Unsuccessful invaders structure a natural freshwater phytoplankton community

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Abstract. Species invasion dynamics are a core topic in ecology and the effects of species invasions on ecosystems are well described. However, the knowledge about the mechanisms during the invasion process itself is limited. Many interactions between invaders and resident species occur during the invasion process. These interactions influence individual species abundance and subsequently community composition. Even though invaders are often unsuccessful, the very short interactions with the resident species can influence species abundances and therefore community composition. The prominent effects of unsuccessful invaders on community composition were already predicted in a theoretical model. To empirically test the effect of such unsuccessful invaders on a resident phytoplankton community, we conducted a mesocosm experiment in a mesotrophic freshwater pond. Six freshwater algae belonging to three taxonomic groups (chlorophyta, cyanobacteria, and bacillariophyta) were added as potential invader species to the natural phytoplankton community of the pond (as single species or as combination of two species of the same taxonomic group). After nine days, the invasion success and the impact on the natural phytoplankton community were estimated. Results show that although all invaders were unsuccessful, they had lasting effects on the resident community. In all communities exposed to invasion, biodiversity (H′) was maintained. In contrast, in control communities, the diversity at the end of the experiment was lower compared to the initial community. Furthermore, communities exposed to invasion were less similar to control communities without invasion than to the other invaded communities. Differences were found in taxonomic group composition as well as in species composition. Additionally, we found evidence for synergistic effects between the combined added species. Our results give clear evidence of strong transient effects of unsuccessful invaders on freshwater phytoplankton community composition.

Key words: community composition; phytoplankton; transient effects; unsuccessful invasion.

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INTRODUCTION

For decades, invasion dynamics of non-native species have been an important topic in ecological research (Elton 1958). Especially now, the alteration of habitats by invading species is of major interest caused by rising awareness of globalization and climate change. Invaders can have major effects on resident ecosystems, for example, the loss of biodiversity, changes in nutrient cycles, and the alteration of the community composition and dynamics (Mack et al. 2000, Ehrenfeld 2003, Levine et al. 2003). For example, the giant hogweed (*Heracleum mantegazzianum*) invasive to northern Europe (Pyšek 1991) caused a 50–60% decrease of vascular plant diversity in central Europe in invaded habitats (Hejda et al. 2009). In particular, resident species, like grass or other low-growing species, were excluded from the resident communities by shading (Thiele et al. 2007). Another example of lasting effects of invasion was shown by Vitousek et al. (1987), who

discovered a change in nutrient cycles in volcanic regions caused by the nitrogen fixating tree *Myrica faya.* Its ability to fix nitrogen increased the nitrogen availability for other plants. Initially, nitrogen-limited plants were supported in their growth, which resulted in an altered community composition. Invasion, however, is not limited to terrestrial ecosystems. Aquatic ecosystems experience invasions of non-native species with a comparable intensity as terrestrial ecosystems (Simberloff et al. 2013, Gallardo et al. 2016). One of the most known examples is the invasion of the crayfish *Orconectes rusticus*, which caused a strong alteration of the community structure and trophic pathways in North American lakes. In the presence of the crayfish, the species richness (SR) and abundance of benthic macroinvertebrates decreased (Nilsson et al. 2012).

These examples show the high impact of successful invaders on ecosystems. During the process, each invading species needs to successfully pass different invasion stages to finally influence an ecosystem. These stages are transport to a new habitat; introduction into the new habitat; establishment by surviving and reproducing in the new habitat; and finally spreading by dispersal (Lockwood et al. 2007, Blackburn et al. 2011). While the described invasion process is a theoretically and empirically well-studied topic (Elton 1958, Lockwood et al. 2007, Blackburn et al. 2011), a mechanistic understanding of the different stages of invasion on the species level still remains unclear. A more detailed concept of invasion based on species interactions during the invasion process is needed. Valéry et al. (2008) described invasion as a mechanism where a species acquires a dominant role compared to a resident species of the established community, which allows it to proliferate and rapidly spread to new habitats. In general, all species—invaders and residents—interact with the abiotic environment, for example, by nutrient uptake or release, as well as with the biotic environment via species interactions.

These interactions can be direct or indirect, both affecting the species development in a community. Through direct interaction, a species could be excluded by another species (predation, competition) or both species could coexist in the same habitat (Tilman 1977). However, natural communities usually contain a large number of different species. The direct interactions between two species will therefore result in indirect effects on other species (Tilman 1987).

An additional, sometimes underestimated aspect of direct interactions (besides the resident and invader interaction) is the direct interaction between several invaders. Simberloff and Von Holle (1999) clearly discussed the aspect that one invading species can have a positive effect on other invading species. This interplay of invading species may have bigger effects than single species have on resident communities. However, the outcome of such interactions is influenced by species traits, which are manifold and give species the ability for, for example, movement, survival, reproduction, and competition (Litchman and Klausmeier 2008).

In previous research, discrepancy between convergent and divergent traits of the invading species to resident species was found (Tilman 2004, Cleland 2011). On the one hand, Tilman (2004) found that invading species with divergent traits (compared to residents) have an invasion advantage, thus allowing a better usage of available resources by slight overlap of common resource requirements. On the other hand, Case et al. (2016) hypothesized that invading species with convergent traits (compared to residents) have an advantage when it comes to invading the resident community, because they are similarly well adapted to the specific environment as resident species are.

Both assumptions imply failure of the invading species as a possibility, as not every invading species might successfully establish in the new habitat (Zenni and Nuñez 2013). However, the competitive advantage of an invading species may influence the resident community even if the invading species fails to establish, as it interacts for a certain time during the invasion process with the resident species and the environment. Such transient effects of a failed invasion on a resident community were shown in two theoretical studies. Case (1995) demonstrated in his model approach that the temporary abundance of an invading species can change the environmental and/or the competitive conditions in the resident community. Even after the disappearance of the unsuccessful invader, the resident community was changed. A theoretical study by Miller et al. (2009) concluded that also a transient species (unsuccessful invader) can

influence the resident community composition in manifold ways, due to possible direct and indirect interactions of species. Changes in the community composition were seen in >50% of the theoretical analyzed communities. Overall, empirical evidence for the scenario of unsuccessful invasion is rare and most studies focus on the effects of successful invasion (Mooney and Cleland 2001, Molnar et al. 2008, Hejda et al. 2009).

