | Chlorophyll fluorescence parameters measured from Mantua lakes system macrophytes. **(A)** maximum quantum yield for whole chain electron transport (α), **(B)** maximal electron transport rate (ETR$_{max}$), **(C)** theoretical saturation light intensity (I$_k$), **(D)** PSII photochemical efficiency (F$_v$/F$_m$), **(E)** coefficient of photochemical quenching (qP), and **(F)** coefficient of non-photochemical quenching (qN).

Ludwigia with respect to the rest of the species (Kruskal-Wallis One Way Analysis of Variance on Ranks; $H = 57.2$; $P < 0.001$; $n = 182$), but their distributions were bimodal, suggesting the presence of two subgroups (**Figure 2**). These separate *Ludwigia* subgroups can partially be explained by the marked differences between 2016 and 2017 samples: ETR$_{max}$ scores for *Ludwigia* are in fact significantly lower in July 2016 than in July 2017, 162.2 ± 15.9 and 743.4 ± 290.2 μmol m^{-2} s^{-1} respectively (Mann–Whitney *U* test; $U = 0$; $P < 0.001$; $n_1 = n_2 = 9$). Due to this difference in ETR$_{max}$ and in I$_k$, the position of *Ludwigia* in the photophysiological PCA was well defined (**Figure 3**).

The photophysiological properties of *Nelumbo* did not differ from those of the local macrophytes, therefore *Nelumbo* aligned with the rest of the species present within the PCA. Further analysis of the photophysiological parameters of the studied macrophytes demonstrated the higher plasticity of *Ludwigia*, *Trapa*, and *Nuphar* compared with *Nymphaea* and *Nelumbo* (**Table 1**).

Pigment Analysis

Chl-a, Chl-b, and Car content of *Nelumbo* and *Ludwigia* on weight basis were significantly higher than those of other species (**Figures 4A–C**; Kruskal-Wallis One Way Analysis of Variance on Ranks; $P < 0.001$). *Ludwigia* Chl-a content was higher in May than in July, 2001 ± 258 compared with 1591 ± 313 μg g$_{fw}$$^{-1}$ (Mann–Whitney *U* test; $U = 11$; $P = 0.01$; $n_1 = n_2 = 9$). Nevertheless, despite the higher pigment content - *Ludwigia* and *Nelumbo* had, on average, 2.8, 2.7, and 2.4 times higher Chl-a, Chl-b, and Car content than *Nymphaea*, *Nuphar*, and *Trapa*, respectively, the Chl-a/ Chl-b ratio (a/b) of *Ludwigia* alone differed significantly ($P < 0.05$) from that of *Nelumbo*, *Nuphar*, and *Nymphaea*, and was similar to that of *Trapa* (**Figure 4D**). The total chlorophyll (Chl-a + Chl-b) to Car ratio (chl/car) of *Nymphaea* was significantly lower (2.18 ± 0.05) than the rest of the species (3.56 ± 0.05). Due to the significant difference of *Ludwigia* and *Nelumbo* from native species in leaf pigments content, their discrimination was clearly defined through PCA (**Figure 5**). Further analysis of pigment content results showed high phenotypic plasticity of Chl-a, Chl-b, and Car content for both *Ludwigia* and *Nelumbo*, although pigment stoichiometry was also variable for *Nuphar* and *Trapa* (**Table 1**).

Spectroradiometric Measurements

After assessing the cross-correlation of tested SIs for the sampled species (**Supplementary Figure S1**), some indices were excluded from further analyses. The list of SIs tested, with their full names and relevant references are included in **Supplementary Table S1**. Revised Photochemical Reflectance Index 2 (PRI$_{r2}$) and Photochemical Reflectance Index combination with Chlorophyll Index (PRI*CI) were excluded because of their high correlation with Photochemical Reflectance Index (PRI; $r = 0.94$ and $r = 0.95$, respectively, **Figures 6**, **7**). Normalized Difference

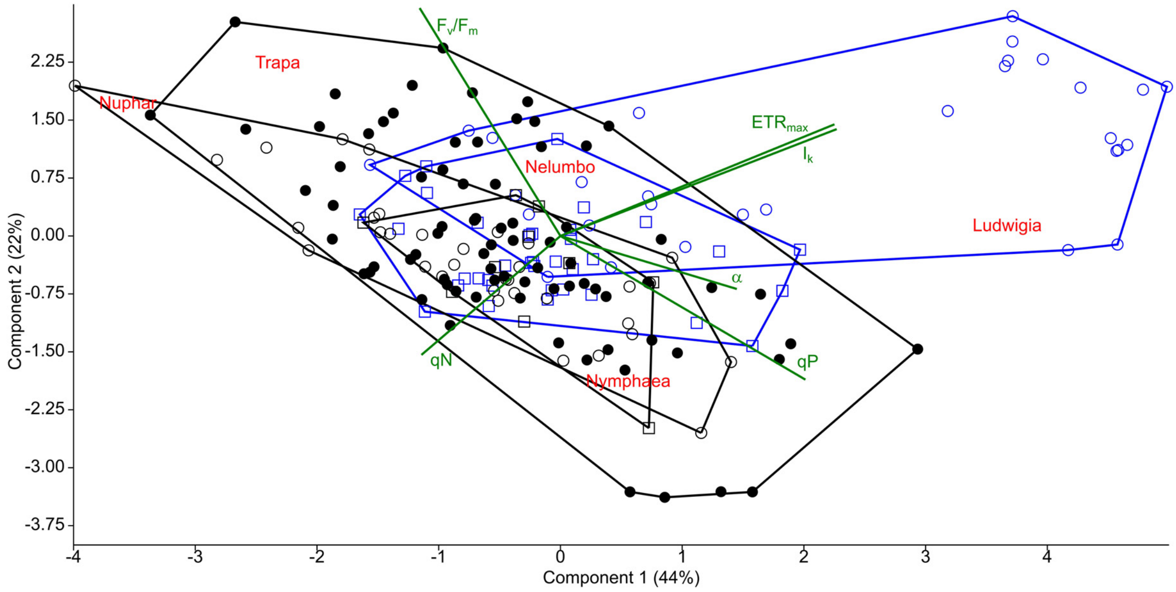

FIGURE 3 | Principal component analysis (PCA) of photophysiological parameters measured from macrophytes of the Mantua lakes system. α, maximum quantum yield for whole chain electron transport; ETR_{max}, maximal electron transport rate; I_k, theoretical saturation light intensity; F_v/F_m, PSII photochemical efficiency; qP, coefficient of photochemical quenching; and qN, coefficient of non-photochemical quenching.

TABLE 1 | Coefficient of variation of the standardized pigment and photophysiological data of Mantua lakes system macrophytes.

Species	chl-a	chl-b	car	a/b	chl/car	α	ETR_{max}	I_k	F_v/F_m	qN	qP
Ludwigia	0.26	0.46	0.18	0.84	0.35	1.14	3.11	1.41	0.75	0.83	0.36
Nelumbo	0.41	0.40	0.33	0.31	0.24	0.34	0.04	0.15	0.53	0.70	0.35
Nuphar	0.05	0.09	0.08	1.08	0.22	1.66	0.05	0.20	1.03	0.57	1.10
Nymphaea	0.02	0.03	0.05	0.41	0.08	0.38	0.01	0.12	0.78	0.40	0.37
Trapa	0.04	0.07	0.04	0.75	0.56	0.93	0.05	0.21	1.35	1.11	1.28

chl-a, chlorophyll a content; chl-b, chlorophyll b content; car, total carotenoids content; a/b, chlorophyll a to chlorophyll b ratio; chl/car, chlorophylls to carotenoids ratio; α, maximum quantum yield for whole chain electron transport; ETR_{max}*, maximal electron transport rate;* I_k*, theoretical saturation light intensity;* F_v/F_m*, PSII photochemical efficiency; qN, coefficient of non-photochemical quenching; and qP, coefficient of photochemical quenching.*

Spectral Index 539,560 ($ND_{539,560}$) was also not considered further due to its high correlation with PRI ($r = 0.92$). Within the macrophyte eco-physiology sensitive SIs, Normalized Difference Spectral Index 659,687 ($ND_{659,687}$) and Normalized Difference Spectral Index 621,692 ($ND_{621,692}$) were found to be highly correlated ($r = 0.91$), so we therefore only retained the latter for further analysis. Finally, we excluded Normalized Difference 750/705 (ND_{705}) due to its high correlation with modified Normalized Difference 750/705 (mND_{705}; $r = 0.84$), and Chlorophyll Index (CI) due to its lower sensitivity to leaf chlorophyll content compared to mND_{705} ($r = 0.41$–0.42 and $r = 0.77$–0.78 respectively).

Within our dataset, there is a significant difference in PRI between the species (Kruskal-Wallis One Way Analysis of Variance on Ranks; $H = 45.8$; $P < 0.001$; $n = 152$). PRI of *Nelumbo* samples was higher (Dunn's Multiple Comparison test, $P < 0.05$) than that of *Trapa*, *Nuphar*, and *Nymphaea*, while *Ludwigia* was not found to be significantly different, although on average PRI was lower. Photochemical Reflectance Index 515 (PRI_{515}) differs from PRI especially for the allochthonous species, and in particular for *Nelumbo*, which shows significantly lower PRI_{515} scores compared to the autochthonous species (Dunn's Multiple Comparison test, $P < 0.05$).

Nelumbo is statistically different from all the other species (Dunn's Multiple Comparison test; $P < 0.05$) in terms of Normalized Difference Spectral Index 546,551 ($ND_{546,551}$), and is different from the autochthonous species (Dunn's Multiple Comparison test; $P < 0.05$) in terms of $ND_{621,692}$. Even though not statistically significant, $ND_{621,692}$ scores for *Ludwigia* are also slightly lower than those of the autochthonous species (0.055 ± 0.021 and 0.076 ± 0.034, respectively, in July 2017).

Anthocyanin reflectance index 2 (ARI_2), which is related to secondary pigment contents, showed no difference between the species (Kruskal-Wallis One Way Analysis of Variance on Ranks; $H = 6.748$; $P = 0.150$; $n = 152$). Rather, significant differences between the species are observed for mND_{705}, related to total chlorophyll content (Kruskal-Wallis One Way Analysis of Variance on Ranks; $H = 31.5$; $P < 0.001$; $n = 152$), with *Nelumbo* showing higher values compared to any other species (Dunn's Multiple Comparison test, $P < 0.05$).

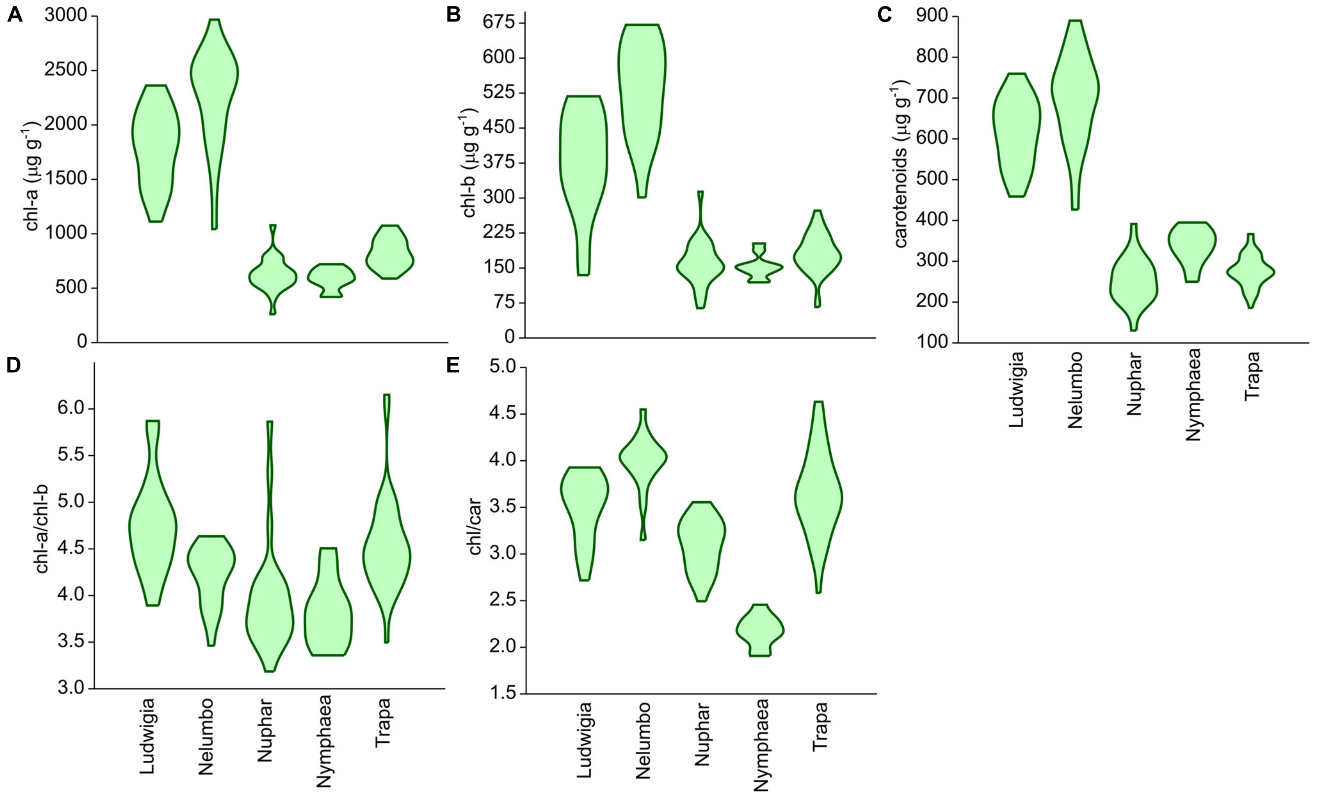

FIGURE 4 | Leaf pigment contents of macrophytes of the Mantua lakes system, reported as a function of fresh weight. **(A)** chlorophyll a content (chl-a; $\mu g\ g_{fw}{}^{-1}$), **(B)** chlorophyll b content (chl-b; $\mu g\ g_{fw}{}^{-1}$), **(C)** total carotenoids content (carotenoids; $\mu g\ g_{fw}{}^{-1}$), **(D)** chlorophyll a to chlorophyll b ratio (chl-a/chl-b), **(E)** chlorophylls to carotenoids ratio (chl/car).

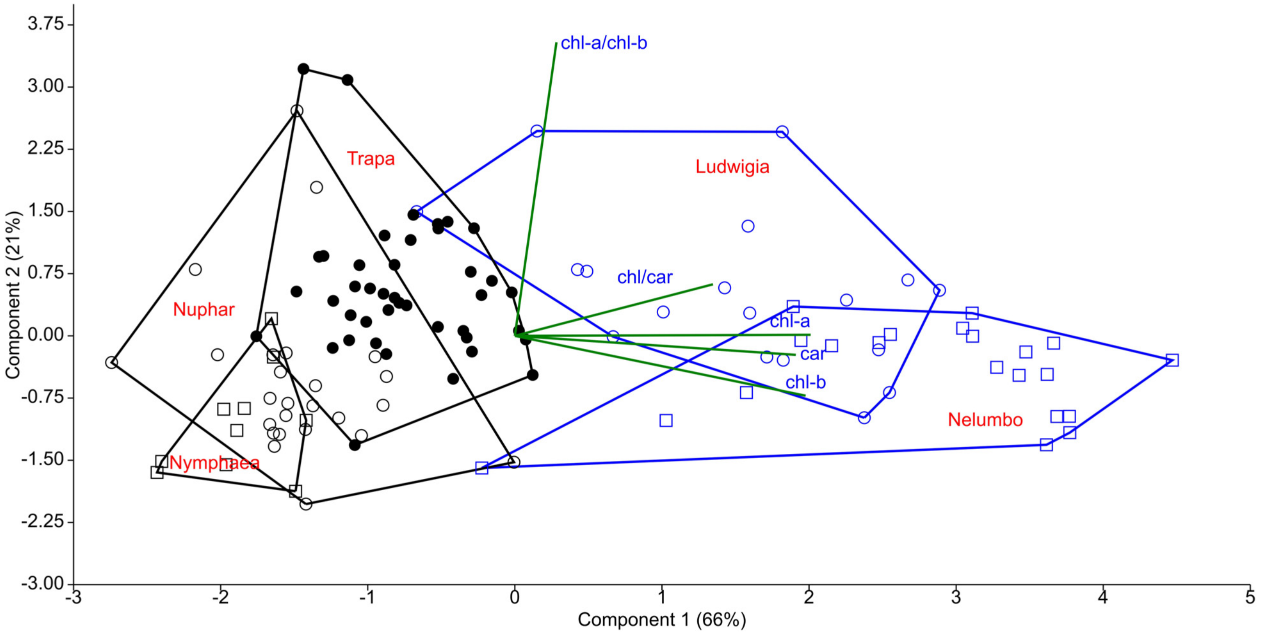

FIGURE 5 | Principal component analysis (PCA) of leaf pigment content of macrophytes of the Mantua lakes system. chl-a, chlorophyll a content; chl-b, chlorophyll b content; car, total carotenoids content; chl-a/chl-b, chlorophyll a to chlorophyll b ratio; and chl/car, chlorophylls to carotenoids ratio.

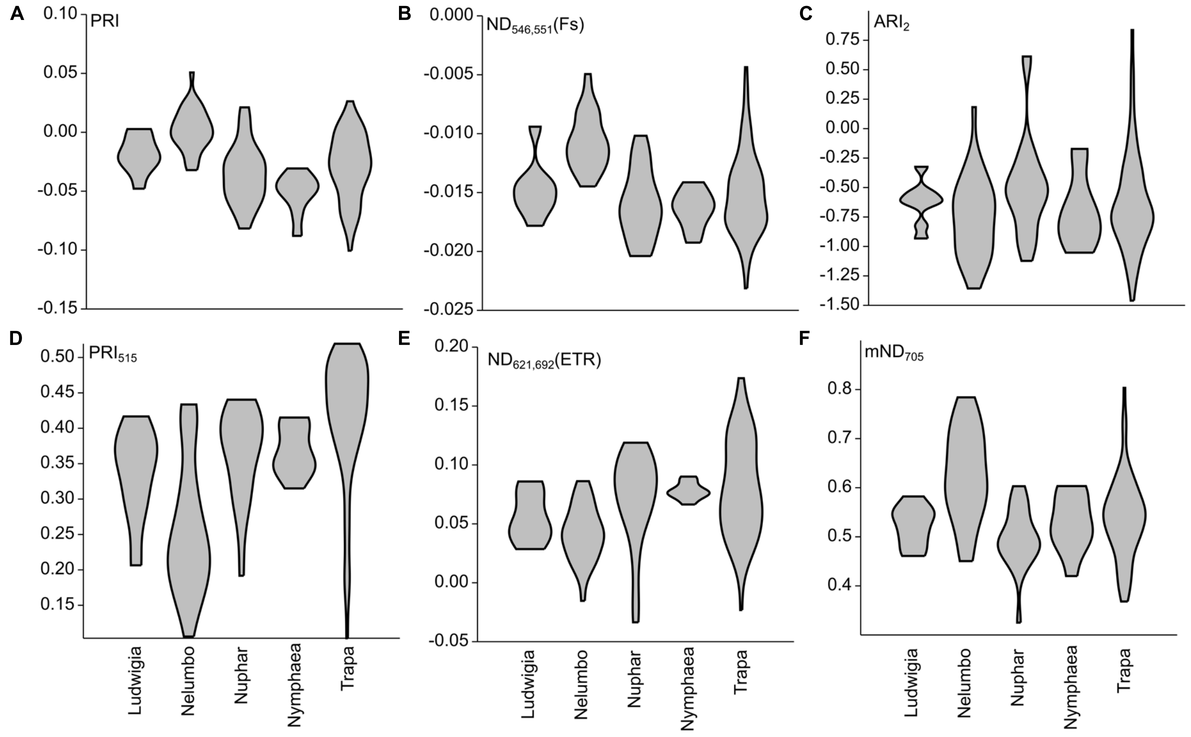

FIGURE 6 | Selected SIs calculated from Mantua lakes system macrophyte leaf reflectance data of. **(A)** Photochemical reflectance index (PRI); **(B)** Normalized difference spectral index 546,551 ($ND_{546,551}$); **(C)** Anthocyanin reflectance index 2 (ARI_2); **(D)** Photochemical reflectance index 515 (PRI_{515}); **(E)** Normalized difference spectral index 621,692 ($ND_{621,692}$); and **(F)** modified normalized difference 750/705 (mND_{705}).

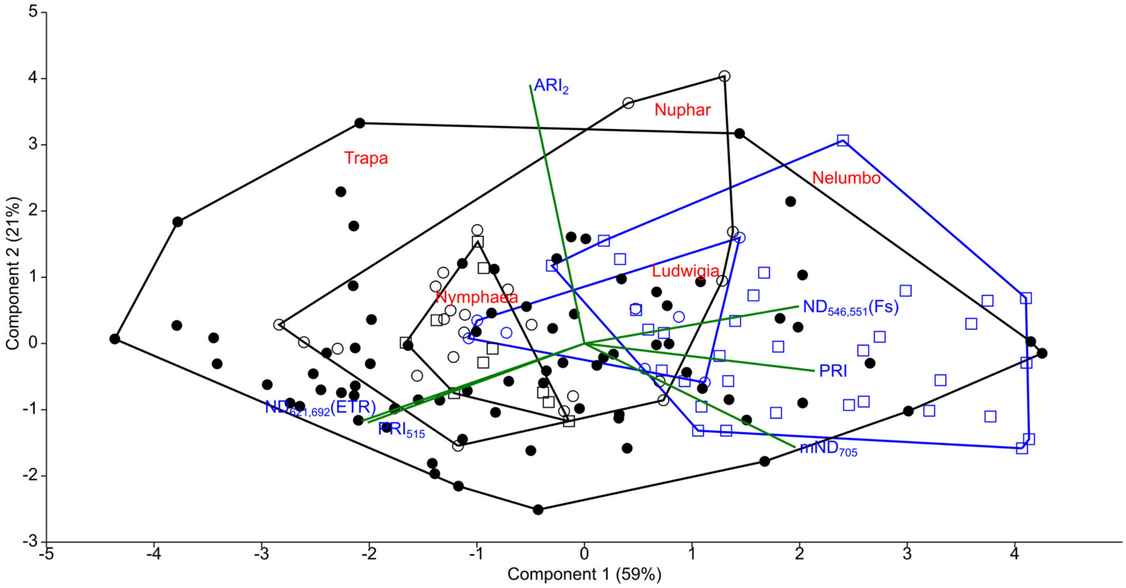

FIGURE 7 | Principal component analysis (PCA) of selected SIs calculated from Mantua lakes system macrophyte leaf reflectance data. PRI, photochemical reflectance index; $ND_{546,551}$, normalized difference spectral index 546,551; ARI_2, anthocyanin reflectance index 2; PRI_{515}, photochemical reflectance index 515; Normalized $ND_{621,692}$, difference spectral index 621,692; and mND_{705}, modified normalized difference 750/705.

Seasonal Dynamics Features

Differences between allochthonous and autochthonous species, in terms of their seasonal dynamics, or phenology metrics – i.e., day of the start (SoS), peak (PoS), and end of season (EoS), as well as the length of growing season – are highlighted in **Table 2**, referring to 2015 growing season. Peak LAI values (LAI_{max}) and LAI growth and senescence rates (LAI_{growth}, $LAI_{senescence}$) were also calculated.

The growing season of allochthonous species was found to be significantly longer than that of native species: 192 ± 15 days for *Ludwigia* (Mann–Whitney *U* test; $U = 12853$; $P < 0.001$; $n_1 = 58$, $n_2 = 2249$), and 172 ± 5 days for *Nelumbo* (Mann–Whitney *U* test; $U = 128812$; $P < 0.001$; $n_1 = 2024$, $n_2 = 2249$). Such differences in season length is mainly driven by prolonged viability of allochthonous macrophytes during autumn months, lasting over water until late October to mid-November. *Nelumbo* also shows morphological advantages, with maximal leaf area index (LAI_{max}) reaching 1.7 m^2 m^{-2} at PoS, significantly higher that of all the other species, reaching 1.0–1.2 m^2 m^{-2} (Mann–Whitney *U* test; $U = 280047$; $P < 0.001$; $n_1 = 2024$, $n_2 = 2307$), as well as fast early season growth, with LAI_{growth} – i.e., the rate of LAI growth from SoS to PoS – higher by 2–3 times compare to that of the other species (Mann–Whitney *U* test; $U = 438004$; $P < 0.001$; $n_1 = 2024$, $n_2 = 2307$).

DISCUSSION

The background and dynamics of invasion processes are still a matter of debate. Nevertheless, studies focusing on morphological and physiological characteristics of native and invasive plants can elucidate the success of the latter (Baruch and Goldstein, 1999; Hufbauer and Torchin, 2008; Van Kleunen et al., 2010). While a given explanation of how morphological and physiological parameters can promote the spread of non-native species might only be applicable to the ecosystem in question, some of the observations could be considered to be more generally valid. For example, higher photosynthetic rates of invasive plants could result in higher biomass accumulation, consequently exhausting resources available for native species and/or shading out native competitors (Baruch and Goldstein, 1999; Hufbauer and Torchin, 2008). To our first hypothesis, we have a partial answer: of all the species investigated, *Ludwigia* had the highest photosynthetic parameters (ETR_{max}, I_k), which is in line with its C_4 nature and high photosynthesis rates (Dandelot et al., 2005; Hussner, 2009), although this does not always translate into higher biomass (Hussner, 2009). It is worth noting that in the Mantua lakes system, *Ludwigia* samples showed a strong response to environmental changes. One of the explanations of the outstanding bimodality of ETR_{max} and I_k for this species could be the inter-seasonal differences between 2016 and 2017, linked to differences in meteorological conditions accumulated up to late July for the 2 years. When compared to 2016, 2017 was much drier (total precipitation 52% lower), hotter (average max temperature higher by 0.9°C, or 4.6%), and with higher incoming radiation (by 5.9%), as shown in **Supplementary Figure S3**. Nevertheless, intra-seasonal differences also reveal that other factors (e.g., water and sediment conditions, prevalence of growth form) should be taken into account to explain *Ludwigia* ecotype specialization (Thouvenot et al., 2013; Gérard et al., 2014). Interestingly, other *Ludwigia* photophysiological parameters (α, F_v/F_m, and qP) did not differ significantly from those of other species; only its qN was slightly lower than the other macrophytes, highlighting the higher efficiency of *Ludwigia* in dissipating excitation energy through photosynthesis.

The photophysiological response of *Ludwigia* to the anomalous meteorological conditions of 2017 can be compared to those of the most-abundant native species in Mantua lakes system: *Trapa*. Both species showed consistent responses to such environmental conditions, in terms of F_v/F_m and qP, with a tendency toward slightly lower scores in 2017 than in 2016. Inversely, the two species reacted differently to the dry and hot weather of 2017, in terms of non-photochemical quenching (qN). Compared to July 2016, *Trapa* samples measured in July 2017 tend to have higher qN, while *Ludwigia* had lower levels of non-photochemical quenching in 2017 than in 2016. The reaction of *Ludwigia* to anomalously dry and hot conditions could in part explain the competitive success and invasiveness of water primrose in temperate areas of Europe.

On the other hand, absolute photosynthesis and biomass accumulation values are not the only features underlying the

TABLE 2 | Synoptic metrics of seasonal dynamics (phenology and LAI growth) for Mantua lakes system macrophytes for 2015, derived from Villa et al. (2018).

	Ludwigia	*Nelumbo*	*Nuphar-Nymphaea*	*Trapa*
SoS (DOY – Julian day)	122.5 ± 6.4	132.4 ± 3.9	115.1 ± 8.0	155.2 ± 16.6
PoS (DOY – Julian day)	222.3 ± 6.9	218.6 ± 4.0	200.6 ± 13.3	215.8 ± 13.0
EoS (DOY – Julian day)	314.8 ± 13.2	304.4 ± 3.9	263.1 ± 20.4	256.7 ± 13.2
Length (days)	192.3 ± 14.8	172.0 ± 5.2	147.9 ± 28.1	101.5 ± 9.7
LAI_{max} (m^2 m^{-2})	1.15 ± 0.28	1.69 ± 0.05	1.06 ± 0.18	1.28 ± 0.13
Growth rate (m^2 m^{-2} d^{-1})	0.018 ± 0.007	0.047 ± 0.008	0.015 ± 0.005	0.023 ± 0.004
Senescence rate (m^2 m^{-2} d^{-1})	0.013 ± 0.004	0.026 ± 0.004	0.018 ± 0.006	0.045 ± 0.018
Area (ha)	2	69	9	74
% cover of total macrophytes	1.3	44.8	5.8	48.1

SoS, day of the start of season; PoS, day of the peak of season; EoS, day of the end of season; length, duration of the growing season (EoS-SoS); LAI_{max}, maximum LAI value; growth rate, rate of increase of LAI during the early growth; and senescence rate, rate of decrease of LAI during the senescence.

success of invasive species. The ability to survive, and even to thrive, in very different environmental conditions could be part of invasive species' advantage, allowing their spread in areas where they have been introduced. We also hypothesized that invasive plant will have higher phenotypic plasticity. Again, when we consider the variability of *Ludwigia*, we found this species to be distinctive, in that its coefficients of variation for ETR_{max} and I_k were at least 62 and 7 times higher than any other species, respectively (**Table 1**). This extreme plasticity could help *Ludwigia* to survive in highly variable conditions encountered in both its native range and in areas to which it is introduced. The other species showing high photophysiological variability was *Trapa* (in terms of F_v/F_m, qP, qN), showing its ecological advantage in the Mantua lakes system and explaining its spread in the study area (i.e., nearly half of the total macrophyte-covered surface). The combination of the above make it evident that, in the end, these traits of *Ludwigia* or *Trapa* would largely determine the structure and diversity of the Mantua lakes system macrophyte communities. The results also showed that higher plasticity in a community could be an important determinant of the diversity of a given species. This plasticity helps the species to overcome environmental factors that might otherwise limit its spread in the given environment (Lankau, 2011; Andersen et al., 2012). Not only does the lower mortality at population level undoubtedly result in either higher density and coverage or spatial spreading, but the high variability of functional traits is considered to be an advantage for species establishment and survival (Levine and HilleRisLambers, 2009; Lankau, 2011).

We also hypothesized that leaf pigment pools of invasive plants are different in size and composition from native ones. Another key feature facilitating invasive plants in their new environments is the lack of top-down pressure (specific herbivores), and thus the absence of structural defense biomass (Feng et al., 2009; Rout and Callaway, 2009). This results in the preferential allocation of N from structural biomass into leaf tissues and photosystems in invasive plants, consequently increasing the non-structural, photosynthetically active biomass. Our results support this theory, since both *Ludwigia* and *Nelumbo* had significantly higher pigment content on weight basis (by at least 2–3 times) compared to the native species. The intensification of photosynthetically active biomass helped the plants to further increase the resources available to them, and consequently to extend their presence in their new areas. Besides having the highest absolute values, *Nelumbo* and *Ludwigia* also showed the most variable pigment content, suggesting that these invasive species could adapt to very different environmental conditions. The high pigment content (especially total chlorophyll) of the leaf tissues also affected the specific reflectance of the invasive species and thus the SIs.

PRI was developed and its effectiveness as a proxy for RUE (Gamon et al., 1992; Garbulsky et al., 2011) was demonstrated. Recent works and meta-analysis have shown that PRI also correlates to the seasonal dynamics of pigments pools, and in particular to the ratio of chlorophylls to carotenoids (Filella et al., 2009; Gitelson et al., 2017), and to the daily variability of photosynthetic rates (Filella et al., 2009; Gamon et al., 2015). The PRI literature mainly focuses on terrestrial plants and crops (Garbulsky et al., 2011), and not much is known on its ranges and sensitivity for aquatic plants. Our results suggest that, among the macrophyte species considered, the invasive *Nelumbo* might have a higher RUE compared to the native species considered (**Figure 6A**). This is possibly due to its higher chl/car ratio (**Figure 4E**). PRI_{515}, a modification of the original PRI concept that is inversely related to total carotenoid content in terrestrial plants (Hernández-Clemente et al., 2011), shows some notable differences from PRI for allochthonous macrophytes. *Nelumbo* in particular has significantly lower PRI_{515} scores, as well as higher pigment (chlorophylls and total carotenoids; **Figures 4A–C**) contents, compared to other species.

Although originally developed for common reed, SIs sensitive to physiological parameters of aquatic plants (Stratoulias et al., 2015) confirm the uniqueness of *Nelumbo* compared to the other species, reinforcing what was already observed for PRI. This species is statistically different from all the others in terms of $ND_{546,551}$, indicating higher chlorophyll fluorescence levels in light-adapted state, and from all the native species in terms of $ND_{621,692}$, which can be attributed to lower values of instantaneous ETR. Even if not statistically significant at the 95% confidence level, *Ludwigia* $ND_{621,692}$ scores are on average slightly lower than those of native species. Additionally, *Nelumbo* mND_{705} scores, related to leaf chlorophyll content in terrestrial plants (Sims and Gamon, 2002), but not yet tested on aquatic vegetation, are significantly higher than any of the other macrophytes. This is similar to the leaf pigment measurements made on our samples (**Figures 4A–C**).

Our results suggest that mND_{705} could be effectively used as a proxy for total chlorophyll content for macrophytes ($R^2 = 0.59$), but estimation error might vary depending on species. In fact, the relationship between mND_{705} and pigment contents for sampled macrophytes seems to be different for autochthonous and allochthonous species (**Supplementary Figures S4, S5**).

It was hypothesized that invasive macrophytes can effectively exploit temporal niches left unoccupied by the seasonal dynamics of native species. Overall, the observed photophysiological and spectral reflectance parameter features point to some notable difference in photosynthetic performance of autochthonous and allochthonous species, either in terms of adaptation to anomalous environmental conditions and maximum photosynthetic rates, in the case of *Ludwigia*, or pigment pool size and higher RUE for *Nelumbo*. In addition, seasonal dynamic features (**Table 2**) show that both *Nelumbo* and *Ludwigia* have marked differences in season length with respect to *Trapa* and Nymphaeids, with the end of season delayed by approximately 40–60 days (i.e., until November), which arguably constitutes an advantage in terms of productivity.

For *Nelumbo*, this might be related to peculiarities in circadian clock family genes, making it easy for this species to adapt to a wide range of climates and day length regimes, with prolonged flowering times (Ming et al., 2013). In addition, morphological and structural traits (bigger leaves, emerging above water in overlapping layers; average $LAI_{max} = 1.69\ m^2\ m^{-2}$) and fast dynamics during the early vegetative phase (May–June) – with $LAI_{growth} = 0.047\ m^2\ m^{-2}\ dd^{-1}$ – provide an advantage for *Nelumbo* in the environmental conditions of the Mantua lakes system, and contribute to the success of this species, now established here for approximately a century.

The notion that invasive species tend to show higher scores of functional traits, which emerged in a recent meta-analysis (Van Kleunen et al., 2010), was generally confirmed by our results for the macrophyte species investigated, especially when dealing with traits related to physiology (e.g., leaf pigment contents, ETR_{max}, RUE), size (LAI_{max}), growth rate (LAI_{growth}), and fitness (phenology timing, growing season length). *Ludwigia* and *Nelumbo*, though, have been shown to outperform autochthonous plants in different group of traits, with the former having a greater advantage in terms of physiology-related traits, and the latter in terms of size and growth rate-related traits.

In summary, marked differences of *Ludwigia* and *Nelumbo* compared to native macrophytes, in terms of pigment contents (Chl-a, Chl-b, Car), growing season length, and end of season timing were observed. Parallel to this, compared to all other species, *Ludwigia* had the highest photophysiological parameters (ETR_{max}, I_k). Differences were detected in *Nelumbo* compared to all other species, in terms of spectral reflectance features (i.e., PRI, PRI_{515}, $ND_{546,551}$, and mND_{705}, connected to higher RUE and pigment pool size), as well as morphological and growth rate features. During the anomalously hot and dry 2017 season, invasive species were found to react differently from native species, as well as between themselves. *Ludwigia* performs differently in terms of α, qN, and $ND_{546,551}$, linked to the variability in photosynthetic efficiency, while *Nelumbo* performs differently in terms of PRI_{515} and mND_{705}, mainly related to pigment contents and composition. As these parameters are good indicators of enhanced productivity, such an outcome suggests that an increase in temperature, as for current climate change projections, may further favor the spread of invasive species in temperate areas.

CONCLUSION

Solar radiation is an essential resource for plants, and whichever species gain ascendancy over its competitors could be dominant. We found that the success of an invasive macrophyte, in terms of persisting and propagating in its new ecosystem, may be the result of multiple ecological strategies employed. The specificities of the new host areas (such as the lack of top-down pressure, lack of pathogens, etc.,) reduce defense costs, thus liberating resources to extend and intensify photosynthesis. This, coupled with their better ability to compete for resources and to tolerate harsh conditions, consequently improves the chances of survival of non-native species. *Ludwigia hexapetala* and *Nelumbo nucifera* have been documented to establish, spread, and alter the Mantua lakes system. Our data show that specific performance-based traits of the invasive macrophytes, photophysiological efficiency, pigment pool size and balance, and leaf spectral reflectance specifically, can describe and explain the success of these species over native ones in the same environment, in terms of both resource competitiveness and tolerance to variability in environmental conditions.

AUTHOR CONTRIBUTIONS

VT and PV designed the concept of the study, organized the databases, performed the visualization of data and statistical analysis, and wrote the first draft of the manuscript. All authors performed the measurements shown in the study, wrote parts of the manuscript, and contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.00647/full#supplementary-material

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ORIGINAL RESEARCH
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Does Soil Nutrient Heterogeneity Improve the Growth Performance and Intraspecific Competition of the Invasive Plant *Myriophyllum aquaticum*?

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Spatial heterogeneity in soil nutrient availability is considered to play an important role in promoting plant invasion success and can affect interspecific competition. Although some clonal plants have been demonstrated to be correlated with resource heterogeneity in terrestrial systems, little is known about how soil nutrient heterogeneity affects the growth of invasive aquatic plants or their population structure. A greenhouse experiment was therefore conducted to study the response of the invasive aquatic plant *Myriophyllum aquaticum* to the spatial heterogeneity of soil nutrients under three plant densities (one, four, or twelve plants 0.28 m^2) with a constant amount of soil nutrients. The results showed that soil nutrient heterogeneity significantly increased the number of shoots in the single-plant density treatment. However, heterogeneous soil nutrient treatment significantly increased the number of shoots at the expense of total biomass and aboveground biomass in the twelve-plant density treatment. The heterogeneous soil nutrient treatment had low effects on other growth traits and intraspecific competition under different plant density treatments. These results indicate that spatial heterogeneity in soil nutrient availability may facilitate the spread of *M. aquaticum*.

