Interactive effects of foundation species on ecosystem functioning and stability in response to disturbance

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Abstract

A major challenge in ecology is to understand determinants of ecosystem functioning and stability in the face of disturbance. Some important species can strongly shape community structure and ecosystem functioning, but their impacts and interactions on ecosystem-level responses to disturbance are less well known. Shallow ponds provide a model system in which to study the effects of such species because some taxa mitigate transitions between alternate ecosystem states caused by eutrophication. We performed pond experiments to test how two foundation species (a macrophyte and a mussel) affected the biomass of planktonic primary producers and its stability in response to nutrient additions. Individually, each species reduced phytoplankton biomass and tended to increase rates of recovery from disturbance, but together the species reversed these effects, particularly with larger nutrient additions. This reversal was mediated by high cyanobacterial dominance of the community and a resulting loss of trait evenness. Effects of the foundation species on primary producer biomass were associated with effects on other ecosystem properties, including turbidity and dissolved oxygen. Our work highlights the important role of foundation species and their interactive effects in determining responses of ecosystem functioning to disturbance.
Introduction

Ecosystems vary in their stability, particularly in response to externally imposed disturbances (1). Disturbances have historically been considered to be processes which are destructive and cause mortality, such as fire or grazing (2, 3), but may also include discrete enrichment events via nutrient addition due to the impact that this can have on demographic rates, and community and ecosystem structure (4, 5). Stability in the face of a disturbance can describe various aspects of a system’s response, including its ability to resist change (resistance) and the rate at which the system recovers once the disturbance has stopped (resilience) (6). Systems may respond smoothly and proportionally to the extent of environmental change, whereas others may show threshold responses – responding abruptly only beyond a critical value of disturbance (1, 7). Importantly, some organisms can profoundly alter how systems respond to disturbance (8), either directly based on their own behavior and ecology, or indirectly, by shaping the biotic community and by establishing particular sets of biotic processes and feedbacks (9-11). Numerous examples in community ecology have demonstrated the important roles that individual species can play in determining community structure and whole-ecosystem properties. These important species include foundation species, which can determine the structure of ecological communities (8, 12), ecosystem engineers, which play a large role in structuring resource flows (9), and keystone species which have disproportionately large effects on a community or ecosystem function relative to their abundance (13). Still, we know of no whole-ecosystem manipulations that provide insight into how such species affect ecosystem functions and stability in response to nutrient addition disturbances and how their effects may interact (11, 14).

Insight from biodiversity-ecosystem functioning research can suggest mechanisms by which important taxa (e.g. foundation species) may impact ecosystem stability via their effects on community structure and community-level trait variation (15-17). Important species and their interactions may select for taxa that are either resistant or susceptible to disturbance, thereby altering the ecosystem
properties affected by these newly dominant taxa (i.e. a 'selection effect', 15, 18). Further, they may
either promote or constrain the taxonomic and trait diversity within communities, and thereby alter
ecosystem stability (17). Communities with greater species diversity or trait variation tend to be more
stable over time due to statistical portfolio effects (19) and/or compensatory dynamics (15, 20, 21),
whereby asynchronous temporal variation among taxa or functional types provides insurance because
when one is doing poorly, another fares well (22). Compensatory interactions, however, may also be
the result of competition among species within a trophic level. If important species (e.g. foundation
species) alter competitive networks so as to generate competitive release, their effects may be
destabilizing (1). Lastly, if most species in a system respond similarly and synchronously to both
disturbance and the impact of important species, then system stability will decrease.