In a recent meta-analysis, Zenni and Nuñez (2013) analyzed why invaders fail to establish and mentioned the necessity for gaining more detailed knowledge of how unsuccessful invaders are affecting resident communities. To analyze the transient effects of invading species, phytoplankton communities are an appropriate study system. These communities continuously face competition with invading species, for example, for nutrients, due to a consistent exchange of phytoplankton species between different water bodies resulting from the high dispersal of phytoplankton species (Kristiansen 1996). The short generation time of phytoplankton (Reynolds 2006) allows investigation of numerical responses within communities. Additionally, several traits (e.g., size, nutrient uptake, growth rate) are known to influence growth, metabolism, access to resources, and other factors (Smayda 1970, Sterner 1989, Litchman and Klausmeier 2008, Litchman et al. 2010). Therefore, traits of both, the resident and invasive species, are very important factors for the success of invasion.

Here, we used a natural phytoplankton community to examine the effects of attempted invasion by added phytoplankton species on community composition. We conducted a field experiment in which freshwater phytoplankton species were allowed to invade a natural phytoplankton community in small mesocosms. A natural phytoplankton community was inoculated with additional species (single or a combination of species), which belonged to different taxonomic groups. The following hypotheses were tested: (1) transient effects of unsuccessful invaders influence the resident community composition due to changing conditions of competition; (2) species that share more convergent traits to resident species might have stronger effects on the resident community; (3) species cell size (resident or invasive) is an important trait influencing species abundances and composition; and (4)

adding a combination of species to a community has stronger effects on the resident community, due to additive effects of these species, than adding these species separately.

METHODS

Experimental set-up

The experiment was carried out in a mesotrophic pond (TP = 13.06 μg/L; 11°C; pH of 8.4 ± 0.2 standard error [SE]), Martinsried (Bavaria, Germany), and set up in dialysis bags (Nadir, Microdyn-Nadir GmbH, Wiesbaden, Germany), which allow diffusion of molecules up to a size of 10–20 kDa. This set-up facilitates a free exchange of dissolved nutrients between the interior and the surrounding water of the bag ensuring an identical nutritional concentration inside and outside of the dialysis membrane. The dialysis membrane therefore enables natural nutrient conditions for phytoplankton communities within the bags for a certain amount of time (four days, Sommer et al. 2005). The bags were filled with 250 mL pond water containing the resident phytoplankton community. To avoid effects of macro- and meso-zooplankton within the dialysis bags, the pond water was previously filtered through 225 μm gauze. Thus, observed effects are mainly based on competitive conditions of the phytoplankton species.

As potential invaders, we chose six different laboratory phytoplankton species belonging to three different common phytoplankton groups. Individual biovolumes ranged from 100 to 700 μm^3 (Table 1). The resident community consisted of 40.6% bacillariophyta composed of 34% *Synedra* sp. (dominant species of the community), 32% chrysophyta, 26% chlorophyta (mainly small coccal chlorophyta), and 0.5% cyanobacteria. All introduced species were cultivated in monoculture in modified Woods Hole Combo growth medium (WC-Medium, Guillard and Lorenzen 1972) under

Table 1. Experimental species used as potential invaders.

Taxonomic group	Added species
Chlorophyta	*Chlamydomonas reinhardtii*
Chlorophyta	*Pediastrum simplex*
Cyanobacteria	*Anabaena cylindrica*
Cyanobacteria	*Pseudoanabaena galeata*
Bacillariophyta	*Fragilaria crotonensis*
Bacillariophyta	*Cyclotella meneghiniana*

laboratory conditions (12:12 h light:dark cycle; 90 μmol photons·m^{-2}·s^{-1}; 20°C).

The control treatment contained only the resident phytoplankton community of the pond. The experimental treatments additionally included the respectively added laboratory phytoplankton species (single species or a combination of two species of the same taxonomic group). The added biovolume of the added species equaled 10% of the initial resident community's biovolume. When two species were added, each species contributed equally to the 10%. The biovolume was measured using a cell counter (CASY 1 Cell Counter and Analyzer system TTC; Schärfe System GmbH, Germany). All treatments were set up in triplicates, which resulted in a total number of N = 30 dialysis bags. The dialysis bags were incubated 40 cm below the water surface at the deepest point of the pond (4 m). In the middle of the experiment (after four and a half days), the dialysis bags were exchanged to minimize clogging of the membrane by bacterial growth. This exchange allowed optimal nutritional conditions for the communities inside the dialysis bags.

At the end of the experiment (after nine days), samples of each phytoplankton community were preserved with Lugol's iodine solution. For analyses of species diversity and phytoplankton community compositions, samples were counted according to Utermöhl (1958) using an inverted light microscope (M40; WILD, Heerbrugg, Switzerland). If possible, at least 100 individuals (or colonies) per category (genus) were counted in two perpendicular transects to keep the counting error below 10% (Lund et al. 1958). Measurements of the respective laboratory cultures were used to estimate the added species' individual cell biovolume. Individual cell biovolumes of the resident species community were estimated according to Kremer et al. (2014). Additionally, we categorized the community in three cell volume-size classes: nanoplankton (10–10^3 μm^3), microplankton (10^3–10^6 μm^3), and mesoplankton (10^6–10^9 μm^3) according to Ignatiades (2015). Based on this, all our potential invader species belong to the nanoplankton group.

Data analysis

Invasion success.—To test whether the experimentally added species were successful at invading the resident communities during the experiment, we calculated invasion success modified after Sperfeld et al. (2010). The invasion success was calculated as follows:

$$\text{Invasion success} = \log 2\left(\frac{\text{ASP}_{\text{end},i} - \text{EASP}_{\text{end},i}}{\text{ASP}_{\text{start},i}}\right)$$

(1)

where $\text{ASP}_{\text{end},i}$ represents the added species i (% of total biovolume) at the end, $\text{EASP}_{\text{end},i}$ the equivalent phytoplankton species to species i (% of total biovolume), and $\text{ASP}_{\text{start},i}$ represents added phytoplankton species i (% of total biovolume) at the start. EASP are species in the resident community, which are morphologically similar to the potential invader species. We found EASP in the initial resident community for all our added species, except for *Pediastrum simplex*. Since a microscopic distinction between EASP and ASP is impossible, we corrected the abundance of ASP in the added species treatment with values of EASP in the control treatment at the end of the experiment. We found no or only very low amounts of EASP (highest % species total biovolume was <0.8%; Appendix S2: Table S1) for *Chlamydomonas reinhardtii*, *Pseudoanabaena galeata*, *Fragilaria crotonensis*, and *Cyclotella meneghiniana*, indicating no interference of these species with our results.