Keywords: soil nutrient heterogeneity, competition, invasive, aquatic plants, *Myriophyllum aquaticum*

INTRODUCTION

Spatial soil heterogeneity is common in natural habitats (Farley and Fitter, 1999; James et al., 2010) and has positive effects on the performance of clonal plants (Day et al., 2003a; Wacker et al., 2008; Zhou et al., 2012; You et al., 2014). Clonal plants respond to resource heterogeneity by concentrating more nutrient-absorbing organs (e.g., roots or ramets) where nutrient levels are relatively high (Wijesinghe et al., 2001; Hodge, 2004; Kroon et al., 2010; Gao et al., 2012). Some studies have shown that soil nutrient heterogeneity increases the growth performance of individual plants or plant populations (Wacker et al., 2008; Zhou et al., 2012; Xue et al., 2016). For example, plant yield can be enhanced through physiological integration in heterogeneous conditions (Wijesinghe and Hutchings, 1997; Hutchings and Wijesinghe, 2008). However, soil nutrient heterogeneity does not always play a positive role in plant growth, and any positive effect may be eliminated if resources become limited (Roiloa and Retuerto, 2006; Zhang and He, 2009; Dong et al., 2015).

Intraspecific competition can affect the growth performance and reproductive values of plant individuals in a population and, as a consequence, the effective structure and size of the population (Heywood, 1986; Hartl and Clark, 1989; Kleunen et al., 2001). For example, intraspecific competition significantly affects the number and size of ramets of *Ranunculus reptans* (Kleunen et al., 2001) and significantly affects the stolon length and dry mass of *Alternanthera philoxeroides* (Zhou et al., 2012).

Soil nutrient heterogeneity can affect the competitive relationship between plants (Ritchie and Olff, 1999; Hutchings et al., 2003; Van et al., 2011; Mommer et al., 2012). The relationship between the degree of nutrient heterogeneity and the intensity of competition changes dynamically (Kume et al., 2006; Maestre and Reynolds, 2007; Van et al., 2011). For example, soil nutrient heterogeneity can increase the intensity of competition (Fransen et al., 2001; Day et al., 2003b) because the nutrient-absorbing organs of neighboring plants would be concentrated in a smaller area of high quality in heterogeneous environments. However, Zhou et al. (2012) and Yu et al. (2018) found that soil nutrient heterogeneity does not increase competition when plants are genetically identical. Another theory is that a significant effect of soil heterogeneity on intraspecific or interspecific competition may be caused by the differences between plants in their ability to concentrate their nutrient-absorbing organs where resource levels are high (Fransen et al., 2001; Bliss et al., 2002; Dong et al., 2015).

Many alien invasive plants have the capacity for clonal growth (Liu et al., 2006). However, the understanding of the responses of invasive clonal plants to soil nutrient heterogeneity remains limited. Thus, to investigate how soil nutrient heterogeneity affects the growth performance and intraspecific competition of invasive clonal plants, we conducted an experiment with the stoloniferous, invasive clonal plant *Myriophyllum aquaticum*. We hypothesized that soil nutrient heterogeneity will significantly increase the growth performance and influence the intraspecific competition intensity of *M. aquaticum*.

MATERIALS AND METHODS

The Species

Myriophyllum aquaticum (Vell.) Verdc., a perennial aquatic clonal plant is widely distributed in tropical and temperate regions such as South America (Sutton, 1985). This species can grow in a broad range of habitats, from semi-moist to semi-submersed conditions (Hussner et al., 2010) and forms dense floating mats by producing creeping stolons over the water surface (Wersal and Madsen, 2011). *M. aquaticum* produces flowers and fruits from April to September (Sutton, 1985). It is increasingly becoming a harmful weed in shallow streams and shallow lakes of North America (Sutton, 1985; Wersal and Madsen, 2011). The *M. aquaticum* plants used in this experiment were collected from natural plant populations at the National Field Station of Freshwater Ecosystem of Liangzi Lake (N 30°05′–30°18′, E 114°21′–114°39′) in Hubei Province, China. All of the collected plant materials were planted in containers (100 cm long × 50 cm wide × 50 cm deep). The containers were filled with clay (TN: 2.77 ± 0.54 $mg.g^{-1}$, TP: 0.79 ± 0.19 $mg.g^{-1}$) and to maintain a moist habitat, 1-cm-deep lake water (TN: 0.6 ± 0.2 mg. L^{-1}; TP: 0.04 ± 0.01 mg. L^{-1}) was maintained above the substrate surface throughout the pre-cultivated period. The plant materials were pre-cultivated in the greenhouse for approximately 2 months before the experiment was set up. On June 20, 2017, we selected 220 morphologically identical plants without shoots. Sixteen plants were randomly selected and dried to determine their initial biomass. The 204 remaining plants (height: approximately 25 cm; initial biomass: mean ± SE, 5.66 ± 0.58 g) were selected for the experiment.

Experimental Design

The experiments used a two-factorial design of plant density treatments and soil treatments. Plants were subjected to three density treatments (one, four, or twelve plants per container) and two soil treatments (homogeneous or heterogeneous; **Figure 1**). Each experimental container was 60 cm in height × 60 cm in diameter. There were six container replicates for each of the six treatments and thus 36 containers in total. The heterogeneous soil treatment was composed of two contrasting patches of equal volumes of clay (total nitrogen content: 2.98 ± 0.64 $mg.g^{-1}$, total phosphorus content: 0.83 ± 0.21 $mg.g^{-1}$, organic material content: 45.11 ± 2.67 $mg.g^{-1}$, mean ± SE) and pure sand. For the homogeneous soil treatment, each container was a mixture of the same total amounts of clay and sand as in the heterogeneous treatments. The total amount of soil nutrients was the same in all treatments.

During the experiment, the experimental units were randomly repositioned every week to avoid the potential effects of environmental heterogeneity (such as light) and 1-cm-deep lake water (TN: 0.6 ± 0.2 mg. L^{-1}; TP: 0.04 ± 0.01 mg. L^{-1}) was

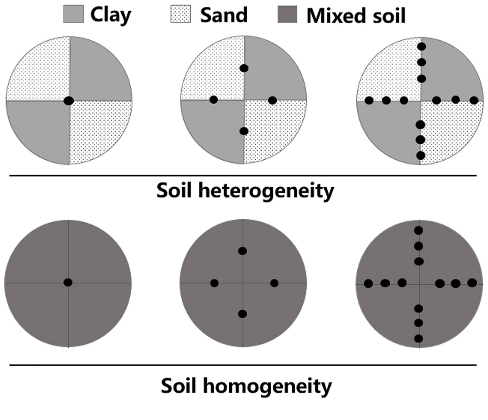

FIGURE 1 | Schematic representation of the experimental design. Soil heterogeneity treatment design: light gray and lightly stippled areas represent high (clay) and low (sand) soil nutrient patches, respectively. Soil homogeneity treatment design: the area in dark gray was filled with an even mixture of the same volume of clay and sand. The total amounts of soil nutrients were thus the same in both treatments. Black dots represent where *M. aquaticum* was planted in three density-level treatments: one, four, or twelve plants per pot.

maintained above the substrate surface. The diurnal variation in temperature, humidity and illumination was recorded each day by a hygrothermograph and an illuminometer in the greenhouse. The mean values of temperature, humidity, and illumination in the greenhouse were 27.58 ± 0.62°C, 64.52 ± 1.22% and 2892.06 ± 321.93 μmol. $m^{-2}.s^{-1}$ (mean ± SE), respectively.

After approximately 90 days of growth, on September 23, 2017, the total number of shoots was recorded, and the total length of shoots and plant height of each plant were measured. Each plant was then divided into aboveground (leaves and stem) and belowground (roots) parts, dried at 70°C for 72 h and weighed. The relative competition intensity (RCI) and log response ratio of biomass (LnRR) were calculated as follows:

$$\text{Relative competition intensity (RCI)} = \frac{(B_{\text{mono}} - B_{\text{mix}})}{Bmix}$$

$$\text{Log response ratio of biomass (LnRR)} = \text{Ln}\left(\frac{B_{\text{mono}}}{B_{\text{mix}}}\right)$$

where B_{mono} is the total biomass in the absence of competition (i.e., one-plant density treatment), and B_{mix} is the average biomass of a plant in each container in the presence of competition (i.e., the four-plant and twelve-plant treatments) (Grace, 1995; Armas et al., 2004).

Statistical Analyses

In this experiment, six experimental treatment groups were analyzed, with six samples in each treatment group. The total biomass, aboveground biomass and belowground biomass were transformed using the $\log_{10}(x)$ function. Thus, all experimental data met the assumptions of normality and homogeneity of variance prior to analysis. Two-way ANOVAs were used to test the effects of plant density and soil treatments on the growth traits and intensity of intraspecific competition of *M. aquaticum*. Duncan's test was used to examine the differences in trait values among the treatments. All data analyses were conducted using SPSS 22.0 (SPSS, Chicago, IL, United States).

RESULTS

Effects of Soil Nutrient Heterogeneity and Density Treatments on the Growth of *M. aquaticum*

The soil substrate type had a significant effect on the total biomass, aboveground biomass and number of shoots (**Table 1**). However, it had no significant effects on belowground biomass, shoot length or plant height (**Figures 2C,E,F** and **Table 1**). For example, in the twelve-plant density treatment, soil nutrient heterogeneity significantly reduced total biomass by 38.94% and aboveground biomass by 35.46% but significantly increased the 1.46-fold number of shoots compared to plants grown in homogeneous soil (**Figures 2A,B,D**). Density treatments had a significant influence on the growth performance of *M. aquaticum*, and the growth traits of the low-density treatment were significantly greater than those of the high-density treatment (**Figures 2A–F** and **Table 1**). For example, soil nutrient heterogeneity significantly increased shoot number by 70.37% in single-plant treatments and shoot length by 87.17% in four-plant treatments compared to plants grown in homogeneous soil.

TABLE 1 | *F*-value and significance of two-way ANOVA results for effects of soil substrate type (S) and plant density (D) on measures of biomass, morphological traits, and intraspecific competition of *M. aquaticum*.

	Substrate type (S)		Plant density (D)		S × D	
	F	*P*	*F*	*P*	*F*	*P*
Total biomass^a (g)	7.394	**0.010**	63.187	**<0.001**	1.076	0.352
Aboveground biomass^a (g)	7.844	**0.008**	55.947	**<0.001**	1.515	0.234
Belowground biomass^a (g)	2.410	0.129	96.868	**<0.001**	1.472	0.243
Number of shoots	33.340	**<0.001**	29.253	**<0.001**	4.007	**0.027**
Shoot length (cm)	2.266	0.141	22.618	**<0.001**	3.295	**0.049**
Plant height (cm)	0.001	0.982	14.272	**<0.001**	0.038	0.963
LnRR	1.554	0.225	27.758	**<0.001**	1.754	0.198
RCI	0.162	0.691	20.750	**<0.001**	0.910	0.350

**Significant P-values are shown in bold.* **That little "a" shown that data were transformed using the* $\log_{10}(x)$ *function.*

Except for the number and length of shoots, the effect of density treatments on the growth performance of *M. aquaticum* showed the same trends in the soil heterogeneity or homogeneity treatments (**Figures 2A–F** and **Table 1**). The interaction effect of soil and density treatment had significant effects on the shoot number and shoot length of *M. aquaticum* (**Table 1**).

Effects of Soil Nutrient Heterogeneity Treatments on the Intraspecific Competition Intensity of *M. aquaticum*

With the increase in plant density, the intraspecific competition intensity increased gradually. For example, the log response ratio of biomass (LnRR) significantly increased, by approximately 51.73% ∼ 87.38%, both when soil nutrients were homogeneous and when they were heterogeneous (**Figures 3A,B** and **Table 1**). These results show that the intraspecific competition of *M. aquaticum* gradually intensified with increasing plant density. Although the intraspecific competition intensity of the high-density treatment was very high, soil nutrient heterogeneity did not aggravate intraspecific competition in *M. aquaticum* (**Figures 3A,B** and **Table 1**).

DISCUSSION

Soil Nutrient Heterogeneity May Be a Promoter of Invasion and Spread in *M. aquaticum*

Heterogeneous soil had no effects on most growth traits except shoot numbers in the one-plant density treatment (**Figure 2**). In addition, plants accumulated less biomass when soil nutrient availability was heterogeneous at high density (**Figure 2**). Few studies have found that clonal plants grew more biomass in the heterogeneous than in the homogeneous

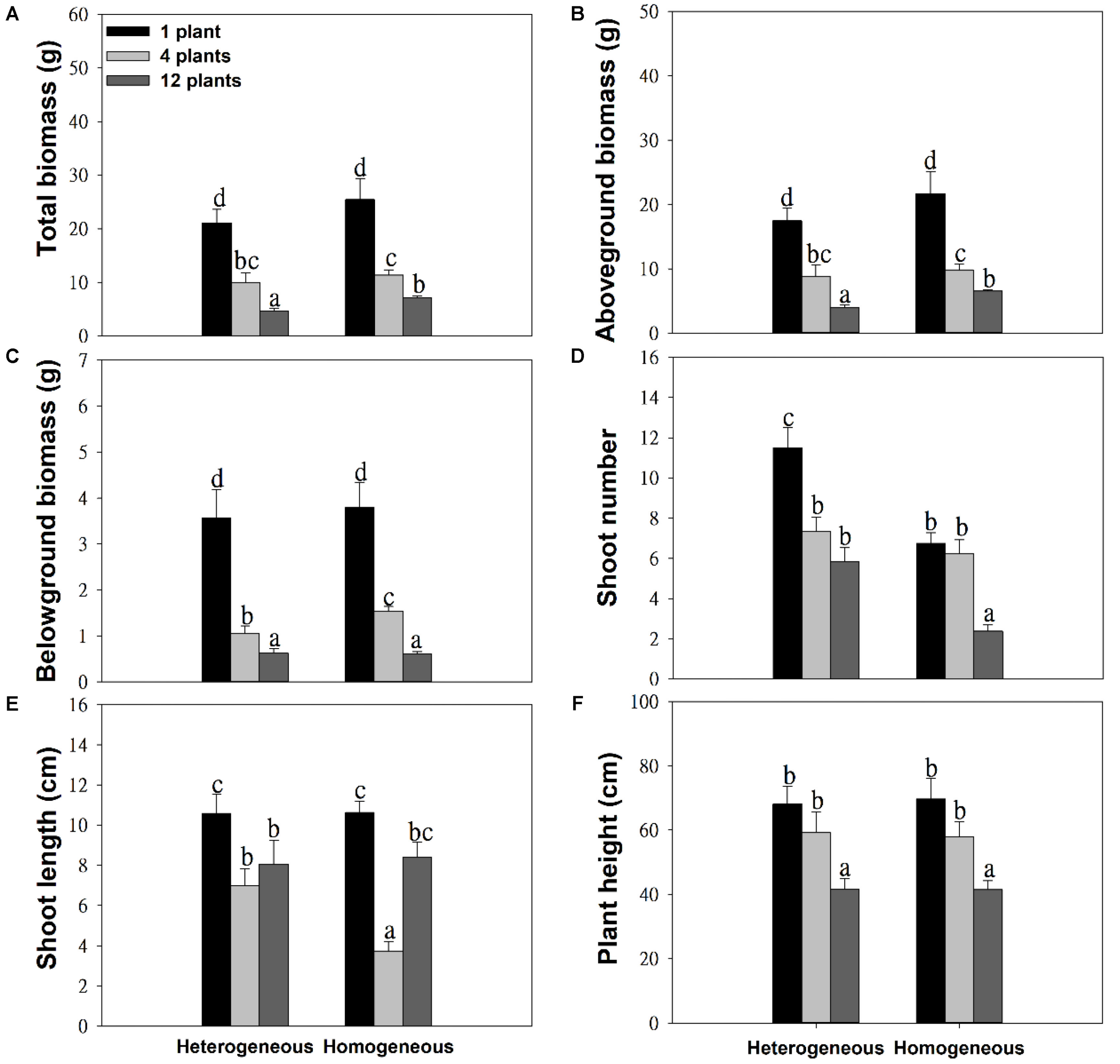

FIGURE 2 | Effects of soil substrate type and plant density on **(A)** total biomass, **(B)** aboveground biomass, **(C)** belowground biomass, **(D)** shoot number, **(E)** shoot length, and **(F)** plant height of *M. aquaticum*. Values represent the mean ± SE. Means with different small letters are significantly different at $P < 0.05$ between the different treatments.

soil treatment (Hutchings and Wijesinghe, 2008; Zhou et al., 2012; You et al., 2014). However, the positive effect of soil nutrient heterogeneity on the growth performance of plants may gradually decrease because the soil nutrients become limited in high-density populations (Day et al., 2003a; Dong et al., 2015). For example, soil nutrient heterogeneity has a short-term effect on the growth cycle of *Cardamine hirsuta*, and it does not have a long-term impact (Day et al., 2003a,b). Furthermore, the long-term response of *Holcus lanatus* was to produce far less shoot biomass in the heterogeneous treatment than expected under the homogeneity treatment (Fransen and Kroon, 2001). In addition, the growth performance of clonal plants in heterogeneous soil conditions may be correlated with the spatial scale of heterogeneity (Wijesinghe and Hutchings, 1997; Wang et al., 2016). For example, the growth of *Glechoma hederacea* was dependent on the spatial scale of soil nutrient heterogeneity (Wijesinghe and Hutchings, 1997), but *Alternanthera philoxeroides* displayed generally similar, scale-independent performance in most traits under different scales of soil nutrient heterogeneity (Wang et al., 2016). We speculate that the benefits of environmental heterogeneity to clonal plants may be correlated with spatial or temporal scale.

However, our study found that *M. aquaticum* produced significantly more and longer shoots in the heterogeneous soil than in the homogenous soil treatment. Morphological plasticity enables the adaptation of clonal plants to heterogeneous environments, which probably benefits clonal plants through the optimization of plant performance (Kroon and Hutchings, 1995). Clonal integration may help *M. aquaticum* adapt to the

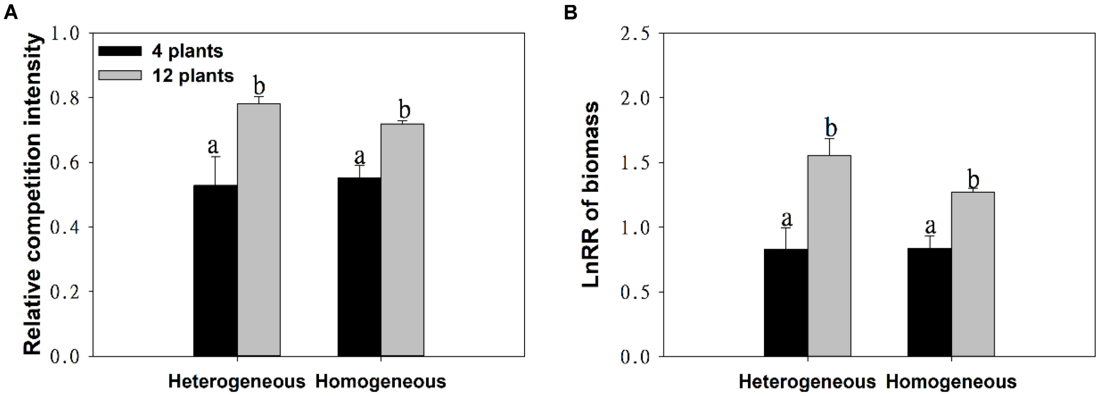

FIGURE 3 | Effects of soil substrate type and plant density on **(A)** relative competition intensity, **(B)** LnRR of biomass of *M. aquaticum*. Values represent the mean ± SE. Means with the different small letters are significantly different at $P < 0.05$ between the different treatments.

heterogeneous distribution of resources. For example, clonal integration can boost the growth of *M. aquaticum* when subjected to heterogeneity in resource supply in changing environments (You et al., 2013). Clonal plants can share photosynthates, mineral nutrients, or water among individual subunits through clonal integration, which increases the survival of clonal plants when they experience heterogeneous distribution of resources (Xiao et al., 2006; Yu et al., 2019). Thus, we predict that the positive response of clonal plants to environmental heterogeneity may be correlated with clonal integration and morphological plasticity.

Except for a higher number of shoots, slight effects and even lower biomass of *M. aquaticum* were found in heterogeneous nutrient treatments. This result is not completely in agreement with the prediction of our hypothesis but is consistent with previous studies, for example, nutrient heterogeneity does not affect the growth of a species of the same genera, *Myriophyllum spicatum* (Li et al., 2016). *Vallisneria natans* and *Prosopis glandulosa* did not show any significant changes in growth performance under spatially heterogeneous conditions (Maestre and Reynolds, 2007; Xie et al., 2007). On the other hand, *M. aquaticum* survival and spread depends solely on vegetative reproduction via fragmentation (Sutton, 1985). The higher number of shoots may aid in the spread of this species. Thus, soil nutrient heterogeneity can have a positive effect on invasive success in *M. aquaticum*, especially through occupying space and spreading.

Soil Nutrient Heterogeneity Could Not Change the Competitive Relationship Among Individuals in the Population of *M. aquaticum*

Spatial heterogeneity in soil nutrient availability can influence interspecific or intraspecific competition (Day et al., 2003b; Van et al., 2011; Mommer et al., 2012; Zhou et al., 2012). Plants can proliferate roots and ramets in nutrient-rich substrate patches to improve nutrient absorption efficiency in heterogeneous environments (Fransen et al., 2001; Day et al., 2003b; Dong et al., 2015). This may lead to fierce competition between plant species in heterogeneous conditions because the foraging organs of neighboring plants would be concentrated in a smaller patch of the soil. A previous study showed that soil nutrient heterogeneity, acting through its effect on competition, is likely to be an important influence on community structure and composition (Day et al., 2003b).

However, in this study, we found that soil nutrient heterogeneity could not alter the intraspecific competition of *M. aquaticum*. This was not due to an absence of competition, as plants grew less at high than at low density treatments both when soil nutrients were homogeneous and when they were heterogeneous. The reasons for these results may be as follows: First, clonal plants can share resources among individual subunits by physiological integration (Hutchings and Wijesinghe, 1997; Hellstrom et al., 2006), which may alleviate the severe competition between individual subunits in nutrient-patches (Novoplansky, 2009; Dong et al., 2015). Second, resource heterogeneity can significantly affect plant competition when individuals are not genetically identical (Day et al., 2003b; Zhou et al., 2012) or may be due to the differences in plant ability to place foraging organs in areas where available resources are high (Wijesinghe et al., 2001; Bliss et al., 2002).

In heterogeneous environments, local adaptation to particular environmental conditions may also occur within plant populations on a much smaller geographical scale (Hangelbroek et al., 2004). Substrate characteristics can determine macrophyte community structure within lakes from a combination of both regional-scale multi-lake studies and smaller-scale studies (Johnson and Ostrofsky, 2004). Thus, our study showed that soil nutrient heterogeneity, especially for fine-scale heterogeneity, does not increase competition between individuals of *M. aquaticum*.

CONCLUSION

We conclude that spatial heterogeneity in soil nutrient availability is likely to be a primary promoter of invasive success in

M. aquaticum. In the case of *M. aquaticum*, the positive effects of soil nutrient heterogeneity are shown mainly in the morphological characteristics of individual clonal shoots and cannot change the competitive relationship of clonal plants such as *M. aquaticum*. Therefore, spatial heterogeneity in soil nutrient availability may have a positive effect on the invasive spread of *M. aquaticum*. In the future, a more intimate knowledge of how diversified environmental heterogeneity due to various ecological factors at different scales affects the invasive performance of alien species is needed. This will enable a better understanding of the dynamic changes in invasive species composition and richness in aquatic ecosystems.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CL and DY designed the experiment and edited the manuscript text. HY and NS performed the experiment. NS and SY wrote the manuscript text and executed statistical analysis.

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REVIEW
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Global Change Sharpens the Double-Edged Sword Effect of Aquatic Alien Plants in China and Beyond

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Many alien aquatic plants are deliberately introduced because they have economic, ornamental, or environmental values; however, they may also negatively affect aquatic ecosystems, by blocking rivers, restricting aquatic animals and plants by decreasing dissolved oxygen, and reducing native biodiversity. These positive and/or negative ecological effects may be enhanced under global change. Here, we examine the impacts of global change on aquatic alien plant introduction and/or invasions by reviewing their introduction pathways, distributions, and ecological effects. We focus on how climate change, aquatic environmental pollution, and China's rapid economic growth in recent decades affect their uses and invasiveness in China. Among 55 species of alien aquatic plants in China, 10 species are invasive, such as *Eichhornia crassipes*, *Alternanthera philoxeroides*, and *Pistia stratiotes*. Most of these invaders were intentionally introduced and dispersed across the country but are now widely distributed and invasive. Under climate warming, many species have expanded their distributions to areas where it was originally too cold for their survival. Thus, these species are (and will be) considered to be beneficial plants in aquaculture and for the restoration of aquatic ecosystems (for water purification) across larger areas. However, for potential invasive species, climate warming is (and will be) increasing their invasion risk in more areas. In addition, nitrogen deposition and phosphorus inputs may also alter the status of some alien species. Furthermore, climate warming has shifted the interactions between alien aquatic plants and herbivores, thus impacting their future spreads. Under climate change, more precipitation in North China and more frequent flooding in South China will increase the uncertainties of ecological effects of alien aquatic plants in these regions. We also predict that, under the continuing booming economy in China, more and more alien aquatic plants will be used for aquatic landscaping and water purification. In conclusion, our study indicates that both human activities under rapid economic growth and climate change can either increase the potential uses of alien aquatic plants or make the aquatic invaders worse in China and other areas in the world. These findings are critical for future risk assessment of aquatic plant introduction and aquatic ecosystem restoration.

Keywords: alien aquatic plants, biological invasions, aquatic ecosystem, global change, China

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INTRODUCTION

Global change includes climate change, nitrogen deposition, changes in land-use patterns, and biological invasions (Lin et al., 2010; Pyšek et al., 2010; Lu et al., 2013). Global change may accelerate the spread of alien plants, alter the species composition of plant communities, and affect the physiological and/or ecological traits of alien plants in aquatic ecosystems (Maki and Galatowitsch, 2004; Hastwell et al., 2008; Sorte et al., 2013; Henriksen et al., 2018). For instance, with rapidly growing international trades, many aquatic alien plants from around the world have been intentionally or unintentionally introduced into China (Ding et al., 2008; Wang et al., 2016), and some of them have become invaders for many reasons, such as the lack of co-evolved natural enemies (Lu et al., 2013; You et al., 2014). More seriously, biological invasions, as a major component of global change, have caused significant ecological and economic impacts on aquatic ecosystems, together with the impacts of other factors, such as global warming, eutrophication, and flooding (Hastwell et al., 2008; Collinge et al., 2011; Thouvenot et al., 2013; Lu et al., 2015).

Aquatic plants, as an ecological group closely dependent on water, have multiple ecotypes, including emergent, floating-leaved, submersed, and free-floating forms, totaling more than 2600 species of aquatic plants belonging to 88 families in the world (Chambers et al., 2008; Li, 2014). Although aquatic plants only account for approximately 2% of the 350,000 angiosperm species, they play key roles in the functioning of aquatic ecosystems (Carpenter and Lodge, 1986; Jeppesen et al., 1998; Hilt et al., 2017). Due to the growing ornamental, horticultural, and aquacultural trades and/or unintentional transport, many aquatic plants have been introduced into new continents or countries from their native range and have become alien species (Ding et al., 2008; Hussner, 2012). Unlike the immobile roots of their terrestrial congeners, aquatic alien plants usually have relatively weaker root systems, and some of them are free-floating macrophytes; thus, the fluctuating water further provides suitable conditions for the dispersal and diffusion of these alien diaspores, especially during flooding (Li, 2014).

Aquatic alien plants may have significant double-edged sword effects with respect to ecology. On the one hand, they provide numerous ecosystem services, including ornamental, landscaping, ecological restoration, food, forage, and green manure uses (Hussner, 2012; Wang et al., 2016). For example, many aquatic alien species of Nymphaeaceae and Alismataceae are introduced into China from America and Europe for use as ornamental or aquarium plants, such as *Victoria regia* and *Echinodorus amazonicus* (Chen et al., 2012). On the other hand, aquatic invasive plants have been shown to cause more serious impacts on their habitats than their terrestrial counterparts (Vila et al., 2009). In freshwater ecosystems especially, once aquatic alien plants successfully become invaders, they hinder river runoff, cause oxygen deficiency, reduce water quality and native biodiversity, and even disturb food web structures (Hussner, 2012; Hussner et al., 2017; Kennedy and El-Sabaawi, 2017; Liu D.S. et al., 2017). Relative to their native accompanying species, many aquatic invaders are opportunistic species that could quickly capitalize on increased resources, thus their growth and reproduction may be enhanced by elevated temperature and precipitation (Blumenthal, 2006; Sorte et al., 2013), which could ultimately alter their positive or negative ecological effects under rapid global change in the future. Therefore, examining the ecological effects of aquatic alien plants under global change is crucial for utilizing biological resources, predicting and preventing aquatic invasions, and protecting native biodiversity in freshwater ecosystems; however, such work has rarely been reported.

Here, we focus on how climate change, aquatic environmental pollution, and China's rapid economic growth in recent decades affect alien aquatic plant uses and invasiveness. China has been experiencing a booming economy and has greatly increased international trades in the past 40 years, which dramatically facilitates aquatic alien plant establishment across this country (Ding et al., 2008; Weber and Li, 2008; Wu et al., 2010). Additionally, there are many types of freshwater bodies (lakes, rivers, estuaries, ponds, etc.) in China, further benefiting the spread and diffusion of aquatic alien plants (Wang et al., 2016). For example, the water hyacinth, *Eichhornia crassipes*, a free-floating aquatic macrophyte native to South America, was initially introduced into China for its ornament value (Qin et al., 2016a), and it also has water purifying properties in many large freshwater bodies of China (Wang et al., 2012, 2013; Liu et al., 2015). However, due to the booming economy and industrial development of China, high amounts of nutrients have been largely deposited into freshwater, accelerating eutrophication (Ding et al., 2008) and facilitating *E. crassipes* growth, which has made this plant the most important aquatic invasive plant in South China (Ding et al., 2006; You et al., 2014). Furthermore, the increasing global ornamental trades and developed hydrographic networks of China have also accelerated *E. crassipes* invasion and dispersal (Gao and Li, 2004; Liu D.S. et al., 2017). As one of the world's worst aquatic weeds, *E. crassipes* has widely invaded into the rest of the world besides China, e.g., in Southeast Asia, Southeastern United States, Central America, and Central and Western Africa, causing serious damages to environment, biodiversity, economy, and human health in invaded regions (Ding et al., 2006; Villamagna and Murphy, 2010).

China is a geographically vast country, spanning 50 degrees of latitude and covering five climatic zones (Wang et al., 2016); thus, with climate change, high latitudinal regions of North China have experienced a larger temperature rise in the last 60 years, and this warming process will continue (Yang et al., 2018). In addition, the precipitation of high latitudes in North China will also increase in the future (Gao et al., 2015). These climate changes may accelerate the distribution of aquatic alien plants into higher latitudes in China. For example, the alligator weed *Alternanthera philoxeroides*, one of China's major aquatic invaders native to South America that was introduced as a fodder species in the 1930s, has now expanded 2° of its northern boundary (from 34.7°N to 36.8°N) along latitudinal gradients in the last decade (Lu et al., 2015). Warming could expand the range of both *A. philoxeroides* and its natural enemy *Agasicles hygrophila*, an insect introduced for its biological control, shifting their interactions, and likely facilitating its invasiveness

(Lu et al., 2013, 2015; Wu et al., 2017a). Together, under rapid global change, aquatic ecosystems would suffer a greater threat from biological invasions (Wu and Ding, 2014), and the double-edged sword of the ecological effects of aquatic alien plants may be further sharpened.

Here, we review the impacts of global change on aquatic alien plants and focus on the geographic origins and introduction pathway of these plants in China. We hypothesize that (1) global change has significant impacts on the double-edged sword effects of aquatic alien plants across the world and (2) specifically, the negative ecological effects of China's aquatic alien plants will be aggravated by global change.

AQUATIC ALIEN PLANTS IN CHINA

In conjunction with previous related studies, we define aquatic alien plants as plant species that were introduced from their origins into new countries or regions due to intentional or inadvertent human involvement; the life cycles of these plant species are almost completely dependent on the water, or these plants are submerged for at least one part of their life history (Cook, 1990; Pyšek et al., 2004; Hussner, 2012; Li, 2014; Wang et al., 2016). By contrast, aquatic invasive plants are species among aquatic alien plants that "cause, or have the potential to cause, harm to the environment, economies, or human health" (GISP, 2003). We only study aquatic alien plants that occur in freshwater habitats and exclude species varieties by artificially breeding.

In total, 55 aquatic alien plant species belonging to 20 families and 29 genera were recorded in freshwater ecosystems in China (**Supplementary Table 1**). Nymphaeaceae had the highest species number of aquatic alien plants (11 spp.), followed by Alismataceae with 10 species and Gramineae with 7 species, while the other 11 families only possessed 1 alien plant species. According to our survey, 55 aquatic alien plant species were intentionally introduced into China through human involvement, and the original purposes were to use them for ornamental, aquatic landscaping, water purification, and forage purposes. However, 10 species among them (nearly 18%) later became invaders, i.e., *Cabomba caroliniana*, *Spartina alterniflora*, *Hydrocotyle vulgaris*, *Azolla filiculoides*, *A. philoxeroides*, *Myriophyllum aquaticum*, *E. crassipes*, *Pistia stratiotes*, *Brachiaria brizantha*, and *Brachiaria mutica*. Among these, five species were introduced from South America, three species were introduced from North America, and two species were introduced from Africa (**Figure 1**). One of the worst invaders, *A. philoxeroides*, has invaded 18 provinces spanning a large latitudinal gradient, followed by *E. crassipes*, which has invaded 16 provinces (**Figure 2**). *S. alterniflora*, *P. stratiotes*, and *M. aquaticum* also have wide distributions and occur in more than nine provinces. Compared with inland provinces, there are more diverse aquatic invasive plants in the coastal provinces of China, such as Fujian, Guangdong, Guangxi, Zhejiang, and Jiangsu provinces. Moreover, the richness of aquatic invasive plant species in China increases toward further east and south (**Figure 2**). This pattern is consistent with the latitudinal diversity gradient (LDG) rule which states that global biodiversity usually declines from tropics to the poles (Fischer, 1960), as the solar radiation and precipitation decrease with increasing latitudes (Lu et al., 2016; Wu et al., 2016). Thus, the low average annual temperature in higher latitude would also be unfavorable for the growth and reproduction of China's aquatic invasive plants, most of which are native to the tropics with a long history of adaptation to higher temperatures.

POSITIVE AND NEGATIVE IMPACTS OF GLOBAL CHANGE ON AQUATIC ALIEN PLANTS

Aquatic alien plants have caused significant double-edged sword effects on the freshwater ecosystems. They may threaten human health by providing habitats for mosquitoes (O'Meara et al., 2003; Chandra et al., 2006). They also hamper recreational activities and disrupt agricultural production, causing great economic losses (Oreska and Aldridge, 2010; Rumlerova et al., 2016; Keller et al., 2018; Tanveer et al., 2018). Alien aquatic plants often compete for space, nutrients, and sediment fertilities with native macrophytes, thus hindering their re-establishment and decreasing diversity (Michelan et al., 2018; Silveira et al., 2018). Some of them aggravate water loss in invaded habitats through extensive transpiration (Fraser et al., 2016); accelerating water pollution by increasing sedimentary organic matter (Cuassolo et al., 2016; Bertrin et al., 2017); and reducing oxygen diffusion across the water–air interface (Chamier et al., 2012), etc.

Alien aquatic plants could also increase regional or local aquatic plant species richness (Gołdyn, 2010; Bolpagni and Piotti, 2015). As ecosystem engineers, they could be used for water purification by reducing water turbidity, decreasing sediment nutrient loading, and intensifying seasonal fluctuations of oxygen and carbon for keeping the balance of benthic nutrients in freshwater ecosystems (Thomaz et al., 2015; Bai and Shang, 2017; Ribaudo et al., 2018). They may also provide shelters for aquatic macroinvertebrates (Rocha-Ramírez et al., 2014) and increase pollinator visitants for native aquatic plants (Stiers et al., 2014), etc.

In this study, to examine the impacts of rapid global change on both of the negative and positive effects of aquatic alien plants, we searched the literatures (article or review) from the ISI Web of Science database, mostly published during 1998–2018s, using the following search terms: ("warming" or "temperature" or "climate warming" or "global warming"), ("eutrophication" or "nitrogen" or "phosphorus"), ("flood" or "precipitation" or "rainfall"), ("trade" or "global trade" or "economic globalization") combined with ("aquatic alien plant" or "alien aquatic plant" or "aquatic exotic plant" or "exotic aquatic plant"). We carefully selected the articles that clearly reported the positive or negative impacts of global change on ecological effects of aquatic alien plants and excluded studies that addressed unrelated topics. In total, we collected data from 102 case studies about the impacts of global change on aquatic alien plants at a global

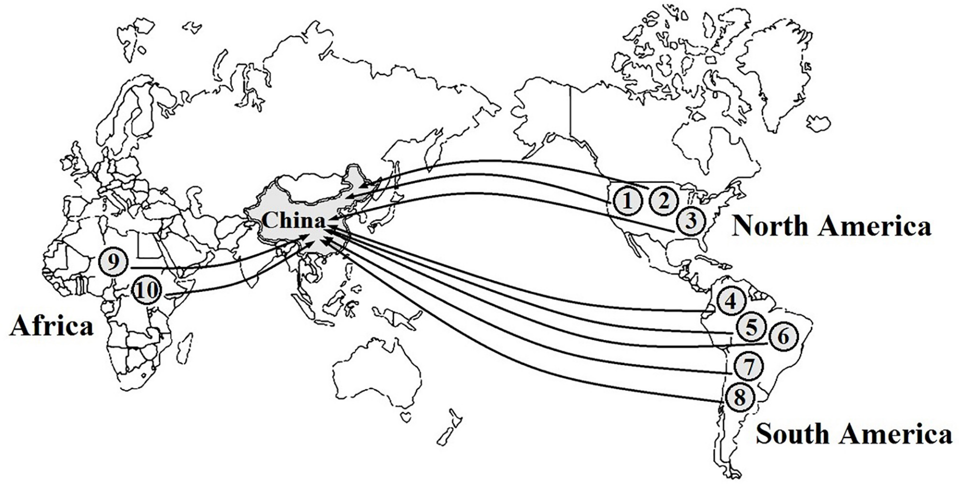

FIGURE 1 | Geographical origins of 10 major aquatic invasive plants in China. Data are from the announcements of "*Inventory of Invasive Species in China* (first–fourth volumes)" which were enacted by the Ministry of Ecology and Environment of China & Chinese Academy of Sciences (2003, 2010, 2014, 2016) (http://www.mee.gov.cn/) and some published literatures (Ding et al., 2008; Yan et al., 2014; Wang et al., 2016). Latin names of species code in this figure are *Cabomba caroliniana* (1), *Spartina alterniflora* (2), *Hydrocotyle vulgaris* (3), *Azolla filiculoides* (4), *Alternanthera philoxeroides* (5), *Myriophyllum aquaticum* (6), *Eichhornia crassipes* (7), *Pistia stratiotes* (8), *Brachiaria brizantha* (9), and *Brachiaria mutica* (10).