In aquatic systems, shallow lakes have been used as a model system in which to investigate the
role of important species in determining whole-ecosystem stability because these systems can
experience regime shifts, particularly in response to increasing nutrient loading (7). For example,
shallow lakes can shift from clear to turbid water as nutrient supply increases, but either state can occur
at intermediate nutrient levels where biotic interactions can determine the stability of alternative states
and transitions between them. For example, both macrophytes and filter feeders such as mussels may
reduce phytoplankton responses to nutrient additions. Specifically, macrophytes promote clear water by
stabilizing sediments, taking up dissolved nutrients, sheltering grazers, and producing allelochemicals
(23, 24). Allelopathic effects may restructure phytoplankton communities because they are taxon-
specific, with cyanobacterial growth generally being most strongly inhibited (25, 26), though this effect
may be reversed in the presence of green algae (27). Furthermore they may alter competitive outcomes
between cyanobacteria and eukaryotic phytoplankton (28). Similarly, mussels are capable of controlling
phytoplankton responses to nutrient loading, and maintaining clear water due to their high filtration
rates (23, 29). However, dreissenid mussels may select for cyanobacterial dominance by releasing small
or low food-quality species undigested in their pseudo-feces (30, 31). Though prior work suggests that macrophytes and mussels are important organisms in determining how aquatic systems respond to nutrients, the potential for their effects to interact and affect ecosystem-level stability in response to disturbance by nutrient addition remains unknown. Such experiments, which include the influence of natural ecological complexity, are crucial to inform management (32).

We performed whole-pond experimental manipulations to investigate the impact of two foundation species on the community structure, ecosystem functioning and stability of freshwater pond ecosystems in response to nutrient addition disturbances. The foundation species are the Eurasian Watermilfoil, *Myriophyllum spicatum* (hereafter ‘Myriophyllum’), and the Zebra Mussel, *Dreissena polymorpha* (a regionally-abundant non-native species, hereafter ‘Dreissena’). Both species have previously been termed ecosystem engineers due to their impacts on physical habitat provisioning and resource availability (10), though in this study their impacts on community structure are more consistent with the concept of foundation species. We tested the hypothesis that these species would modify the phytoplankton community composition and thereby affect ecosystem functioning and stability in response to nutrient additions. Specifically, we expected both foundation species to affect the relative abundance of cyanobacteria and eukaryotic phytoplankton, with Myriophyllum selecting for a lower abundance of cyanobacteria and Dreissena selecting for greater abundance. The main ecosystem function of interest was the standing stock of primary producer biomass. Ecosystem stability can be defined in many ways, and changes in community structure may not affect them all in the same way (33). Here we focused specifically on resistance – the degree to which a system remains unchanged after disturbance – and resilience – the rate of recovery from a disturbed state (6). We also tested whether the foundation species affected temporal autocorrelation of primary production, with greater autocorrelation being consistent with a slower rate of return from disturbance and therefore lower stability (34, 35). Finally, we investigated the indirect effects of these foundation species on numerous
biotic and abiotic ecosystem properties including dissolved nutrient availability, water turbidity, dissolved oxygen and the abundance of grazers.

We predicted that both foundation species would individually reduce and stabilize phytoplankton biomass production, in particular by limiting the extent of its response to nutrient addition, increasing the rate of recovery post-disturbance, and reducing temporal autocorrelation. We expected that both species together would further synergistically reduce and stabilize the impact of nutrient disturbance on primary production through their combined bottom-up and top-down control of phytoplankton abundance. Lastly, we predicted that the effects of the foundation species on primary producer biomass and other ecosystem properties would be mediated by changes in the community structure and trait variation of the phytoplankton community, with greater taxonomic and trait variation leading to greater stability.