If the invasion success estimates are significantly greater than zero, an invader was successful. An invasion success estimate significantly lower or equal to zero indicates an unsuccessful invader. We calculated the arithmetic mean and the 95% confidence intervals (CI) from replicates ($n = 3$) of the invasion successes for each treatment, except for one case (*C. meneghiniana* in combined treatment) where no experimental species could be found in two of the three replicates.

Diversity, evenness, species richness, and similarity.—We calculated Shannon diversity (H′), Pielou's evenness (E; Krebs 1985), and estimated SR of final phytoplankton communities in all treatments. Additionally, we calculated arithmetic mean and the 95% CI of H′ and E for the taxonomic groups of added species. In order to understand the magnitude of the differences between treatments and the control of H′ and E, we calculated the effect size (Cohen's d) of all treatments, where 0.2 is a small, 0.5 a medium, 0.8 a large, and 1.3 a very large effect (Cohen 1988). We also calculated the differences in SR of the final phytoplankton communities for each treatment and the

control against the initial values. We performed one-way ANOVAs to analyze differences of H′ and E of the added species taxonomic groups and SR between experimental treatments and control, when applicable with Holm-Sidak post hoc tests for multiple pair wise comparisons.

To estimate similarity between experimental treatments and control communities, we calculated the Bray–Curtis similarity coefficient (Bray and Curtis 1957) based on species log $(x + 1)$-transformed species biovolume proportion data.

The multivariate similarity percentages analyses (SIMPER) were used to estimate the percentage contribution and ranking of each species to the average dissimilarity among start, control, and experimental (invaded) communities. Similarity percentages analyses was used to determine the species contribution to the dissimilarity between experimental treatments and control communities.

Species abundances and cell sizes.—Differences in taxonomic group and species abundance (based on their biovolume) between each pair of experimental treatments and control were calculated. Additionally, we categorized all experimental communities in three cell volume-size classes: nanoplankton ($10–10^3$ μm^3), microplankton

($10^3–10^6$ μm^3), and mesoplankton ($10^6–10^9$ μm^3) according to Ignatiades (2015). To analyze differences between experimental treatments and control, the arithmetic mean and the 95% CIs were calculated (mean $\pm$ 1 CI, $n = 3$).

Statistical analyses were performed with Sigma-Plot (Version 11; Systat Software, San Jose, California, USA), Primer (OLIGO Primer Analysis Software Version 7; Molecular Biology Insights, Cascade, Colorado, USA), and PAST (Version 2.17c; Hammer et al. 2001).

Results

Invasion success of added species

None of the added species were successful at invading the resident communities, which was indicated by a negative value for the calculated invasion success and indicated by 95% CI not overlapping with the zero line (Fig. 1). The zero line indicates the added amount of the potential invaders. Added *Anabaena cylindrica* (cyanobacteria) showed the lowest value of invasion success, in the treatment with two combined added cyanobacteria (-15.94 ± 0.65). Also compared to all other unsuccessful invading species, besides
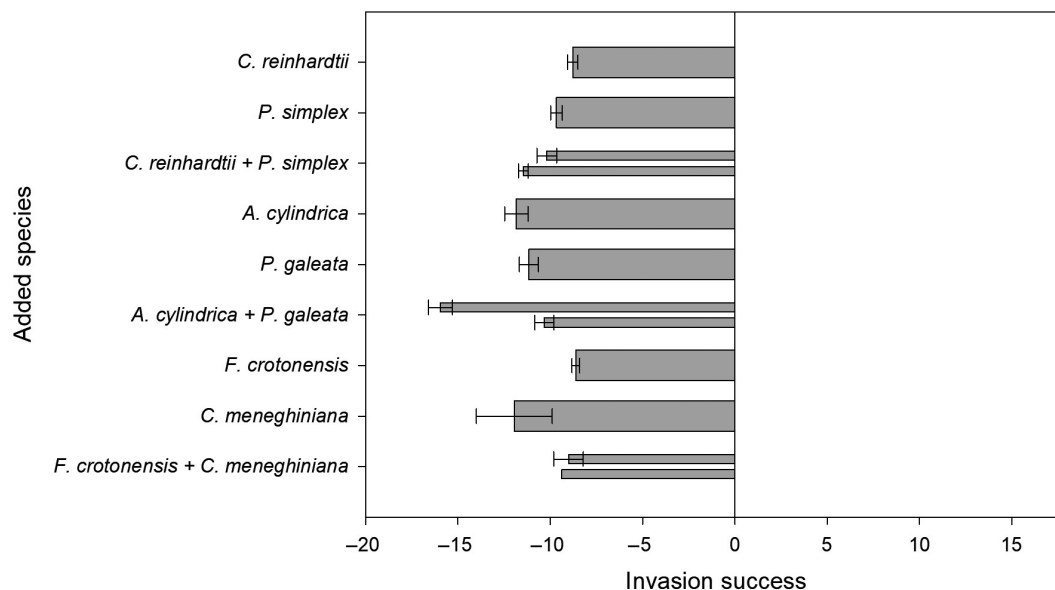

Fig. 1. Invasion success of added species at the end of the experiment (mean $\pm$ 1 confidence interval [CI], $n = 3$). In the combined added species groups, the first bar represents the species stated first. Zero line indicates the initially added amounts of potential invaders. In the combined added treatment, *Cyclotella meneghiniana* had only one replicate (mean $\pm$ 1 CI, $n = 1$).

single added *Cyclotella meneghiniana* (indicated by 95% CI), single added *A. cylindrica* showed the lowest invasion success. Single added *Fragilaria crotonensis* (bacillariophyta; -8.62 ± 0.20) showed among the unsuccessful invading species the less negative invasion success. Furthermore, we found in two out of three cases, that one added species showed a lower abundance when they were added in combination with the second species of the same taxonomic group. Added *A. cylindrica*

and *Pediastrum simplex* decreased in their abundance more strongly when they were added in combination with the second species compared to when they were added as single species.