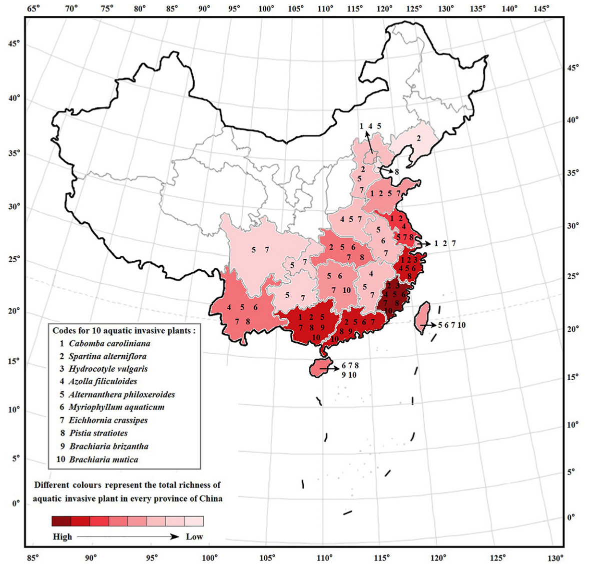

FIGURE 2 | Interprovincial distributions of China's 10 major aquatic invasive plants. Data are from the Chinese books including "*The Chinese Aquatic Plants*" (Chen et al., 2012), "*The Checklist of the Invasive Plants*" (Ma, 2013), "*Inventory Invasive Alien Species in China*" (Xu and Qiang, 2007), and "*Illustrations of Alien Invasive Plants in China*" (Yan et al., 2016); Chinese databases including "Chinese Virtual Herbarium" (http://www.cvh.ac.cn/), "Flora Reipublicae Popularis Sinicae" (the online version, http://frps.eflora.cn/), and "National Specimen Information Infrastructure of China" (http://mnh.scu.edu.cn/); and some published literatures (Ren et al., 2004; Ding et al., 2008; Zhang and Meng, 2013; Yan et al., 2014; Wang et al., 2016; Wu et al., 2016).

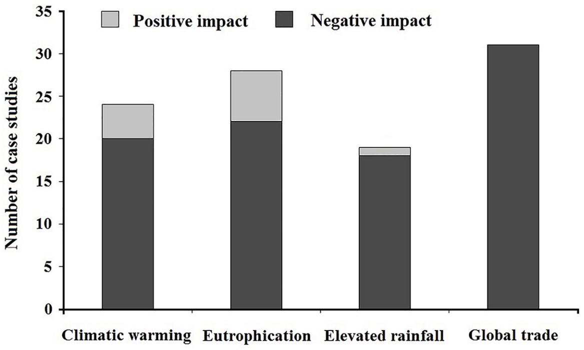

FIGURE 3 | Impacts of global change on the ecological effects of aquatic alien plants at the global scale. Data are from the related 102 published studies that are listed in the **Supplementary Material** (see **Supplementary Table 2**).

scale (see **Supplementary Table 2**, the reference list). We found that, most of studies on climatic warming, eutrophication and elevated rainfall reported negative impacts and a few addressed positive impacts, while studies for global trade only dealt with the negative impacts, i.e., ecological effects of aquatic alien plants (**Figure 3**).

One of the positive impacts of climatic warming on aquatic alien plants is water purification through an increase in the inhibition by certain aquatic alien plants of harmful algae, and through improving aquatic landscaping, as a result of increasing in adaptability of alien ornamental plants to aquatic environments. The negative impacts of warming include the reduction in native species diversity through increased interspecific competition, accelerated water pollution through increased alien plant litter decay rates, and aggravated aquatic alien plant invasions through an increase in their biomass and overwintering (**Figure 4**). Some aquatic invaders such

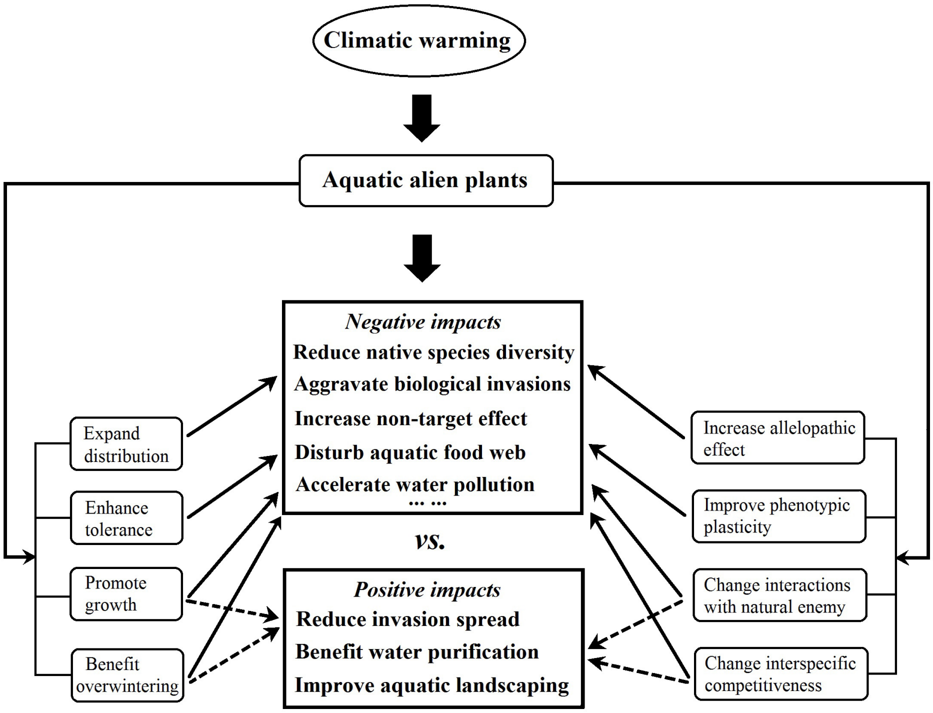

FIGURE 4 | Positive and negative impacts of climatic warming on the ecological effects of aquatic alien plants. The thin solid arrow indicates that climatic warming will facilitate the negative ecological effects of aquatic alien plants, while the thin dotted arrow indicates that warming will facilitate their positive ecological effects. Data are from the related 24 published studies that are listed in the **Supplementary Material** (see **Supplementary Table 2**).

as *P. stratiotes* and *E. crassipes* are floating plants and overwinter with floating vegetative tissues; the warmer water temperature could prevent the leaves and roots from being killed by frost in the winter, and their overwintering vegetative biomass respond quickly to the elevated temperatures, thus climate change will enhance their invasion and increase their negative impacts (Santos et al., 2011; Hussner et al., 2014; You et al., 2014).

Eutrophication increases the applicability of aquatic alien plants for water purification and can decrease the relative competitive ability of certain aquatic invasive plants compared with their native accompanying plants (Qin et al., 2016b; Xu et al., 2017). However, eutrophication strongly benefits the reproduction, clonal spread, compensatory growth, metabolic enzyme activity, and nutrient assimilation of many aquatic invasive plants around the word, which would further aggravate their invasiveness (Coetzee et al., 2011; Li and Wang, 2011; You et al., 2014).

Elevated rainfall was reported to reduce the salinity and increase the water level of Lake Naivasha in Kenya, which was favorable for the survival and establishment of several exotics such as *Cyperus papyrus* and *Potamogeton distinctus*, improved the native biodiversity (Lamb et al., 2003). However, the flooding caused by elevated rainfall accelerates the downstream movement of seedlings or propagules of alien plants and may wash away many small native hydrophytes, resulting in a decrease in the resistance of freshwater ecosystems to invasion (Holmes et al., 2008; Hofstra et al., 2010; Wu et al., 2017b). Extreme hydrological events also increase the landscape connectivity of water bodies in aquatic ecosystems, increasing external nutrient inputs in freshwater discharged from agricultural areas and sewage effluents and thus accelerating aquatic invasions (Espinar et al., 2015; Anufriieva and Shadrin, 2017).

Global trade (particularly aquarium and ornamental trade) has been identified as the major pathway for aquatic alien plant introductions, and the rapid spread of propagules and/or seedlings of nonindigenous aquatic plants that caused by trade may accelerate their invasions worldwide (Ding et al., 2008; June-Wells et al., 2012; Oele et al., 2015). High numbers of potential aquatic invaders have been massively introduced into many countries for sale through global trade without strict legal regulations (Thum et al., 2012). Global trade also facilitates mutualistic invasion; for instance, the invasive *Physa acuta* was carried into Thailand and Laos in the introduction process of many alien aquatic ornamental/invasive plants (Ng et al., 2018). Furthermore, with the rapid development of global online trade, alien aquatic ornamental plants can be bought online easily and cheaply through the unregulated market, and some of them are invaders, such as the notorious *E. crassipes* (Brundu et al., 2013). In general, the negative impacts of global change on aquatic alien plants are much greater than its positive impacts at a global scale.

WARMING IS EXPANDING THE DISTRIBUTIONS OF AQUATIC ALIEN PLANTS ACROSS CHINA

The Earth's climate has warmed by nearly 1.0°C over the past 100 years, and it is predicted that the average temperature will continue to increase by 3°C at the end of the 21st century, however, it could breach 1.5°C between 2030 and 2052 if climate warming continues at its current rate (IPCC, 2018; Tollefson, 2018). In China, the average temperature has increased by approximately 0.4°C per decade in the last 40 years, and the high latitudinal regions in North China will have a greater temperature increase under global warming (Walther et al., 2002; Ding et al., 2007). Increasing water temperature has caused profound impacts on the establishment, growth, phenology, and distribution of aquatic plant species in freshwater ecosystems, especially alien species, because aquatic alien plants usually have more active responses to climatic warming compared with native co-occurring plants (Sorte et al., 2013; Hussner, 2014; You et al., 2014). Thus, warming may increase the risk of aquatic alien plants transforming into invaders, such as the alien *Thalia dealbata* in China (Chen and Ding, 2011), and accelerate aquatic invasive plants spreading to more new regions (especially higher latitudes), such as the invasive *A. filiculoides* in Spain, *P. stratiotes* in Germany, and *Egeria densa* in the United States; the invasiveness and overwintering of these invaders were greatly promoted by warming (Santos et al., 2011; Hussner et al., 2014; Espinar et al., 2015).

In China, climate warming has also increased the net photosynthetic rate and morphological plasticity of invasive *A. philoxeroides* (Lu et al., 2013; Chu et al., 2014; Wang et al., 2017), as well as accelerated its spread to higher latitudes of North China. Warming also increased enemy release from the bio-control beetle *A. hygrophila* (**Figure 5**), because *A. philoxeroides* tolerated cold better than its natural enemy *A. hygrophila* and expanded more fast to the higher latitudes, while *A. hygrophila* failed to overwinter in the low temperature of Northern China, thus, geographical gap between *A. philoxeroides* and *A. hygrophila* would be shifted to higher latitudes under warming, further benefiting plant invasion (Lu et al., 2013). However, warming could also affect the biotic interactions among *A. hygrophila*, *A. philoxeroides*, and its native congener *A. sessilis* in China, as warming significantly increased the plant performances (e.g., aboveground biomass, flower, and seed numbers, etc.) of *A. sessilis* relative to the co-occurring invader in the presence of *A. hygrophila*, and the beetle abundance on *A. philoxeroides* was higher than that on *A. sessilis* under elevated temperature, thus relatively increased the biotic resistance of native *A. sessilis* to *A. philoxeroides* invasion (Lu et al., 2016).

Warming significantly increases the growth of *E. crassipes*, which is one of the major aquatic invaders in China (You et al., 2014). Consistent with findings in other countries (Hussner, 2014; Espinar et al., 2015; Vojtkó et al., 2017), invasion by *P. stratiotes*, *A. filiculoides*, and *C. caroliniana* in China would also be continuously aggravated by climatic warming. According to the latest survey, *C. caroliniana* has expanded its northern distribution boundary to the 40°N region of Beijing City, i.e., at higher latitudes (Zhang and Meng, 2013). Warming could also improve the adaption of some aquatic alien plants used in landscaping (such as *Nymphaea rubra*) and thus increase the species diversity of aquatic vegetation (Hussner and Lösch, 2005; Vojtkó et al., 2017). Warming could even weaken the spread of certain aquatic invasive plants, such as *Elodea canadensis* in Polish lakes, as warming strengthened the thermal stratification of water columns and thus might reduce the nutrient cycles between deeper waters and surface, which would weaken the nutrient supply to *E. canadensis* (Kolada and Kutyła, 2016; Vasconcelos et al., 2019). Furthermore, the asymmetry of global warming between day and night may further exacerbate the invasion of aquatic alien plants and cause considerable detrimental impacts (Harvey, 1995; Walther et al., 2002; Chu et al., 2014). For example, the submerged plant species *Myriophyllum spicatum*, which is native to Europe, Asia, and has invaded to North America, has prolonged its growing season under climate warming, thus the increasing abundance in freshwater ecosystems significantly increased its biomass and carbon stock (Velthuis et al., 2018). In China, many aquatic invaders were introduced from the tropics with higher thermal tolerance,

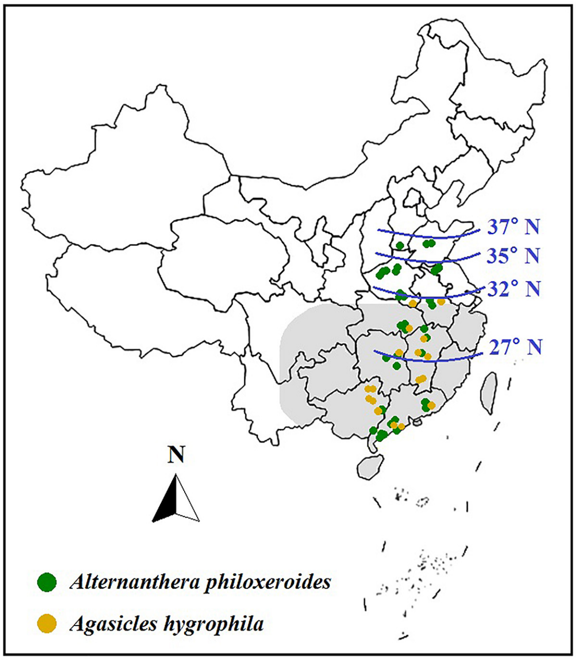

FIGURE 5 | Dynamic distributions of aquatic invasive *A. philoxeroides* and its biocontrol beetle *Agasicles hygrophila* in China under global warming. In 1995, the potential northern boundaries of *A. philoxeroides* and *A. hygrophila* were predicted to be at 31.5°N (as the shadowing shows) and 27°N, respectively. In 2001, the northern boundaries of *A. philoxeroides* and *A. hygrophila* were found at 32°N and 35°N, respectively. In 2013, the northern boundary of *A. hygrophila* was still limited at 32°N (as the yellow dots show); however, *A. philoxeroides* had expanded to approximately 37°N (as the green dots show). Data were from related literatures (Julien et al., 1995; Ma, 2001; Lu et al., 2013).

and they are distributed in large geographical ranges of China (**Figures 1**, **2**), warming may increase their invasion risk at high latitudes in the future.

EUTROPHICATION PROMOTES THE APPLICABILITY AND INVASION RISKS OF ALIEN HYDROPHYTES

Water eutrophication is the consequence of human activities caused by depositing high levels of organic compounds and/or nutrients (nitrogen and phosphorus) into freshwater ecosystems (Carpenter et al., 1998; Yu et al., 2018). In China, a large amount of domestic and industrial wastewater is discharged without treatment, which seriously intensifies eutrophication (Ding et al., 2008; Le et al., 2010). At present, more than 70% of the major lakes in China have undergone severe eutrophication (Jin et al., 2005; Yu et al., 2018). For these reasons, there is increased demand for certain aquatic alien plant for water purification in China, such as *E. crassipes*, *P. stratiotes*, *M. aquaticum*, *A. philoxeroides*, *T. dealbata*, *S. alterniflora*, which all have excellent phytoremediation properties, with a high nutrient removal ability and allelopathic inhibition of harmful algae (Zuo et al., 2012; Qin et al., 2016a); consequently, water transparency and the control of cyanobacterial blooms could increase.

On the other hand, the growth rates, photosynthetic capacity, and phenotypic plasticity of aquatic alien plants usually increase more intensively than those of native hydrophytes in nutrient-rich waters (Riis et al., 2010; Yu et al., 2018), and most aquatic alien plant species become successful invaders. In China, eutrophication increases biomass accumulation, asexual reproduction, compensatory growth, and photosynthetic leaf areas of *A. philoxeroides* (Shen et al., 2007; Jiang et al., 2010; Zuo et al., 2012; Ding et al., 2014); promotes the growth rate, clonal propagation, nitrate reductase, and glutamine synthetase activities of *E. crassipes* (Li et al., 2008, Li and Wang, 2011); and increases the biomass, shoot length, and nutrient uptake of *M. aquaticum* (You et al., 2013; Liu S.B. et al., 2017), which intensify the interspecific competition of these three aquatic invaders with respect to native plants. In addition, interactions between warming and eutrophication have been shown to significantly improve the overwintering ability of *E. crassipes* in China (You et al., 2014). Excessive growth of aquatic invasive plants in nutrient-rich waters may exacerbate the deposition of their dead matter and reduce the dissolved oxygen in invaded habitats. Indeed, more than a hundred rivers with eutrophication have been blocked by *E. crassipes* and/or *A. philoxeroides* in southern China over the last 30 years (Ding et al., 2008). Similarly, eutrophication in other countries also severely accelerates aquatic plant invasions, such as *E. canadensis* in New Zealand and *Glyceria maxima* in Australia (Loo et al., 2009; Riis et al., 2010). Moreover, the annual bulk deposition of nitrogen in China has increased by 8 kg per hectare in the last 30 years (Liu et al., 2013); excessive nitrogen flowing into waters would aggravate the eutrophication of freshwater ecosystems and thus further accelerate China's aquatic plant invasions.

INCREASING RAINFALL ACCELERATES THE SPREAD OF AQUATIC ALIEN PLANTS IN CHINA

With ongoing climate change, extreme global climatic events become more frequent, such as floods, and China is under a high risk of heavy rainfall (Piao et al., 2010; Diez et al., 2012). For instance, summer precipitation has significantly increased in southern China since the 1960s, which readily causes severe flooding (especially in the Yangtze River basin) (Piao et al., 2010), while the frequency of moderate rain has increased in the high latitudes of northern China, and extreme rainfall may increase over most of China in the future (Gao et al., 2015). This elevated rainfall improves the adaptation and survival of aquatic alien ornamental/landscape plants in arid regions of northern China; however, this would thus promote the transport of alien plant propagules across China, particularly those of free-floating species (such as *E. crassipes*, *P. stratiotes*, and *A. filiculoides*), as well as provide more suitable aquatic environments for the spread and establishment of aquatic invasive plants at high latitudes. It has been reported that some aquatic plant invasions (e.g., *A. philoxeroides* and *M. aquaticum*) in China are significantly correlated with precipitation, as rainfall increases their biomass or species coverage (You et al., 2013; Wu et al., 2017b). For instance, elevated rainfall increased peroxidase and superoxide dismutase activities, species coverage, and new leaf numbers of *A. philoxeroides*, while water level fluctuation increases *A. philoxeroides* shoot length but reduces intraspecific competition (Yu, 2011; Chen et al., 2016). Elevated rainfall/water level fluctuations can also increase clonal integration, the number of branches, and the stolon length of the invasive species *M. aquaticum* (You et al., 2013; Chen et al., 2016), facilitating its invasion in China. Flash flooding due to elevated rainfall also reduces the biotic resistance of native aquatic plants, aggravates eutrophication through fertilizer runoff, and improves the connectivity of waters, increasing the invasion of freshwater ecosystems by aquatic alien plants (Collinge et al., 2011; Espinar et al., 2015; Anufriieva and Shadrin, 2017). In Florida, elevated rainfall increases the water content in the leaf axils of aquatic alien plants, which improves habitats for the growth of mosquitoes and thus exacerbates potential harms caused to human health (O'Meara et al., 2003). In addition, large-scale hydraulic projects, such as China's South-to-North Water Diversion Project (SNWD), may provide an express channel for the spread of aquatic invasive plants, especially during flooding (Liu D.S. et al., 2017), and elevated rainfall could even interact with climatic warming to accelerate the spreading of aquatic invaders (Espinar et al., 2015). Together, these findings indicate that rainfall will increase the uncertainties of the ecological effects of alien aquatic plants.

BOOMING GLOBAL TRADE AGGRAVATES CHINA'S ONGOING AQUATIC INVASIONS

Since China implemented the landmark "reform and opening" policy in 1978, its gross volume of import and export trade increased rapidly. After China joined the World Trade Organization (WTO) in 2001, it has become the second largest importing country in the world, such that the total value of China's imports and exports increased from approximately RMB 35.5 billion to RMB 27,800 billion over the period 1978–2017, which is a 782-fold increase, and its trading partners expanded from 40 to 231 countries/regions (National Bureau of Statistics of China [NBSC], 2017). Under global change, such phenomenal growth in the international trade of China also aggravated aquatic plant invasions (Ding et al., 2008; Weber and Li, 2008). Global trade effectively promotes the distribution and spread of many of these species, through horticultural, ornamental, and aquarium trades; the dumping of ballast water and the burgeoning unregulated Internet trades; and many aquatic alien ornamental plants that come from international trades usually have higher growth rates, cold tolerance, and dispersal ability (Pemberton and Liu, 2009; Martin and Coetzee, 2011; Azan et al., 2015). For example, the invasive species *E. crassipes* and *C. caroliniana* were introduced into China through global ornamental and aquarium trades without risk assessment and have subsequently severely invaded and threatened native plant diversity in Southern China (He et al., 2011; You et al., 2014). Other aquatic alien plants, such as *T. dealbata* and *E. densa*, which also have great potential invasiveness, are still imported into China through international trading (Chen and Ding, 2011). Recently, China started another huge international trade project, "The Belt and Road (B&R)," in 2013. Under this project, the total value of China's imports and exports from the B&R reaches RMB 3.32 billion in 5 years, with a growth rate that is 1.4% higher than that of the national average level (National Bureau of Statistics of China [NBSC], 2017). These growing global trades may thus continuously increase the invasion risk of aquatic alien plants in China.

CONCLUSION

Our study shows that alien aquatic plants have caused both positive and negative ecological effects on freshwater ecosystems and global change, such as climate warming, eutrophication, elevated rainfall, and global trade could increase or decrease those effects. In China, eutrophication increases the demands for aquatic alien plants for use in water purification and landscaping, while climate warming may improve the adaption of aquatic alien ornamental plants and even increase the biotic resistance of native plants to aquatic invaders. However, warming, eutrophication, and elevated rainfall could increase the invasiveness of many aquatic invasive plants, and booming global trade may accelerate the dispersal of aquatic invaders across this country. In brief, global change is sharpening the double-edged sword effect of China's aquatic alien plants, increasing the utilization of aquatic alien plant resources, and aggravating their invasion risk.

Our study also indicates that human activities under rapid economic development and climate change can either accelerate aquatic alien plants establishment or the spread of invaders. It is necessary to intensify the risk assessment before introduction and prediction of the potential distributions of invading species. Our findings may assist in predicting aquatic plant invasions and the rational utilization of aquatic plant resources, as well as provide important implications for native plant biodiversity protection under rapid global change.

AUTHOR CONTRIBUTIONS

JD conceptualized the overall structure. HW collected and analyzed all the data. HW and JD wrote the manuscript and approved this final version of the manuscript to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.00787/full#supplementary-material

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Mutual Facilitation Among Invading Nuttall's Waterweed and Quagga Mussels

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Nuttall's waterweed (*Elodea nuttallii*) is the most abundant invasive aquatic plant species in several European countries. *Elodea* populations often follow a boom-bust cycle, but the causes and consequences of this dynamics are yet unknown. We hypothesize that both boom and bust periods can be affected by dreissenid mussel invasions. While mutual facilitations between these invaders could explain their rapid parallel expansion, subsequent competition for space might occur. To test this hypothesis, we use data on temporal changes in the water quality and the abundance of *E. nuttallii* and the quagga mussel *Dreissena rostriformis bugensis* in a temperate shallow lake. Lake Müggelsee (Germany) was turbid and devoid of submerged macrophytes for 20 years (1970–1989), but re-colonization with macrophytes started in 1990 upon reductions in nutrient loading. We mapped macrophyte abundance from 1999 and mussel abundance from 2011 onwards. *E. nuttallii* was first detected in 2011, spread rapidly, and was the most abundant macrophyte species by 2017. Native macrophyte species were not replaced, but spread more slowly, resulting in an overall increase in macrophyte coverage to 25% of the lake surface. The increased abundance of *E. nuttallii* was paralleled by increasing water clarity and decreasing total phosphorus concentrations in the water. These changes were attributed to a rapid invasion by quagga mussels in 2012. In 2017, they covered about one-third of the lake area, with mean abundances of 3,600 mussels m^{-2}, filtering up to twice the lake's volume every day. The increasing light availability in deeper littoral areas supported the rapid spread of waterweed, while in turn waterweed provided surface for mussel colonization. Quantities of dreissenid mussels and *E. nuttallii* measured at 24 locations were significantly correlated in 2016, and yearly means of *E. nuttallii* quantities increased with increasing mean dreissenid mussel quantities between 2011 and 2018. In 2018, both *E. nuttallii* and dreissenid abundances declined. These data imply that invasive waterweed and quagga mussels initially facilitated their establishment, supporting the invasional meltdown hypothesis, while subsequently competition for space may have occurred. Such temporal changes in invasive species interaction might contribute to the boom-bust dynamics that have been observed in *Elodea* populations.

Keywords: macrophyte, lake, invasional meltdown hypothesis, competition, invasive species

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INTRODUCTION

Positive interactions can play a decisive role in shaping communities and regulating ecosystem structure and function (Halpern et al., 2007; Brooker et al., 2008; Soliveres et al., 2015). Based on the widespread occurrence of positive interactions between non-native species, Simberloff and Von Holle (1999) developed the "invasional meltdown hypothesis (IMH)" suggesting that non-indigenous species can facilitate one another's invasion in various ways, increasing the likelihood of survival and/or of ecological impact, and possibly the magnitude of impact. A review of invasions in the Great Lakes by Ricciardi (2001) indeed showed that direct positive (mutualistic and commensal) interactions among introduced species were more common than negative interactions. Recently, Braga et al. (2018) used the hierarchy-of-hypotheses approach to differentiate key aspects of the IMH. While the majority of studies supported the IMH, studies at the community or ecosystem level were rare, especially for cases where two non-native species interact and both species are affected. There are also only few studies on indirect positive interactions, as these are often difficult to detect and measure, and thus often neglected (White et al., 2006).

Here, we present an observational field study on the rapid invasion of two non-indigenous species and their potential direct and indirect interactions during this process in a temperate polymictic freshwater lake. Similar to many other freshwater bodies, Lake Müggelsee (Germany) had almost completely lost its submerged macrophytes due to eutrophication during the last century. Following significant reductions in nutrient loading, macrophytes slowly started re-colonizing the lake, but deeper littoral areas still remained sparsely covered or devoid of macrophytes (Hilt et al., 2013, 2018). During the last decade, two major invaders entered the lake, Nuttall's waterweed (*Elodea nuttallii*, **Figure 1B**) and the quagga mussel (*Dreissena rostriformis bugensis*, **Figure 1D**), and were both first detected around 2011/12. We hypothesize that mutual facilitation among these invaders could explain their rapid parallel expansion.

E. nuttallii, native in Northern America, was introduced into Europe in 1939. It spread rapidly and became the most abundant non-indigenous aquatic plant species in several countries, owing to its ability to easily colonize new areas by fragments (Wolff, 1980; Hussner, 2012). It is also a common pioneer species colonizing lakes after restoration measures such as biomanipulation of the fish community (Hilt et al., 2018). Excessive growth can cause severe nuisance for fishery and tourism in lakes (Hilt et al., 2006; Zehnsdorf et al., 2015) and can decrease biodiversity by out-competing less robust native macrophyte species and by releasing allelochemicals (Barrat-Segretain, 2005; Kelly and Hawes, 2005; Erhard and Gross, 2006). *Elodea* species often follow a boom-bust dynamic (Simberloff and Gibbons, 2004; Strayer et al., 2017); however, the causes and consequences of these dynamics are yet unknown. In principle, *E. nuttallii* could facilitate an invasion of *D. r. bugensis* directly by providing surface for attachment of mussels and indirectly by oxygen production. Survival of quagga mussels is strongly reduced at low oxygen concentrations (De Ventura et al., 2016). While native macrophytes also provide surface and oxygen, invasive macrophytes are often faster in colonizing newly available habitats after disturbances (Chytry et al., 2008) and *E. nuttallii* is expected to spread fast into deeper littoral areas that have begun to receive sufficient light for macrophyte growth after a quagga mussel invasion. Invasive macrophytes have also been found to specifically facilitate the establishment of invasive bivalves (Michelan et al., 2014), but the mechanisms remain unknown. In contrast, dense macrophytes can have negative effects on phytoplankton abundance (Scheffer et al., 1993) and thus may restrict food availability for mussels (Reusch and Williams, 1999).

D. r. bugensis, native in the Ponto-Caspian region, was first detected in Western Europe in 2004 (Paulus et al., 2014). It is one of the world's most problematic biological invaders and has been shown to affect the biogeochemistry, flora, and fauna of lakes and rivers across North America and Eurasia. It can shift aquatic food webs and energy flow from pelagic-profundal to benthic-littoral energy pathways (Higgins and Vander Zanden, 2010). *D. r. bugensis* could indirectly facilitate the invasion of *E. nuttallii* by increasing the light availability at the littoral of lakes due to its high filtration capacity. Direct effects of dreissenid mussels on submerged macrophytes through nutrient relocation into littoral areas seem possible, but appeared to be less important than the positive effects associated with increased light penetration (Zhu et al., 2007).

We tested the hypothesis of mutual facilitation between these two non-native species using data from Lake Müggelsee on the coverage and quantity of submerged macrophytes from 1999–2018 and of *E. nuttallii* and *D. r. bugensis* during their establishment from 2011–2018, and water quality parameters such as water transparency, concentrations of total phosphorus (TP) and chlorophyll *a.*

MATERIALS AND METHODS

Lake Müggelsee

Lake Müggelsee is the largest lake in Berlin (Germany, 52°26′, 13°39′, **Figure 1A**). It has a surface area of ~7.4 km^2, a volume of 36,560,000 m^3, a water retention time of around 100 days (Gillefalk et al., 2018), and a catchment area of 7,000 km^2. It is a shallow, polymictic lake with a mean depth of 4.9 m and maximum depth of about 8 m (Hilt et al., 2013) with extensive shallow areas at the northeastern, eastern, and southeastern shores. In 1970, the lake had almost completely lost its submerged macrophytes as a result of eutrophication. The external loading of nitrogen (N) and phosphorus (P) to Lake Müggelsee decreased significantly between the late 1970s and 2016. Total N (TN) loads decreased from 140 ± 38 g TN m^{-2} a^{-1} in the 1980s to 30 ± 11 g TN m^{-2} a^{-1} in the decade 2007–2016. Total P (TP) loads decreased from 6 ± 1 to 2 ± 0.4 g TP m^{-2} a^{-1} in the same period (Shatwell and Köhler, 2019). Macrophytes started re-colonizing the lake from 1990 onwards along with reductions in external nutrient loading. Re-colonization, however, was slow and the macrophyte community was dominated by a few species,

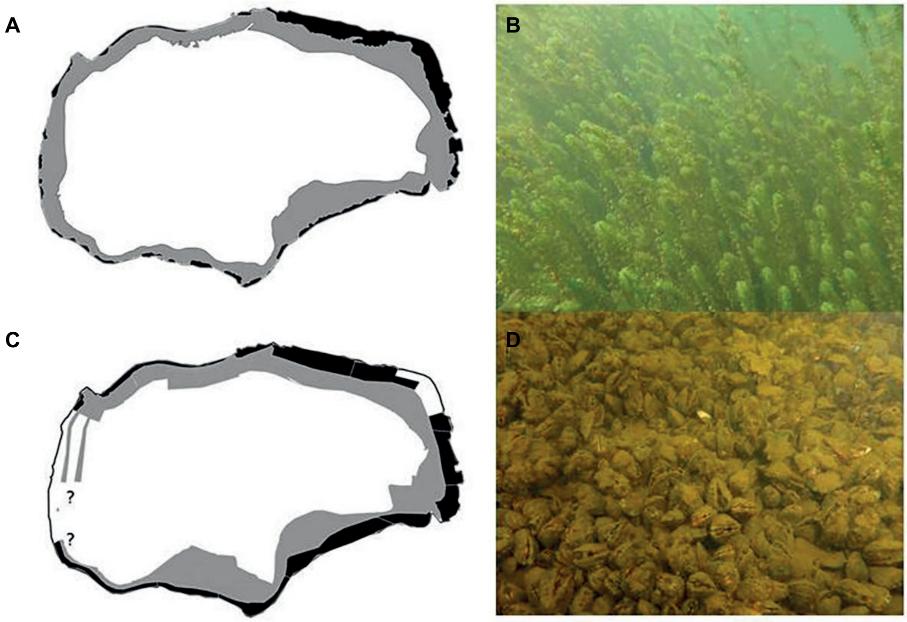

FIGURE 1 | Colonized area of submerged macrophytes **(A)** in 1999 (black area) and 2017 (black and grey area) and of dreissenid mussels in 2017 **(C)** in Lake Müggelsee. Black area in **(C)**: hypothetical distribution of *Dreissena polymorpha* before *D. r. bugensis* invasion down to 2 m due to restriction to hard substrates, black area + grey area in **(C)**: combined distribution of both dreissenid species in 2017. ?: uncertainties in the dreissenid distribution in 2017 due to restricted accessibility *via* motorboat. Photos: *Elodea nuttallii* **(B)** and *D. r. bugensis* **(D)** in Lake Müggelsee in 2017.

mainly sago pondweed [*Stuckenia pectinata* (L.) Börner] for about 20 years (Hilt et al., 2013, 2018).

From 1978 onwards, water samples have been taken and Secchi disk transparency in the water column recorded weekly (summer) or biweekly (winter). Volumetrically weighted integrated samples were taken from 21 subsamples from five different points on the lake since 1987 and concentrations of total phosphorus (TP) and chlorophyll *a* (chl *a*) have been determined [for details see Shatwell and Köhler (2019)]. We have used data for the last 20 years (1999–2018).

Submerged Macrophytes

We used two different approaches for macrophyte mapping: (1) a transect-based mapping which allowed us to follow the detailed development of species, their abundances in different depth zones, and maximum colonization depth (MCD) between 1999 and 2018, and (2) an entire lake mapping of the abundance of macrophytes and maximum colonization depth (MCD) of macrophytes to estimate the total lake coverage in 1999 and 2017.

Transect data are available for 8 years, and macrophytes were always mapped in June or early July. In 2006, 2011, and 2014–2018, submerged macrophytes were mapped at eight transects covering the diversity of habitats in the lake (in 2006 only 5 of those) according to the PHYLIB method developed for the implementation of the EU Water Framework Directive in Germany (Schaumburg et al., 2004). In addition, data of a detailed mapping in 1999 (Körner, 2001) were transformed into the PHYLIB method for the eight selected transects, which was possible due to the low abundance and low MCD. In 1999 and 2006, mappings were performed using wading and aquascopes because MCD (determined by aquascopes and extensive raking of deeper areas from a boat) were low, while scuba diving has been used since 2011.

Maximum colonization depths were recorded for each transect, and abundances were estimated in up to four depth zones (0–1, 1–2, 2–4, and 4–6 m) based on a five-degree scale (1: very rare; 2: rare; 3: common; 4: frequent; and 5: abundant). To obtain at macrophyte quantities, abundance data were exponentiated with 3 to reflect the three-dimensional development of macrophytes (Schaumburg et al., 2004).

Entire lake mappings of macrophytes were performed in 1999 by wading with an aquascope and GPS (for details see Körner, 2001) and in 2017 by using an underwater camera (see Van de Weyer et al., 2007).

Abundance and Quantities of Dreissenid Mussels

Data on dreissenid mussel abundance are only available since 2011. We also used two different approaches for dreissenid mussel mapping: (1) a transect-based mapping which followed their abundances in different depth zones (same as for macrophytes, see section "Submerged Macrophytes") in 2011 and 2015–2018, and (2) an entire lake mapping of the dreissenid mussel abundance and their maximum colonization depth, used to estimate the total lake coverage and to calculate filtration activities in 2017 (see section "Determination of Filtration Rates by Dreissenids"). For the transect-based mapping, two approaches were applied. In 2011, 24 sediment samples were taken at different water depths using a Van Veen grab (sampling 600 cm^2) and mussels were counted at eight transects in parallel to the macrophyte mapping. In 2015–2018, the abundance of dreissenid mussels was recorded by scuba divers in parallel to macrophyte mapping at eight transects using four abundance classes: 0: no dreissenids, 1: up to 33% sediment coverage, 2: 33–66%, and 3: 66–100% sediment coverage. To transform mussel numbers from 2011 into these abundance classes, divers collected dreissenid mussels at 13 locations with different depths and abundance classes at three transects from an area of 170 cm^2 in 2017. A significant linear regression between mussel numbers and abundance class ($y = 10{,}680x$, $R^2 = 0.52$, $p = 0.003$; **Figure 2**) allowed us to calculate threshold levels for the transformation of mussel numbers in 2011 into abundance classes (0: no mussels, 1: 1–10,680 dreissenid mussels m^{-2}, 2: 10,681–21,360 dreissenid mussels m^{-2}, 3: >21,361 dreissenid mussels m^{-2}). Mean quantities were calculated by averaging (square-root) dreissenid abundances measured at the respective transects in different depths inside of each depth class.

Determination of Dreissenid Species and Densities

To determine densities and species distributions of dreissenids, 40 samples were taken from different water depths at 13 transects (3–5 samples per transect) and along the northern shore littoral between October 2017 and January 2018 using an Ekman-Binge sediment sampler (Hydro-Bios®) with a sample area of 225 cm^2. Locations with dreissenid coverages of 66–100% were selected to take sediment samples at water depth intervals of 1 m (±0.5 m). Five locations with dense dreissenid coverage on rock fill or shallow sandy parts were sampled with a shovel. Samples were sieved (0.5 mm) and stored in 1 L wide neck plastic containers at 5°C until further treatment (maximum 20 days). Samples were separated into living and dead *D. r. bugensis* and *Dreissena polymorpha*, respectively, based on morphological identification and each fraction was counted. Living individuals were measured and assigned to size-classes of <1.0, 1.0–2.0, and >2.0 cm.

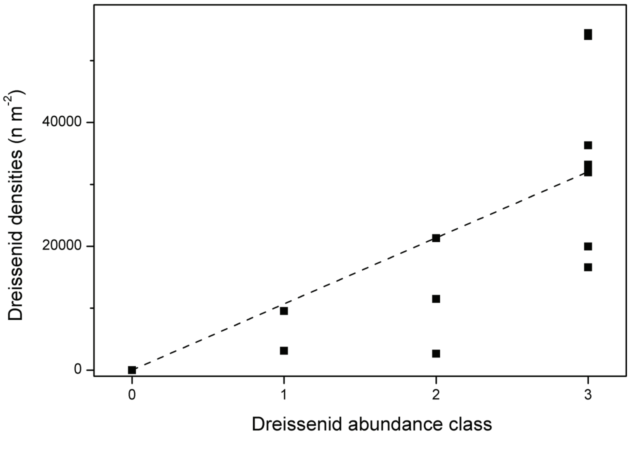

FIGURE 2 | Dreissenid mussel densities per abundance class at three transects in Lake Müggelsee in 2017.