**Materials and Methods**

We factorially manipulated the presence and absence of Dreissena and Myriophyllum in 15 m$^3$ artificial ponds and replicated all treatments 4 times. We refer to the treatment without either foundation species as the “Control”. We compared the treatments with foundation species to the Controls to evaluate the impacts of the foundation species on community composition and trait evenness, phytoplankton biomass, stability and numerous ecosystem properties. Each of these 16 ponds received the same nutrient disturbance regime, characterized by five nutrient addition events of increasing magnitude (described further below). We chose this disturbance regime to investigate both responses to individual disturbance events, as well as the cumulative effects of multiple disturbances over time. Four additional ponds received neither foundation species nor nutrients, and we refer to these as “Oligotrophic” ponds. We used these ponds to evaluate the effect of nutrient addition as a
disturbance. All 20 ponds are located in Eawag’s research facility in Dübendorf, Switzerland (47.4038° N, 8.6098° E). We randomized the treatments across ponds. The ponds are identical, dug into the ground, and lined with fiberglass. Each pond has a shallow-end (0.5 m) and a deep-end (1.5 m), with overflow pipes in each end. Ponds with the foundation species received either 100 live macrophytes (a mean addition of 19.84 g of dry biomass), 100 live mussels (a mean addition of 632.67 mg of soft tissue dry biomass, i.e. without the shell) or both. For detailed methods on how we cleaned, filled and inoculated the ponds with phytoplankton and invertebrates, as well as how we established the experimental foundation species treatments, please see the Supporting Methods. The experiment was started at the end of June 2016, and sampling began in the first week of July and continued until the end of February 2017.

**Sampling**

We performed weekly sampling from aluminum bridges transecting each pond. We constructed a custom-made ‘Leibold-sampler’ for every pond to collect water. A Leibold-sampler consists of a 180 cm long PVC tube (Ø 5 cm) and a rubber ball attached to a rope that passes through the length of the tube. Using the sampler, we haphazardly sampled three vertical profiles of the water column in the deep end (10 L per pond/sampling day). Due to freezing of the pond surfaces in the winter, we sampled through a floating Styrofoam ring, which maintained an ice-free hole on the surface of each pond (Ø 280 mm x H 225 mm, ‘Eisfreihalter’ by Pontec). Due to low temperatures and productivity in the ponds during the winter, we reduced the sampling frequency to bimonthly in November and monthly in December, January and February. For each pond, we took a 1 L sub-sample for chlorophyll-a extractions. We filtered the samples onto Whatman GF/F filters and kept them frozen (-20°C) until extraction (36). During extraction, we added 8 mL of 90% ethanol to each tube, vortexed them, sonicated them in an ice-water bath for 15 min.
and stored them at 4 °C overnight in the dark. We filtered the extracted samples with 0.2 μm cellulose acetate syringe filters (Whatman FP 30/0.2 CA) to remove particles. We measured absorbance between 665-750 nm using a Hitachi U 2000 spectrophotometer.

Samples for phytoplankton microscopy, scanning flow cytometry (CytoBuoy) and biomass stoichiometry were pre-filtered with a 95 μm mesh to remove large zooplankton. We fixed 30 mL sub-samples with 3 mL of Lugol’s iodine solution (Merck/Sigma-Aldrich) for microscopy. Sub-samples were settled in an Utermöhl chamber (HYDRO-BIOS), and phytoplankton were identified and counted using an inverted microscope (Zeiss Axiovert 135) at 640 x total magnification. Identifications were performed to the greatest possible level of taxonomic precision (generally genus or species). The total biovolume of each species was calculated by estimating species’ average cell volumes (N≥10) using geometric formulae based on cell shape (37) and multiplying the cell volumes by cell counts.