Diversity, evenness, and species richness

Final diversity (H′) and evenness (E) of the control and experimental communities were lower compared to the initial community at the start of the experiment (Fig. 2a). Additionally, significant
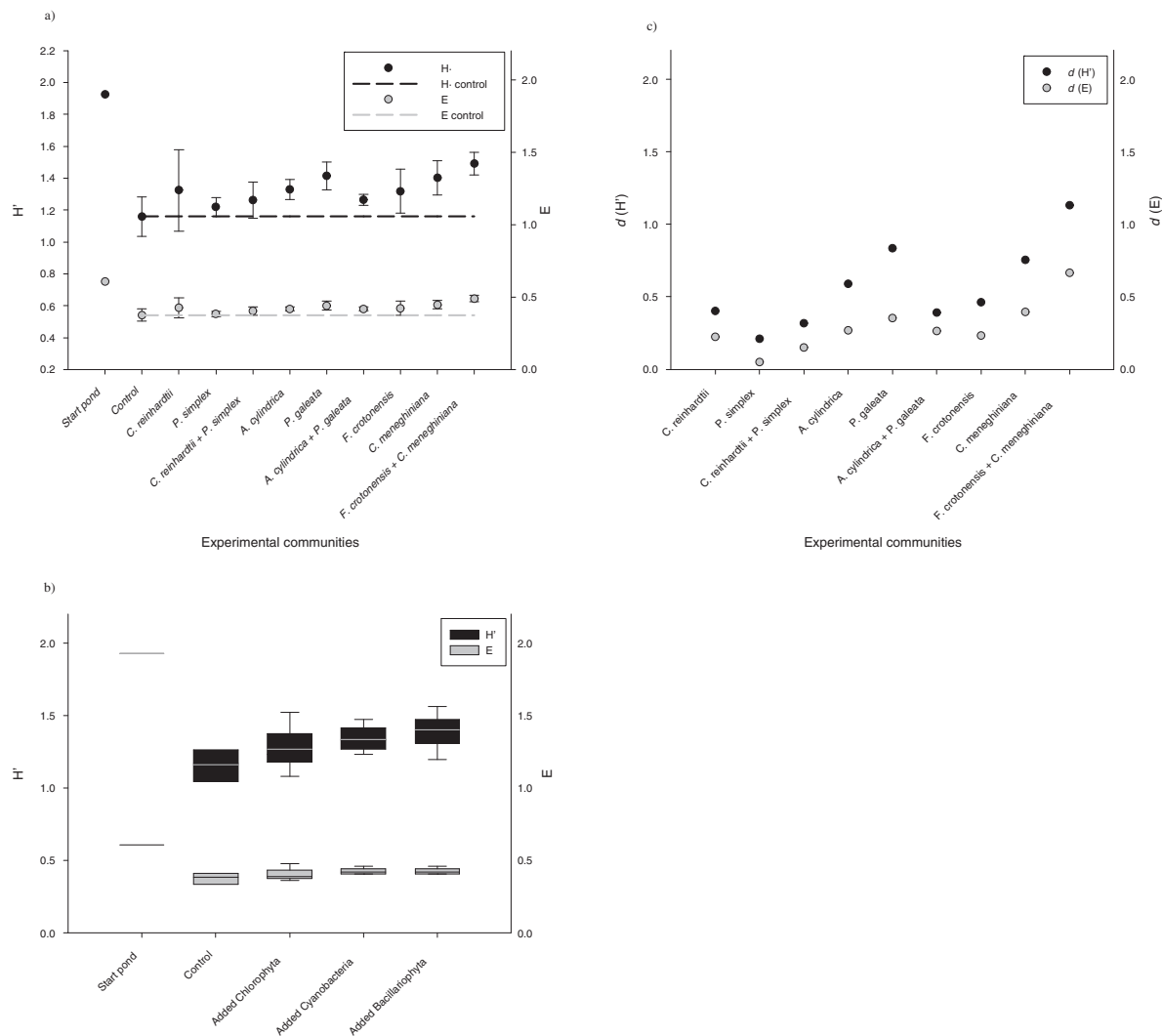

Fig. 2. (a) Biodiversity (H′) and evenness (E) of start pond, controls, and experimental treatments (mean $\pm$ 1 confidence interval [CI], n = 3) at the end of the experiment. (b) Biodiversity (H′) and evenness (E) of start pond, controls, and experimental treatments combined in their taxonomic group (mean $\pm$ 1 CI, n = 3) at the end of the experiment. (c) Effect size (d) of H′ and E of experimental treatments compared to control at the end of the experiment.

differences in H′ (one-way ANOVA $F_{(9,20)}$ = 4.44, P = 0.01) and E (one-way ANOVA $F_{(9,20)}$ = 5.14, P = 0.01) between taxonomic groups of the experimental treatments and control were observed. After Holm-Sidak post hoc tests, added bacillariophyta treatments (H′ = 1.40 ± 0.11 standard deviation [SD]; $P \leq 0.05$) and added cyanobacteria treatments (H′ = 1.34 ± 0.08 SD; $P \leq 0.05$) showed significantly higher values in H′ compared to the control (H′ = 1.16 ± 0.11 SD). Only added bacillariophyta treatments showed significantly higher values in E (0.45 ± 0.04 SD; $P \leq 0.05$) compared to the control (E = 0.38 ± 0.04 SD). All other experimental communities showed no significant differences in H′ and E compared to the control (Fig. 2b). Further, the magnitude of the differences between invaded and uninvaded communities was calculated with Cohen's effect size (d). Thus, we saw for H′ a large to very large effect in the combined added treatment of *F. cortonensis* and *C. meneghiniana* (d = 1.1), a large effect on H′ in single added species treatments of *C. meneghiniana* (d = 0.8) and *Pseudoanabaena galeata* (d = 0.8), and a medium effect on H′ in single added treatments of *F. crotonensis* (d = 0.5) and *A. cylindrica* (d = 0.6). Only the

combined added treatment of *F. cortonensis* and *C. meneghiniana* (d = 0.7) showed a medium to large effect on E. The other experimental treatments showed no or only a small effect on H′ and E (Fig. 2c) at the end of the experiment.

Species richness showed lower values compared to start values in the control, and six experimental treatments (*Chlamydomonas reinhardtii* treatment, combined *C. reinhardtii*, *P. simplex* treatment, combined *A. cylindrica*, *P. galeata* treatment, single *F. crotonensis* and single *C. meneghiniana* treatment and combined *F. crotonensis*, *C. meneghiniana* treatment), during the experiment (Fig. 3). In the comparison between the control and the additionally added phytoplankton treatments, an allover significant difference in the mean of the relative values of SR was observed (one-way ANOVA $F_{(9,20)}$ = 3.43, P = 0.01). The multicomparison of SR showed no significant difference between any experimental treatment and control.