Genetic Verification of Differentiation Between Dreissenid Species

Species determination of dreissenids based on morphological characteristics has been proven difficult or inconsistent due to habitat-specific growth patterns (May and Marsden, 1992; Beggel et al., 2015). Beggel et al. (2015) suggested applying molecular methods to differentiate between *D. r. bugensis* and *D. polymorpha*, and we therefore sequenced the standard mitochondrial DNA Cytochrome c oxidase I (*cox1*) barcode sequence to verify our morphological identifications (Marescaux and Van Doninck, 2013). Total genomic DNA was extracted from 42 *Dreissena* individuals sampled in 2017 of one eastern, one western, and one southwestern location in depths of 2.20, 2.30, and 2.50 m, respectively, using the Dneasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. A 658-bp fragment of *cox1* was amplified following Marescaux and Van Doninck (2013) with the following modifications. Amplifications were performed in a total volume of 25 μl (with 1 μl of DNA, 1× mi-Taq only reaction buffer (green cap; Metabion, Planegg/Steinkirchen, Germany), 200 μM of dNTPs (Metabion), 0.4 μM of each primer, and 1 U of mi-Taq only DNA polymerase (Metabion). PCR cycling conditions were 95°C for 4 min, 30 cycles of 95°C for 45 s, 49°C for 45 s and 72°C for 45 s, and 72°C for 10 min. Sanger sequencing in both directions of COI fragments was performed by LGC Genomics GmbH (Berlin, Germany). Sequences were merged and quality-trimmed using Geneious 10.0.6 (Biomatters ApS, Aarhus, Denmark) and then sequences were used as queries in searches of the NCBI nucleotide database using the *blastn* alorithm[1].

Comparison with NCBI database indicated that our morphological identification was correct in each case. There were >100 bp changes between the two species, and no differences among individuals sampled from Lake Müggelsee. Our *D. r. bugensis* haplotype matched that of individuals from the Meuse River in eastern France, and the *D. polymorpha* haplotype was an exact match to individuals sampled from Poland and North America (New York, Ontario).

Determination of Filtration Rates by Dreissenids

In November 2017, dreissenid coverage of the sediments was mapped in the entire lake using an underwater camera mounted on a motorboat. A SELVAG® OC-1 outdoor surveillance camera attached to an 8-m long monitor microphone TS cable was directed toward the lake bottom. The analogue camera image was converted to a digital signal using a USB-audio and video converter and displayed on a

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[1] https://blast.ncbi.nlm.nih.gov/Blast.cgi

computer screen. Dreissenid mussel sediment coverages were assessed while looking at the live camera image and assigned to the four coverage classes (see section "Abundance and Quantities of Dreissenid Mussels") simultaneously with coordinates, water depth, date, and time. To map areas deeper than 5 m, where visibility was usually lower, the boat was anchored at depth-intervals of 0.5 m to enable a steady image of the lake bottom.

We subdivided the whole lake into six radial sub-areas (in terms of cardinal directions) while ensuring that a minimum of four samples (see section "Determination of dreissenid species and densities") was located in each sub-area. Median population densities of dreissenids were determined for each sub-area. Population densities were transformed (square-root) to achieve normal distribution, and the effect of sub-area on population densities was analyzed *via* one-way ANOVA. A subsequent *post-hoc* unequal n Tukey's HSD-test was used to determine significant differences among sub-areas regarding the mussels' median population densities. Sub-areas with representative median population densities were then intersected with the coverage classes of dreissenids to yield dreissenid abundance for each lake-sub-area.

Since samples were generally retrieved from locations with dreissenid sediment coverages of 3 (66–100%), population densities of sub-areas with sediment coverages of 2 (33–66%) were multiplied with the correction factor 0.6 and those of sediment coverages classes 1 (1–33%) with factor 0.3. By this means, dreissenid abundances per lake-area were calculated and ultimately summated to a whole-lake abundance of each species, as well as both species combined. For the calculation of the filtration capacity of all dreissenids, we used filtration rates (FR) determined for *D. polymorpha* in Reeders et al. (1989) in two Dutch shallow lakes (Ijsselmeer and Markermeer). Reeders and Bij de Vaate (1990) found that the effect of season and temperature only set a wide limit to the filtration activity, but water temperature below 5°C significantly decreased FR. Hence, FR were adjusted by duration (weeks) of water temperature of Lake Müggelsee with values >5°C (79%) and ≤ 5°C (21%) in 2017. Summed, seasonally adjusted FR were multiplied by median total abundance of both dreissenid species combined. To arrive at the fraction of lake water volume filtered per day, the product of total filtration per day was divided by the water volume of Lake Müggelsee. Mei et al. (2016) legitimized the application of FR reported for zebra mussels to quagga mussels.

Statistics

We tested for significant correlations between the quantities of *E. nuttallii* and native macrophytes measured at 24 sites over 6 years ($n = 144$) using Spearman correlations. We also tested for significant correlations between the quantity of *E. nuttallii* and native macrophytes in the depth zone 2–4 m with MCD of macrophytes.

Relationships between the quantities of dreissenids and *E. nuttallii* or native macrophytes were investigated with Spearman correlations using the data available for eight transects and three depth zones ($n = 24$) separately for each of the years 2011 and 2015–18. Additionally, we tested for significant relationships between the yearly mean quantities of dreissenid mussels and *E. nuttallii* or native macrophytes for the period 2011–18 ($n = 5$) by best fit non-linear regressions as well as between dreissenid mussels and Secchi disc transparency in winter (October to March) and spring (April to June) by linear regressions. All statistical analyses were performed using SPSS 19.

RESULTS

Submerged Macrophytes

Total coverage of submerged macrophytes in Lake Müggelsee increased from 5% in 1999 to 25% in 2017 (**Figures 1A,C**). The six most abundant submerged macrophytes in Lake Müggelsee during its re-colonization were the native species *S. pectinata, Potamogeton perfoliatus, Najas major, Fontinalis antipyretica*, *Ceratophyllum demersum*, and (from 2011) the invasive *E. nuttallii*. Until 2006, only *S. pectinata, P. perfoliatus,* and *N. major* were present in significant quantities and hardly any macrophytes were found in depth zones below 2 m (**Figures 3A–C**). High mean quantities were only reached by *S. pectinata* in the shallowest depth zone (0–1 m). *E. nuttallii* was first discovered with low quantities at two transects at the south-eastern shore in depth zones 1–2 and 2–4 m in 2011. Subsequently, it spread to the entire lake and was present at all eight transects in 2017. Its quantity per site increased between 2011 and 2017 along with that of other native macrophyte species, and their quantities at the investigated sites were significantly positively correlated (Spearman correlation, $R = 0.445$, $p < 0.001$, $n = 144$). The number of native macrophyte species per site also increased during this period and was positively correlated to the quantities of native macrophytes and *E. nuttallii* (Spearman correlations, $R = 0.691$, $p < 0.001$; $R = 0.504$, $p < 0.001$, respectively, $n = 144$). In 2017, *E. nuttallii* became the most abundant macrophyte species in Lake Müggelsee, while *S. pectinata* used to dominate the macrophyte community before 2017 (**Figures 3A–C**). Highest mean quantities of *E. nuttallii* were observed in depth zones 1–2 and 2–4 m (**Figures 3B,C**). In 2018, mean *E. nuttallii* quantities significantly declined as compared to 2017 (**Figures 3A–C**, Wilcoxon test, $p = 0.05$). Mean quantities of most native submerged macrophytes increased, in particular that of *N. major* in depth zones 0–1 and 1–2 m, of *C. demersum* in depth zones 1–2 and 2–4 m and of *F. antipyretica* in depth zone 2–4 m (**Figures 3A–C**). Mean maximum colonization depth (MCD) of macrophytes at eight transects increased from 0.9 ± 0.2 m in 1999 to 3.6 ± 0.3 m in 2018 (**Figure 4A**). Quantities of both, *E. nuttallii* and native macrophytes in the depth zone 2–4 m, were positively correlated with MCD (Spearman correlations, $R = 0.463$, $p = 0.001$; $R = 0.441$, $p = 0.002$, respectively, $n = 48$).

Dreissenid Mussels

In 2017, dreissenid mussels covered about one third of the sediment surface of Lake Müggelsee. Grab samples taken in

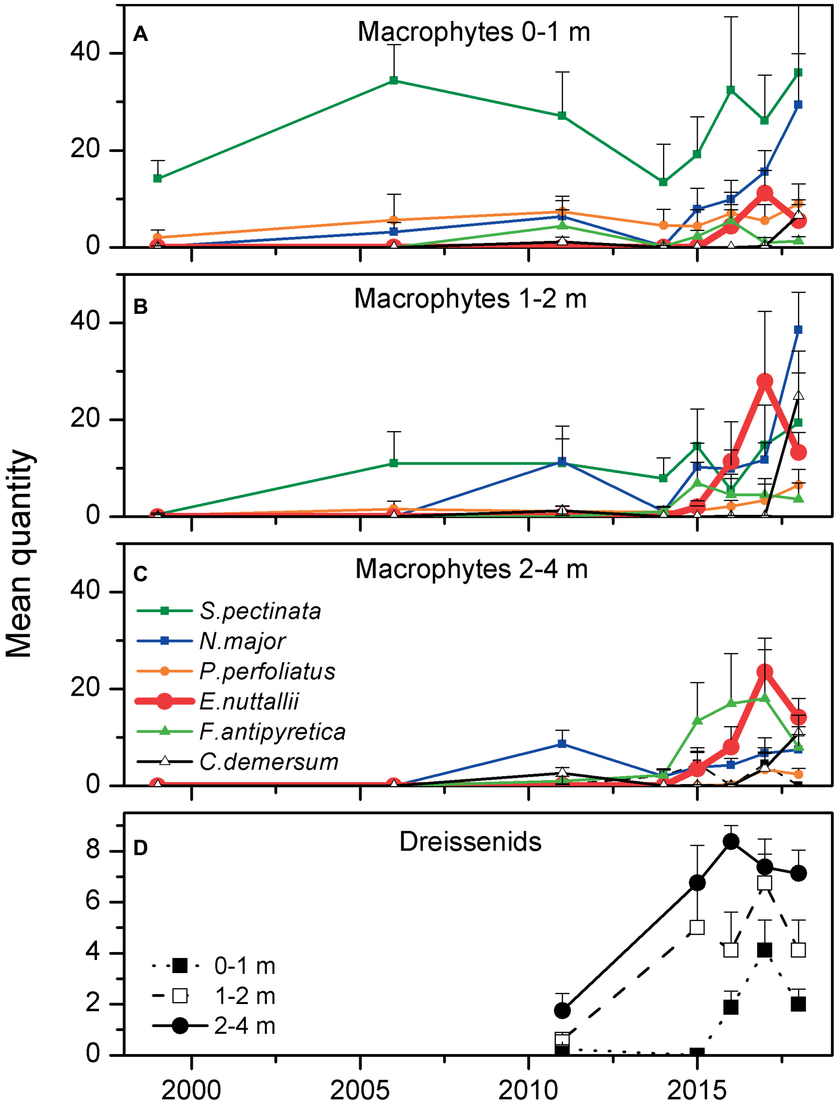

FIGURE 3 | Mean quantity (see section "Submerged Macrophytes") of the six most common submerged macrophyte species (+standard error) measured at eight transects in three different depth zones (**A**: 0–1 m, **B**: 1–2 m, **C**: 2–4 m) and mean quantity of dreissenid mussels (+standard error) in the different depth zones **(D)** at eight transects in Lake Müggelsee between 1999 and 2018.

2017 were composed of 97.3% *D. r. bugensis* and 2.7% *D. polymorpha*. A larger proportion of *D. polymorpha* (11.0% of all dreissenids) was only found in nearshore samples taken from rock fill and sand with high contents of stones. Maximum colonization depth was on average 5.3 m (**Figure 1C**). Population densities of dreissenids varied between 1,600 and 46,000 mussels m^{-2}, with a median of 12,800 $mussels^{-2}$. Weighted by the share of coverage classes, whole-lake density was 3,600 dreissenid mussels m^{-2} (**Table 1**). The effect of lake-sub-area on dreissenid mussel densities was significant ($F_{5,34}$ = 4.56, p = 0.003). Two main sub-areas were considered by merging (1) N, NE, and E sub-areas into one (~42% of whole lake-area) with a median density of ~10,000 mussels m^{-2} (abundance ~8.6 × 10^9) and (2) SE/S, SW/W, and NW sub-areas (58% of whole lake-area) with median densities of ~20,000 mussels m^{-2} (abundance ~19.6 × 10^9). The total abundance of *D. r. bugensis* of ~28.2 × 10^9 resulted in the clearance of filtered lake water about 1.9 times per day.

In 2011, dreissenid mussels comprised only *D. polymorpha* and 50% of the investigated 24 locations (three depth zones at each of the eight transects) did not have any dreissenid mussels. Abundance class 3 did not occur and abundance class 2 was only measured in depth zone 2–4 m at three transects. From 2015 (no data are available for the period 2012–14) onwards, much higher mean quantities were observed in all depth zones and highest values were reached in the depth zone 2–4 m (**Figure 3D**). In 2018, mean dreissenid quantities dropped as compared to 2017 (**Figure 3D**, Wilcoxon test,

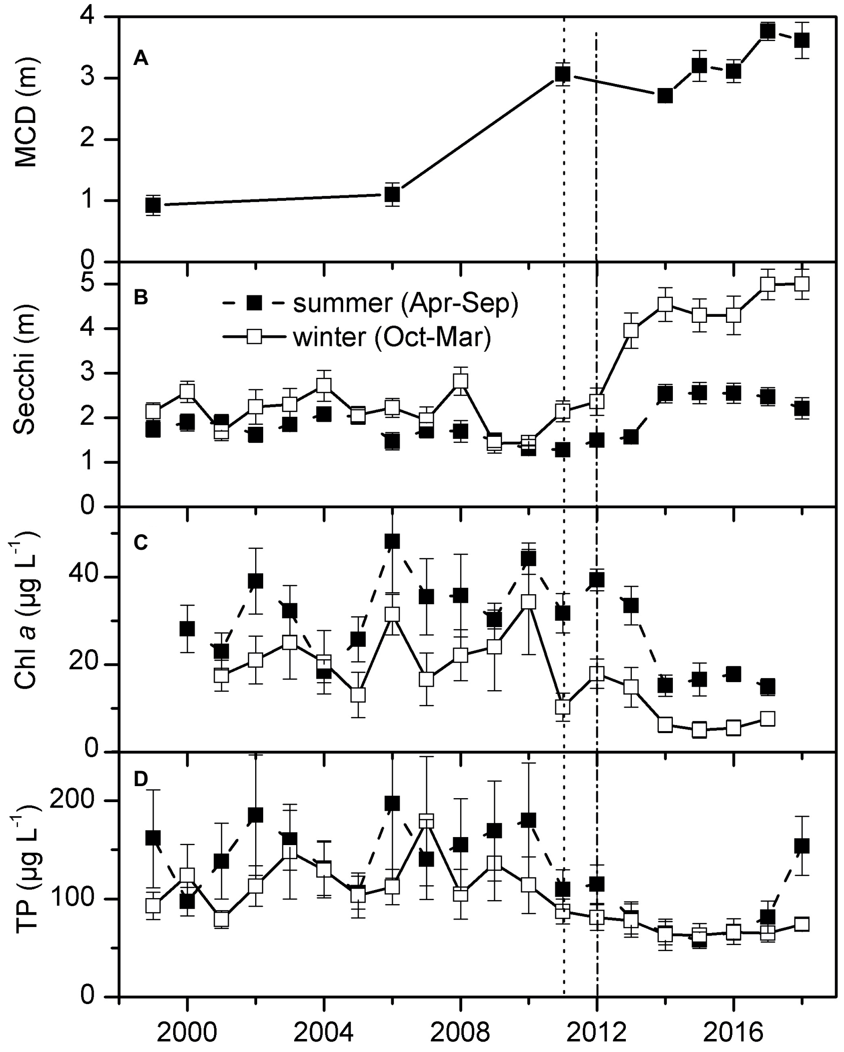

FIGURE 4 | Maximum colonization depth of submerged macrophytes (MCD, measured at the end of June at 8 transects) **(A)**, summer (April–September) and winter (October–March) Secchi disk transparency **(B)** and concentrations of total phosphorus (TP) **(D)** and chlorophyll (chl) *a* in Lake Müggelsee [means of weekly (summer) or biweekly (winter) measurements ± standard error] **(C)**. Vertical lines represent first detection of *E. nuttallii* (dots) and assumed start of invasion of *D. r. bugensis* (dash-dot).

$p = 0.05$). When separately testing data of each year, a significant positive correlation was found between quantities of dreissenids and *E. nuttallii* in 2016 (Spearman correlation, $R = 0.47$, $p = 0.02$, $n = 24$), while quantities of native macrophytes did not show significant correlations with quantities of dreissenids. Using yearly means of all 24 locations, a significant correlation (non-linear regression: $y = 0.06x^{3.2}$, $R^2 = 0.96$, $p = 0.004$) was found between quantities of dreissenids and *E. nuttallii* (**Figure 5A**).

Lake Water Quality

Secchi disk transparency in Lake Müggelsee showed low interannual variability and were only slightly higher in winter than in summer between 1999 and 2010. A significant increase in winter Secchi disk transparency was observed between 2010 and 2012, the period when *E. nuttallii* and *D. r. bugensis* colonized the lake. Mean summer Secchi disk transparency only increased significantly between 2013 and 2014 (**Figure 4B**). A significant linear regression was found between yearly mean quantities of dreissenids and mean Secchi disk transparency in April–June for the period 2011–18 (**Figure 5B**, linear regression: $y = 0.43x + 0.89$, $R^2 = 0.8$, $p = 0.04$). Concentrations of Chl *a* (**Figure 4C**) and TP (**Figure 4D**) decreased in parallel with increased Secchi disk transparency (**Figure 4B**).

DISCUSSION

Our results provide empirical evidence for IMH during the initial establishment of two important aquatic invaders and

TABLE 1 | Comparison of population densities of quagga mussels (*Dreissena r. bugensis*) in different lakes.

Lake	Investigated depths (m)	Mean quagga mussel density (n m^{-2})	Reference
Szczecin Lagoon (PL)	3.8–8.5	4,000 ± 355	Woźniczka et al., 2016
Lake Mead (USA)	2–112	747 ± 398	Wittmann et al., 2010
Lake Müggelsee (DE)	0.7–5.6	3,600 ± 1,200	This study
Lake Eem (NL)	2.1	2,300	Noordhuis et al., 2016
Lake Simcoe (CA)	2–20	334 ± 65	Ginn et al., 2018
	>20	39 ± 21	
Lake Ontario (CA)	5–20	9,400 ± 7,200	Wilson et al., 2006
Lake Huron (USA)	46–73	72–811	French et al., 2009
Lake Michigan (USA)	20–45	6,900 ± 4,500	Nalepa 2010
Lake Erie (USA)	0–>24	380 ± 40	Karatayev et al., 2014

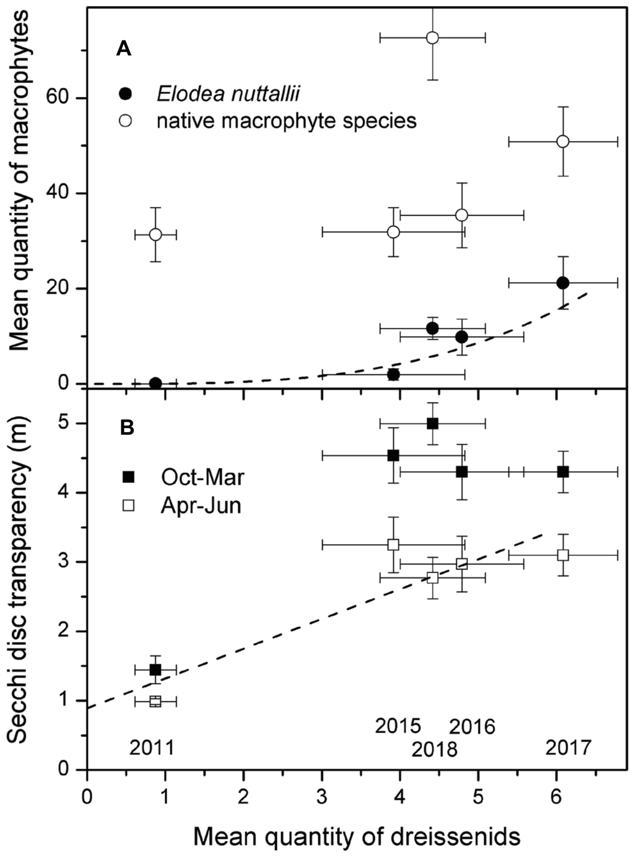

FIGURE 5 | Relationships between the yearly mean quantities of dreissenid mussels and *E. nuttallii* or native macrophytes for the period 2011–2018 ($n = 5$) **(A)** and between yearly mean quantities of dreissenid mussels and mean Secchi disc transparency in winter (October to March) and spring (April to June) **(B)**. Lines represent significant regressions, years are given in **(B)**.

suggest a subsequent change of the invader-invader-interaction into competition for space. We observed a significant correlation between quantities of dreissenids and *E. nuttallii* at the site level in 2016 and a significant non-linear regression between yearly means (2011–2018) of quantities of dreissenids and *E. nuttallii* at the whole-lake level, indicating a mutual facilitation between the establishment of quagga mussels (97% of dreissenids in 2017) and the waterweed in a temperate freshwater lake during the first 4–6 years of their invasion. Mussel filtration induced an increase in water clarity, supporting macrophyte colonization in deeper littoral zones, while macrophytes provided substrate for the attachment of young mussels and produced oxygen which may help to prevent hypoxia during summer months. We propose that the invasive *E. nuttallii* was able to rapidly and efficiently make use of the newly available habitat in deeper littoral areas once light availability increased compared to native macrophytes. This is due to its ability to spread by fragments, its rapid growth rates, survival into winter months, and compensation of losses by herbivory through branching. This can give *E. nuttallii* a temporal advantage over native macrophytes. About 5 years after starting the invasion, *E. nuttallii* dominated the submerged macrophytes, covering 25% and quagga mussels colonizing 33% of the lake's sediment surface. In 2018, quantities of both dreissenids and *E. nuttallii* decreased, suggesting a competition for space in the lake which could be a reason for the boom-bust dynamics the latter species is known for. The spread of native macrophytes can re-inforce this process.

Invasion of Waterweed and Quagga Mussels and Mutual Facilitation

Nuttall's waterweed invaded Lake Müggelsee at some point between 2006, when it was not present, and 2011, when it was detected for the first time at two transects at the southeastern shore. This location suggests that *E. nuttallii* entered the lake *via* River Spree. The establishment in the lake took about 5 years until 2016 and 2017, when *E. nuttallii* was found at 6 and 8 out of 8 transects, respectively. Native macrophyte quantities increased at a slower pace, which confirms findings of Kelly et al. (2015) in Irish lakes, but contrasts laboratory experiments that show *E. nuttallii* can outcompete other submerged species (Barrat-Segretain, 2005). Despite a parallel increase in native macrophyte abundance, *E. nuttallii* was initially faster in colonizing the new habitat in deeper littoral areas after increased light availability than native macrophytes. We assume that this was due to a combination of traits such as rapid spread by fragments (Hussner, 2012), rapid growth rates, being wintergreen and growth at low temperature (Kunii, 1981) and compensating biomass losses by herbivory through branching (He et al., 2019). In addition, *E. nuttallii* can take up phosphorus (P) *via* shoots and roots (Angelstein and Schubert, 2008) and thus can directly make use of the P translocated by quagga mussels from pelagic to littoral areas ("benthic shunt", Hecky et al., 2004). However, P was not a limiting factor for macrophyte growth in Müggelsee, as this would require much lower P concentrations in the water (Gross, 2009).

E. nuttallii invaded the lake when Secchi disk transparency was still rather low in summer and winter, while maximum colonization depths of macrophytes were already deeper than 3 m, but total macrophyte quantities in the depth zone 2–4 m were still lower than in shallower parts of the lake. From winter 2013/14 onwards, winter water clarity suddenly was about twice higher than before and from 2014 onwards, water clarity was also higher in summer. Quagga mussel invasion (see below) is assumed to be responsible for this effect. *E. nuttallii* has been observed to grow even at 4°C, shoot elongation starts in spring at about 10°C (Kunii, 1981) and green shoots have been found washed ashore at Lake Müggelsee in December 2017 (S.H., personal observation). Thus, it could probably take most advantage of the higher light availability in winter and spring and increase its abundance into deeper water before most other competing native species also spread into this depth zone. The native species *F. antipyretica* is also wintergreen (Maberly, 1985) and its strong increase in quantity in the depth zone 2–4 m suggests that this species also gained from higher water clarity in winter. However, due to its lower growth rate as compared to *Elodea* (Sand-Jensen and Madsen, 1991), the invasive *E. nuttallii* at least temporarily dominated the submerged vegetation of Lake Müggelsee in 2017.

Existing knowledge on mechanisms of invasions of aquatic plant communities is still limited (Fleming and Dibble, 2015). However, several examples also suggest a link between the spread of invasive macrophytes and *D. polymorpha* induced turbidity reductions in lakes (Skubinna et al., 1995; MacIsaac, 1996; Zhu et al., 2006). Because quagga mussels can colonize all regions of a lake, and form larger populations, they may filter larger water volumes and may thus have even greater effects on macrophyte abundance than *D. polymorpha*, which are restricted to shallower portions of lakes (Karatayev et al., 2015). In the Dutch Lake Eem, the establishment of quagga mussels was also paralleled by an increased macrophyte abundance, but only by native species (Noordhuis et al., 2016).

Quagga mussels in Lake Müggelsee constituted 97% of the total *Dreissena* population in 2017, indicating that their invasion started around 2012 based on a spread model for *D. r. bugensis* in Western Europe by Heiler et al. (2013). The lake has been invaded by the congener *D. polymorpha* decades earlier. This species, however, was limited to littoral areas with hard substrates (Karatayev et al., 2015). In contrast, *D. r. bugensis* can also colonize soft sediments (Karatayev et al., 2015) and thus could reach a mean maximum colonization depth of 5.3 m and a total coverage of about a third of Lake Müggelsee's sediment in 2017. Quagga mussel densities in Lake Müggelsee were high in 2017, but similarly high values have also been found in other lakes (**Table 1**). Filtration capacities in Müggelsee were comparable to those found in Dutch shallow lakes (Noordhuis et al., 2016) and Lake Erie (Vanderploeg et al., 2002).

Although not directly measured, *E. nuttallii* were supposed to have supported quagga mussel invasion by provision of surface for attachment. A number of studies indicate that macrophytes provide a suitable substrate for dreissenid mussel attachment. Körner et al. (2002) found *D. polymorpha* being the most abundant invertebrates on submerged macrophytes in Lake Müggelsee. Musko and Bako (2005) reported that *D. polymorpha* represented 2–85% of all animals on submerged macrophytes and their density ranged between 9 and 2,000 individuals g^{-1} macrophyte dry mass. Although studies on quagga mussel abundance on macrophytes are lacking, we assume that macrophytes support zebra and quagga mussel attachment in a similar way. Diggins et al. (2004) reported submerged macrophytes as a refuge for zebra mussels during quagga mussel invasion in the North American Great lakes.

E. nuttallii (together with the native macrophytes) may also have supported quagga mussels by oxygen production. Low oxygen concentrations (De Ventura et al., 2016) limit the survival of dreissenids and regular annual hypoxia (oxygen concentrations below 2 mg L^{-1}) excluded dreissenids (Karatayev et al., 2018a). Assuming an average increase in *E. nuttallii* biomass in June by about 100 g dry weight m^{-2} in dense stands (Cook and Urmi-König, 1985) and a carbon content of 33% dry weight (Velthuis et al., 2017), the additional oxygen production amounts to about 20 mg L^{-1} in a 4 m water column in 30 days. In comparison, phytoplankton would produce about 4 mg O_2 L^{-1} (means of net production in 2011–2017, Köhler, unpublished data). Available studies show that macrophyte biomass can be the most influential environmental factor on the fluctuation of dissolved oxygen concentrations in the bottom water of lakes (Haga et al., 2006; Vilas et al., 2017). While aquatic plants are usually thought of as providing oxygen to aquatic environments, they can also engineer extremely low values (Caraco et al., 2006). Vilas et al. (2017) report on the development of night-time anoxic conditions close to the sediments when macrophytes occupied at least 50% of the water column and induced stratification.

Competition for Space

Karatayev et al. (2015) suggested that quagga mussels provide additional space and food for many invertebrates in the littoral zone, and thus have overall positive impacts on the benthic community by increasing diversity, density, and biomass of invertebrates. In the profundal zone, however, quagga mussels compete for space and food resources with most of native invertebrates decreasing their overall diversity, density, and biomass. The decline in quantities of dreissenids and waterweed in Müggelsee in 2018 suggests that there can be competition for space among quagga mussels and macrophytes also in the littoral zone. According to Nalepa (2010), *D. r. bugensis* usually has a more even distribution and rarely forms large druses on soft sediments of the profundal. In Lake Müggelsee, dense carpets of quagga mussels were observed between 2 and 4 m (**Figure 1D**). These mussel carpets are assumed to prevent an attachment of *E. nuttallii* shoots to the sediments by roots. *Vice versa*, dense stands of *E. nuttallii* (**Figure 1B**) or native macrophytes could prevent a successful establishment and survival of quagga mussels due to particle retention (Vermaat et al., 2000) and allelopathic inhibition of phytoplankton (Erhard and Gross, 2006) resulting in insufficient food availability for quagga mussels. Indeed, low phytoplankton abundances (**Figure 4C**) might have affected the growth, recruitment and survival of quagga mussels in Müggelsee. Declining dreissenid recruitment and growth has been found following declines in food availability in the hypolimnion of Lake Erie (Karatayev et al., 2018b), so it would be worth following size-frequency distributions in the future.

Higgins (2014), however, did not find evidence for diminished effects of dreissenids on ecosystems within two decades after their establishment in US waters.

We conclude that our observational study indicates a mutual facilitation between *E. nuttallii* and quagga mussels during the first years of their invasion, which subsequently turns into a competition for space. Observational approaches are re-emerging in ecology and have demonstrated their capability in testing hypotheses by correlating variables, comparing observed patterns to output from existing models and exploiting natural experiments (Sagarin and Pauchard, 2010). Ideally, they would be combined with experimental manipulations to isolate fine-scale ecological mechanisms. In our study, a full understanding of the interactions between dense dreissenid populations and macrophyte stands in lakes requires further detailed analyses, in particular on fluxes and mass balances of dissolved inorganic carbon and oxygen. For *E. nuttallii*, several other potential invasion mechanisms have been suggested, including enemy release, novel weapons/allelopathy, phenotypic plasticity, fluctuating resources, and opportunity windows (Fleming and Dibble, 2015 and references therein). In the case of Lake Müggelsee, none of these seems to be more likely than invasional meltdown.

AUTHOR CONTRIBUTIONS

SH conceived the presented idea and wrote the manuscript together with BW. BW conducted the dreissenid mapping in 2017 and KvdW the macrophyte and dreissenid mapping. JK provided lake and phytoplankton data. EF and MM performed molecular analyses. AK and MG provided background information on the lake and supported statistical analyses. All authors contributed to discussions and the writing of different parts of the text.

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Allelopathic Effects of Native Versus Invasive Plants on One Major Invader

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Allelopathy is defined as the effects (stimulatory and inhibitory) of a plant on the development of neighboring plants through the release of secondary compounds. Autoallelophaty is the beneficial or harmful effect of a plant species on itself. The allelopathic potential belonging to a native species could induce a biotic resistance against invasive plants, whereas allelochemicals released by exotic species could favor the establishment of invasive species (invasional meltdown). The aim of our study was to examine the potential allelopathic effect of four plant species on the target species *Ludwigia hexapetala* using two experiments. In the first experiment, we tested the allelopathic effect of root and leaf leachates of the two congeneric exotic species *Ludwigia hexapetala* and *Ludwigia peploides* on *L. hexapetala,* while in the second experiment, we studied the allelopathic effect of root and leaf leachates of a sympatric exotic species *Myriophyllum aquaticum* and of one native species *Mentha aquatica* on *L. hexapetala*. We measured the stem length to calculate the relative growth rate and four physiological traits (nitrogen balance index and flavonol, chorophyll, anthocyanin indices) of the target plants on a weekly basis. At the end of the experiment, we determined the aboveground and belowground biomass. We also counted the number of lateral branches and measured their lengths. We found that the root leachates of *L. peploides* and of *Myriophyllum aquaticum* had stimulated the synthesis of flavonols of *L. hexapetala.* Leaf leachate of *L. hexapetala* also stimulated its own flavonol synthesis. Also, the root leachate of *L. peploides* had stimulated the total biomass and length of lateral branches of *L. hexapetala,* whereas the production of lateral branches had been stimulated by root leachates of both *Ludwigia* species and by leaf leachate of *Myriophyllum aquaticum.* The autoallelopathy of *L. hexapetala* could explain its invasiveness*.* Both leachates produced by *Mentha aquatica* had no effect on the physiological and morphological traits of the invasive *L. hexapetala* and indicated no biotic resistance in the recipient community*.* The two invasive plant species *Myriophyllum aquaticum* and *L. peploides* could favor the establishment of *L. hexapetala*. These results suggested an "invasional meltdown."

Keywords: **plant-plant interactions, invasive species, *Ludwigia hexapetala*, *Ludwigia peploides*, *Myriophyllum aquaticum*, *Mentha aquatica***

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INTRODUCTION

Many aquatic plant species have been introduced to other continents either accidentally or voluntary for ornamental purposes for example. An invasive species is one that spreads outside their natural range and may impact the native diversity and overall structure and function of ecosystems. Invasive species often establish monospecific patches in their introduced ranges but coexist with neighbors in their native habitat (Ridenour and Callaway, 2001). Many studies suggest that allelopathy may contribute to the ability of exotic species to form dense stands in invaded ecosystems (Hierro and Callaway, 2003). Allelopathy, defined as the chemical interactions between plants or plants and microorganisms, could have either positive or negative effects on the performance of neighbors (Rice, 1984). Apart from affecting the establishment of coexisting species, allelopathic species can also affect their own establishment and self-regeneration. When the target plant is also the donor, the phenomenon is called autoallelopathy, which is a type of intraspecific interaction. Few studies showed a positive effect of autoallelopathy on the growth of the plant itself (Zhu et al., 2015; Bardon et al., 2017). The suspicion that allelochemicals, released by a root or leaf, may interfere with neighbors has been the subject of different theories on biological invasions such as the "novel weapon hypothesis" (NWH, Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004) or the "Biotic Resistance" hypothesis (Elton, 1958). However, many ecosystems often contain combinations of exotic species. These communities of invaders could be driven by facilitation or mutualistic interactions between exotic species, according to the theory of "invasional meltdown" (Simberloff and Von Holle, 1999). An invasional meltdown is defined by the interactions which leads one invasive species to favor the invasion of one or more other exotic species. Moreover, plants that have co-evolved with a species with an allelopathic ability may be less susceptible to allelochemicals, while newly exposed species may exhibit less resistance (theory NWH, Callaway and Aschehoug, 2000). Consequently, allelochemicals released by native plants could also affect the growth of invasive species and would, thus, constitute a biotic resistance against plant invasion (Christina et al., 2015).

However, some exotic species may limit the establishment of other exotic species. Indeed, exotic species that have not co-evolved with the invasive one could be sensitive to allelochemichals (Callaway and Aschehoug, 2000), whereas sympatric species could be favored by secondary compounds released by the donor species (Ehlers and Thompson, 2004). Moreover, the secondary metabolite composition of plants is phylogenetically determined (Grutters et al., 2017) and two close species may produce similar chemical compounds. Consequently, two conspecific species are less susceptible to allelochemicals released by their own individuals and by those of the other species. The allelopathic effects of native plants on exotic plants and on the interactions between conspecific and heterospecific invasive plants have rarely been studied.

This paper is focused on a major invader in wetlands, the water primrose *Ludwigia hexapetala* (Hook. and Arn.) (syn. *L. grandiflora* subsp. *hexapetala*). Native to South America, this species was introduced into France in 1830 and spread initially within the Mediterranean region of France and later into Europe (Thouvenot et al., 2013). The water primrose has been listed on the European List of Invasive Exotic Species since July 2016. The rapid and extensive development of *Ludwigia* spp. populations can block waterways, irrigation ditches, and canals; impact human activities (navigation, hunting, fishing, irrigation, and drainage); reduce biodiversity (Stiers et al., 2011); and degrade water quality (Thouvenot et al., 2013). *L. hexapetala* is a perennial aquatic plant which forms very dense mats. It grows horizontally in water (or mud) and can break the water surface. It is mainly aquatic, but is also able to colonize terrestrial habitats such as riverbanks and wet meadows (Thouvenot et al., 2013). The terrestrial form of *L. hexapetala* has recently invaded wet meadows along the Atlantic Coast of France leading to the depreciation of the fodder value of meadows, resulting in the abandonment of pasture (Billet et al., 2018). In a previous study, we established that *L. hexapatala* stimulated its own germination and could promote its own population persistence (Santonja et al., 2018). *L. hexapetala* produces allelochemicals (Dandelot et al., 2008; Santonja et al., 2018; Thiébaut et al., 2018) and these substances could be implicated in the outcome of the interactions between the water primrose and the surrounding species, be they native or exotic.

The aim of our study was to test whether individuals of *L. hexapetala* would modulate their morphological and physiological traits after exposure to the leachate of different species. The morphological traits were related to plants' ability to grow, to regenerate, and to colonize new habitats. The physiological traits were indicative of plant's allocation of resources to growth or to defenses, the plant's ability to photosynthesize, and an indicator of an exposure to stress. In other words, we investigated the autoallelopathy of *L. hexapetala* and whether a native species and two sympatric species have the ability to promote or to inhibit the establishment of the water primrose through allelopathy. The first hypothesis was that the leachate of the congeneric plant *Ludwigia peploides* (Kunth) Raven ssp. *montevidensis* (Spreng.) Raven have no negative effect on the performance of *L. hexapetala*, because these two species have a common historic exposure to the allelochemicals (theory NWH) and that the leachates of *L. hexapetala* promote the growth of itself. The second hypothesis was that the putative allelochemicals released by the native species *Mentha aquatica* L. have a negative effect on the growth of the invasive species *L. hexapetala* (Biotic Resistance Hypothesis), whereas the putative secondary compounds produced by the sympatric species *Myriophyllum aquaticum* (Vell.) Verdc have a positive effect on *L. hexapetala* (invasional meltdown theory).

MATERIALS AND METHODS

Donor Species

Native to South America, *L. peploides* (primrose-willow) was imported into France around 1830 from the South East as an ornamental plant (Dutartre, 2004). It is now a widespread species and has been listed on the European List of Invasive Exotic Species since July 2016. *L. peploides* often forms monospecific stands and outcompetes other aquatic species

(Dutartre, 2004). It is a creeping emergent macrophyte. It can root in the substrate and send out long prostrates or ascending stems that freely root and branch at nodes and often create dense mats. Dandelot et al. (2008) showed that *L. peploides* possess an allelopathic activity that induces a seedling chlorosis, a decrease in germination and an increase in mortality for watercress *Nasturtium officinalis* R. Br.