We took a 10 mL sub-sample for each pond for scanning flow cytometry, and preserved it using 100 μL of a fixative solution (final concentration = 0.01 % paraformaldehyde and 0.1 % glutaraldehyde). Samples were stored in the dark at 4°C until processing. Samples were diluted 1:3 in water before running them on a Cytobuoy scanning flow cytometer. The Cytobuoy was run using an internal flow rate of 1 μL·s⁻¹ and a trigger threshold of 10 mW on sideways scatter (SWSTrigger). We extracted the raw data files and converted them into csv files using the Cytoclus 3 software (CytoBuoy BV©v 3.7.16.9). We used a custom R script to separate particles of phytoplankton from background noise, debris and bacteria (38). We then used the eight most important flow cytometry parameters encompassing variation in size, shape and pigmentation that enable unsupervised clustering of phytoplankton functional groups using machine learning (Fig. 2b from 38) to calculate a multivariate measure of phytoplankton trait evenness (hereafter called “TED”) from the scanning flow cytometry data. TED provides an estimate of the evenness of the distribution of phytoplankton individuals within multivariate trait space (39).
We filtered samples onto a set of two pre-ashed (500 °C) Whatman GF/F filters (Ø25 mm) for algal biomass stoichiometry, one for carbon and nitrogen and one for phosphorus. Filters were dried at 60 °C. The carbon and nitrogen filters were and packed into tin capsules for analysis using an Elementar vario PYRO cube® EA-IRMS system. The phosphorus filters were placed into 15 mL conical tubes and digested in 10 mL of digestion solution (10 g of K₂S₂O₈ and 1.5 g of NaOH in 1 L of water). Digested samples were diluted 1:20, and analysed using a Skalar San++ Continuous Flow P/N analyser. Due to low algal biomass in the winter, stoichiometry was not estimated after December 2016.

Zooplankton were sampled biweekly by collecting 10 L of pond water and filtering it through 30 µm Nitex mesh. The zooplankton were then rinsed from the mesh with 100% ethanol into a 50 mL jar. The zooplankton were then counted and identified under a dissecting microscope at 4x magnification.

We used handheld sondes to measure turbidity (HACH® TSS), temperature and dissolved oxygen (WTW MultiLine® Multi 3630 IDS SET G) at 1 m depth. We took a 1 L sub-sample to measure the concentrations of dissolved nutrients and pH. Before measuring dissolved nutrients (PO₄-P and NO₃-N), we filtered the water through 0.45 µm nitrocellulose filters (Sartorius, Stedim Biotech).

Orthophosphate-phosphorus (PO₄-P) was measured spectrophotometrically using Varian Cary 50 bio (detection limit of 1.0 µg/L (±0.5)) after reaction to phosphorus molybdenum blue complex (40).

Nitrate-nitrogen was also measured on a Varian Cary 50 bio (detection limit of 0.05 mg/L (± 0.02 mg/L)), after evaporating the sample to dryness, reacting with sodium salicylate (C₇H₅NaO₃) in sulfuric acid solution (H₂SO₄), and converting to nitrosalicylic acid (C₇H₅NO₅) (41). We measured pH in the lab with a Metrohm 809 Titrando conductivity-pH meter (Metrohm Schweiz AG, Zofingen).

On March 03, 2017, at the end of the experiment, we performed visual surveys to ensure the presence of live foundation species in the ponds in which they were introduced. We successfully observed live organisms with one exception; live mussels were not seen in Pond 2C, a Myriophyllum and Dreissena treatment pond. This pond did not show any obvious differences from other replicates in
terms of chlorophyll-a or cyanobacterial dominance, and was therefore included in all further analysis.

More quantitative assessments of abundance or biomass were not possible, but regular visual observations suggested no major increases in the abundance of either.

**Nutrient additions**

After 8 weeks of sampling, when we observed that chlorophyll-a had stabilized relative to initial measurements, we began the nutrient additions (Fig. 1a). At each nutrient addition, we added nitrogen and phosphorus in form of KNO$_3$ and K$_2$HPO$_4$, respectively, to all except the oligotrophic ponds. We increased phosphorus additions from 10 µg/L to 20, 30, 40 and finally 50 ug/L. We also added nitrogen at double the Redfield ratio (i.e. 32:1 molar ratio of N:P) because nitrogen can be lost from aquatic systems via denitrification but phosphorus cannot. Nutrients were added every 2-2.5 weeks, starting on July 12, 2016 and ending on October 10, 2016. We chose this interval because the chlorophyll-a data suggested that most ponds had experienced disturbance and some degree of recovery, indicated by a return towards the pre-disturbance state, within this time-frame.