Community group composition

At the end of the experiment, the control phytoplankton communities consisted of mainly bacillariophyta (67.3% ± 1.3 SE), followed by chlorophyta (31.2% ± 1.2 SE), cyanobacteria
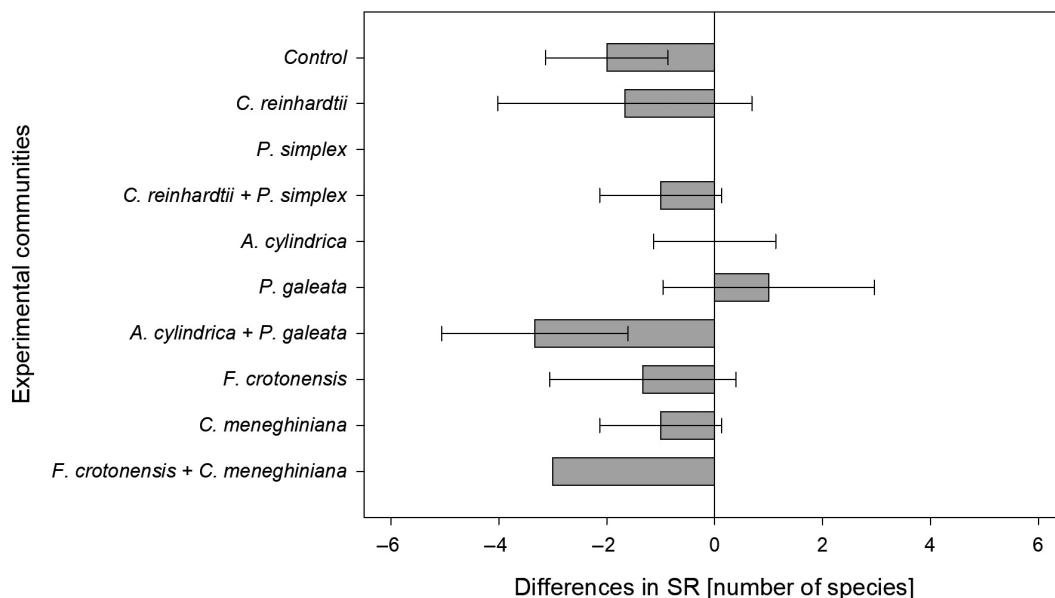

Fig. 3. Differences in species richness (SR) of control and experimental treatments against the start community (mean ± 1 confidence interval [CI], n = 3) at the end of the experiment. Zero line indicates the initial value of SR.

(0.6% ± 0.08 SE), and chrysophyta (0.4% ± 0.2 SE). This result indicates an increase in bacillario-phyta and a decrease in chrysophyta. However, there was an overall loss of chrysophyta in all treatments in the experiment.

The treatment with a combination of two added bacillariophyta species (combined *F. croto-nensis, C. meneghiniana* treatment) showed the lowest (77.5% ± 4.2 CI) similarity while the treatment with two combined added chloro-phyta species (combined *C. reinhardtii, P. simplex* treatment 92.4% ± 0.5 CI) showed the highest similarity with the control (Fig. 4).

The taxonomic group composition showed small significant differences between experimen-tal treatments and control (indicated by 95% CIs not overlapping with zero; Fig. 5). For instance, two out of three experimental treatments with added bacillariophyta showed a higher abun-dance of resident chlorophyta (*C. meneghiniana* treatment: 10.5% ± 2.7 CI, combined *F. crotonen-sis, C. meneghiniana* treatment: 10.53% ± 8.7 CI), but a lower abundance of resident bacillario-phyta (*C. meneghiniana* treatment: −10.42% ± 2.5 CI, combined *F. crotonensis, C. meneghiniana* treatment: −12.17% ± 8.3 CI) compared to the control (Fig. 5). Very similar shifts in the taxo-nomic group composition were found in two out of three treatments with added experimental cyanobacteria (single and combined species). In treatments with added single *P. galeata* and a com-bination of two cyanobacteria species, the resident chlorophyta abundance was higher (*A. cylindrica* treatment: 10.3% ± 9.3 CI; *P. galeata* treatment: 7.6% ± 6.9 CI) compared to the control. Accord-ingly, resident bacillariophyta abundance was lower in these experimental treatments (*A. cylin-drica* treatment: −11.5% ± 10.3 CI; *P. galeata* treat-ment: −9.2% ± 7.2 CI) in comparison with the control. The abundance of resident cyanobacteria showed only significantly higher values in treat-ments with added cyanobacteria (single and com-bined species, *A. cylindrica* treatment: 0.8% ± 0.4 CI, *P. galeata* treatment: 0.9% ± 0.7 CI, *A. cylin-drica* and *P. galeata* combination treatment: 1.8% ± 0.6 CI) and one treatment with added chlorophyta (*C. reinhardtii* treatment: 0.4% ± 0.12 CI) compared to the control.
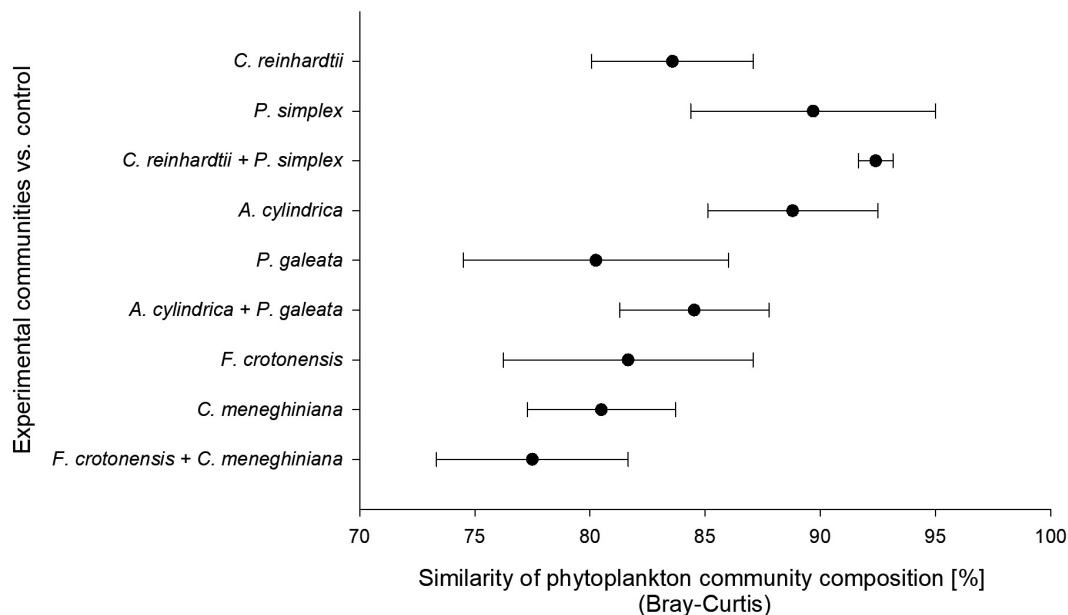