Originating in South America, the creeping emergent Parrot's Feather *Myriophyllum aquaticum* was introduced into Europe, more specifically, into France, in the 1880s (Sheppard et al., 2006). It was imported for use in aquaria and garden ponds but escaped into the wild. The European Union has banned the sale and planting or keeping of this plant, even in isolated ponds. Regulations are met for *Myriophyllum aquaticum*. The plant's stems may float out over the surface to form dense stands, from which the emergent shoots rise, making impenetrable mats (Hussner, 2009). Once introduced into a new region, the plants easily spread downstream mainly in the form of vegetative fragments. It is often found in eutrophic waters (small water bodies, irrigation channel networks, and small streams). This species has demonstrated a potential inhibitory effect on neighboring plants (Elakovich and Wooten, 1989). Saito et al. (1989) showed a significant inhibitory activity on growth of the blue-green algae *Microcystis aeruginosa* f. *aeruginosa* (strain number NIES-44) and *Anabaena flos-aquae* f. *flos-aquae* (NIES-73).

Mentha aquatica is a perennial plant from the northern temperate regions of Europe. It has a creeping rhizome with submerged leaves and the erect stems possess aerial leaves. *Mentha aquatica* is typically associated with permanently wet habitats adjacent to open water, often partially or wholly submerged. The invasive *L. hexapetala* and the native *Mentha aquatica* can co-occur in the wild in European aquatic ecosystems. The watermint *Mentha aquatica* is recognized as having an allelopathic effect (Santonja et al., 2018).

Experimental Design

Two experiments were conducted with the target species *L. hexapetala*. In the experiment 1, the two donor species were the two congeneric species *L. hexapetala* and *L. peploides,* whereas in the experiment 2, the donor species were *Myriophyllum aquaticum* and *Mentha aquatica*. In the experiment 1, the aim was also to test the potential autoallelopathy of *L. hexapetala* on its growth.

Experiment 1

In mid-May 2018, 50 shoots of *L. hexapetala* were collected from Apigné pond (01°44′25.2″W, 48°05′41.4″N). For each shoot, an apical shoot (hereafter called individual), without buds or lateral stems, was cut to a length of 8 cm. In the laboratory, the individuals were washed to remove invertebrates, algae, and debris. They were acclimatized for 2 weeks in deionized tap water at room temperature (19°C). They float free in deionized water. The individuals produced roots during this acclimatization period. After these 2 weeks, each individual was planted in a pot (7 cm in diameter and 8 cm in height), containing 50% fertile agricultural soil (NPK = 14:10:18 kg/m^3, pH = 6) and 50% of sand.

Leaves and roots of *L. hexapetala* and of *L. peploides* were selected in the spring of 2018 from the Apigné pond in Brittany for *L. hexapetala* and the Brière Marshland (02°26′41″W, 47°32′63″N) for *L. peploides*. Only, the small, round, floating leaves (i.e., those in contact with water) were collected. The leaves were detached from the stems, washed to remove benthic invertebrates and filamentous algae, and stored in the dark at 4°C. Leaf and root leachates of *L. hexapetala* and *L. peploides* were separately prepared by soaking 10 g of fresh leaves and 10 g of fresh roots (equivalent dry weight) in 1,000 ml of deionized water for 12 h in darkness. These 1% aqueous solutions were then filtered through a filter paper (Whatman #1). The leaf and root leachates were then stored at 4°C for 24 h prior to the experiment.

At the start of the experiment, each individual of the target species *L. hexapetala* was watered either with 15 ml of deionized water for the control (C) or with 15 ml of a leaf/root leachate (1%) of the donor species, either *L. peploides* or *L. hexapetala*. Target individuals were watered with leaf or root leachate only once at the beginning of the experiment. Each treatment and the control had 10 replicates. Pots were randomly positioned in a growth chamber (Photon Flux Density 100 μmols^{-1} m^2, 14 h light/10 h dark cycle) at 21°C for 28 days. The bottom of the pots was kept in tap water (*ca*. 1–2 cm depth). Individuals of the target species *L. hexapetala* were watered with deionized water to maintain by to keep the substrate wet, once each week for 4 weeks.

Measurement of Morphological and Physiological Traits

We used a functional trait approach to study the responses of individuals of *L. hexapetala* after an exposure to root and leaf leachates. We measured both physiological and morphological traits. Four physiological traits were measured simultaneously *in vivo* using a non-destructive measurement device called the Dualex Scientific+™ sensor. This is a hand-held leaf-clip sensor (Cerovic et al., 2012; Bürling et al., 2013) that measures flavonols (Flav.), anthocyanin (Anth.), and chlorophyll (Chl.) indices and calculates the nitrogen balance index (NBI). The NBI is more of an indicator of C/N allocation changes due to N deficiency (from 0 to 100) than a measure of leaf nitrogen content *per se*. The Chl. index related to the chlorophyll content (between 0 and 150) is an indicator of the photosynthetic yield. The Flav. index related to the flavonol content or to phenolics accumulation is an indicator of the defense mechanisms against pathogens and herbivores. The Anth. index related to anthocyanin is an indicator of an exposure to stress (shading conditions, nutrient deficiencies, temperature stress etc.). The measurements of physiological traits were taken from two apical leaves per individual of *L. hexapetala* per pot. We repeated these physiological measurements on five individuals (i.e., 10 measurements per week).

Five morphological traits were measured. We counted lateral branches and measured their length (Barrat-Segretain et al., 1998). Based on the number of roots, we evaluated plant ability to colonize. To obtain a proxy of the apical growth, we measured stem length and then calculated the relative growth rate (RGR; d − 1) according to Hunt (1990):

$$\text{RGR stem} = (\ln \text{L2} - \ln \text{L1}) / (\text{T2} - \text{T1})$$

where L1 and L2 represent total shoot length, at the beginning (T1) and end of the experiment (T2), respectively.

At the end of the experiment, the main shoot length and the lateral shoot length were measured, the lateral branches were counted, and the roots and shoots were harvested. The above and belowground vegetative parts of the plants were dried separately at 65°C for 72 h and weighed. The ratio of belowground to aboveground mass was calculated.

Experiment 2

Leaves and roots of the two donor species *Mentha aquatica* and *Myriophyllum aquaticum* were collected once more in mid-June of 2018 from two different ponds in Apigné in Brittany, France. We also collected 50 individuals of the target species *L. hexapetala*. At these sampling sites, the two donor and the target species did not co-occur. The leaf and root leachates of each species (*Mentha aquatica, Myriophyllum aquaticum*) were prepared according to the protocol followed in Experiment 1. The same experimental design was applied and the same morphological and physiological traits were measured as in Experiment 1. The duration of this experiment was 3 weeks.

Data Analysis

Longitudinal data in both experiments, RGR, and the physiological parameters NBI, chlorophyll, flavonols, and anthocyanin were analyzed on the basis of repeated measures. Whenever data met parametric test assumptions, a linear mixed model with a split-plot design for repeated measures was applied, including each plant as a random factor; otherwise data were analyzed by means of non-parametric testing (Naguchi et al., 2012). We double checked that data met the parametric test assumptions graphically and by means of the Shapiro-Wilk test (residuals normality) and Breusch-Pagan test (homoscedasticity test). A type III ANOVA model was used to test hypothesis in parametric repeated measures analyses to RGR data in both experiments and NBI, chlorophyll, and flavonols in Experiment 2. Whenever the interaction between treatment effect and sampling time was significant, a *post hoc* pairwise comparison was performed among treatments for each sampling period individually. We used *t*-test comparisons for parametric datasets and Mann-Whitney-Wilcoxon test for non-parametric datasets; in both cases, *p's* were subsequently corrected by means of Benjamini-Hochberg False Discovery Rate test for multiple comparisons with a 10% acceptance level (Benjamini and Yekutieli, 2001).

The effects of the leachate source upon total mass and root/stem mass ratio were tested with a one-way ANOVA test, the model II, owing to an imbalance among the number of plants in the treatments in the two experiments. The root/stem mass ratio was in each occasion transformed with logit transformation to improve normality and homoscedasticity of residuals. Total biomass log transformation was needed only for the *L. hexapetala* dataset in the first experiment. In both experiments, the effects of the leachate origin upon the number of branches, including plants without branches in the datasets, were analyzed with a generalized linear model following a Poisson error distribution. The effect of the leachate origin upon the total length of branches was tested with a one-way ANOVA, type II, excluding from the analyzed dataset the plants without branches. All analyses were performed with R software (R Core Team, 2016).

RESULTS

Effects of Root and Leaf Leachates of *L. hexapetala* and of *L. peploides* on *L. hexapetala* (Experiment 1)

The interaction of two factors (time and leachate origin) was significant for the synthesis of flavonols in leaves of *L. hexapetala* after 3 and 4 weeks, respectively ($p = 0.01$, **Figure 1E, Table 1**). The root leachate of *L. peploides* and the leaf leachate of *L. hexapetala* significantly stimulated the synthesis of flavonols of *L. hexapetala* after 3 and 4 weeks, respectively. There was no significant effect of both leaf and root leachates of both *L. hexapetala* and *L. peploides* on the NBI, chlorophyll, and anthocyanin indices (**Figures 1A–D, Table 1**).

There was no effect of the leaf and root leachates of *L. hexapetala* and *L. peploides* on the RGR of *L. hexapetala* (**Table 1**). All the morphological traits were significantly influenced by the type of leachate (**Table 2**). The total biomass of *L. hexapetala* was stimulated by the root leachate of *L. peploides* ($F = 4.80$; $p = 0.003$, **Figure 2A**). The ratio below/aboveground mass was not significantly affected by the leachates (**Figure 2B**). The number of branches of *L. hexapetala* was stimulated both by the root leachate of *L. hexapetala* (Chi = 36.93; $p < 0.0001$) and *L. peploides* (Chi = 32.97; $p < 0.0001$, **Figure 2C**). The lengths of lateral shoots of *L. hexapetala* were longer after exposure to the root leachate of *L. peploides* ($F = 4.69$; $p = 0.003$, **Figure 2D**). There was no effect of leaf leachates of both *Ludwigia* species on the morphological traits of *L. hexapetala* (**Figure 2**).

The root leachate of *L. hexapetala* did not affect its own physiological traits, apical growth, biomass, and branching (**Figure 2**).

Effects of Root and Leaf Leachates of *Mentha aquatica* and *Myriophyllum aquaticum* on *L. hexapetala* (Experiment 2)

The interaction of two factors (time and leachate origin) was significant for NBI and the chlorophyll content in *L. hexapetala* at the beginning of the experiment (**Figure 1F, Table 1**). The individuals watered with deionized

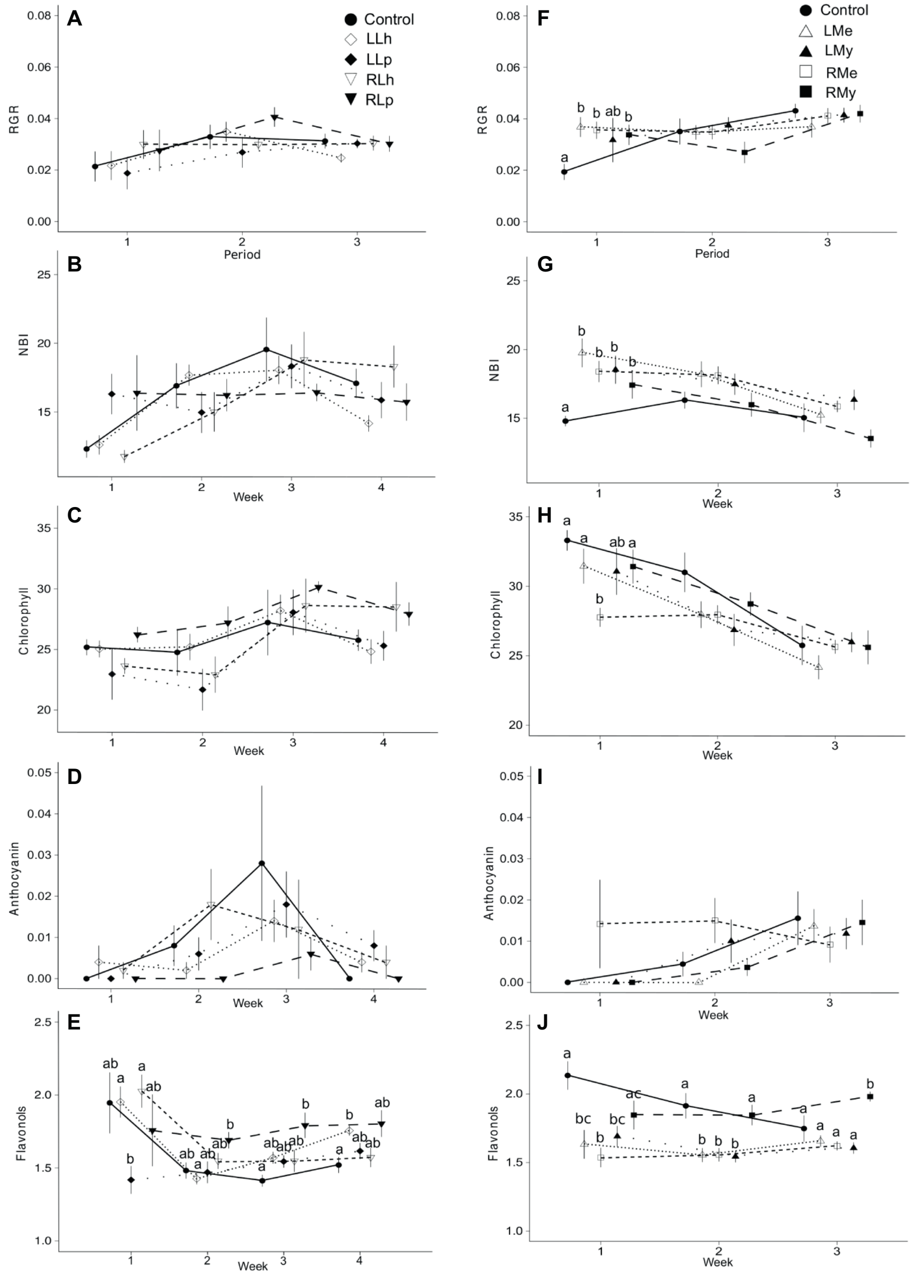

FIGURE 1 | Mean values plus standard error of longitudinal data from experiments 1 and 2. **(A–E)** show the effects of root and leaf leachates of *L. hexapetala* and *L. peploides* plants upon *L. hexapetala* plants, and **(F–J)** show the effects of root and leaf leachates of *Myriophyllum aquaticum* and *Mentha aquatica* upon *L. hexapetala* plants. RGR refers to relative growth rate assessed between two consecutive sampling times. In **(A–E)**, solid black circles = control values, white rotated squares = *L. hexapetala* leaf leachate (LLh), solid black rotated squares = *L. peploides* leaf leachate (LLp), white inverted triangles = *L. hexapetala* root leachate (RLh), solid black inverted triangles = *L. peploides* root leachate (RLp). In **(F–J)**, solid black circles = control values, white triangles = *Mentha aquatica* leaf leachate (LMe), solid black triangles = *Myriophyllum aquaticum* leaf leachate (LMy), white squares = *Mentha aquatica* root leachate (RMe), solid black square = *Myriophyllum aquaticum* root leachate (RMy). Letters set the significance of pairwise comparisons (significance threshold of 0.05).

water (control) were characterized during the first week by a lower RGR and NBI than the individuals watered by root or leaf leachates of *Mentha aquatica* or *Myriophyllum aquaticum* (**Figures 1F,G, Table 1**). The chlorophyll content in control was higher than that in the individuals exposed to root leachates of *Mentha aquatica* during the first week of the experiment (**Figures 1H,J**). There was no effect of root and leaf leachates of *M aquatica* and *Myriophyllum aquaticum* on the anthocyanin synthesis of *L. hexapetala* (**Figure 1I, Table 1**). The interaction of two factors (time and leachate origin) was significant for the synthesis of flavonols of *L. hexapetala* (p = 0.0001, **Figure 1J, Table 1**). After 3 weeks,

TABLE 1 | Effects of leaf/root leachates on physiological traits of *L. hexapetala* (experiment 1: leachates of *L. peploides* or *L. hexapetala*; experiment 2: leachates of *Myriophyllum aquaticum* or *Mentha aquatica*).

Physiological traits		**Experiment 1**			**Experiment 2**		
		Statistic	**df**	***p***	**Statistic**	**df**	***p***
RGR	Treatment	4.93	4	0.4	13.30	4	**0.009**
	Time	2.46	1	0.1	21.16	1	**<0.0001**
	Treatment × Time	2.40	4	0.7	11.60	4	**0.02**
NBI	Treatment	0.16	3.08	0.9	18.49	4	**<0.0001**
	Time	0.92	2.41	**<0.0001**	0.05	1	0.8
	Treatment × Time	1.89	6.40	0.07	11.48	4	**0.02**
Chl	Treatment	2.44	3.37	0.06	5.86	4	**0.003**
	Time	10.49	2.61	**<0.0001**	23.48	1	**<0.0001**
	Treatment × Time	0.74	6.86	0.6	9.68	4	**0.05**
Flav	Treatment	4.16	3.40	**0.004**	42.11	4	**<0.0001**
	Time	7.35	1.96	**0.007**	18.75	1	**<0.0001**
	Treatment × Time	2.92	5.47	**0.01**	23.22	4	**0.0001**
Anth	Treatment	1.36	2.93	0.3	1.77	3.62	0.1
	Time	6.25	2.47	**0.0008**	18.39	1.63	**<0.0001**
	Treatment × Time	1.44	6.52	0.2	1.70	5.31	0.1

Longitudinal data analysis results based on repeated measures analysis. RGR, relative growth rate; NBI, nitrogen balance index; Chl, chlorophyll index; Flav, flavonol index; Anth, anthocyanin index. Significant p's at 5% significance level are in bold.

TABLE 2 | Effects of leaf/root leachates on morphological traits of *L. hexapetala* observed at the end of the experiment (experiment 1: leachates of *L. peploides* or *L. hexapetala*; experiment 2: leachates of *Myriophyllum aquaticum* or *Mentha aquatica*).

Morphological traits	**Experiment 1**			**Experiment 2**		
	Statistic	**df**	***p***	**Statistic**	**df**	***p***
Total biomass	4.05	4	**0.003**	0.84	4	0.5
Below/aboveground mass ratio	4.15	4	**0.006**	2.04	4	0.1
Lateral branches length	3.47	4	**0.02**	1.63	4	0.2
Number of branches	36.93	4	**<0.0001**	14.30	4	**0.006**

Significant p's at 5% significance level are in bold.

root leachate of *Myriophyllum aquaticum* stimulated the synthesis of flavonols in *L. hexapetala* (**Figure 1J**).

There was no effect of leaf and root leachates of *Mentha aquatica* on the morphological and physiological traits of the target species (**Figures 2E–H, Table 2**). There was effect of leaf and root leachates of *Myriophyllum aquaticum* on the total dry biomass, on ratio below/aboveground mass and on the length of branches (**Figures 2E,F,H, Table 2**). The number of branches of *L. hexapetala* was stimulated by the leaf leachate of *Myriophyllum aquaticum* (**Figure 2G**).

DISCUSSION

Allelopathic Effects of Leaf and Root Leachates of *L. peploides* and of *L. hexapetala* on the Traits of *L. hexapetala* (Experiment 1)

The leachates of *L. peploides* and of *L. hexapetala* affected the physiological and morphological traits of *L. hexapetala*. Leachates from leaves of *L. hexapetala* and from roots of *L. peploides* stimulated the flavonol synthesis of *L. hexapetala*. The increase of flavonols content in the epidermis of *L. hexapetala* is a surrogate of leaf dry mass per area. The epidermis of the leaves of all plants contains flavonoids that protect against UV-B radiation (280–320 nm). These compounds absorb light in the UV-B range but allow visible light to pass through uninterrupted for photosynthesis and consequently enhanced photosynthesis. Flavonoids act as signal molecules to take preventive measures against attack and were consequently implied in the mechanisms of defenses (Samanta et al., 2011). They increased in *L. hexapetala*, whereas no apical growth (RGR) was established. The allocation of energy to defense versus growth ("The dilemma of plant," Herms and Mattson, 1992) is particularly important for the invasive plant persistence in field. Harms et al. (2017) reported strong herbivory damage in *L. hexapetala* in spring. However, despite the diverse assemblage of herbivores and fungi associated with *L. hexapetala*, damage was relatively low and the plant continues to persist as an invasive species (Harms et al., 2017). The root leachates of both *Ludwigia* spp. increasing the synthesis of flavonols contributed to the resistance of *L. hexapetala* to herbivores. This represents an efficient strategy for *L. hexapetala*.

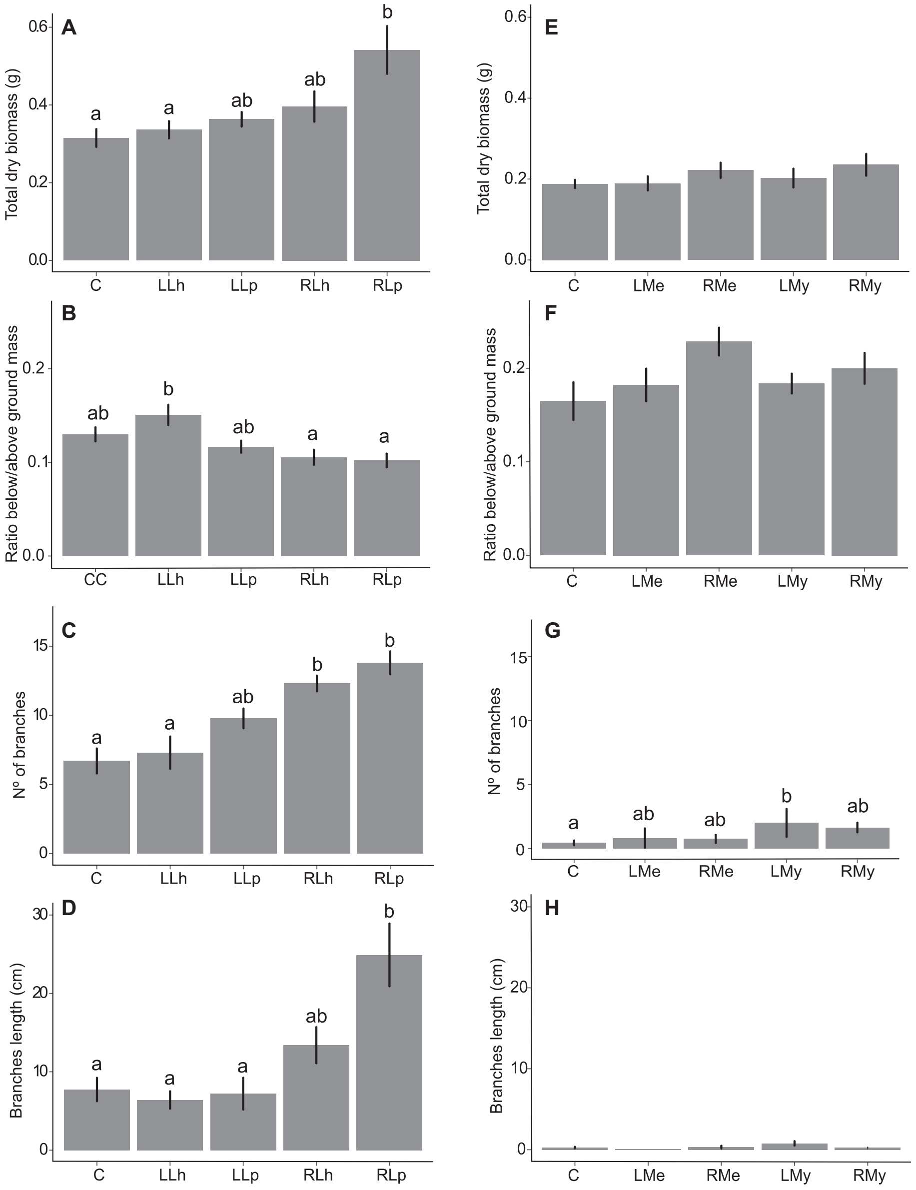

FIGURE 2 | Mean values plus standard error of morphological traits data from Experiment 1 **(A–D)** and 2 **(E–H)**. Leachate treatments are represented in the abscissa axis, where control is always identified by **(C)**. In **(A–D)**, *L. hexapetala* leaf leachate is identified by LLh, *L. peploides* leaf leachate by LLp, *L. hexapetala* root leachate by RLh, and *L. peploides* root leachate by RLp. In **(E–H)**, *Mentha aquatica* leaf leachate is identified by LMe, *M. aquatcum* leaf leachate by LMy, *Mentha aquatica* root leachate is identified by RMe, and *Myriophyllum aquaticum* root leachate by RMy. Letters set the significance of pairwise comparisons (significance threshold of 0.05).

The ability of both *Ludwigia* spp. to release allelochemicals by roots into the soil may increase nutrient availability (Bardon et al., 2017; Thiébaut et al., 2018) and consequently stimulate the branching of *L. hexapetala*. More specifically, the lateral growth (number and length of lateral branches) and the total biomass of *L. hexapetala* were enhanced in the presence of the root leachate of *L. peploides*. The stimulation of branching and biomass increased the vigor, the regeneration, and the colonization abilities of *L. hexapetala*. These positive effects of root leachate of *L. peploides* could be considered as facilitation interactions and "invasional meltdown." Though rare, literature data established that facilitation among congeneric plants does occur and is referred to as "intraspecific" facilitation (Loayza et al., 2017). The strong allelopathic potential of two *Ludwigia* species leads to think that water-soluble compounds released from these plants play a significant role in the successful invasion of these aquatic macrophytes. A similar result was reported with two invasive aquatic plants of *Alternanthera* species (Abbas et al., 2016). Our first hypothesis on facilitation effect of congeneric species *L. peploides* on the growth of *L. hexapetala* was validated.

We also found a positive autoallelopathy of *L. hexapetala* leachates on the flavonols synthesis and on the production of lateral shoots. These results are congruent with a previous work in which leaf leachates of *L. hexapetala* have been reported to stimulate seed germination of itself (Santonja et al., 2018). Similarly, Zhu et al. (2015) showed that root extracts of *Ailanthus altissima* stimulated seed germination, elongation of radicle extension, and elongation of seedlings of itself. Few examples of positive effects of autoallelopathy on plant's growth were reported (Zhu et al., 2015; Bardon et al., 2017) and on their implication to the ecosystem functioning. For example, Bardon et al. (2017) showed that *Fallopia* spp. complex (Asian knotweeds) produce high quantities of procyanidins that they were not considered to be self-toxic. The release of procyanidins by *Fallopia* spp. themselves induced a higher biomass allocation below ground and increases the lateral root production in *Fallopia* spp. and could also inhibit denitrification, thus improving nitrogen availability in nutrient-poor soils (Bardon et al., 2017). Thus, autoallelopathy of *L. hexapatala* could promote plant spread by increasing its competitive ability. Our first hypothesis on a positive effect of autoallelopathy of *L. hexapetala* on itself was validated.

Plant species may have developed resistance against allelochemicals from plants in the same habitat by co-evolution (Reigosa et al., 1999). Resistant species co-occurring with the donor plant could even benefit from the production of allelochemicals by the plant (Hilt et al., 2006). Many secondary metabolites, despite playing a primary role in defending the plant against pathogens or herbivores, can be considered to play secondary roles in plant-plant interactions, by which they nevertheless enhance the competitive potential of the plant (Reigosa et al., 1999). The putative allelochemicals released by the roots of *L. peploides* could directly favor the lateral growth of *L. hexapetala* or could also indirectly affect its development by modifying the chemical and physical properties of the soil and by regulating the soil microbial community (Walker et al., 2003) and by favoring the nutrient availability or altering pH (Blum et al., 1993). However, we have no evidence that the positive effect of root leachate of *L. peploides* on *L. hexapetala* traits results from a common past history and from co-evolution (it is possible that the two *Ludwigia* species introduced in France did not co-occur in their native range). Our study paved the way for future research about the allelopathic effects of *L. peploides* on *L. hexapetala* in both native and introduced ranges.

Effects of Root and Leaf Leachates of *Mentha aquatica* and of *Myriophyllum aquaticum* on *L. hexapetala* (Experiment 2)

No effect of root leachates was observed on the morphological traits of *L. hexapetala*. Parrot's Feather *Myriophyllum aquaticum* has the same biological type with both aquatic and terrestrial forms as *L. hexapetala* and share the same niche. We suspected that to limit niche overlapping of both species, allelochemicals released by roots of *Myriophyllum aquaticum* do not affect the nutrient availability of soil and consequently they do not favor the nutrient acquisition and biomass of *L. hexapetala*. However, our results showed that the root leachate of *Myriophyllum aquaticum* had a positive effect on the flavonols in the leaves of *L. hexapetala*. Flavonoids are phenolic compounds that may be employed by plants as visual and olfactory attractants. Indeed, the leaf leachate of *Myriophyllum aquaticum* slightly stimulated the production of the lateral branches of *L. hexapetala*. The production of secondary metabolites by plants is determined by the genetic characteristics of the species producing them. Allelopathic plants may involve genetic changes within nearby growing plants. It may suggest that genotypes that are sensitive to allopathic chemicals have been removed from the gene pool, due to the continuous selection pressure of selective allelopathic chemicals, especially phenolic acids released by aquatic plants (Abbas et al., 2014). The two invasive species *Myriophyllum aquaticum* and *L. hexapetala* can coexist in field in their introduced range. The release of allelochemicals is also determined by the environmental conditions in which the plants are found (Reigosa et al., 2013). Variables such as temperature, humidity, and light intensity, added to the effects of the biota and the physicochemical structure of the soil, can affect not only the production of metabolites but also the chemical structure and degree of activity of substances released into the environment. Our hypothesis of "invasional meltdown" between the two sympatric species was validated.

In contrast, the hypothesis about a negative effect of the watermint *Mentha aquatica* leachates on the traits of *L. hexapetala* was invalidated. No effect of *Mentha aquatica* leachate on the *L. hexapetala* traits could be explained by the season and by the nature of the secondary compounds. Furthermore, the absence of effect of the watermint leachates could be due to the degradation of the allelochemicals after a short time. Secondary compounds can be degraded after they have been released into the soil; the half-life of allelochemicals varies from a few hours to a few months (Cheng and Cheng, 2015). This is mainly associated with the allelochemical concentration, soil type, and soil microbial population (Cheng and Cheng, 2015). Further studies are required to determine the concentration of these compounds in *L. hexapetala* soil and the stability of those compounds in the soil.

CONCLUSIONS

The leachates of *L. hexapetala* favored its own synthesis of flavonols and its branching. This autoallelopathy could partly explain the water primrose invasiveness. The two invasive species *L. peploides* and *Myriophyllum aquaticum* stimulated the flavonols synthesis and the branching of the water primrose. These results suggested an "invasional meltdown." Stimulation of the lateral growth and defense mechanisms by sympatric invasive species mediated by allelochemicals could potentially favor the persistence of *L. hexapetala* populations in invaded communities. The native *Mentha aquatica* leachate had no impact on the performance of the invasive *L. hexapetala*, showing no "biotic resistance." These preliminary results must be taken carefully, while invasive plant growth was also determined by the interference between plants of the same or different species in the field. Deepening the understanding on plant-plant interactions has important implications for the management and the restoration of ecosystems that are both resistant and resilient to invasive species.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

AUTHOR CONTRIBUTIONS

GT and MT designed the experiment and conducted them. HR-P analyzed the data. GT wrote the manuscript with contributions from all the authors.

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Modeling Top-Down and Bottom-Up Drivers of a Regime Shift in Invasive Aquatic Plant Stable States

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The evidence for alternate stable states characterized by dominance of either floating or submerged plant dominance is well established. Inspired by an existing model and controlled experiments, we conceptually describe a dynamic that we have observed in the field using a simple model, the aim of which was to investigate key interactions of the shift between invasive floating and invasive submerged plant dominance, driven by the rapid decomposition of floating plants as a consequence of herbivory by biological control agents. This study showed that the rate of switch between floating and submerged invasive plant dominance, and the point in time at which the switch occurs, is dependent on the nutrient status of the water and the density of biological control agents on floating plant populations. Therefore, top-down invasive plant biological control efforts using natural enemies can affect systems on a wider scale than the intended agent – plant level, and can be significantly altered by bottom-up changes to the system, i.e., nutrient loading. The implications of this are essential for understanding the multiple roles invasive plants and their control have upon ecosystem dynamics. The results emphasize the importance of multi-trophic considerations for future invasive plant management and offer evidence for new pathways of invasion. The model outputs support the conclusion that, after the shift and in the absence of effective intervention, a submerged invasive stable state will persist.

Keywords: floating macrophytes, submerged macrophytes, invasion, biological control, resilience

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INTRODUCTION

Regime shifts in ecological systems can occur rapidly and suddenly, causing changes in key structures and functioning that can threaten sustainability and be difficult to reverse (Scheffer et al., 2003; MacNally et al., 2014; Rocha et al., 2015). These shifts, such as switches in lakes from clear water to algal blooms, can result from relatively small changes in environmental pressures but once a critical threshold is passed, the key mechanisms maintaining the system are disrupted or broken, altering the system trajectory toward a new regime. New feedback mechanisms then develop, allowing the new regime to become stable (Beisner et al., 2003; Scheffer and Carpenter, 2003; Folke et al., 2004; Walker and Meyers, 2004; Kinzig et al., 2006; Biggs et al., 2009).

The existence of alternate stable states with basins of attraction dominated by floating and submerged plant species is a classic example of a regime shift and is well documented in freshwater lakes, supported experimentally, observationally, and theoretically (Scheffer et al., 2003; Folke et al., 2004; Netten et al., 2010). Scheffer et al.'s (2003) seminal paper presents a mathematical model describing the key interactions among the main variables. The model, although contextually broad, explores the asymmetry between floating and submerged plant stable states with regards to their competition for resources, where submerged plants are able to access nutrients in the sediment not available to the floating plants, but are less able to compete for light (Scheffer, 2009). The switch between states can occur rapidly, and the subsequent changes in aquatic plant community structures have trophic cascade effects, resulting in altered water, sediment, and nutrient cycling regimes (Blindow et al., 1993; Yarrow et al., 2009; Havel et al., 2015).

Invasive macrophytes, whose establishment and spread continues to be one of the leading threats to global freshwater ecosystems, significantly alter ecosystem structure and functioning whilst limiting access to vital ecosystem services (Lovell et al., 2006; Hussner et al., 2017). South Africa, in particular, has been heavily impacted by floating invasive macrophytes such as water hyacinth (*Eichhornia crassipes* Mart. Solms (Pontederiaceae) and water lettuce [*Pistia stratiotes* L. (Araceae)], which form dense mats on the water's surface as a result of nutrient loading, release from natural enemies, and a relatively small native macrophyte species diversity with which to compete (Coetzee et al., 2011b). These mats reduce biodiversity, limit access to potable freshwater, increase both siltation of rivers and flood risks, drown livestock and damage vital infrastructure (Janse and Van Puijenbroek, 1998; Scheffer et al., 2003; Caraco et al., 2006). Classical biological control (CBC) initiatives using host specific natural enemies have successfully reduced many of these invasions to the extent they are now regarded as being under control (Hill and Coetzee, 2017).

The past decade has seen an increase in the establishment of multiple invasive submerged plant species following the control of floating macrophytes, which is a major concern for the future safeguarding of South Africa's freshwater (Hill and Coetzee, 2017). Notorious submerged invasive species such as *Myriophyllum spicatum* L. (Haloragaceae), *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae) and *Egeria densa* Planch. (Hydrocharitaceae) have successfully established far more widely than previously thought (Madeira et al., 2007; Coetzee et al., 2011b; Martin and Coetzee, 2011; Weyl and Coetzee, 2014). Globally, the biological control programs associated with floating or emergent macrophytes have been highly successful, but similar biological control of submerged plant species has proved more challenging (Schmitz and Schardt, 2015). For example, the first biological control agent against *H. verticillata* was released in the United States in 1988 and over a quarter of a century later, it is still considered the most problematic aquatic plant in the United States (Gu, 2006; True-Meadows et al., 2016).

Although biological control has effectively reduced populations of floating invasive plants, the effect this has on the submerged plant community structure is relatively unknown. The majority of biological control programs traditionally investigate the direct interactions between a potential agent and its target species, while plant interaction experiments focus on changes within a single trophic level (Van et al., 1999; James et al., 2006; Martin and Coetzee, 2014). However, studying the indirect effects of the agents on the competitive interactions of the target species, as well as multitrophic cascading effects of biological control, would paint a more holistic picture of the impacts they can have on a system (Harvey et al., 2010). We thus propose that, as floating invasive plants decompose due to herbivory pressure from biological control agents, nutrients, light, and space become available to submerged plants, which successfully capitalize on this new abundance of resources and proliferate (Chimney and Pietro, 2006; James et al., 2006; Shilla et al., 2006; Longhi et al., 2008). However, the relative paucity of native submerged plant species, as a result of few natural freshwater systems in the South African landscape, combined with external nutrient loading, means that invasive submerged plants are more likely to establish than native ones. Once the invasive submerged plants are established, their ability to rapidly grow and capitalize on available nutrients allows them to dominate the system (Szabo et al., 2010). In other words, the system has two basins of attraction, one dominated by floating invasive plants and the other by submerged invasive plants, where biological control induces the shift in dominance (**Figure 1**; Strange et al., 2018). The interactions between the three key variables of floating plants, nutrients and agents results in more favorable conditions

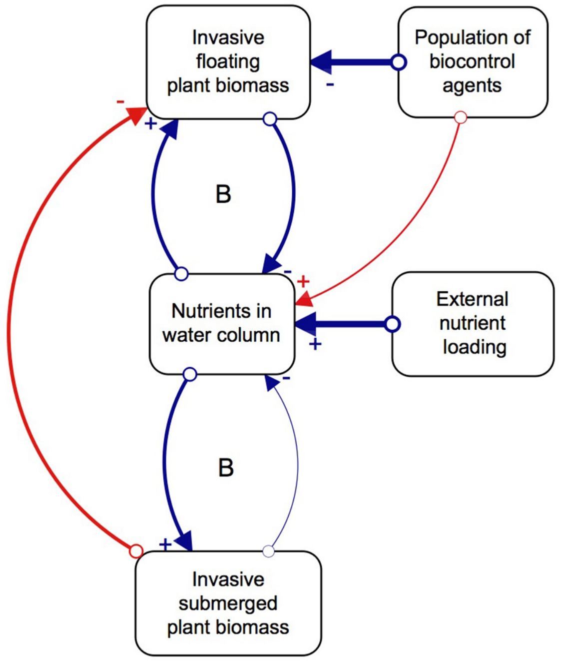

FIGURE 1 | Direct (blue) and indirect (red) relationships between key factors and processes of the regime shift, and the feedback mechanisms that balance (B) the system. Created in STELLA Professional (iSEE systems Inc., Version 1.0.3). The strength of interactions is depicted by the thickness of the arrows connecting the variables that have a positive (+) or negative impact on the other (–). Revised from Strange et al. (2018).

for invasive submerged plant communities which lock up the available nutrients in the system, sustained by continued external nutrient loading.

The proposed shift is supported by existing theories on ecosystem invasibility such as the fluctuating resource hypothesis of invasion that assumes plant communities become more susceptible to invasion following increased availability of unused resources (Davis et al., 2000). Beyond theoretical support, we have documented multiple cases in the field of the shift in dominance from floating to submerged invasive plants following successful biological control of numerous floating species across South Africa (**Table 1** and **Figure 2**). To further support these field observations, we explored the competitive interactions and relationships between three species in controlled mesocosm experiments, each representing the potential dominant states in a South African context; the floating invasive *P. stratiotes*, the submerged invasive *E. densa*, and the confamilial, trophically analogous native *Lagarosiphon major* Ridl. Moss ex Wager (Hydrocharitaceae). Differences in the responses of the native and non-native submerged species to the biological control of the floating plants, using the *P. stratiotes* control agent, *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae) supported the hypotheses of nutrient loading and biological control acting as key drivers between states (Strange, 2017; Strange et al., 2018).