**Data analysis**

To understand how the foundation species affected community and ecosystem level properties, we compared treatment effects to the Control using Dunnett’s tests (glht function in the multcomp package in R v. 3.5.1). The Control ponds received nutrient additions but not live foundation species. To assess how impacts of treatments changed over time and in response to nutrient additions, we broke the time-series up into pre-, during- and post-nutrient addition periods. The pre-nutrient addition time points included the three weeks before the first nutrient addition, the during- time points included all weeks after the first and before the last nutrient addition, and the after- time points included all weeks after the last nutrient addition. Within each time-period, the Dunnett’s tests were performed on linear
mixed effects models where chlorophyll-a (a proxy for primary producer biomass) and various ecosystem properties were modeled as a function of experimental treatment, with time as a random effect nested within pond. Additionally, we calculated the log response ratios (‘LRR’s) of the effect of each foundation species and their interactive effects, relative to the control for each of the community and ecosystem response variables (ESM Figs. 1 & 2). We compared the interactive effect to the additive expectation (sum of separate effect sizes), and considered the interaction to be non-additive when its 95% confidence interval did not overlap the additive expectation.

To investigate how phytoplankton community biovolume structure changed over time, we plotted the change in Hellinger-transformed phytoplankton biovolumes over time in a PCA and estimated the significance of the treatments using a phenotypic trajectory analysis (PTA) (42). PTA constructs an empirical null distribution of model parameters from a MANOVA. It permutes the residuals of a model of interest (i.e. \( y^{\text{time*treatment}} \)) against a simpler model (i.e. without the interaction), and calculates the likelihood of the observed trajectories of change (length, shape and direction) given the null distribution.

To evaluate how the stability of chlorophyll-a responded to the nutrient additions and the presence of foundation species, we calculated log response ratios of chlorophyll-a over time:

\[
LRR = \log \frac{\text{Chlorophyll} - a_t}{\text{Chlorophyll} - a_{t-1}}
\]

Positive LRRs indicate increases in chlorophyll-a and negative LRRs indicate decreases. LRRs relativize absolute changes to initial values, so that changes can be compared across time and across time-series, where changes are relative to different starting values, i.e. the value of chlorophyll-a just prior to the nutrient addition. For each nutrient addition, we calculated LRRs for all values after a nutrient addition and prior to the next one, and then selected the maximum LRR as an indication of the maximum rate of increase after the disturbance (inverse of resistance). We calculated the minimum LRR as an indicator of the rate of recovery (resilience), with the stipulation that positive values indicate no recovery at all. We
also estimated the degree of temporal autocorrelation of chlorophyll-a with a lag of 1 week within each pond. These analyses were performed on the chlorophyll-a values of each pond, after subtracting the mean chlorophyll-a of the Oligotrophic treatment for each time-point. This was done to account for seasonally-driven variation in primary producer biomass. We then tested whether these measures of stability responded to the foundation species treatment using linear models.

Lastly, we used a structural equations models which included temporal autoregressive linear models in order to test causal hypotheses (pSEM function in piecewiseSEM in R v. 3.5.1) (43). Because we expected that the foundation species would cause changes in the abundance of various phytoplankton groups, we started with an initial model in which the biovolume of all major groups could be affected by the experimental foundation species treatments. In turn, both chlorophyll-a and phytoplankton trait evenness could be driven by the biovolume of individual phytoplankton groups. We included temperature as a driver of each algal group to account for effects of seasonal change. Lastly, we checked for evidence of our hypothesis that chlorophyll-a and trait evenness affected biotic and abiotic ecosystem properties including the concentration of dissolved nutrients, turbidity, dissolved oxygen, pH, and the density of grazers (i.e. daphnia and copepods). We used data from all time points up to the end of November 2016 and all ponds to test the model. Each equation within the pSEM is a linear mixed effect model with Pond as a random effect and accounting for temporal autocorrelation of the dependent variable within Pond. We followed a progression of including missing causal links (identified by tests of directed separation, d-sep tests), as well as removing non-significant pathways (43), iterating this process until all identified significant paths were included (see Supporting Methods for the starting and selected models). When a path was identified by a d-sep test where the directionality made more biological sense when reversed, we reversed the causal path. When a path was identified as significant, but we had no hypothesis regarding the relationship between the variables, we included the relationship as a correlation. We selected the model with the lowest AIC. Since we
expected that the main and interactive effects of Myriophyllum and Dreissena would be driven largely by relative changes in cyanobacteria and eukaryotic biovolume, we also tested a model in which algal biovolume was only separated into cyanobacterial or eukaryotic groups (details in the Electronic Supplementary Material). Results were broadly consistent with the outcome of the original model, with less variance explained in chlorophyll and turbidity in the simplified model, so the original model selection with effects on all taxonomic groups was retained.