Fig. 4. Comparison of phytoplankton community composition via Bray–Curtis similarity (100% = total simi-lar; 0% = total dissimilar) based on log (X + 1)-transformed species biovolume (mean ± 1 confidence interval [CI], n = 3). Data show similarity (%) of experimental treatments with added species compared to control treat-ment without added species.
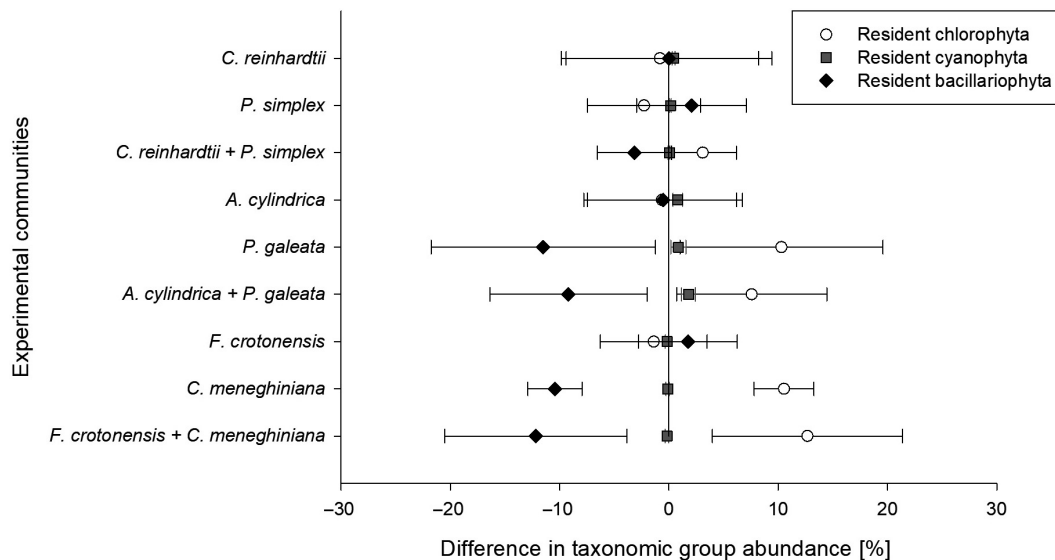
Fig. 5. Differences in abundance (% of total phytoplankton biovolume) of the most abundant resident taxonomic groups between control and experimental treatments (mean ± 1 confidence intervals [CI], $n = 3$). Zero line indicates the control values. Significant differences were indicated by 95% CIs not overlapping with the zero line (black straight line).

Species composition changes in resident communities

Not all experimental treatments with added species showed major changes in their resident species composition. Four of the treatments showed only very minor changes (a shift in species abundance of about 0.1%); however, five of the nine treatments showed significant changes (shifts in species abundances up to 15.8%) in their resident species composition, mainly regarding their dominant species (Appendix S1: Fig. S1).

In all treatments where we added bacillariophyta species, either added as single species or in combination of both bacillariophyta species, the same resident species, *Synedra* sp., *Ankistrodesmus* sp., *Scenedesmus* sp., were always affected. One of these resident species was dominant in the resident communities (*Synedra* sp.). According to the SIMPER analyses, *Synedra* sp. (microplankton) was one of the major contributors to the overall dissimilarity (18.0%, *F. crotonensis* treatment; 24.3%, *C. meneghiniana* treatment; 32.1%, combined *F. crotonensis, C. meneghiniana* treatment) to the controls. *Synedra* sp. showed lower abundances in all treatments where we added bacillariophyta (*F. crotonensis* treatment −3.5% ± 1.7 CI; *C. meneghiniana* treatment −9.2% ± 0.8 CI;

combined *F. crotonensis, C. meneghiniana* treatment −15.8% ± 5.9 CI) compared to the control (64.0% ± 1.8 CI). The resident chlorophyta *Ankistrodesmus* sp., nanoplankton, according to the SIMPER analyses, was also a main contributor to dissimilarity (17.6% in *F. crotonensis* treatment, to 38.0% in *C. meneghiniana* treatment and 28.5% in combined *F. crotonensis, C. meneghiniana* treatment). Different to *Synedra* sp., *Ankistrodesmus* sp. showed higher abundances after adding bacillariophyta species (*F. crotonensis* treatment 6.4% ± 3.6 CI; *C. meneghiniana* treatment 14.7% ± 2.6 CI; combined *F. crotonensis, C. meneghiniana* treatment 14.0% ± 5.5 CI). Additionally, four species, *Cyclotella* sp., *Fragillaria* sp., *Crucigenia* sp., and small coccal chlorophyta, were also affected in single and/or combined added bacillariophyta treatments. Two out of these species were more (shifts higher than 5%) affected than the other two (shifts lower than 1.6%). *Fragilaria* sp., nanoplankton, showed higher abundances in both treatments where only *F. crotonensis* was added or in combination with the other baccilariophyta (*F. crotonensis* treatment 8.5% ± 1.4 CI; combined *F. crotonensis* and *C. meneghiniana* treatment 6.3% ± 0.9 CI) compared to control communities. Based on the SIMPER analyses, *Fragilaria* sp. was

the main species contributing to the dissimilarity between the control and added single bacillariophyta treatment (*F. crotonensis* treatments) with 23.3%, and in combined *F. crotonensis* and *C. meneghiniana* treatment with 12.7%. Small coccal chlorophyta (<5 µm, nanoplankton), also a dominant resident species, had lower abundances in treatments with single added *F. crotonensis* (−8.4% ± 6.6 CI) and single added *C. meneghiniana* (−7.1% ± 3.9 CI) in comparison with controls. According to the SIMPER analyses, the reduction of small coccal chlorophyta abundance in treatments where we added bacillariophyta contributed with 23.2% (*F. crotonensis* treatment) and 18.2% (*C. meneghiniana* treatment) to dissimilarity to the control.

In treatments with added cyanobacteria species, only one resident species was strongly affected (shifts higher than 10%) especially by the addition of *P. galeata* and the combined addition of both cyanobacteria species (*A. cylindrica* and *P. galeata*). The abundance of *Ankistrodesmus* sp. was higher (14.7% ± 3.9 CI *P. galeata* treatment and 10.5% ± 0.5 CI combined *A. cylindrica, P. galeata* treatments) when compared to control treatment and was the major contributor (37.2% *P. galeata* treatment; 35.4% combined *A. cylindrical, P. galeata* treatment) to dissimilarity to the control. Additionally, eleven other species showed small shifts in abundances (from 3.3% to 1.1%) in treatments where single and/or combined cyanobacteria were added compared to the control.