Mathematical models of ecological systems cannot incorporate the full scope of natural processes, and compromises are made regarding spatial or temporal elements, but they are still useful for disentangling the individual mechanisms that lead to overall system changes (Bulling et al., 2006; Scheller et al., 2010; Chatzinikolaou, 2013). Subsequently, the aim of this study was to develop a qualitative, dynamic model that might offer initial theoretical support for a shift from invasive floating to invasive submerged macrophyte dominance, based on the hypothesis that nutrient loading and the application of biological control are the main drivers of species dominance.

MATERIALS AND METHODS

Study Species

Pistia stratiotes was targeted for biological control in the 1980s with the introduction of the weevil *N. affinis* following the success of this control method in Australia (Harley et al., 1990). This species was chosen as the model invasive floating macrophyte due to the short timeframes required to achieve total control by herbivory from the agent (Coetzee et al., 2011b). Further still, a switch in states from dominance of *P. stratiotes* to submerged invasive macrophytes has been observed in a number of locations across South Africa as a result of biological control (**Figure 2** and **Table 1**). *Egeria densa* was selected as the invasive submerged species as it has been identified as the most widespread submerged aquatic invader in South Africa (Coetzee et al., 2011a; Smith et al., 2019) and has been recorded in multiple sites where floating invaders previously dominated (**Figure 2** and **Table 1**).

Model

The following equations were developed, based on Scheffer et al.'s (2003) original model, to describe the main interactions emerged from previous experiments (Strange, 2017; Strange et al., 2018) between the key variables of the proposed regime shift between floating and submerged species, driven by biological control:

$$\frac{dF}{dt} = -l_F F + a_F N F - c_F(N) F^2 - BF$$

$$\frac{dS}{dt} = -l_S S + a_S N S - c_S(N) S^2$$

TABLE 1 | Site numbers (corresponding to **Figure 2**), names and coordinates where a switch from floating invasive to submerged invasive plant dominance has been observed in the field following biological control of floating plant species (Coetzee, unpublished data, from Rhodes University annual aquatic weed surveys conducted from 2008 to 2015).

No.	Site name	Coordinates	Floating spp.	Control agent(s)	Submerged spp.
1	Riverlea, Ashburton	–29.676780, 30.462460	*Salvinia molesta*	*Cyrtobagous salviniae*	*Egeria densa*
2	Cato Ridge Golf Course, Cato Ridge	–29.754497, 30.593318	*S. molesta*	*C. salviniae*	*E. densa*
3	Bluff Nature Reserve, Durban	–29.938398, 30.992749	*S molesta*	*C. salviniae*	*Ceratophyllum demersum*
4	Vaalharts Weir, Warrenton	–28.114557, 24.927286	*Eichhornia crassipes*	*Neochetina eichhorniae; N. bruchi*	*Myriophyllum spicatum*
5	Nahoon River, East London	–32.964137, 27.913206	*E. crassipes*	*N. eichhorniae; N. bruchi; Eccritotarsus catarinensis*	*E. densa*
6	Etna Farm Dam, Gonubie	–32.924842, 27.993539	*Pistia stratiotes*	*Neohydronomus affinis*	*C. demersum*
7	Swartkops River, Port Elizabeth	–33.790993, 25.420586	*E crassipes*	*N. eichhorniae; N. bruchi*	*E. densa*
8	St Francis Marine, Cape St Frances	–34.148490, 24.815530	*S. molesta*	*C. salviniae*	*E. densa*
9	Breede River, Robertson	–33.823270, 19.865260	*S. molesta*	*C. salviniae*	*C. demersum*
10	Liesbeeck River, Cape Town	–33.93942, 18.47841	*E. crassipes*	*N. eichhorniae; N. bruchi*	*E. densa*
11	Mocke River, Cape Town	–34.044140, 18.474640	*E. crassipes*	*N. eichhorniae; N. bruchi*	*C. demersum*
12	Zandvlei, Cape Town	–34.085306, 18.461542	*E. crassipes*	*N. eichhorniae; N. bruchi*	*C. demersum*
13	Westlake River, Cape Town	–34.081266, 18.455327	*P. stratiotes*	*N. affinis*	*C. demersum*
14	Keyser's River, Cape Town	–34.066997, 18.460870	*P. stratiotes*	*N. affinis*	*C. demersum*
15	Kogmanskloof River, Montagu	–33.793216, 20.105881	*S. molesta*	*C. salviniae*	*C. demersum*

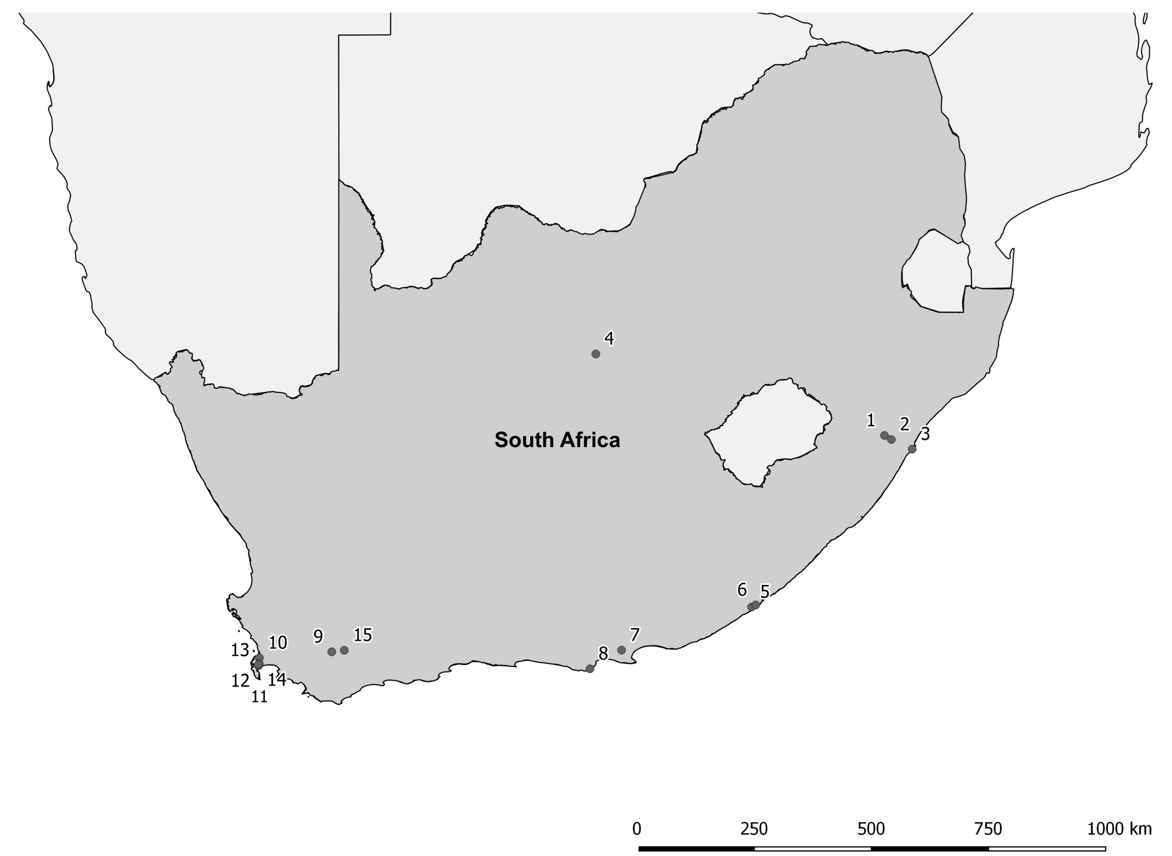

FIGURE 2 | Distribution map of locations where a switch from floating invasive to submerged invasive plant dominance has been observed following biological control of the floating plant species (Coetzee, unpublished data, from Rhodes University annual aquatic weed surveys conducted from 2008 to 2015). For details of each site see **Table 1**.

where the changes over time (t) of the biomass of invasive floating plants (F) and invasive submerged plants (S) were modeled as a function of their mortality rates, l_F and l_S, modified by the rates of nutrient uptake, a_F and a_S, and their intraspecific and nutrient-dependent competitive abilities, $c_F(N)$ and $c_S(N)$. This dynamic is based on multiple studies demonstrating the impact of nutrients on intra- and interspecific competition of submerged (e.g., Martin and Coetzee, 2014; Strange, 2017; Strange et al., 2018) and floating (Njambuya et al., 2011) macrophyte populations. In the present model intraspecific competitive abilities $c_F(N)$ and $c_S(N)$ were modeled using the following equations:

$$c_F(N) = c_{F0} exp(-e_F N)$$

$$c_S(N) = c_{S0} exp(-e_S N)$$

where intraspecific competition decreases with available nutrients in the water column, e_F and e_S represent the strength of this decay, and c_{F0} and c_{S0} the maximum competition. Interspecific competition is modeled through different uptake rates (a_F and a_S) and different effect on nutrients in the water column, m_F and m_S. In fact, available nutrients in the water column N changes with floating and submerged plant biomass dynamics according the following equation:

$$N = N_0 - \ m_F(F - F_0) \ - m_S(S - S_0)$$

where N_0, F_0, and S_0 are, respectively, the initial nutrient availability, floating plant, and submerged plant biomass. The effect of biological control, B, was incorporated into the model for floating plants as an additional mortality rate.

The parameters for the model (**Table 2**) were qualitatively chosen using a combination of the trends observed experimentally (Strange et al., 2018) and from the literature of the original floating to submerged shift model (Scheffer et al., 2003). Model dynamics are robust to the specific values chosen. Each plant population began with the same initial biomass. Mortality rates were assumed to also be equal as there are no specific data indicating otherwise (Scheffer et al., 2003). The floating plants were given a higher value for intraspecific competition compared to the submerged plants, as reflected in the results of previous experiments due to competition for light and space (Strange, 2017). Submerged plants are very efficient in locking up nutrients from the water column thus were assigned a higher value for such effect (Barko et al., 1988;

TABLE 2 | Summary of model variables and parameters, their definitions and dimension.

Parameter	Description	Dimension
F	Biomass of floating invasive plant	Biomass
S	Biomass of submerged invasive plant	Biomass
N	Availability of nutrients in the water column	Mass
B	Biological control mortality rate	1/Time
e_F	Relationship of nutrients and competition (floating)	1/Mass
e_S	Relationship of nutrients and competition (submerged)	1/Mass
c_{F0}	Intraspecific competition (floating)	1/(Biomass Time)
c_{S0}	Intraspecific competition (submerged)	1/(Biomass Time)
l_F	Natural mortality rate (floating)	1/Time
l_S	Natural mortality rate (submerged)	1/Time
a_F	Rate of nutrient uptake (floating)	1/(Mass Time)
a_S	Rate of nutrient uptake (submerged)	1/(Mass Time)
m_F	Effect on nutrients (floating)	Mass/Biomass
m_S	Effect on nutrients (floating)	Mass/Biomass

Chen and Barko, 1988; Rattray et al., 1994; Mazzeo et al., 2003; Scheffer et al., 2003). The rate of nutrient uptake was set to be higher for floating plants than submerged as floating plants are better competitors for light, which increases plant growth and affects the size of roots, providing more surface area for nutrient uptake (DeBusk et al., 1981; Akinbile and Yusoff, 2012). In the experiments upon which the observations were made, nutrients (manipulated at different levels within the mesocosms in the form of NH_4NO_3) were determined to be a key driver (Strange et al., 2018) and the model was developed specifically to explore the interrelationship between the model species, biological control and nutrients, thus other environmental variables such as light were not directly modeled.

The model was implemented within the STELLA® Professional software environment (iSEE systems Inc., Version 1.0.3). STELLA models use stocks, flows and converters to produce time-series simulations. The model outputs trace the temporal changes in populations (stocks), as effected by the external pressures and parameters (converters), according to the equations which are used to describe their interactions (flows). Values were assigned to each stock (F_0, S_0, and N_0), which provides the initial populations, and to each converter, which provides parameter values. The flows contain the model equations describing interactions and therefore have no numeric values.

RESULTS

The simulation outputs show temporal changes in the biomass of floating and submerged plant populations, with increased biomass indicating plant dominance, as well as changes in the levels of nutrients within the water column. For the first series of simulations, the initial starting level of nutrients was set to a relative high value (**Figures 3A–C**). The first output from this simulation (**Figure 3A**), where the biological control rate of the floating plants was set to zero, shows a sharp initial increase followed by a plateau in biomass of the floating plants. Whilst the submerged plants also initially increased in biomass, the growth was slower and plateaued a short while after the point in which the floating plant population peaked. This indicates the floating plants become dominant and are able to maintain dominance in the system. The second output of this simulation (**Figure 3B**), where nutrients remained high but the biological control rate was increased, reveals the same overall trend of floating plant dominance, but the relationship between the two plant biomass changed. There is a longer initial period of increased nutrients in the system (a product of the biological control of the floating plants and subsequent senescent plant material), and the submerged plant population demonstrates a much sharper increase before leveling off. The final output of this simulation (**Figure 3C**) shows a complete switch between the plant populations; as the biological control slows the growth of the floating population and increases the levels of nutrients in the system, there is an initial phase of co-existence between the plant populations. Eventually a point is reached, where the floating plants are completely limited by the biological control, and the submerged plant population is able to grow to a point that surpasses the floating plant biomass and maintain dominance.

The second series of model simulations (**Figures 3D–F**) shows outputs for systems, where the initial level of nutrients in the system was set to a lower initial value. The three outputs were once again set to a sequential increase in the extra mortality by biological control agents on the floating plants. The results show the same overall relationship between the floating and submerged plant populations, with a switch in dominance once a critical level of biological control agents has been crossed. However, there are some differences in the interactions. Firstly, in the reduced nutrient setting of these simulations the switch occurs less suddenly, with reduced slopes, indicating a slower rate of change for both plant populations. Secondly, the required rates of biological control that led to a subsequent shift in plant dominance were reduced in the lower nutrient setting. Lastly, there is a reduced disparity between the two plant biomasses when the biological control rates are at the lowest and highest setting compared to the high nutrients.

DISCUSSION

This model estimates, for the first time, the effects that current species-level management of floating invasive plants have upon wider community-level interactions, in a South African context, supporting the hypothesis that the switch between floating invasive and submerged invasive plant dominance can be influenced by the biological control of floating plants. While bottom-up driven changes to plant-herbivore interactions in aquatic systems have been demonstrated in multiple cases (e.g., Coetzee and Hill, 2012; Maseko et al., 2019), the model also supports the theory that top-down pressures (that affect ecosystems on a wider scale than the intended control agent and target plant level) can be significantly altered by bottom-up changes to the system (nutrient loading).

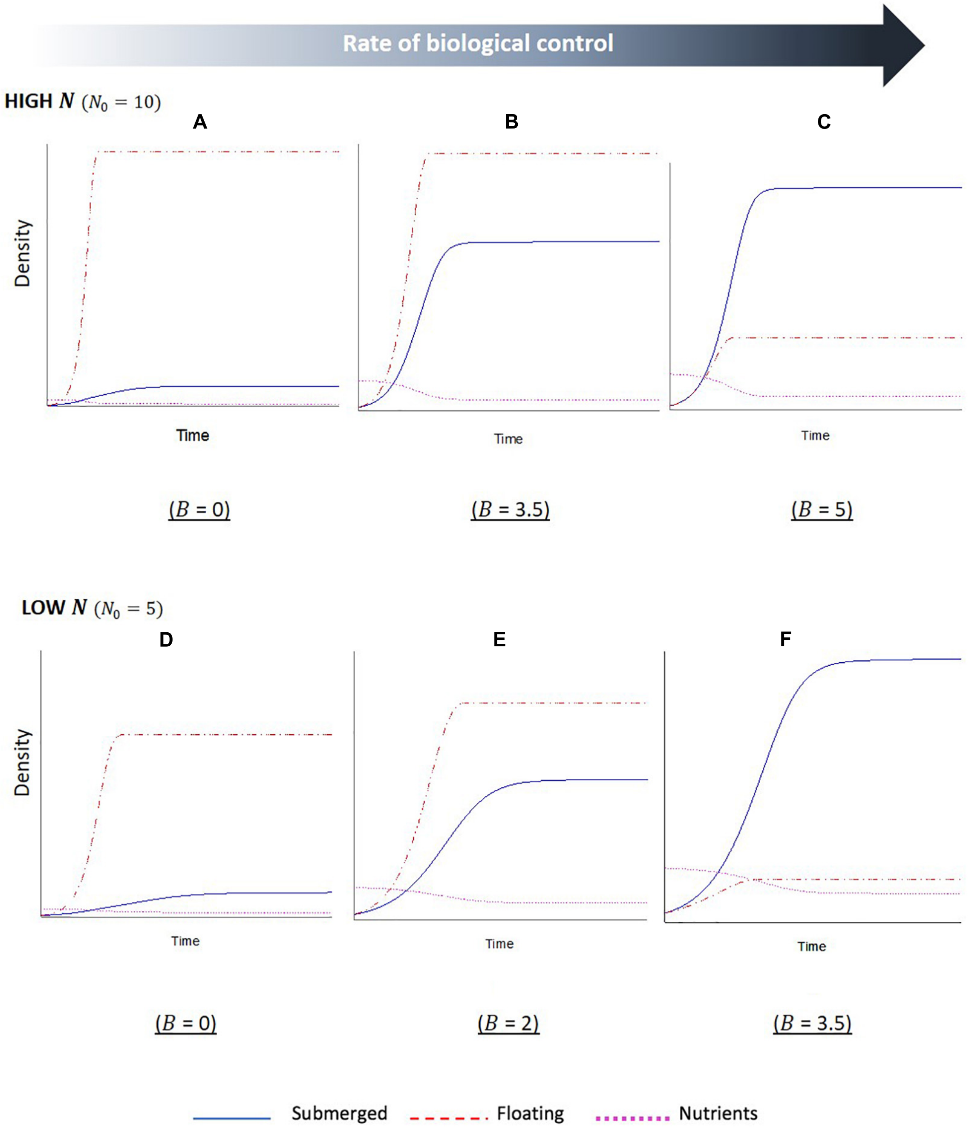

FIGURE 3 | Stella simulation outputs showing temporal changes in floating (*F*, red dashed line) and submerged (*S*, blue solid line) plant biomass and nutrient (*N*, magenta dotted line) levels in scenarios with **(A–C)** high initial nutrients and **(D–F)** low initial nutrients. Within each nutrient condition, the rate of biological control (*B*) was altered, as indicated beneath each graph. Scales are not fixed. Initial conditions: $F_0 = S_0 = 1$. Parameter values: $l_F = l_S = 1$, $a_F = 1$, $a_S = 0.5$, $c_{F0} = 1$, $c_{S0} = 0.5$, $e_F = e_S = 1$, $m_F = 0.01$, $m_S = 0.1$.

Reduced nutrient loading significantly increased the success of biological control of water hyacinth (*E. crassipes*) (Heard and Winterton, 2000; Coetzee and Hill, 2012); as less nutrients were available, macrophytes were not able to recover as quickly from herbivory damage (McNaughton, 1983), therefore plant mortality can be achieved at a lower density of biological control agents. This pattern was reflected in our model system outputs; the level of biological control required to alter plant dominance was reduced in scenarios where initial nutrient loading was lower. Center and Dray (2010) explored the effects of nutrient loading on the relationship between water hyacinth (*E. crassipes)* and two associated biological control agents (*Neochetina eichhorniae* Warner and Neochetina *bruchi* Hustache). Their results showed population growth for both agents was affected by plant quality; plants grown in high nutrient conditions were superior hosts for *N. bruchi* and there were significant increases in reproductive outputs of *N. bruchi*. They conclude that previously developed models aiming to simulate biological control of *E. crassipes* fell short because bottom-up drivers were under-estimated and overlooked. Our study supports this viewpoint and both underline how wider understanding of multi-trophic dynamics, explored using theoretical models can be applied to the practical aspects of invasive species control. Center and Dray (2010) present experimental data to develop a conceptual model to can aid future integrated invasive plant management strategies; as models become more accessible to a wider range of practitioners through more user-friendly interfaces, their role in applied decision making will inevitably become more prominent (Plagányi, 2007; García-Llorente et al., 2008; McCallum, 2008; Chatzinikolaou, 2013). Rightfully, there remain reservations regarding the application of modeling outputs to real life scenarios, such as over simplification of complex systems which highlight the importance of controlled and field-based experiments to describe the finer mechanisms of system changes and increase model validity.

Schroder et al. (2005), in reviewing the direct evidence for alternate stable states, concluded that future research in the field should focus primarily on the specific mechanisms behind switches in ecological states. Manipulation experiments may be bound by spatial and temporal constraints, but small-scale experiments can be crucial to help explain large-scale patterns, and can be a powerful way to show that a system has alternate attractors (Scheffer and Carpenter, 2003; Benton et al., 2007). Although Schroder et al. (2005) report a bias in the literature toward laboratory experiments, there is a paucity in multi-trophic experiments within the field of invasive plant research overall (Harvey et al., 2010; Villamagna and Murphy, 2010; Schultz and Dibble, 2012), yet they are essential for understanding internal ecosystem processes and they have been labeled as an over-looked asset in the exploration of regime shifts (Chase, 2003; Anderson et al., 2009). The validity of evidence claiming to support the existence of multiple stable states has been the subject of increased scrutiny and debate (Schroder et al., 2005; MacNally et al., 2014; Capon et al., 2015). Capon et al. (2015) argue that empirical field-based studies are severely lacking and report common false associations between theoretical constructs with results that do not support them. In agreement with this review, we recognize that whilst the model we present is neither predictive nor quantitative, it offers insight into the multitrophic consequences of invasive species control and the interplay between bottom-up and top-down drivers of ecosystem change. It is now crucial to identify whether or not the switch between floating invasive and submerged invasive plant dominance, instigated by biological control, is occurring in the field. If so, future studies are crucial to determine whether the management of these systems can be executed in a way that might reduce the likelihood of this shift, whilst increasing system resilience; and whether increasing native submerged plant populations prior to floating macrophyte removal could curb invasive submerged plant establishment. Restoration studies, where community assemblies are purposefully altered by selecting native species determined by resource-use traits to occupy vulnerable systems, have been shown to increase resistance of a community to successful invasion (Funk et al., 2008), and following the results of this study, indicate that this should be a priority to managers of invaded aquatic systems.

The efforts of this study aimed to bring together the theoretical concepts of alternative stable states and community change with the practical and applied domain of invasive species management and control. As with all models there are limitations that must be taken into consideration when drawing conclusions. The model is not quantitative and as such cannot be used as a predictive or diagnostic tool (and indeed this was not the intention). Future experiments could be used to develop and further parameterize the model by including more variables we know to be important in plant community structure such as light, water chemistry and presence of decomposers. The original experiments upon which the model was based used only the species described in this manuscript and exploring the same competitive dynamics between other plant species (of differing growth forms, families, etc.) would be both valid and interesting. The model was developed to offer initial theoretical support, along with field and laboratory-based observations, to the proposed multi-trophic consequences of floating invasive species control. In light of this the results presented do have the potential to better inform management of South Africa's freshwater systems and highlight the benefit of continuing multi-trophic considerations for future invasive plant management worldwide, as well as opening up a multitude of possibilities for research into the mechanisms of submerged plant invasions and resilience of native macrophyte communities in South Africa, and further afield. Based on the findings presented in this manuscript, we recommend further investigations to increase understanding of the multi-trophic consequences of invasive species control and removal. Further, and more specifically to aquatic macrophyte invasions, we recommend a more holistic approach to the management of floating invasive plants including commitment to nutrient amelioration and post-control community restoration.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

ES conducted the experimental studies whose results were used for the simulation models. PL and ES developed and refined the models. JH and JC conceptualized and supervised the work, and commented on and edited the manuscript.

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The Ecophysiological Response of Two Invasive Submerged Plants to Light and Nitrogen

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Two submerged *Elodea* species have small differences in their ecophysiological responses when exposed to individual environmental factors. However, field observations showed that under eutrophic conditions with low light availability, *Elodea canadensis* could be displaced by *Elodea nuttallii*. Here we investigated the combined effect of environmental factors on the ecophysiological response of the two species in order to explain the differences in their invasion successes. We cultivated the plants in aquaria containing five different nitrogen (N) concentrations and incubated at five different light intensities. For both species increasing nitrogen concentrations resulted in increased relative growth rate, chlorophyll concentration, and actual photochemical efficiency of photosystem II (Φ_{PSII}), however, they produced less roots. Lowering light intensity resulted in a lower relative growth rate, root production, and nutrient removal. In contrast, chlorophyll concentration in the leaves, and Φ_{PSII} increased. The main difference between the two *Elodea* species was that the light compensation point (I_c) and weight loss in the dark were significantly higher and photochemical efficiency and chlorophyll concentration were significantly lower for *E. canadensis* than for *E. nuttallii*, indicating that the latter can survive under much more shady and hypertrophic conditions. The change in nitrogen concentration of the media and in tissue concentration of the plants indicated that *E. nuttallii* has a higher nitrogen removal capacity. The ecophysiological differences between the two species can be an explanation for invasion success of *E. nuttallii* over *E. canadensis* and thus may explain why the latter is replaced by the first.

Keywords: ***Elodea*, growth rate, interaction, nutrient removal, photochemical efficiency**

INTRODUCTION

Light and nutrient availability are key factors governing the distribution and growth of submerged macrophytes in aquatic ecosystems (Chambers, 1987; Chambers and Kalff, 1987; Best et al., 2001). Eutrophication changes the availability of nutrients and light for submerged macrophytes and this might be beneficial for some macrophytes but harmful to others (Cao et al., 2011). Rooted submerged plants grow under relatively low light conditions at the start of the growing season, and their growth is accelerated when temperature rises. Due to their apical elongation, they grow towards the water surface and become exposed to higher light levels which is favorable for photon capture (Kuni, 1982). The relative competitive advantage of submerged macrophytes depends on their capacity to take up nutrients and to capture light (Szabó et al., 2010). The sooner a species reaches the higher light level, the better this species can shade out and finally displace others (Barrat-Segretain, 2005), and this is especially important in eutrophic and turbid waters. Light availability for submerged plants is not only influenced by shading of neighboring plants but also by the presence of free-floating vegetation (Scheffer et al., 2003; Morris et al., 2004; Lu et al., 2013; van Gerven et al., 2015). Floating algal bed, planktonic and periphytic algae can additionally reduce inherently poor light conditions under the water and lead to the decline of aquatic vegetation (Phillips et al., 1978; Hillebrand, 1983). Ultimately, plants that have a lower light compensation point or have a higher shade tolerance are expected to be better survivors in turbid water or under the shade of floating macrophytes. Consequently, eutrophication opens the window of opportunity for submerged macrophytes that have those traits (James et al., 1999). Frequently, species with those traits invade new regions where they are more competitive than native species (Vilà and Weiner, 2004; Espinar et al., 2015).

An interesting case is the introduction of congeneric exotic water plant species like *Elodea canadensis* and *Elodea nuttallii*. Both species are native to North America and were introduced to Europe. *E. canadensis* arrived to the British Isles in the middle of the 19th century. After the first records of *E. nuttallii* in 1966, this new invader spread rapidly all over England and displaced *E. canadensis* even at sites where the latter was well-established (Lund, 1979; Simpson, 1984; Simpson, 1990). This displacement was relatively rapid, taking place over a period of years, and is consistent with displacements elsewhere in Western Europe (Barrat-Segretain, 2001). From 1870 onwards *E. canadensis* was also present in Central European waters while *E. nuttallii* arrived in the beginning of the 21th century in Central European waters i.e. Slovakia (Ot'ahel'ová and Valachovič, 2002), Hungary (Sipos et al., 2003; Király et al., 2007), Croatia, (Grudnik and Germ, 2013; Grudnik et al., 2014; Kočic et al., 2014) and it was questionable whether *E. canadensis* would be displaced.

Several authors suggested that the displacement of *E. canadensis* by *E. nuttallii* was due to differences in their ecophysiological responses to environmental variables. However, observed differences in their ecophysiological responses like growth rate along a single gradient of temperature (Kuni, 1982; Kuni, 1984), nitrogen and phosphorus accumulation (Robach et al., 1995; James et al., 2006), photosynthesis and respiration (James et al., 1999), allelopathic activity against algae (Erhard and Gross, 2006; Lürling et al., 2006) all seemed too slight to induce such a displacement. Also, the tiny differences in life-history traits like fragment regeneration, colonization, and palatability seem also not plausible to explain the observed displacement of *E. canadensis* by *E. nuttallii* (Barrat-Segretain et al., 2002; James et al., 2006). Interestingly, Barrat-Segretain (2005) concluded from an experiment that *E. nuttallii* seemed to be the better competitor of the two for light. Furthermore, data from The Netherlands and Central European waters suggested that the replacement of *E. canadensis* by *E. nuttallii* was especially pronounced in ditches in agricultural areas, where total nitrogen input was much higher (Knoben and Peeters, 1997; Király et al., 2007; Grudnik et al., 2014). A recent study showed that light and nitrogen jointly triggered the development of those phenotypic characteristics that makes *E. nuttallii* a more successful invader in eutrophic waters than *E. canadensis* (Szabó et al., 2019). These two factors (light availability and nitrogen) are strongly related to eutrophication. The responses to these factors separately have been well documented (light: Sand-Jensen and Madsen, 1991; Madsen and Sand-Jensen, 1994; Angelstein and Schubert, 2009; nutrients: Barrat-Segretain, 2004; James et al., 2006), but their combined impact has been investigated only on the phenotypic characteristics (Szabó et al., 2019).

In this study we go further. We hypothesize that small ecophysiological differences between the two *Elodea* species become more pronounced under increasing nitrogen concentrations and decreasing light conditions and this may contribute to the invasion success of *E. nuttallii*. The present study aims to evaluate this hypothesis by investigating the combined effects of light and nitrogen on the ecophysiological responses of the two *Elodea* species in an indoor experiment. Since both *Elodea* species may strongly modify light conditions if they are grown in co-cultures (Barrat-Segretain and Elger, 2004; Barrat-Segretain, 2005), both species were cultivated separately in order to exclude these effects.

METHODS

Plant Collection, Preincubation

E. nuttallii shoots were collected from the Eastern Principal Channel, (N 47.860911°, E 21.382270°) and *E. canadensis* from the River Bodrog (N 48.172491°, E 21.363358°) Hungary. The selected apical shoots were preincubated for 18 days under experimental conditions. Shoots were set in five plastic boxes containing 20-L culture medium (Barko and Smart, 1985). The supply of phosphorus was ensured by adding K_2HPO_4 to the final concentration to 0.2 mg P L^{-1} and supply of micronutrients by adding TROPICA Supplier micronutrient solution. Final concentration of the solutions for nitrogen varied from oligotrophic (0.05 mg L^{-1}), mesotrophic (0.25 mg L^{-1}), eutrophic (0.5 mg L^{-1}), and hypertrophic (2.5 and 5 mg L^{-1})

among the treatments by adding NH_4NO_3 stock solution to the medium (Szabó et al., 2019). The culture medium was renewed every second day. The length of the shoots was reduced to 65 mm after preincubation. Subsamples of three initial shoots of each species from each nitrogen concentration was measured for fresh and dry weight (W_0).

Laboratory Experiment

Elodea plants (three shoots) were set on a plastic grid and placed in 2-L aquaria containing the culture media described above. All aquaria were put into a controlled temperature (23–25°C) water bath with renewing of the medium every second day. For both *Elodea* species, the five different N treatments were incubated in a 16:8 h L/D regime at five different light intensities varying from complete dark to well-illuminated conditions: 0, 10, 28, 80, and 180 μmol m^{-2} s^{-1} PAR photon flux density (Szabó et al., 2019). Illumination was carried out by 400 W metal halogen lamps and by using plastic gauze above the aquaria. Each treatment was replicated four times meaning that 200 aquaria were used. The plants were harvested after 12 days of incubation.

Relative Growth Rate

Six leaves were taken from each shoot (18 leaves per aquarium) and divided into two portions. One portion was used for fresh weight and chlorophyll determination, the other for dry weight determination (W_{leave}). For a single measurement per aquarium, dry weight of the three shoots and their roots of each aquarium were measured. Samples were dried at 65°C for 2 days. The root–shoot ratios were expressed on a dry-weight basis. The relative growth rate (RGR) of the plants was calculated as $\text{RGR} = (\ln W_t - \ln W_0)/t$, where W_0 represents the initial and W_t the final dry weight (in g) of the three plants in each aquarium and t is the growing time in days. The light compensation point for growth I_c (μmol m^{-2} s^{-1}) was estimated according to Sand-Jensen and Madsen (1991). Weight loss in the dark (RGR_d, 10^{-3} day^{-1}) was measured for plants incubated in the dark for 12 days (0 μmol m^{-2} s^{-1}) compared to initial weight (W_0).

Photochemical Efficiency

The actual photochemical efficiency of photosystem II (Φ_{PSII}) was measured by means of chlorophyll fluorescence with MINI-PAM fluorometer (Walz, Germany). The measurement on the plants was carried out using the middle part of the apical shoots from each aquarium as described by Snel et al. (1998). The shoots were placed in a 25 ml glass tube and faced to the common end of the optical fiber of the fluorometer. The actual photochemical efficiency of photosystem II was calculated as $\Delta F/F_m' = (F_m' - F_s)/F_m'$ with F_m' the maximal fluorescence and F_s is the steady-state fluorescence of the illuminated shoots (Genty et al., 1989). Steady-state fluorescence (F_s) was achieved after exposure to actinic light for 10 min. Maximum-fluorescence under steady-state conditions (F_m') was determined by applying pulses of the saturating light when the actinic light was on (Marwood et al., 2001). The duration of the saturating light pulses was 500 ms and the pulses were given every 60 s. The average of three chlorophyll fluorescence measurements represented the photochemical efficiency of the plants in each aquarium.

Chlorophyll Concentration

For a single measurement per aquarium, chlorophyll of the nine leaves from each aquarium was extracted in a test tube containing 6 ml 95% ethanol for 24 h at 4°C in the dark. Total chlorophyll concentrations were measured by spectrophotometry (T80+ Spectrometer, PG Instruments Limited, UK) and were calculated according to Lichtentaler (1987). Leaf chlorophyll concentrations were expressed on a dry-weight basis of the leaves (W_{leave}).

Elemental Composition

Samples were taken from the water at the end of the experiment. We first recorded pH and thereafter samples were filtered (mesh size 0.45 μm) and analyzed for NO_3^-–N, NO_2^-–N, NH_4^+–N (Technicon Auto Analyzer). At the end of the experiment nitrogen and carbon concentration of the dried plants had been grown at 10–180 μmol m^{-2} s^{-1} light intensity (160 samples) was analyzed by dry combustion using a Vario Max Cube elemental analyzer (Elementar GMBH, Germany).

Statistical Analysis

Normality of the variables was checked by the Kolmogorov–Smirnov test. RGR, chlorophyll concentration of the leaves, root–shoot ratio and nitrogen concentration of the plants were all normally distributed ($P > 0.05$). Data of actual photochemical efficiency of photosystem II and C/N ratio were log-transformed for normality. A general linear model was used to test the significance of the factors (light, nitrogen, species identity) and their interactions on the variables. Residuals were checked for normality and homogeneity of variances was evaluated by Levene's test. Tukey post-hoc tests were used to evaluate which treatments differed from each other. Pairwise comparisons were used to test the variables for significant differences between species where the mean difference (MD) + standard error were indicated. All analyses were done using SPSS 16.0 software.

RESULTS

Relative Growth Rate

Species identity, nitrogen concentration, light intensity, and their interactions significantly influenced the RGR (**Table 1**). The RGR of both *Elodea* species increased with increasing light intensity and with increasing N concentration (**Figure 1**). Furthermore, pairwise comparisons showed that the differences between the species were statistically significant (**Table 1**). Growth seemed to be saturated above 80 μmol m^{-2} s^{-1} for *E. nuttallii* at all N concentrations except for the lowest one, and in contrast, light stimulated the growth of *E. canadensis* up to the highest light intensity (180 μmol m^{-2} s^{-1}). The RGR measured at the highest light intensity was significantly higher for *E. canadensis* than for *E. nuttallii* (MD 0.025 ± 0.002 Pairwise comparisons $P < 0.001$). However, under low light levels (0–10 μmol m^{-2} s^{-1}) *E. nuttallii* showed a significantly higher growth rate than *E. canadensis* (MD 0.012 ± 0.002 Pairwise comparisons $P < 0.001$). Weight loss in the dark differed significantly (pairwise

TABLE 1 | Analysis of variance of the relative growth rate (RGR), actual photosynthetic efficiency of PSII, chlorophyll concentration (Chl cc), root–shoot ratio of *Elodea* (*E. canadensis*, *E. nuttallii*) cultures grown in aquaria under different nitrogen concentrations in the water combined with different light intensities.

Source/Trait	*df*	Mean Square	*F*	Sig.
RGR				
Species	1	0.00	7.91	0.01
Light	4	0.10	1,739.81	<0.01
Nitrogen	4	0.01	112.24	<0.01
Light * Species	4	0.00	57.06	<0.01
Nitrogen * Species	4	0.00	4.72	<0.01
Light * Nitrogen	16	0.00	8.94	<0.01
Error	150	0.00		
Photosynthetic efficiency				
Species	1	0.01	32.21	<0.01
Light	4	0.86	1,903.57	<0.01
Nitrogen	4	0.06	134.30	<0.01
Light × Species	4	0.03	70.56	<0.01
Nitrogen × Species	4	0.00	9.44	<0.01
Light × Nitrogen	16	0.01	17.82	<0.01
Error	150	0.00		
Chl cc				
Species	1	1,975.04	544.42	<0.01
Light	4	892.66	246.06	<0.01
Nitrogen	4	2,112.38	582.28	<0.01
Light × Species	4	49.04	13.52	<0.01
Nitrogen × Species	4	77.45	21.35	<0.01
Light × Nitrogen	16	54.07	14.90	<0.01
Error	150	3.63		
Root–shoot ratio				
Species	1	0.02	144.82	<0.01
Light	4	0.12	841.85	<0.01
Nitrogen	4	0.02	161.74	<0.01
Light × Species	4	0.00	18.45	<0.01
Nitrogen × Species	4	0.00	15.86	<0.01
Light × Nitrogen	16	0.00	33.89	<0.01
Error	150	0.00		

comparisons $P < 0.001$) between the two *Elodea* species and was higher for *E. canadensis* than for *E. nuttallii* (MD 0.015 ± 0.002). The increase of N concentration from 2.5 to 5 mg L^{-1} did not cause differences in the growth rate of either species (**Figure 1**).

The light compensation point (I_c) decreased sharply with increasing N concentration and was significantly higher (MD 5.500 ± 2.131 pairwise comparisons, $P = 0.033$) for *E. canadensis* than for *E. nuttallii* (**Figure 2**).