Results

Nutrient additions and the presence of foundation species either alone or together had strong effects on phytoplankton biomass (estimated as chlorophyll-a) (Fig. 1a), community composition (Fig 1b), and phytoplankton trait evenness (Fig. 1c). The nature of the effects of foundation species differed, depending whether they occurred before, during or after the nutrient additions (Fig. 1, ESM Table 1a-c). Both before and during nutrient additions, Myriophyllum and Dreissena independently reduced chlorophyll-a relative to the control treatment (Fig. 1a, ESM Table 1a and Fig. 1a). Over the same time-period, the treatment with both Myriophyllum and Dreissena treatment (MD) was not different from the Control treatment. However, in the three weeks following the last nutrient addition we observed a strong positive effect of the MD treatment on chlorophyll-a (Fig. 1a, ESM Table 1a), and this effect was generally greater than the additive expectation (ESM Fig. 1a). The ponds with both species also had a significantly greater dominance of community biovolume by cyanobacteria (mostly Synechochoccus) (Fig. 1b, ESM Table 1b). Ponds with only Myriophyllum also showed slightly greater cyanobacterial dominance than the Control ponds (Fig. 1b, ESM Table 1b). The high dominance of cyanobacteria in the MD treatment was also associated with a significant drop in trait evenness to near zero following the nutrient additions (Fig. 1c, ESM Table 1c). This effect was significantly lower than the additive expectation (ESM Fig. 1c). Ponds receiving either Myriophyllum or Dreissena were less heavily
dominated by cyanobacteria than the ponds receiving both (Fig. 1b, ESM Fig. 3), and had a trait evenness that was not significantly different from the Control ponds (Fig 1c, ESM Table 1c). The foundation species treatments also had a significant effect on the phytoplankton community trajectory in terms of its direction (PTA summary statistic=955.967, p<0.001, ESM Fig. 3). In the MD treatment the community-level changes in cyanobacterial dominance and reduced trait evenness were accompanied by a marginally non-significant increase in the C:N ratio of the phytoplankton community relative to the Control (p=0.07, Fig. 1d, ESM Table 1d).