Two resident species were mainly affected by adding chlorophyta species. Similarity percentages analyses showed a main contribution of *Chlamydomonas* sp. (17.6% *C. reinhardtii* treatment; 12.3% combined *C. reinhardtii, P. simplex* treatment) and small coccal chlorophyta (30% *C. reinhardtii* treatment; 24.1% combined *C. reinhardtii, P. simplex* treatment) to the dissimilarity compared to control communities. The abundance of *Chlamydomonas* sp. was higher (5.1% ± 3.2 CI *C. reinhardtii* treatment) compared to the control, whereas the abundance of small coccal chlorophyta was lower (−8.7% ± 7.7 CI *C. reinhardtii* treatment). Additionally, nine other species showed only small shifts in their abundances (0.1–2.6%) in treatments where single and/or combined chlorophyta were added compared to the control.

The cell size composition of all treatments at the beginning and at the end of the experiment showed mainly nanoplankton species ($10–10^3$ µm^3). Only seven species (*Amphora* sp., *Synedra* sp., *Tabellaria* sp., *Botryococcus* sp., *Mougeotia* sp., *Synura* sp., and *Gymnodinium* sp.) out of the 29 total species belonged to microplankton ($10^3–10^6$ µm^3). However, we were able to detect shifts in cell size composition in communities with added species compared to the control communities based on the species abundance at the end of the experiment (Appendix S1: Fig. S1). In six (all added cyanobacteria and all bacillariophyta treatments) out of nine treatments, the nanoplankton species, *Ankistrodesmus* sp., increased in abundance compared to the control. In two (*F. crotonensis* treatment and combined *F. crotonensis, C. meneghiniana* treatment) out of three bacillariophyta treatments, the microplankton *Synedra* sp. showed, however, reduced abundances compared to the control. In two bacillariophyta treatments (*F. crotonensis* treatment, *C. meneghiniana* treatment) and one chlorophyta treatment (*C. reinhardtii* treatment), small coccal chlorophyta (nanoplankton, small greens) showed reduced abundances. In all chlorophyta treatments (single or combined added chlorophyta), *Cyclotella* sp. showed reduced abundances.

DISCUSSION

Failed invasion

None of the six added species (added single or in combination) was able to establish within the natural phytoplankton community in our experiment. A possible explanation might be the abrupt environmental change, from the artificial laboratory conditions (growth medium, temperature, light) to the natural environmental conditions. The inoculated laboratory species might not be able to adapt quickly enough to the new conditions of the natural pond, which might have caused their decline. A similar decline of an invasive phytoplankton species entering a new habitat was observed in a study by Weithoff et al. (2017). The authors tested which mechanism (consumptive or competitive resistance) prevents a successful invasion of three isolates of the cyanobacterium *Cylindrospermopsis raciborskii* in a natural freshwater system. Two isolates of *C. raciborskii* were unable to invade the community, but the temporary abundance of

C. raciborskii significantly changed the resident communities with and without grazing pressure (Weithoff et al. 2017). Under natural conditions, a rapid change of environmental conditions is likely and can act as a selective force during an invasion process (Weithoff et al. 2017) leading to unsuccessful invasions.

Changes in community composition

We found major alterations in community composition at the end of the experiment. Differences in diversity, evenness, and similarity, but not in species richness, were found between the resident community with added species (potential invaders) and a control community without invasion. Diversity and evenness were significantly higher in communities with added bacillariophyta and resulted in reduced similarity compared to control communities. In particular, after disturbance the evenness of the communities responded more rapidly than just species numbers (Mattingly et al. 2007). SR in the combined added bacillariophyta treatment showed a stronger decrease compared to the control (Fig. 3). For E, a medium effect was found for this treatment compared to the control (Fig. 2c). Besides E, we found a very large and a large effect on H′ between invaded bacillariophyta treatments and uninvaded controls (Fig. 2c). However, the majority of our treatments showed small effects on H′ and E, and the medium and large to very large effects on H′ and E indicate major influences of the added bacillariophyta on the resident community.

We observed shifts (increase and decrease) in the taxonomic group and species abundances caused by different species influencing the dissimilarities between the control and experimental treatments. Although the dominant species (e.g., *Synedra* sp.) was still highly abundant, its reduced abundance, due to the addition of species in experimental communities, contributed the most to the dissimilarity of the communities compared to controls. Thus, initially dominant resident species were negatively affected which enabled other species to increase in abundance. This finding is in line with Emery and Gross (2007) who hypothesized that dominant species are more likely to interact with other species since they are more likely to get in contact with other species due to their high abundance.

So far, potential effects of unsuccessful invaders on resident communities are mostly neglected. However, two theoretical models of Case (1995) and Miller et al. (2009) predicted crucial effects of unsuccessful invaders on resident communities and one experimental study found, after a failed invasion, lasting effects on the resident phytoplankton community (Weithoff et al. 2017). According to the results of the model of Case (1995), we also found a changed competition situation in the resident community, which favored a non-dominant species (e.g., *Ankristodesmus* sp.). Furthermore, all our findings reinforce the importance of species interactions. Miller et al. (2009) assume that the effects of changes in the community are the result of indirect effects of few unsuccessful species in the community. In our study, direct and indirect interactions of unsuccessful invading species and resident species led to different community compositions compared to non-invaded ones. Consequently, these interactions could be driving forces for community composition shifts in the resident community. We suggest three possible explanations for that: (1) direct influence of added species on resident species belonging to the same taxonomic group by competition for the same resources; (2) substitution of one resident species by another resident species within the same taxonomic group; and/or (3) indirect influence of the added species on resident species from another taxonomic group.

First, direct competition between species for the same resource will be strongly defined by the different competitive abilities of the individual species for a specific resource: Hereby, similar species (e.g., belonging to the same taxonomic group) are more likely to have similar resource requirements and compete for those (Tilman 1977, Sommer 1984, Kneitel and Chase 2004). However, species belonging to the same taxonomic group may still differ in other functional traits (e.g., motility) and therefore have slightly different utilization abilities, as well as competitive abilities (Litchman 2007, Litchman and Klausmeier 2008). Our results provide evidence that competition among species from the same taxonomic group and size class, nanoplankton, results in a lower abundance of dominant resident species belonging to the same taxonomic group as the experimentally added species. Such

an effect is seen in the treatment where we added *Chlamydomonas reinhardtii*, which lowered the abundances of other resident chlorophyta species (Appendix S1: Fig. S1).