Photochemical Efficiency

Light intensity, nitrogen concentration, and their interactions had a significant impact on the actual photochemical efficiency of photosystem II (Φ_{PSII}) (**Table 1**). Both *Elodea* species showed the highest Φ_{PSII} at low and medium light levels (**Figure 3**, 0–28 µmol m^{-2} s^{-1}) and the lowest Φ_{PSII} at the highest light level within each nitrogen concentration. Actual photochemical efficiency of photosystem II increased significantly at lower light intensities ($P < 0.001$) and at higher nitrogen

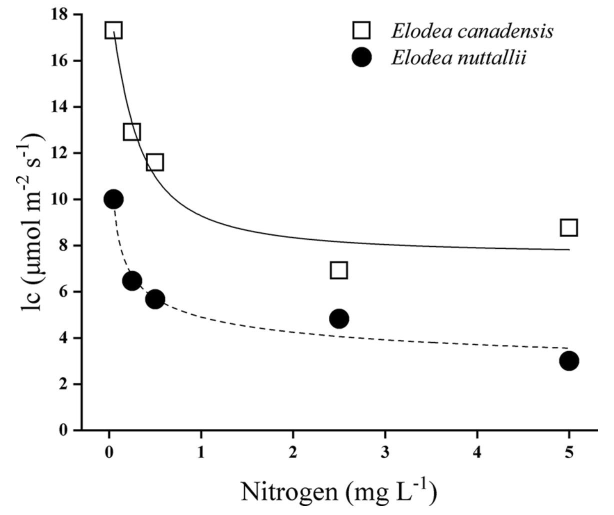

FIGURE 2 | Light compensation point (I_c) of *E. canadensis* and *E. nuttallii* cultures grown at different nitrogen concentrations. I_c values were estimated according to Sand-Jensen and Madsen (1991).

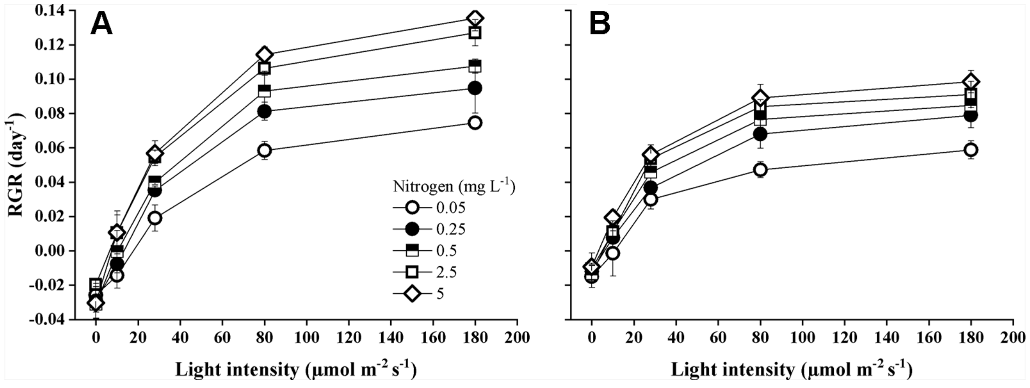

FIGURE 1 | Relative growth rate (RGR) of *E. canadensis* **(A)** and *E. nuttallii* **(B)** cultures grown at different nitrogen concentrations and light levels (mean ± SD, $n = 4$).

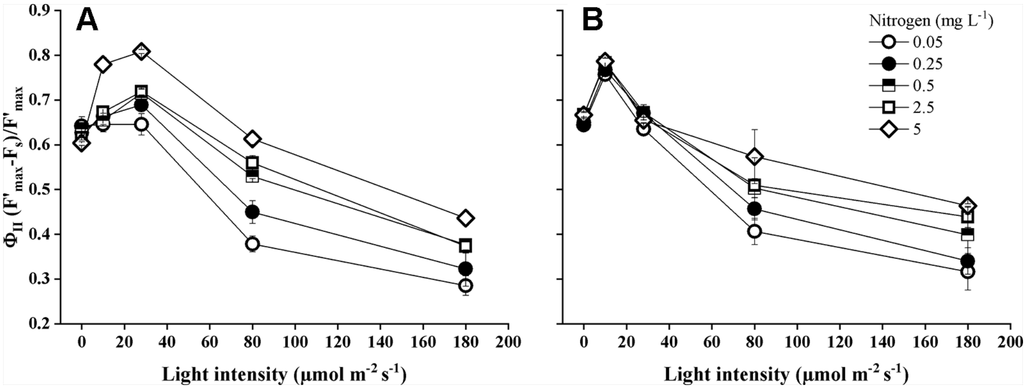

FIGURE 3 | Actual photochemical efficiency of photosystem II (Φ_{II}) of *E. canadensis* **(A)** and *E. nuttallii* **(B)** cultures grown at different nitrogen concentrations and light levels (mean ± SD, $n = 4$).

concentrations ($P = 0.022$). Photochemical efficiency differed significantly (pairwise comparisons $P < 0.001$) between the two *Elodea* species and was higher for *E. nuttallii* than for *E. canadensis* (MD 0.017 ± 0.003). Under low light levels (0–10 µmol m^{-2} s^{-1}), the difference for Φ_{PSII} between the two *Elodea* species was even higher (MD 0.063 ± 0.003) (**Figure 3**).

Total Chlorophyll Concentration

Species identity, nitrogen concentration, light intensity, and their interactions significantly influenced total chlorophyll concentrations of the leaves (**Table 1**). Chlorophyll concentration was significantly higher (pairwise comparisons, $P < 0.001$) in *E. nuttallii* than in *E. canadensis* (MD 6.28 ± 0.27). According to the Tukey test, both species demonstrated that increasing light intensity resulted in significantly ($P < 0.001$) lower chlorophyll concentrations, but this was also depending on the N concentration (**Figure 4**). Increasing the light intensity, *E. canadensis* showed a much stronger drop in chlorophyll concentration than *E. nuttallii*. Lowering the nitrogen concentration significantly ($P < 0.001$) reduced chlorophyll concentration (**Table 1**) in both species (**Figure 4**).

Root–Shoot Ratio

Species identity, light intensity, and the statistical interaction between light intensity and N concentrations had a significant effect on root-shoot ratios (**Table 1**). Root–shoot ratios were the highest (0.17–0.22) at lower nitrogen concentrations (0.25–0.5 mg N L^{-1}) and higher light intensities for both species (**Figure 5**). *E. canadensis* had significantly higher (pairwise comparisons, $P < 0.001$) root–shoot ratio than *E. nuttallii* (MD 0.021 ± 0.002). The difference in root–shoot ratios was even higher (MD 0.046 ± 0.006, $P < 0.001$) under the highest light level (180 µmol m^{-2} s^{-1}).

Chemical Composition of the Plants

Nitrogen concentration of the water, light intensity, and their interactions significantly influenced carbon/nitrogen ratio (C/N ratio) of the plants (**Supplementary Table 1**). C/N ratio of both *Elodea* species decreased with increasing N concentration and with decreasing light intensity (**Figure 6**). At the highest light intensity, tissue N concentration (mg N g^{-1}) was significantly higher for *E. nuttallii* than for *E. canadensis* (MD 6.043 ± 0.342, pairwise comparisons $P < 0.001$) (**Figure 7**).

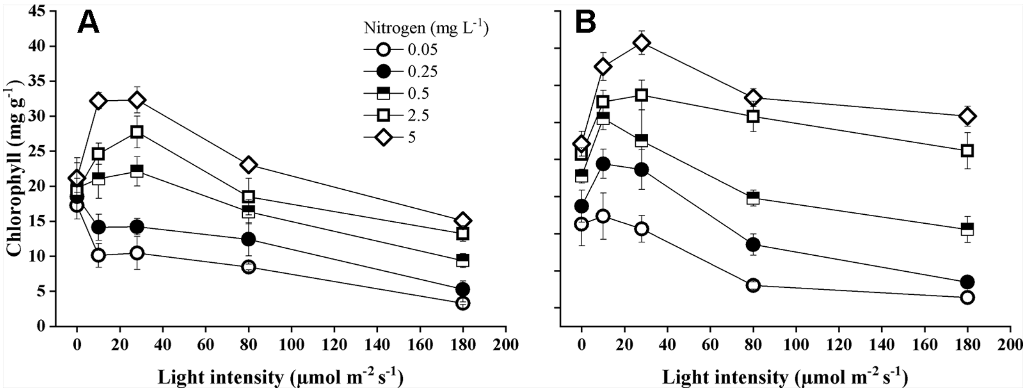

FIGURE 4 | Chlorophyll concentration (µg mg^{-1}) in the leaves of *E. canadensis* **(A)** and *E. nuttallii* **(B)** cultures grown at different nitrogen concentrations and light levels, (mean ± SD, $n = 4$).

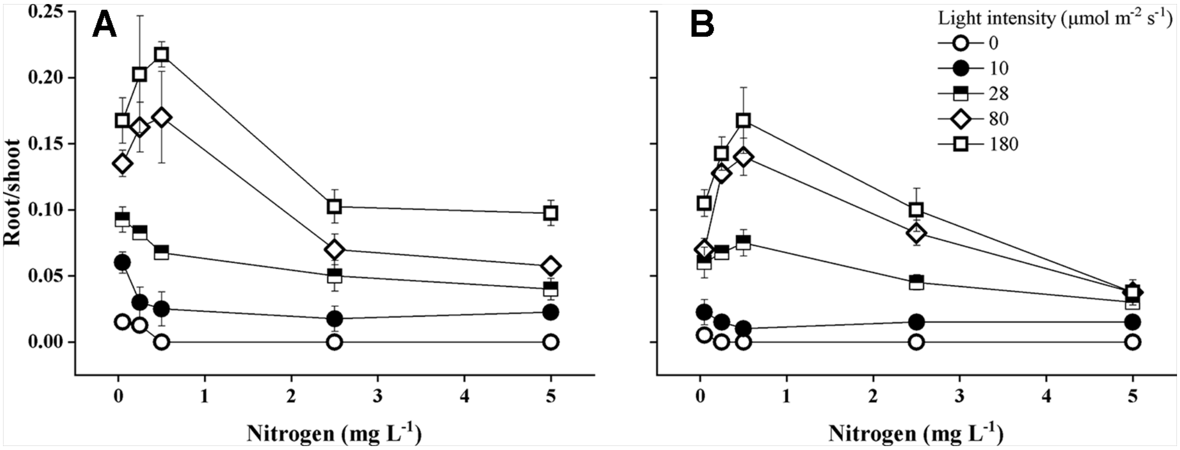

FIGURE 5 | Root–shoot ratio of *Elodea canadensis* **(A)** and *E. nuttallii* **(B)** cultures grown at different nitrogen concentrations and light levels, (mean ± SD, n = 4).

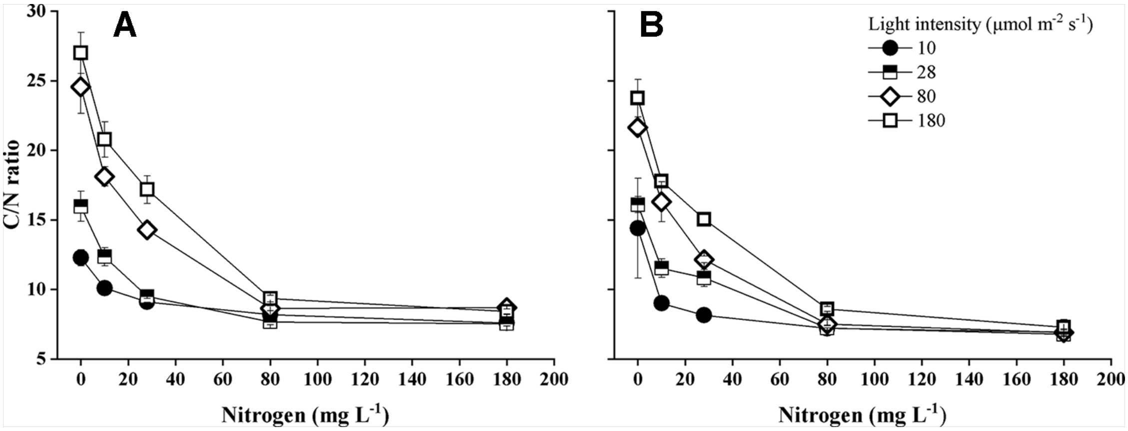

FIGURE 6 | Carbon/nitrogen ratio (C/N ratio) of *E. canadensis* **(A)** and *E. nuttallii* **(B)** cultures grown at different nitrogen concentrations and light levels (mean ± SD, n = 4).

Chemical Composition of Water

Total nitrogen removal by the two examined *Elodea* species (expressed by a drop in total nitrogen concentration of the water) decreased with decreasing light intensities. The final N concentration was significantly lower with *E. nuttallii* than with *E. canadensis* (pairwise comparison $P < 0.001$; MD 0.451 ± 0.021) when initial concentration was higher than 0.25 mg N L^{-1}. Under the highest light intensity and the highest initial nitrogen level (5 mg L^{-1}), the difference in the final nitrogen concentration between the two species was even higher (pairwise comparison $P < 0.001$; MD 2.558 ± 0.057) (**Figure 8**). At the highest light intensity, *E. nuttallii* increased the pH more markedly (10.41) than *E. canadensis* (9.74). We found the highest at the highest light level. The pH decreased drastically at reduced light intensities.

DISCUSSION

Interactive Effects of Light and Nitrogen

Interactive effects of various factors on plant growth have previously been observed more often for light and inorganic carbon, and for nitrogen and inorganic carbon (Madsen and Sand-Jensen, 1994; Madsen et al., 1998), light and temperature (Barko et al., 1982), light and nitrogen (Szabó et al., 2019), temperature and phosphorus (Peeters et al., 2013), and nitrogen and phosphorus (Cao et al., 2011; Li et al., 2016). In this study, light and nitrogen both affected the performance of the two *Elodea* species. Interactive effects of light and nitrogen were also obvious: increase in light stimulated growth twice as much under high nitrogen concentrations (2.5 and 5 mg L^{-1}) than at lower N levels (0.05 mg L^{-1}). Therefore, the same growth rate can be obtained under different conditions: reduced

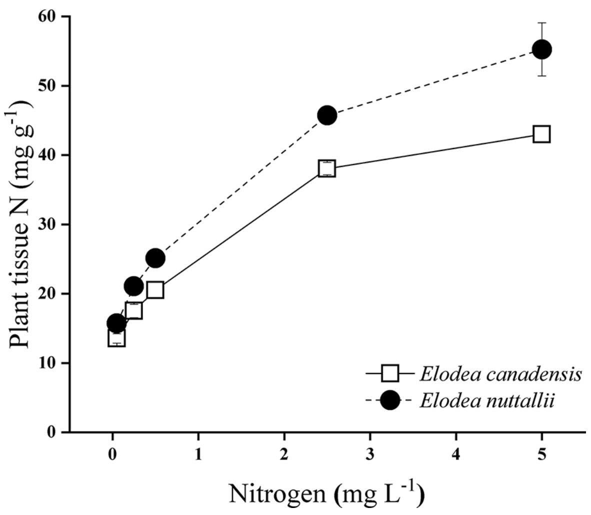

FIGURE 7 | Plant tissue nitrogen concentration of *Elodea* species grown at different nitrogen concentrations at the highest light intensity (180 µmol m^{-2} s^{-1}) (mean ± SD, *n* = 4).

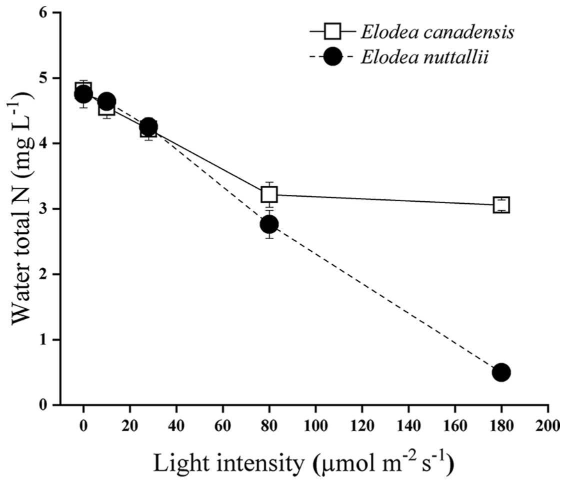

FIGURE 8 | Total nitrogen concentration of the medium with *Elodea* species grown under 5 mg L^{-1} initial N concentration at different light levels (mean ± SD, *n* = 4).

light conditions but with more nitrogen gave similar yields as lower nitrogen and more light.

Light Compensation Point, Weight Loss in the Dark

The light compensation (I_c) point of *E. canadensis* grown under optimal nitrogen supply in the present study closely approximates the results of Madsen and Sand-Jensen (1994) who kept the plants under an optimal carbon supply. They also found a sharp increase in the light compensation point under lowered inorganic carbon supply as with nitrogen levels in the present study. *E. canadensis* had a much higher light compensation point (I_c) than *E. nuttallii*. This indicates that the latter species may survive better under more shaded conditions, such as turbid water, periphytic algae, or below a dense mat of floating plants (Angelstein and Schubert, 2009; van Gerven et al., 2015). Weight loss in the dark of *E. canadensis* was approximately two times higher than recorded by Madsen and Sand-Jensen (1994), which can be explained by dark respiration being lower at low temperatures. Interestingly, *E. nuttallii* showed lower weight loss in the dark than *E. canadensis,* and this might be an important indication that *E. nuttallii* can tolerate darkness for a longer period.

Change in Chlorophyll Concentration

Since nitrogen is a crucial component of chlorophyll, it is not surprising that the total chlorophyll concentration of both *Elodea* species was strongly affected by the availability of nitrogen. Therefore, our results are in line with the findings of many authors (Szabó et al., 2005; Szabó et al., 2010; Zhao et al., 2010). In general, *E. nuttallii* had a higher chlorophyll concentration than *E. canadensis* and plants showed a peak in chlorophyll under rather low light conditions (10–28 µmol m^{-2} s^{-1}) as was also found by Madsen and Sand-Jensen (1994). In complete darkness, however, both species lost some chlorophyll content since they did not receive sufficient energy to sustain chlorophyll synthesis and maintaining the photosynthetic apparatus for a long period is uneconomical (Raven, 1984).

Change in Photosynthetic Efficiency

The actual photosynthetic efficiency of both *Elodea* species was slightly decreased under low nitrogen supply which is in line with the studies of Cruz et al. (2003) and Huang et al. (2004) and indicates that nitrogen deficiency causes damage to PSII reaction centers (Verhoeven et al., 1997; Lu et al., 2001). Furthermore, nitrogen is essential for protein synthesis in order to sustain or rebuild the photosynthetic apparatus (Evans, 1989). Under low light levels (0–10 µmol m^{-2} s^{-1}) parallel to the RGR results, *E. nuttallii* showed marginally but significantly higher actual photosynthetic efficiency than *E. canadensis*. Higher Φ_{PSII} efficiency of *E. nuttallii* can be taken as a characteristic related to shade tolerance only in combination with the lower lc which the primary factor here. Remarkably, after twelve days of incubation in total darkness, both *Elodea* species still showed significant photosynthetic activity indicating that if oxygen is not limited, they can tolerate dark conditions for extended periods.

Morphological and Ecophysiological Strategies

Both *Elodea* species showed a high ability to acclimate to various light and nutrient levels, and they shared many similarities in their ecophysiological and morphological responses (**Figure 9**). Under high nitrogen levels the plants seem to invest more energy in photon capture than in nutrient uptake as evidenced by the reduced root-shoot ratio (James et al., 2006), light compensation point, increased chlorophyll concentration and photochemical efficiency which is in line with the studies of Li et al. (2016) and Gautam et al. (2016). An alternative explanation could be that

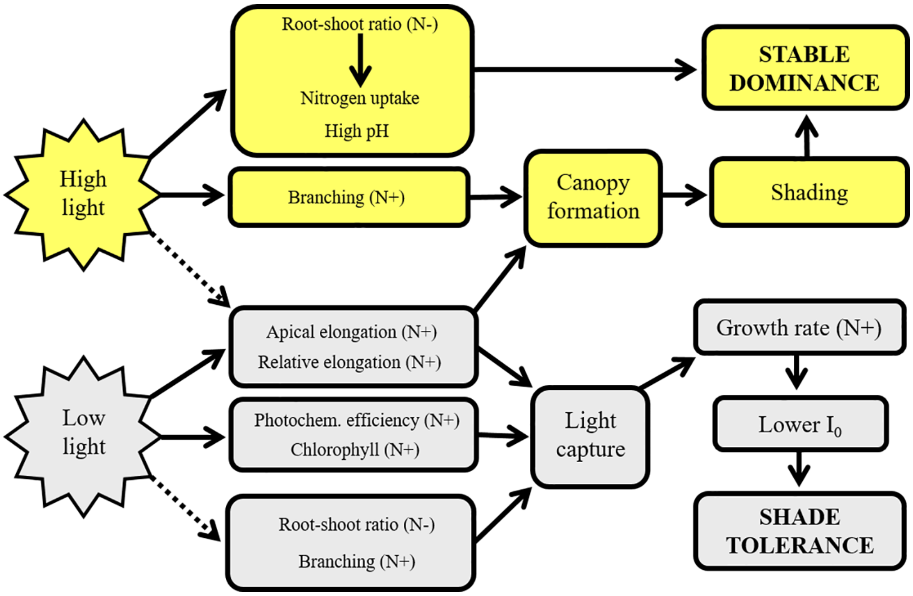

FIGURE 9 | The effect of light levels and nitrogen on the ecophysiological and phenotypic traits of *Elodea nuttallii* resulting in invasion success. Solid lines represent stimulation, dashed lines represent inhibition processes. N+ and N– indicate the stimulating or inhibitory impacts of increased nitrogen supply, I_0 light compensation point.

plants invest less in root biomass since less below-ground biomass is necessary for nutrient uptake in N rich water (Madsen and Cedergreen, 2002). At low light levels, similarly as high nitrogen levels, the plants also redirected resources towards a more efficient photon capture rather than nutrient uptake as proved by the increase in the chlorophyll concentration in the leaves together with a higher actual photochemical efficiency of PSII, increased shoot elongation per unit biomass and reduced allocation to root formation and reduced nutrient removal (**Figure 9**). At low light levels, *E. nuttallii* the stronger invader showed drop in branching.

Increase in chlorophyll concentration with lowering light is well in line with the results of Angelstein and Schubert (2009). In our study, both morphological (root–shoot ratio) and ecophysiological (actual photochemical efficiency of PSII, chlorophyll concentration) changes were similar to those observed in response to decreased light intensities caused by shading from floating vegetation which has been found in other submerged macrophytes (Janes et al., 1996; Forchhammer, 1999; Lu et al., 2013).

Displacement Mechanisms

Our laboratory study showed that *E. canadensis* had a slightly higher growth rate than *E. nuttallii*, supporting the view that both species responded similarly to changing light and N-levels. The directions of the differences were always similar; however the magnitude was different (**Supplementary Table 2**). The replacement of *E. canadensis* by *E. nuttallii* indicates that there must be differences between both species. From our laboratory experiment we are not able to infer the competitive abilities of the two *Elodea* since we had no mixed cultures (McCreary, 1991). However, it is evident that in a competition experiment, the competition itself may mask relevant mechanisms but also small differences between the two species. The data of tissue N concentration and the decrease in nitrogen concentration in the water indicated that under higher light intensity *E. nuttallii* has much stronger nitrogen removal capacity than *E. canadensis* (**Figure 8**, **Supplementary Table 2**). This trait may especially advantageous in waters with suboptimal nitrogen concentration. Furthermore, *E. nuttallii* exhibited less reduction in biomass and showed lower light compensation point than *E. canadensis* and seemed thus more shade tolerant (**Supplementary Table 2**). In hypertrophic ditches and ponds, thick layers of floating algal mat reduce light conditions (Hillebrand, 1983) in spring whereas in summer floating vegetation may cause dense shade on submerged plants reducing their growth (Scheffer et al., 2003; van, Zuidam and Peeters, 2013; van Gerven et al., 2015). In addition, at high trophic levels *E. nuttallii* has much less periphytic algal biomass than *E. canadensis* (James et al., 2006) thus the new invader may have an even greater advantage for light capture.

Our former results pointed out that *E. canadensis* tends to produce dense canopy with numerous branches even under low nitrogen and light levels. Thus, *E. canadensis* shows less apical growth that might be a disadvantage in the competition for light with *E. nuttallii*. On the contrary, *E. nuttallii* invests much more on apical shoot elongation and thereby gain a better position for light capture (Szabó et al., 2019) (**Figure 9**, **Supplementary Table 2**). Under low light levels, *E. nuttallii* is able to elongate much faster due to its higher elongation and lower branching degree abilities and lower light compensation point (**Figure 9**). Thus, the shoots of *E. nuttallii* are able to achieve optimal light conditions sooner. Near to the water surface under high trophic level, they can form a dense canopy (Kuni, 1984) due to their increased branching degree, resulting in a strong shading for other submerged plants (**Figure 9**). Therefore, under hypertrophic conditions stands of *E. nuttallii* may develop sooner than that of *E. canadensis* and the stronger invader can sustain its stable dominance not only against other submerged plants but against algae and floating vegetation as well (Szabó et al., 2010).

Field observations showed that the replacements of *E. canadensis* by *E. nuttallii* occurred under hypertrophic conditions with nitrogen concentrations above 2 mg L^{-1} (Knoben and Peeters, 1997; van Zuidam and Peeters, 2013; Kočic et al., 2014). Actually, in this study, the niche for light requirement of *E. nuttallii* was narrower than that of *E. canadensis*. Therefore, our results partly contradict the idea of Higgins and Richardson (2014) who concluded that stronger invaders have broader physiological niches. However, this kind of adaptation to achieve fitness and invasiveness was more optimal under shaded conditions. These ecophysiological differences between the two species provide insights that could improve the understanding of the mechanisms of invasion processes under varying light and nutrient levels (**Supplementary Table 2**, **Figure 9**).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

SS designed and performed the experiments. PN analyzed the chemical composition of the plants. SS, EP, and BL analyzed the data. SS, BL, GB, SV, and EP wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.01747/full#supplementary-material

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Evaluation of Habitat Preferences of Invasive Macrophyte *Egeria densa* in Different Channel Slopes Using Hydrogen Peroxide as an Indicator

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Egeria densa is an often-found invasive species in Japan, which has spread widely in the past two decades in rivers where no macrophytes had previously been found. As a result, these ecosystems have now become dominated by *E. densa*. The habitat preference for *E. densa* colony formation was investigated using the tissue concentrations of hydrogen peroxide (H_2O_2: a reactive oxygen species) under varying conditions in rivers and laboratory conditions. The empirical equations that can describe the macrophyte tissue H_2O_2 formation under various velocity and light conditions were produced. The H_2O_2 concentrations of dark-adapted plants are proportional to the flow velocity, and the surplus H_2O_2 concentration in the light-exposed condition corresponded to the photosystems produced H_2O_2. When the H_2O_2 concentration exceeds 16 μmol/gFW, plant tissue starts to deteriorate, and biomass declines, indicating the critical values required for long-term survival of the plant. The empirically obtained relationships between flow velocity or light intensity and the analysis of H_2O_2 concentration for different slopes and depths of channels found that the H_2O_2 value exceeds the critical H_2O_2 concentration in channels with above 1/100 at around 0.6 m depth. This agrees with the observed results where colonies were not found in channels with slopes exceeding 1/100, and biomass concentration was the largest at depths of 0.6 to 0.8 m. H_2O_2 concentration is quite applicable to understanding the macrophyte condition in various kinds of macrophyte management.

Keywords: invasive species, environmental stress, oxidative stress, river vegetation, empirical equations

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INTRODUCTION

The mid-streams of large Japanese rivers were characterized as gravel beds during the post-World War II era. Fine sediment beds were extremely limited. Thus, the ecosystems of gravel beds, characterized by rich hyporheic flows and biota, such as insect larvae and salmonid fish, were maintained for long periods (Hauer et al., 2016). Except for some emergent species, *Phragmites japonica* (Asaeda et al., 2009), almost no submerged macrophyte colonies existed in the main streams of major rivers (Kadono, 2004). Since then, dams and weirs have been frequently constructed and most of the waterways have been regulated. Therefore, almost all the gravel particles introduced upstream are trapped before entering the midstream, thus the supply of gravel to the midstream and downstream is completely curtailed. In addition, gravel was mined for use as construction materials from the 1960s to 1970s. The amount of gravel, therefore, substantially reduced in the midstream, compared to that of previous years (Asaeda and Sanjaya, 2017). In contrast, fine sediment inflows continued from the mid to downstream catchments. They were transported and settled, filling interstices on downstream gravel beds. Thus, the midstream beds are now partially covered with fine sediments that bury stones.

In the past two decades, invasive macrophyte *Egeria densa* began to form colonies in many rivers (Ministry of Lands Infrastructure Transportation and Tourism in Japan [MLIT], 2016). It often covers extensive areas of the channel bed and completely changes the ecosystem there (Collier et al., 1999; Yarrow et al., 2009). Financially, this causes substantial losses to inland fisheries, particularly in the yield of Ayu fish (*Plecoglossus altivelis altivelis*), a grazer of benthic algae (Asaeda et al., 2018). *E. densa* was cultivated in aquariums in the early 19th century, but it was disposed into natural freshwater bodies and became naturalized in the 1940s. However, it had not spread into rivers as they were gravelly in those days and were not in a suitable condition to support submerged macrophytes, however, it has been found in some lakes of western Japan since the 1970s (Kadono, 2004). Besides Japan, *E. densa*, spread widely in other continents (Champion and Tanner, 2000; Santos et al., 2011). It affected stream ecosystems extremely, retarding flow velocity, increasing sedimentation (Collier et al., 1999), and exile of native species (Santos et al., 2011; Gillard et al., 2017). Therefore, though the effects were particularly eminent in Japanese gravel rivers, the invasion of *E. densa* is a worldwide problem.

As several changes occur simultaneously in natural rivers, it is not easy to elucidate the primary reason that prevented earlier macrophyte colonization or their increase today. The habitat preferences of macrophytes are normally evaluated by monitoring their growth rate or biomass (Barko et al., 1991; Riis et al., 2012; O'Hare et al., 2018). However, there are various potentially influential factors in the natural environment and each factor changes from time to time during the period of the macrophytes' growth. The existing conditions are, thus, considered to be a result of the integrated environmental conditions experienced previously, and the casual observation that is mainly practiced in vegetation management is not necessarily appropriate for evaluating their habitat preference.

In natural water, macrophytes are subjected to environmental stresses, such as flow velocity, high solar radiation, excessive high or low temperature, etc. In cell organelles, then, reactive oxygen species (ROS) are generated based on the intensity of the stresses, photosynthesis and metabolic activities (Zaman and Asaeda, 2013; Asaeda and Rashid, 2017; Parveen et al., 2017a). A part of these ROS is scavenged relatively quickly by antioxidant activities, and the homogeneity of ROS in tissues is maintained by a balance between the ROS and the antioxidants. However, under excessive stress, this balance collapses as oxidative stress surpasses the antioxidant capacity of the plant. The existence of ROS in plant tissues leads to oxidative stress, and when critical levels are exceeded, the plants tend to deteriorate (Sharma et al., 2012; Choudhury et al., 2017). The most common ROS is hydrogen peroxide (H_2O_2), which is generated by the superoxide dismutase by in the superoxide (Asada, 2006; Sharma et al., 2012). The H_2O_2 concentration is relatively stable and can be easily analyzed chemically (Satterfield and Bonnell, 1995; Zhou et al., 2006). The amount of tissue H_2O_2 concentration, therefore, has potential for use as an indicator to monitor the instantaneous environmental stress intensity on macrophytes (Asaeda et al., 2018).

During the daytime, the total amount of H_2O_2 generated in plant tissues is, therefore, primarily the sum of the H_2O_2 generated in response to environmental stress, photosynthesis, and other nonstress metabolic products. This process can be generalized as the following simple equation:

$$H_2O_{2(\text{Total})} = H_2O_{2(\text{Photosynthesis})} + H_2O_{2(\text{Metabolic byproduct and respiration})} + \sum H_2O_{2(\text{Environmental stress})} \tag{1}$$

Although there are some interactions between the different environmental stressors and opposing trends in some combinations of stressors (Rivero et al., 2014), the share of H_2O_2 concentration of stresses is separated from metabolic, respiration, and photosynthesis produced H_2O_2 (Mittler, 2002). However, the plant oxidative stress is determined by cumulative H_2O_2 content present in the cells regardless of the source.

Several types of stressors are acting on submerged macrophytes in natural rivers. In the relatively steep non-polluted rivers, the major stressors include the mechanical stress introduced by high current velocity/turbulence, solar radiation, and temperature (Yarrow et al., 2009; Riis et al., 2012). As these are based on different physical quantities, it is difficult to compare the magnitude of each stressor on the submerged macrophytes. However, it is possible to differentiate photosynthesis produced H_2O_2 from the total accumulated H_2O_2 by dark adapting the plants (Asaeda et al., 2018). In addition, when other stresses are eliminated under controlled conditions in the laboratory, it is possible to quantify each type of stress by the produced H_2O_2.

Considering the facts that, it can be hypothesized that (1) there is a relationship between induced H_2O_2 concentration in *E. densa* and the intensity of each stress given by the habitat metrics, such as water velocity, temperature, and the solar radiation of the habitat. (2) *E. densa* growth reduced and is deteriorated in the condition in which H_2O_2 concentration exceeds a threshold value. (3) The habitat metric condition for *E. densa*, therefore, remains to be a H_2O_2 concentration less than the threshold value. Then, the habitat preference and adaptability of *E. densa* is studied in terms of H_2O_2 formation under varying riverine conditions and the controlled conditions in the laboratory, focusing on obtaining empirical relationships of factors on the tissue H_2O_2 contents.

As the tissue presence of H_2O_2 can be used to evaluate the plant condition, and the plant H_2O_2 content can be evaluated in a short period, it has the potential to be adopted in macrophyte monitoring practices. In the present study, we focused the H_2O_2 production of *E. densa* over various field and laboratory conditions. However, the methodology is widely applicable for various types of macrophyte managements, such as the identification of the optimum condition in the endangered species' restoration, or in the extermination of alien species.

MATERIALS AND METHODS

Field Observations

Several rivers that are highly colonized by *E. densa* were selected from the species distribution records in Japan (Ministry of Lands Infrastructure Transportation and Tourism in Japan [MLIT], 2016). In 2016 and 2017, observations were conducted in rivers for location data of *E. densa* colonization. Sampling activities were conducted on fine days (days with clear sky and no rain expected) in different seasons from Eno (Go), Saba and Hii Rivers, including their tributaries (**Table 1**). In each river, the surveys reached approximately 20 to 50 km from the upstream to the downstream area, and five to ten sites where more than one third of the bed was covered with pure *E. densa* colonies were selected for the study, including the most upstream colony in the main channel.

At each sampling site, there were several *E. densa* patches, and each patch was composed of several plants. Thus, more than five samples were collected from overlying shoots of different plants of a same patch in light-exposed (under natural conditions) and dark-adapted conditions to differentiate photosynthesis generated H_2O_2 from environmental factors and metabolism induced H_2O_2 of tissues. The dark exposure treatment was performed by placing a black plastic sheet (3 m × 3 m) floating over the *E. densa* colonies for 30 min. The 30 min pre-dark period was determined from laboratory experiments, which were conducted to determine the optimum pre-darkness duration. The plastic sheets were tied to fixed metal poles that were inserted in the riverbed, allowing the sheets to float on the water surface without causing mechanical disturbances to the macrophytes or altering the water flow. The PAR intensity under the sheet was found to be zero. The light-exposed samples were collected adjacent to the darkness treated samples. The collected samples were put in resealable plastic bags and quickly stored in a cool box containing dry ice until they were transferred to the laboratory to be stored at −80°C. Biomass was obtained from a 50 cm × 50 cm quadrant of each sampling point.

At each sampling point, the water velocity was measured with an ultrasonic velocimeter and recorded for more than 1 min (Tokyo Keisoku Co. Ltd., Japan), at 20% (reference velocity) and 80% (depth of the colony) of the total water depth. Turbulence velocity component was calculated as a root mean square deviation from the mean velocity, from the velocity record. Photosynthetically active radiation intensity (PAR intensity) in the water was measured with a portable quantum flux meter (Apogee, MQ-200, United States) at 10 cm depth intervals.

On October 23, 2018, the lateral configuration of a channel was investigated to derive the effect of the *E. densa* colony on the environment, including the distributions of depth, sand depositions thickness, *E. densa* biomass, particle size, and the *E. densa* burying condition in trapped sediments at a point 45 km upstream from the river mouth of the Yahagi River. During the summers of 2016, 2017, and 2018, surveys were conducted from the upstream to the downstream areas in other rivers where the existence of *E. densa* was recorded, then the locations and the depth of the rivers were recorded and the channel slope was obtained from a topographic map [Geographical Survey Institute of Japan, the (Gsi), 2018].

Laboratory Experiments

The pre-darkness period in the field observation was investigated as follows. The apical cuttings with an average length of 10 cm were obtained from the stock culture and planted in two tanks (50 cm × 35 cm × 35 cm) with thoroughly washed commercial river sand (90% of <0.2 mm particle size; washed using tap water several times until all organic materials are washed away and finally, washed using distilled water to further remove nutrients). In each tank, twenty *E. densa* cuttings were planted and maintained in a temperature-controlled room maintained at a constant 23 ± 3°C temperature. Each tank was exposed to approximately 100 μmol/m^2/s PAR with a 12 h/12 h light and dark period. Nutrients were supplied via a 5% Hoagland nutrient solution. After a two-month acclimatization period, one of the two tanks was covered entirely by a black plastic sheet, providing darkness. *E. densa* tissues from different samples were collected at 10 min intervals for 2 h and then collected at 6, 12, and 24 h. Light-exposed samples were collected, simultaneously. To avoid the stress of cutting, the tissues were collected from fresh tips during each sampling activity. The experiment was conducted in triplicates with different samples. The H_2O_2 concentrations of tissues were then analyzed. The H_2O_2 concentration of *E. densa* gradually declined with the dark duration, taking the lowest value at 30 min, then slightly increasing later for all cases. Therefore, in the field experiment, 30 min of darkness was adopted for the dark-adapted samples.

The effect of temperature on H_2O_2 generation was investigated, using four tanks similar to those in the previous experiments. After an acclimatization period of 2 months, the temperature regimes of the tanks were set to 10, 15, 25, and 30°C, respectively, using an aquarium water temperature controlling system (Aquarium cooler ZC-100α, Zensui Corporation, Japan). Light intensities, 220, 320 and 680 μmol/m^2/s were obtained with the combination of several LED lamps. Then, a 5 m long flume equipped with a straightening plate at the upstream end was used to check the velocity effect. The central part was lightened with 200 μmol/m^2/s of PAR intensity by a LED lamp. The velocity was adjusted at 23 cm/s to maintain the low turbulence intensity condition, less than 2 cm/s of turbulence velocity, following Asaeda et al. (2018), and the normal flow velocity of the *E. densa* habitat (Champion and Tanner, 2000). The temperature conditions were maintained for 7 days, and plants were sampled for the chemical analyses. The experiment was conducted in triplicates with different samples for each condition.

TABLE 1 | River channel data where large colonies of *E. densa* were found.