Second, we found an unexpected effect of substitution in our results, where one species is replaced by another possibly through its superior competitive abilities (Sommer 1983, Tilman and Sterner 1984). In the treatment where *Fragilaria crotonensis* was added, a resident chlorophyta (*Ankistrodesmus* sp.) had a higher abundance whereas another resident chlorophyta (small coccal chlorophyta) showed lower abundance (Appendix S1: Fig. S1) compared to controls. The exchange of the two species abundances is only seen on species level and hidden on taxonomic group level, due to an almost stable total abundance of the taxonomic group abundance. This highlights the importance to analyze the mechanism of interactions on the species level.

Third, the direct interaction of two species, for example, competing for open niches, based on different competitive abilities (Litchman 2007) can cause an indirect effect on a third species (Wootton 1994). The indirectly affected species might use available niches, for example, resources or space, which become available through the direct interaction of competing species which also diminish the competitive strength of the dominant species. This mechanism can be a possible explanation for an observed higher abundance of a specific resident taxonomic group (chlorophyta) in treatments where we added species of another taxonomic group compared to controls (bacillariophyta; Fig. 5). Similar results on community composition were found in treatments with added cyanobacteria. These treatments show a lower abundance of dominant resident bacillariophyta (e.g., *Cyclotella* sp.) and higher resident chlorophyta abundance compared to controls (Appendix S2: Fig. S1).

Role of convergence of added species to resident species

The taxonomic convergence of the added species to the resident species could be neglected in our study. We observed the same influences of added species independent of whether they had more convergent or divergent traits compared to the resident species pool. For example, adding bacillariophyta, which had more convergent traits to the bacillariophyta dominated resident community resulted in comparable effects as adding cyanobacteria, which have very divergent traits to the resident species pool (Fig. 5). This is in accordance with earlier findings of Warren et al. (2003), who tested different resident protist communities invaded by other protist species. Independent of which protist species invaded, the same final community, was reached.

Cell size

We found evidence for the importance of species cell size for the outcome of competition. A possible explanation for the observed higher abundances of *Ankistrodesmus* sp. (nanoplankton) and lower *Synedra* sp. (microplankton) abundances in combined added bacillariophyta treatments, compared to the single added treatments, could be their corresponding cell size. Smaller cells can have distinct advantages over larger cells. For example, smaller cells are more efficient in the uptake of limiting nutrients due to a higher surface to volume ratio (Litchman and Klausmeier 2008). In our study, the combination of the two nanoplankton species (*F. crotonensis* and *Cyclotella meneghiniana*) reduced the abundance of microplankton species (*Synedra* sp.) possibly by enabling better resource utilization via their smaller size. The impact of the size and species contributions to the dissimilarity between invasion treatments and the control indicate that species with a smaller cell size could be better competitors (Litchman and Klausmeier 2008). Mainly *Ankistrodesmus* sp. benefitted from the temporary abundance of another species. These results support our assumption that the trait cell size is important for competition mechanisms affecting community composition and species interactions. However, other mechanisms such as limiting resources might play an additional role for our observed changes in community composition. Therefore, further analyses of how species are interacting might give more insight in the mentioned process and how dominance might change within a community in the presence of added species.

Combined added species effect

An evidence for a higher effect of combined added species on the resident species could only be observed for the combined added

bacillariophyta (Appendix S1: Fig. S1). Resident bacillariophyta in the treatment with combined added *F. crotonensis* and *C. meneghiniana* showed lower abundance than in the treatment were bacillariophyta were added as single species. Our results rather support the effect of the invasion meltdown hypothesis by Simberloff and Von Holle (1999), which suggests that one invader potentially, facilitates the establishment of another species resulting in a synergetic effect on the resident community. In our case, the two added species are not successful at establishing; however, both have effects on the resident community, which are even stronger when the two species are added in combination.

Possible impact on freshwater communities

In our study, the overall influences of unsuccessful invaders on the resident community are rather small and depend on the individual added species. Still, several changes of the resident community are significant. We are aware that the experimental duration was short- and long-term effects could not be described. However, the failed invasion changed the competition condition in reducing the dominance of a single species, which in return might alter long-term community composition in primary producers.

Changes in phytoplankton community composition can have further effects on higher trophic levels such as shifts in the food spectrum available for herbivores. The reduction of a dominant species (microplankton *Synedra* sp.) favored the growth of other species from other taxonomic groups (small chlorophyta). This can have consequences for zooplankton due to changing morphology and indigestibility of the phytoplankton. Small chlorophyta (e.g., *Ankistrodesmus* sp.) are a good food source for many filter feeders (Arnold 1971, Schindler 1971, Henning et al. 1991), whereas bigger bacillariophyta (e.g., *Synedra* sp.) are less indigestible (Post and McQueen 1987, Sommer and Stibor 2002).

Two possible biotic resistance mechanisms exist that can prevent a successful invasion. Competitive resistance due to species interactions favoring strong competition conditions for the invader during the invasion process or the consumptive resistance due to predation. The consumption resistance to prevent a successful invasion is often more important in freshwater systems than the competitive resistance (Alofs and Jackson 2014). This is due to potentially stronger trophic cascades in freshwater systems than in terrestrial ones (Weithoff et al. 2017). It was shown that consumptive resistance makes natural phytoplankton communities more resistant to a successful invasion of *C. raciborskii* (Weithoff et al. 2017). In contrast, we were interested in the producer level and the impact of unsuccessful invaders and their influence on the resident community. Therefore, we needed to uncouple the consumptive and competitive conditions by the exclusion of grazing pressure. Conclusively, our study excluded macrozooplankton and showed competitive conditions between phytoplankton species (resident and/or added species). Further studies could give more insight into direct effects on consumers, shifts in food sources, or possible indirect effects on resident producers.

Conclusion

Our study examines effects of unsuccessfully invading species on the community composition of a freshwater phytoplankton community. Indications for possible underlying mechanisms of direct and indirect species interactions were observed under natural conditions. Our experiments clearly show the importance of unsuccessful invasions resulting in hidden effects on the composition of phytoplankton communities. All effects were observed ones and general statements can therefore not be deduced. However, we found evidence that mainly the dominating resident species is influenced by added species and that some small phytoplankton species have an advantage over larger species due to the temporary presence of the invaders. Effects of unsuccessful invaders on resident communities can be hidden on taxonomic group level, but are clearly present on the species level. To fully understand dynamics of natural plankton communities, potential effects of unsuccessful invasions have to be studied in more detail.

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