	River or tributary	Distance from the river mouth or conjunction (km)	Channel bed slope	Maximum channel depth at normal water level (cm)	Approximate depth of *E. densa* colony (cm)
1	Yahagi River	45.2	1/800	120	30–100
2	Yoshii River	83.5	1/200	50	40–50
3	Asahi River, Nakatsui River Tributary	14.6	1/270	50	40–50
4	Ashida River	79.1	1/210	40	40
5	Ashida River, Takaya River Tributary	0.5	1/1800	40	30–40
6	AshidaRver, Mitsugi River Tributary	9.3	1/220	50	40–50
7	Eno (Gono) River, Mainstream[a]	89.3	1/400	130	50–100
8	Eno (Gono) River, Tajibi River Tributary[a]	0.5	1/120	80	40–80
9	Eno (Gono) River, downstream of Haji Dam[a]	92.5	1/250	60	30–60
10	Eno River, upstream of Haji Dam	160.8	1/280	50	30–50
11	Eno (Gono) River, Saijo River tributary	5.6	1/320	40	30–40
12	Eno (Gono) River, Joge River Tributary	40.5	1/150	40	30–40
13	Ohta River, Misasa River Tributary	3.1	1/400	70	50–70
14	Hii River, Small Tributary	5.0	1/400	50	30–50
15	Takatsu River	57.3	1/160	50	50
16	Takatsu River, Tsuwano River Tributary	18.3	1/180	40	40
17	Saba River, Shimaji River Tributary[b]	10.8	1/190	110	50–110

[a] Sampling was conducted on May 24 and 25, 2016; September 16, 2016; April 18, 2017; and June 11–13, 2017. [b] Sampling was conducted on May 25 and 26, 2016; June 17, 2016; September 17, 2016; April 18, 2017; June 13–15, 2017; and August 7–9, 2018. [c] Sampling was conducted on October 11, 2016.

Chemical and Biomass Analyses

The tissue H_2O_2 was estimated colorimetrically using spectrophotometry (Asaeda et al., 2018). Plant chemicals were extracted into ice-cold phosphate buffers (50 μmol/L, pH 6.0) by crushing approximately 100 mg of the plant in the presence of polyvinylpyrrolidone (PVP). The extractions were centrifuged at 5000 *g* for 15 min at 4°C. The enzyme extraction of 750 μL was then mixed with 2.5 mL of 0.1% titanium sulfate in 20% (v/v) H_2SO_4, and the mixture was centrifuged at 2500 *g* for 15 min at 20°C. The optical absorption at a wavelength of 410 nm was measured using spectrophotometry (UV-1200, UV-Visible Spectrophotometer, Shimadzu, Japan), and the H_2O_2 concentrations (μmol/gFW) were estimated using a standard curve.

The dry weight of biomass was estimated by oven drying the collected biomass samples at 70°C for 72 h or until the weight became stable. The dried biomass was weighed and expressed in units of gDW/m^2.

Statistical Comparison

The linear or power law (for solar radiation intensity) correlation between parameters was tested by Pearson's correlation analysis and the statistical significance between observations were tested with Student's *t*-test. The statistical comparisons of field data were performed to obtain the relationship between the H_2O_2 content and the external factors (such as velocity, turbulence velocity, light intensity, biomass, and depth), and the relationship between the factors (turbulence and mean flow). For H_2O_2 concentration and velocity or temperature relationship, statistical comparisons were performed for the different study sites and/or sampling time groups, which have different temperatures and solar radiations, to obtain the interaction between stresses and the H_2O_2 concentration. The gradient of the regression line was obtained for the whole set of data in the relation between each stress component and H_2O_2 concentration. Then, for each study site and sampling time groups, statistical analysis was conducted to check the significance of the regression. For the relationship between H_2O_2 concentration and light intensity, the power law regression of the excessive H_2O_2 concentration of the light exposed samples and dark-adapted samples was conducted to obtain the light intensity at zero H_2O_2 concentration. Then the data scattering was compared with the standard deviation.

All the statistical tests were performed using IBM SPSS Statistics Version 25. The regression lines and equations were obtained using the inbuilt regression function feature of Microsoft Excel 2016.

RESULTS

The relationship between H_2O_2 concentration and water temperature, obtained from both laboratory experiments (PARs were 220, 320, and 680 $\mu mol/m^2/s$ with 0 cm/s of velocity; 200 $\mu mol/m^2/s$ of PAR and 23 cm/s of flow velocity), and dark-adapted condition of the field observation (PAR = 0 $\mu mol/m^2/s$) is shown in the **Figure 1**. The recorded temperatures of field studies ranged between 10 and 25°C depending on the sampling seasons and rivers, and the fluctuations were observed to be 1–2°C in the same sampling condition groups. Thus, the extrapolated regressed lines of each group to zero velocity were used here (**Figure 1**). H_2O_2 concentration has a negative correlation with temperature

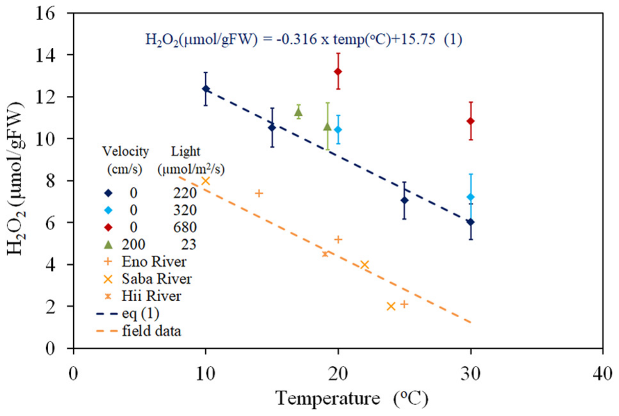

FIGURE 1 | The concentration of H_2O_2 in *E. densa* tissue as a function of water temperature. The error bars represent the standard deviation (*n* = 3). Eno, Hii, and Saba Rivers data are values extrapolated to zero velocity of the regression line with –0.316 (µmol/gFW cm/s) gradient, shown by dashed lines in **Figure 2**.

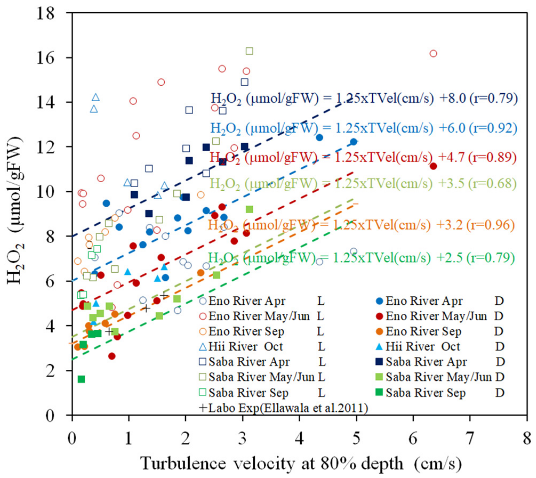

FIGURE 2 | The concentration of H_2O_2 in *E. densa* tissue as a function of turbulence velocity at 80% depth in light-exposed (L) and dark-adapted (D) conditions. The "Labo Exp" represents data from the laboratory experiment (Ellawala et al., 2011). The dashed lines indicate the linear regressed lines with the gradient of 1.25 µmol/[gFW (cm/s)] of the dark-adapted conditions of the same colored symbols of as those of the sampling rivers and seasons, respectively. H_2O_2 is given in units of µmol/gFW, and while the *TVel* (turbulence velocity) are in cm/s. The dates May, Sep (September), and Oct (October) represent data obtained in 2016 while Jun (June) and Apr (April) represent data of 2017. Eno, Saba, and Hii represent Eno, Saba, and Hii rivers including their tributaries, respectively.

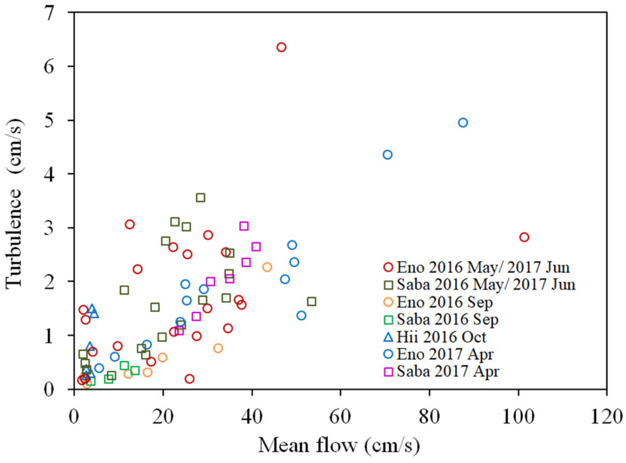

FIGURE 3 | Relationship between the turbulence velocity and the mean flow velocity of each sampling site of different rivers. The sample collected month is represented by the month indicated in the legend. Eno, Saba, and Hii represent Eno, Saba, and Hii rivers, including their tributaries, respectively.

in the 10 to 30°C temperature range and was regressed to lines with a gradient of −0.316 µmol/gFW/degree, for all light intensity groups. The regression equations are shown in **Figure 1** compared to observed data (R = 0.982, $P < 0.01$ for 220 µmol/m²/s PAR; R = 0.963, $P < 0.01$ for field observation, 0 µmol/m²/s PAR). There was no overlapping among data from different groups. Thus, the effect of interaction between temperature and light intensity is sufficiently small.

Figure 2 presents the H_2O_2 contents of the field observation samples with respect to turbulence velocity. Under light exposure, the H_2O_2 contents are always higher than those in the corresponding dark-adapted samples by 5–10 µmol/gFW. However, the scattering was greater than that in the dark-adapted samples. Dark-adapted samples are composed of different temperature groups, which depend on the sampling time and rivers.

The H_2O_2 contents of dark-adapted samples were highly correlated with the turbulence velocity, with a gradient of 1.25 µmol/ [gFW (cm/s)] (R = 0.796, $P < 0.01$). The regression lines with the same gradient are shown in **Figure 2** for the dark-adapted samples of different sampling time and river groups, compared to observed data. For each group, the H_2O_2 contents were highly regressed to the lines (R = 0.917, 0.885, 0.964 for April, May/June and September sampling at the Eno River, respectively, 0.76 for the Hii River, and 0.84, 0.68, and 0.63, respectively, for the sampling during each season, respectively, at the Saba River for all $P < 0.01$). This finding indicates that the H_2O_2 concentration dependence on turbulence velocity is independent of temperature.

There is a significant positive correlation between the mean flow and turbulence velocity (R = 0.722, $P < 0.01$), as shown in **Figure 3**. Also, the correlation between the H_2O_2 content of dark-adapted samples with the mean velocity is also positive and significant (**Figure 4**, R = 0.571, $P < 0.01$). The relationships can be explained with linear regression equations [Eq. (2) for the 0–60 cm/s mean flow velocity range]. The line in **Figure 4** included the cases in which the turbulence intensity was particularly high because of the large gravel beds. Although these values provide slightly higher H_2O_2 concentrations compared to those at normal sites, the relatively

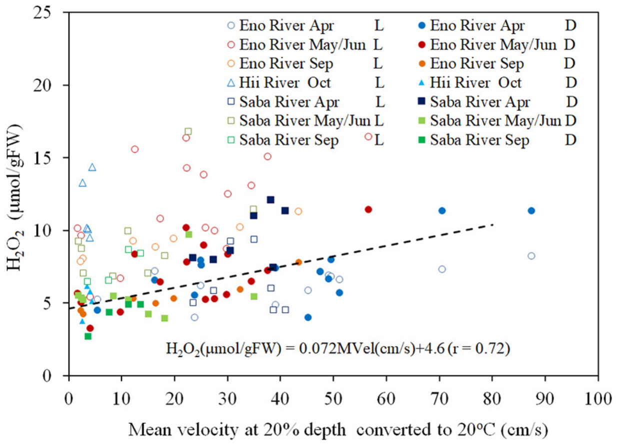

FIGURE 4 | The concentration of H_2O_2, converted to 20°C, in *E. densa* tissue as a function of the mean velocity at 20% deep thin light-exposed (L) and dark-adapted (D) conditions. The dashed line indicates the linear trend of the dark-adapted condition. The dates May, Sep (September), and Oct (October) represent data obtained in 2016 while Jun (June), Apr (April) represent data of 2017. Eno, Saba, and Hii represent Eno, Saba, and Hii rivers including their tributaries, respectively. H_2O_2 is given in units of µmol/gFW, and the *MVel* (mean velocity) is in cm/s.

proportional relationship with turbulence velocity indicates that the mean flow velocity is available as a reference of the mechanical stress due to flow velocity (Asaeda et al., 2017).

$$H_2O_2\ (\mu\text{mol/gFW}) = 0.072 MVel\ (\text{cm/s}) + 4.6 \qquad (2)$$

The H_2O_2 concentration of the light-exposed samples fluctuated heavily, but always exceeded the value of the corresponding dark-adapted samples. The excessive H_2O_2 content is light induced H_2O_2 content, postulated as a function of light intensity in **Figure 5**. There is a positive correlation between the light intensity and the H_2O_2 concentration, however, the increasing rate of the excessive H_2O_2 decreases with increasing light intensity. When the light intensity is lower than 40 µmol/m^2/s, the excessive H_2O_2 was nearly 0, similar to experimental results obtained by Hussner et al. (2010) and Rodrigues and Thomaz (2010). Therefore, power low regression analyses were conducted for the excessive H_2O_2 concentration with respect to the surplus light intensity from 40 µmol/m^2/s, as Eq. (3) in the figure (R = 0.738, P < 0.05). Although, the scattering is large, most of the data are distributed within the standard deviation from the Eq. (3) (1.57 µmol/gFW) without any systematic deviation regardless of rivers and sampling seasons, where the solar radiation intensity ranged from 40 to 600 µmol/m^2/s, and temperature from 10 to 25^{o}C.

$$H_2O_2\ (\mu\text{mol/gFW}) = [Light\ Intensiy - 40]^{\frac{2}{3}}/10 \qquad (3)$$

Diurnal H_2O_2 Variation of *E. densa*

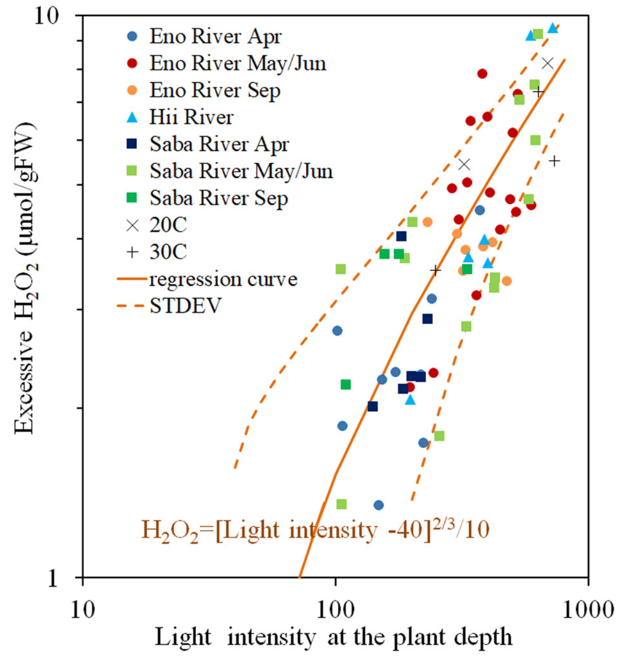

FIGURE 5 | Photosynthesis-produced H_2O_2 concentration (Excessive H_2O_2) of *E. densa* tissue as a function of light intensity. The sample collected month is represented by the year and the month indicated in the legend. Eno, Saba, and Hii represent Eno, Saba, and Hii Rivers, including their tributaries, respectively. When the light intensity is lower than 40 µmol/m^2/s, the excessive H_2O_2 concentration was 0 µmol/gFW. The regression curve in the excessive H_2O_2 concentration and the excessive light intensity from 40 µmol/m^2/s condition is postulated by a rigid line, and the standard deviation is shown by the dashed line. "Light" is the light intensity at the sample depth (µmol/m^2/s), and the excessive H_2O_2 concentration, *H_2O_2rad* (*Temp*), is expressed in µmol/gFW. The "*H_2O_2rad*" denotes the light induced H_2O_2 content. The Exp 20°C and Exp 30°C represent the excessive H_2O_2 concentration quantified in experiments under 20 and 30°C controlled temperatures.

In the diurnal observation, the light intensity during the 2018 sampling was higher for the 2018 observation day (∼1500 µmol/m^2/s at the shoot height) compared to that for the 2017 observation day (<1000 µmol/m^2/s at the shoot height). This high solar radiation intensity condition continued for several weeks before the 2018 observation day, while rainy or cloudy conditions had persisted for several weeks before the 2017 observation day. Therefore, samples from the 2018 observation were exposed to high solar radiation for several weeks, as opposed to samples from the 2017 observation (**Figure 6A**).

The diurnal variation of tissue H_2O_2 concentration followed the diurnal solar radiation intensity in 2017 and for healthy samples at 2018 S2 and S4 in 2018 (**Figure 6B**). Then, during the day, the H_2O_2 concentration rose up to nearly 16 µmol/gFW by noon and then, declined in the afternoon with the decline in solar radiation. In contrast, the values were substantially lower for the degraded samples in the 2018 observation. Then, samples at 2018 S2-2 and 2018 S3, showed less than 10 µmol/gFW of H_2O_2 concentration around noon. *E. densa* colonies remained healthy in the 2017 observation, while in the 2018 observation, shoots close to the water surface were degraded and appeared to be starting to die at S2-2 and S3.

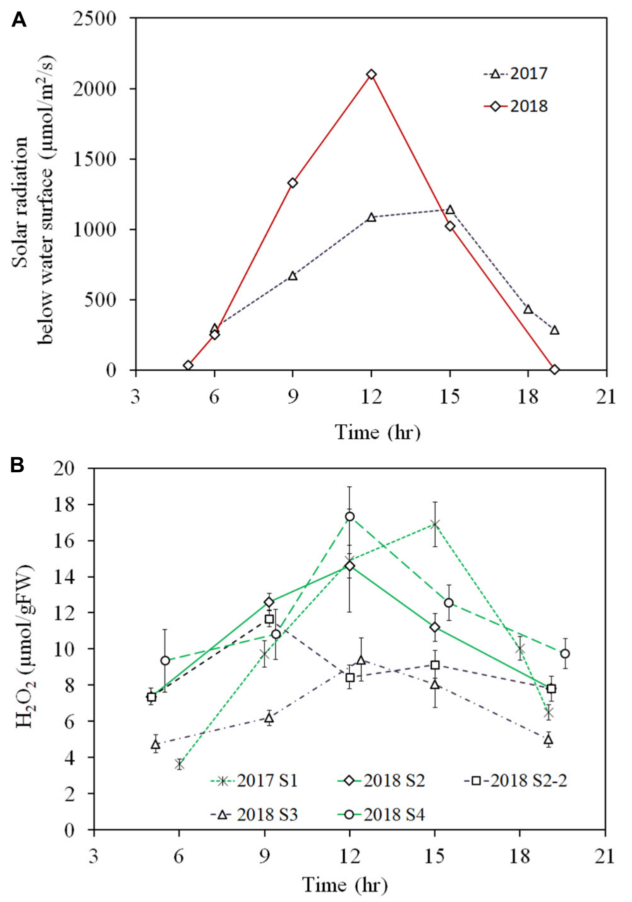

FIGURE 6 | Diurnal variation of light intensity just below the water surface of 2017 and 2018 observations **(A)** and *E. densa* tissue H_2O_2 concentration **(B)**, observed in the years 2017 (moderate radiation), and 2018 (excessive radiation). 2017 S1 represent the healthy shoots of 2017 observation. 2018 S2-2 and 2018 S3 represent degraded shoots under high solar radiation exposure at shallow water (<10 cm deep) in 2018, and 2018 S2 and 2018 S4 represent healthy shoots exposed to moderate radiation in deep depth sites (S2 30 cm deep and S4 50 cm deep) of the observation. The error bars represent the standard deviation.

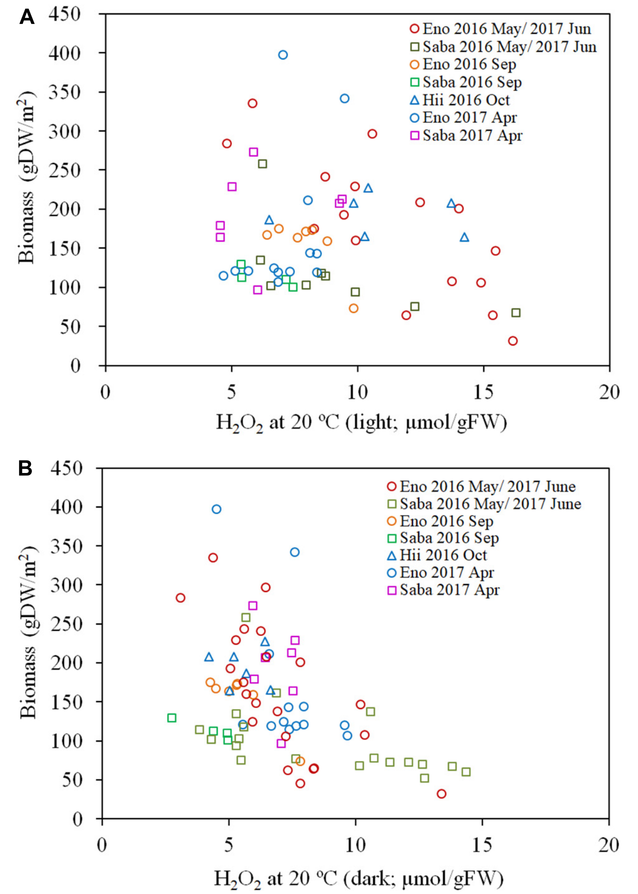

FIGURE 7 | Biomass of *E. densa* as a function of light exposed H_2O_2 concentration **(A)** and dark-adapted H_2O_2 concentration **(B)**. The H_2O_2 concentration was corrected to 20°C. The sample collected month is represented by the year and the month indicated in the legend. Eno, Saba, and Hii represent Eno, Saba, and Hii rivers, including their tributaries, respectively.

As per **Figures 7A,B**, the H_2O_2 content for both the light-exposed and dark-adapted samples were negatively correlated with the biomass ($R = -0.474$, $P < 0.01$ for light-exposed and $R = -0.504$, $P < 0.01$ for dark-adapted). It was observed that there were no samples with an H_2O_2 content exceeding the 16 µmol/gFW range. The approximate channel slope of the river in which large colonies of *E. densa* were formed in the running water was between 1/120 and 1/1800. No *E. densa* colony was found in the further upstream reaches, with channels steeper than 1/100, unless weirs were constructed to regulate the water flow (**Table 1**). The stress on plants generated by the flow velocity is more intensified at deeper sites; however, a high biomass concentration was found in deeper zones in the channel rather than in the shallow zones. The biomass of the colonies was highly correlated with the depth of the water column, peaking at a depth of 80 cm, and gradually declining as the depth increased further (**Figure 8**). The biomass distribution exhibited a common trend irrespective of the river or the observation site.

DISCUSSION

Environmental Stressors on *E. densa*

There are several types of stressors acting on submerged macrophytes in natural rivers. As these are based on different physical quantities, it is difficult to compare the magnitude of the effect of each stressor on the submerged macrophytes. The difference in H_2O_2 concentrations between the continuous light-exposed and 30 min pre-shaded samples differentiate the stress-induced H_2O_2 from photosynthesis generated H_2O_2. Based on the outcome of the laboratory experiment, we developed a relationship between temperature and the H_2O_2 generation in plant tissues. When the fractions of H_2O_2 corresponding to photosynthesis and temperature effects were eliminated from the continuous light-exposed samples of field studies, the observed

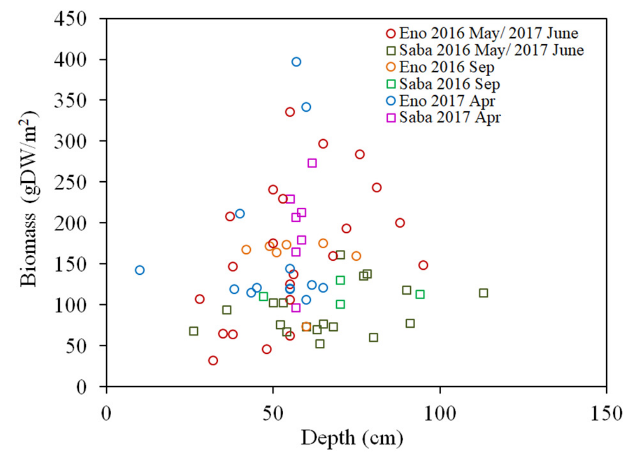

FIGURE 8 | Biomass of *E. densa* as a function of water depth. The sample collected month is represented by the month indicated in the legend. Eno and Saba represent Eno and Saba rivers, including tributaries, respectively.

trend of H_2O_2 was similar to the result of the oscillating grid laboratory experiment, in which the amount of H_2O_2 in macrophyte tissues was proportional to the root mean square velocity of the turbulence as is shown in **Figure 2** (Asaeda et al., 2017).

Under the zero-turbulence velocity, the tissue H_2O_2 concentration corresponds to a combination of photosynthesis, metabolic activities, and environmental stresses. The difference between light and dark treatment experiments distinguishes the amount of H_2O_2 generated by photosynthesis from the remaining stressors. Further, the variation in solar radiation intensity during the day is reflected in the H_2O_2 concentrations, which varied according to the light intensity from approximately 2 to 10 μmol/gFW (**Figure 5**). There were parallel relationships between the H_2O_2 concentration and the temperature for different light intensity groups with the same velocity (**Figure 1**), which explains the independency of H_2O_2-temperature relationship trend from the light intensity but simultaneously elevates the trend due to excessive H_2O_2 production (**Figure 5**). The temperature-H_2O_2 dependency linear relationship provides the temperature generated H_2O_2 concentration, which is reportedly around ∼10 μmol/gFW in the *E. densa* habitat temperature range of 10–35^{o}C, in the present observation and previous reports (Hanamoto and Ikushima, 1988.; Yarrow et al., 2009; Gillard et al., 2017).

H_2O_2 concentration of dark-adapted samples had linearly increasing trends with respect to turbulence velocity, regardless of different sampling months and river groups, which differentiate the temperatures (**Figure 2**). The H_2O_2 concentration extrapolated to zero velocity had the same relationship with the temperature dependency on the H_2O_2 concentration (**Figure 1**). Thus, the increasing rate of the H_2O_2 concentration with respect to turbulence effects indicates the turbulence induced H_2O_2.

These H_2O_2-solar radiation and H_2O_2-temperature relationships exhibit almost the same trends for photosynthetic rates obtained by the outdoor experiments with different temperatures and light intensities (Riis et al., 2012). Therefore, the H_2O_2 relationship with the light intensity and temperature conditions, can be considered as common trends for *E. densa*. In the practical application, H_2O_2 concentration can be applicable to determine the different types of stressors in the same manner and to compare their relative magnitudes.

Compared to the H_2O_2 induced by metabolic activities, which is ∼4 μmol/gFW given in the field experiments of dark-adapted samples at zero velocity, in the relatively steep non-polluted rivers, the major stressors include the mechanical stress introduced by high current velocity/turbulence, solar radiation, and temperature. The river water quality is relatively good, and there is no salinity in the midstream of Japanese rivers. However, in eutrophic water, organic matter accumulates at the bottom of stagnant zones, which creates an anoxic zone in the sediment layer; however, the bottom sediment anoxia contributed only ∼5 μmol/gFW of H_2O_2 (Parveen et al., 2017a). As for the biotic stress, toxic strains of cyanobacteria, *Microcystis*, for instance, generate only 1.5 μmol/gFW of H_2O_2 (Amorin et al., 2017). Therefore, the amount of H_2O_2 generated by photosynthesis, temperature and flow velocity is relatively large compared to other stresses, and these stresses are considered as major stresses, which control its colonization.

The contribution of each stress to the total H_2O_2 of the plant can be distinguished with this method and can be adapted to determine the total level of environmental stress on macrophytes. The combination of different stresses sometimes imposes two opposing demands on the plant (Choudhury et al., 2017) or interact each other. However, the parallel relationship among the major stresses in natural rivers, namely, solar radiation, flow velocity, and water temperature and the interactive effects seems to be sufficiently small. The reason for the relatively lower interactive effects among stressors is not clear.

H_2O_2 is generated by the surplus number of electrons. In the photosynthesis process, the surplus amount of electrons are generated on the thylakoid membrane by strong energy (Asada, 2006), while the consumption of electrons is decreased under low temperature due to the suppressed CO_2 fixation by the inactivation of Rubisco in Calvin cycle (Nishiyama and Murata, 2014), or the mechanical damage of organelles in turbulent flow (Atapaththu et al., 2015). Therefore, the sites that can cause the electron surplus is different between stresses.

Threshold Condition of *E. densa* Mortality

Egeria densa colonies of 2017 observation remained healthy, while in the 2018 observation, shoots close to the water surface were degraded and appeared to be starting to die. The intensive oxidative stress caused by high solar radiation for several days should be the reason for the degradation of 2018 colonies. The results indicate the depression of plant metabolism owing to solar radiation exceeding the tolerable levels. When the H_2O_2 concentration became higher than 16 μmol/gFW in hypoxia and hydrogen sulfate exposure experiments for *E. densa*, plants deteriorated, and the total chlorophyll concentration and the H_2O_2 concentration substantially declined compared to other samples (Parveen et al., 2017a,b,c). Also, with the exposure of Fe, *E. densa* exhibited lowest growth rate, chlorophyll content and photosystem efficiency at around 16 μmol/gFW of H_2O_2 content and beyond the level, healthy plants did not exist (unpublished data). Same as the other observations, the 2018 observations show the H_2O_2 level of colonies peaked beyond the 16 μmol/gFW during the daytime. Therefore, the H_2O_2 concentration of 16 μmol/gFW can be considered as a critical value for the survival of *E. densa*, regardless of the types of stressors. Exceeding the threshold level, would lead to the deterioration of plants due to oxidative damage.

Empirical Expression of Habitat Preference and Colonizable Conditions Simulation

The H_2O_2 concentration of macrophytes can be used to explain the expected macrophyte distribution in a river. When Eq. (4) is considered, the H_2O_2 generated by each stressor can be expressed as follows:

The total H_2O_2 concentration (H_2O_2tot) at a particular temperature ($Temp$) is given by:

$$H_2O_2tot(Temp) = H_2O_2rad(Temp)$$

$$+H_2O_2vel(Temp) + H_2O_2met(Temp) \qquad (4)$$

where H_2O_2rad is the H_2O_2 generated by solar radiation exposure, H_2O_2vel is the H_2O_2 generated by the flow velocity, and H_2O_2met is the H_2O_2 generated by metabolism.

The light intensity at a particular depth of water, z, can be calculated using Eq. (5) (Middelboe and Markager, 1997):

$$I = I_0 exp(-kz) \qquad (5)$$

where k is the attenuation coefficient in water, which was 0.035 (/cm) in the observed rivers. The solar-radiation-induced H_2O_2 content has an increasing relationship with the light intensity, given by Eq. (3). Considering Eq. (3), the H_2O_2 concentration generated by the solar radiation under a particular temperature (within the temperature range 10–30°C), therefore, can be expressed as Eq. (6):

$$H_2O_2rad(Temp) = [I_0 exp(-kz) - 40]^{2/3}/10$$

$$for\ I_0\ exp\ (-kz) \geq 40\ \mu mol/m^2/s;$$

$$H_2O_2rad(Temp) = 0\ for\ I_0\ exp\ (-kz) < 40\ \mu mol/m^2/s. \qquad (6)$$

Direct mechanical stress is generated by turbulence rather than the mean flow (Atapaththu et al., 2015; Asaeda and Rashid, 2017; Asaeda et al., 2017); however, there is a close relationship between these two quantities, particularly in straight uniform channels with uniform roughness. It is assumed that the mean velocity U (m/s) under the uniform flow is empirically given by Manning's law [Eq. (7)]:

$$U = \frac{1}{n} R^{2/3} S^{1/2} \qquad (7)$$

where R is the hydraulic radius, approximately given by the depth "H" (m), the channel bed slope "S," and Manning's roughness coefficient "n." The effects of longitudinal configuration, vegetation, etc., are added in "n." Therefore, considering Eq. (2), the H_2O_2 accumulation due to velocity stress and metabolism at a particular temperature (within the temperature range 10–30°C) can be expressed as follows (Eq. 8):

$$H_2O_2vel\ (Temp) = 0.072 \frac{1}{n} H^{2/3} S^{1/2} + 4.6 \qquad (8)$$

Subsequently, the total H_2O_2 concentration within the temperature range 10–30°C can be given as follows (Eq. 9):

$$H_2O_2\ (Temp) = \left[I_0 exp\ (-kz) - 40\right]^{2/3}$$

$$/10 + 0.072 \frac{1}{n} H^{2/3} S^{1/2} + 4.6$$

$$\text{for } I_0 \text{ exp } (\text{-}kz) \geq 10\ \mu\text{mol/m}^2\text{/s;}$$

$$H_2O_2\ (Temp) = 0.072 \frac{1}{n} H^{2/3} S^{1/2} + 4.6 \qquad (9)$$

$$\text{for } I_0 \text{ exp } (\text{-}kz) < 10\ \mu\text{mol/m}^2\text{/s.}$$

Figure 9 shows the simulated H_2O_2 concentration generated by using Eq. (9), for different channel slopes and depths. I_0 is assumed to be 2000 μmol/m²/s, as the highest solar radiation experienced in the observation area on a fine day during summer and when the canopy top was assumed to be located at the 80% depth, which is the average canopy height of the observed *E. densa* colonies. Manning's roughness coefficient for the channels is approximated at 0.08. Channel slope was obtained from the

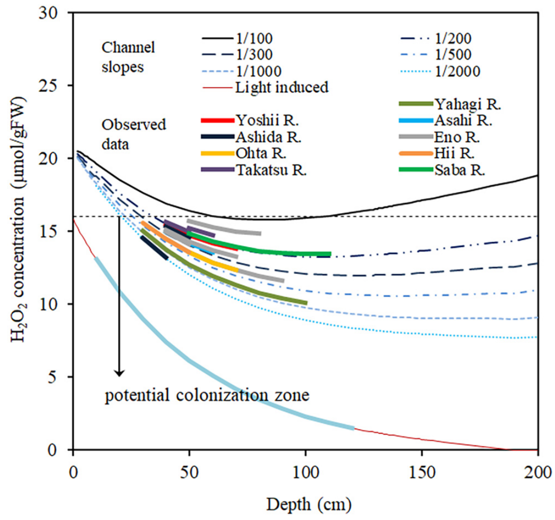

FIGURE 9 | Simulated H_2O_2 concentration in the plant body of *E. densa* changes with the water column depth for different channel slopes and actual slope data of several Japanese rivers. The line "Light-induce" represents the H_2O_2 concentration induced by light and change with the depth. The downward arrow "potential colonization zone" represents the depth zone of different channel slopes suitable for *E. densa* colonization.

topographic map (ArcGIS, 2019, Esri, New York Street, Redlands, California). The target area was characterized by steep basins, and the difference in elevation between the channel bed and the riparian zone is nearly constant thus the riverbed gradient is nearly the same as that of the riparian zone. The simulated results of **Figure 9** are consistent with the observed data of the rivers. The H_2O_2 concentration generally declines with depth, due to declining light intensity. In the steep channel, the H_2O_2 concentration increases again with a further increase in depth as the flow velocity rises. On gentler slopes, due to the lower flow velocity, H_2O_2 concentration is lower, and there is a wider range of depths in which *E. densa* colonies potentially form. However, the shallow depths become unsuitable for colonization of *E. densa* due to the higher light intensity.

E. densa Colonization in Rivers

In rivers steeper than 1/100, the upstream to midstream areas are originally filled with gravel or boulders, and fine sediment beds are rare as they are easily flushed away during flooding. The flow velocity depends on the channel slope, depth, and roughness. Roughness is determined by the bed sediment size as well as other factors related to the channel configuration, such as longitudinal morphology and bars (Parker and Peterson, 1980). The formation of *E. densa* colonies poses a physical disadvantage due to the high flow velocity and high light intensity as well as the high turbulence caused by the gravel bed as long as gravel sediments are supplied. However, when the gravel supply is decreased, the gravel-to-sand transition is extended further upstream (Singer, 2008), and the sand bed area increases in the former gravel zones. Sand is transported mostly as a suspended sediment load along the channel. *E. densa* communities accumulate suspended sediments efficiently, as they have a complex, dense stem structure that is widely distributed over the bed with thicker stems and denser whorls compared to similar native species, *Hydrilla verticillata*, and common native submerged species, *Elodea* sp., *Myriophyllum spicatum*, and *Potamogeton crisps* (Sand-Jensen, 1998; Vermaat, et al. 2000; Statzner et al., 2006). At the same time, water intake weirs have been frequently constructed along rivers in the last 30 years (personal communication). Then, stagnant water was produced in the upstream zone. Thick *E. densa* colonies were developed in the upstream area of many weirs in the observation (data are not shown). With the *E. densa* dispersal ability via fragmentation, it spread increasingly into the downstream (Casati et al., 2000, 2002; Redekop et al., 2016). Then, the dense shoot morphology leads to the formation of many sandy patches in the former complete gravel beds of the downstream. When fine sediments are supplied, it is easier to fix roots and take nutrients from the ground than from the stony bed (Barko et al., 1991). Buried by a sediment layer, the *E. densa* complex shoots reinforce the sand layer, which is otherwise easily washed away by floods. Once the *E. densa* colonies develop, the sandy spaces increase widely on the gravel bed, accommodating more macrophytes, including other species. Subsequently, the ecosystem changes to a macrophyte-dominated ecosystem. A similar phenomenon was observed, after the occurrence of a large flood in the Yahagi river (in July 2017), sandy sediments accumulated in colonies burying *E. densa* up to 20 to 30 cm on a bed that was originally gravelly (**Supplementary Figure 1**). The *E. densa* biomass was relatively dense, up to 150–400 gDW/m^2, in these sites, as the accumulated sand layer was reinforced with *E. densa* stem structures. This layer was thus rigid and could not be easily flushed away during moderate floods (**Supplementary Figure 2**). In contrast to the original gravel bed, the sandy surface was smooth, and turbulence generation was also reduced. Further, in field observations, an accumulation of fine sediments was always found inside the *E. densa* colonies of all rivers.

CONCLUSION

The high concentrations of H_2O_2 introduced by high flow velocity and high solar radiation in summer inhibited the formation of large colonies in the gravel channel, owing to the high oxidative stress. The accumulation of H_2O_2 in *E. densa* showed a significant relationship for both flow velocity and solar radiation. The critical H_2O_2 concentration to maintain a healthy population of *E. densa* can be considered as 16 μmol/gFW, which corresponds to the termination of biomass accumulation. Under the strongest solar radiation on summer days, the H_2O_2 level often exceeds the critical condition, leading to the deterioration of *E. densa* and ultimately, the H_2O_2 concentrations decline as the plant tissues start to deteriorate. H_2O_2 concentrations of *E. densa* were estimated for channels with different slopes and different depths. The H_2O_2 concentration is higher than the critical value in shallow water and increases in steeper channels, exceeding the critical value at a channel slope larger than 1/100. Once colonized, *E. densa* accumulates sandy suspended sediment efficiently and creates a preferable environment for further colonization. The present methodology can be applied to predict the area that can be conveniently colonized by *E. densa* within a short time period, which has been determined based on a prolonged monitoring activity.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

TA conceived the study. TA and LV performed the experiments. TA and MS analyzed and interpreted the data, and wrote the manuscript. All authors reviewed the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00422/full#supplementary-material

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