The foundation species treatments and nutrient additions had significant impacts on a number of abiotic ecosystem-level properties including dissolved nitrate and phosphate (Figs. 2a & b, respectively), turbidity (Fig. 2c), dissolved oxygen (Fig. 2d), pH (Fig. 2e). Nitrogen and phosphorus in the form of dissolved nitrate and phosphate respectively, were more available in the Dreissena treatment after nutrient addition (ESM Table 1f & 1g). At the same time, the turbidity of ponds with both species (i.e. MD) was significantly higher than the Control ponds post-nutrient additions (Fig. 2c, ESM Table 1h), as was dissolved oxygen (Fig. 2d, ESM Table 1i; including only 3 time-points post disturbance). By contrast, turbidity was lower in ponds with only Dreissena or Myriophyllum than in Control ponds during the nutrient additions (Fig. 2c, ESM Table 1h). As a result, turbidity was (non-additively) greater in the MD treatment than expected immediately post-nutrient addition (ESM Fig. 2c), as were dissolved oxygen and pH (ESM Fig. 2d & e). Despite the high primary producer biomass in the MD treatments however, the grazer populations did not show similar increases (ESM Table 1k & 1l). Daphnia were generally low in abundance in all treatments throughout the experiment (ESM Fig. 4a), but during the period of nutrient additions, when abundances rose above Oligotrophic levels, densities were lower in the Control and the MD ponds than in the ponds with only Myriophyllum (ESM Table 1k) or Dreissena (ns). Copepods were more abundant than Daphnia overall (ESM Fig. 4b), but treatment effects on copepods were not significant (ESM Table 1).
We used piecewise structural equation modeling (pSEM) to investigate hypothesized causal relationships among the measured biological and environmental variables. The best model suggests that the presence of both Myriophyllum and Dreissena had significant and positive effects on the (log) total biovolume of both dinoflagellates and cyanobacteria (Fig. 3, ESM Table 2). The model indicates that the biovolume of cyanobacteria had a relatively strong negative causal relationship with community-level trait evenness (coefficient=-0.63), which itself had a negative influence on chlorophyll-a (coefficient=-0.25) and turbidity (coefficient=-0.21), and a positive causal relationship with the remaining dissolved phosphate-phosphorus (coefficient=0.30). At the same time, the model suggests that trait evenness is positively associated with greater daphnia (Fig. 4, coefficient=0.51) and copepod abundance (Fig. 4, coefficient=0.37). Overall, there is an indirect positive relationship between cyanobacterial biovolume and total chlorophyll-a and turbidity, but a negative relationship with grazer abundance, mediated via a negative influence on trait evenness. The model also supports a strong positive effect of chlorophyll-a on dissolved oxygen (Fig. 3, coefficient=0.85) and turbidity (Fig. 3, coefficient=0.34). The (log) biovolumes of golden algae, green algae and cyanobacteria declined with increasing temperature (ESM Table 2), while the biovolume of diatoms increased with increasing temperature. PH was positively associated with green algal biovolume (coefficient=0.58, ESM Table 2), while dissolved nitrate-nitrogen was negatively associated with dinoflagellate biovolume (coefficient=-0.41, ESM Table 2), and turbidity was negatively associated with diatom biovolume (coefficient=-0.27, ESM Table 2). The model also supported direct causal links between Myriophyllum and dissolved oxygen (coefficient=0.34) and pH (coefficient=0.31) which were not mediated via influences on the phytoplankton community or trait evenness. The model most successfully explained variance in turbidity (79%), chlorophyll-a (66%), dissolved oxygen (55%), and community trait evenness (45%).

Foundation species influenced the responses of phytoplankton biomass to nutrient additions over time (Fig. 4a & 4b). The Control and MD ponds had a higher standing stock of chlorophyll-a before
nutrient additions (Fig. 4a, ESM Table 1a), and had a higher cumulative chlorophyll-a production over
time than the ponds with only Myriophyllum or Dreissena (Fig. 4b). Log response ratios (hereafter
“LRRs”) directly after nutrient additions were positive for all treatments, indicating that nutrient
additions acted as a disturbance and resulted in increases in chlorophyll-a (Fig. 4c). Most ponds also
displayed negative LRRs (consistent with recovery) two or more weeks post-disturbance (Fig. 4c & 4d),
though in some cases no recovery occurred before the next nutrient addition (e.g. Control and MD after
the 10 µg·L$^{-1}$ nutrient addition, Fig. 4c). We used linear models to test whether treatment and the size of
nutrient disturbance had an effect (and/or interaction) on the maximum and minimum LRR within each
nutrient addition window. For a given disturbance event, larger maximum LRRs indicate larger positive
effects of the disturbance event (lower resistance), whereas lower minimum LRRs indicate more rapid
rates of recovery (higher resilience) following the peak chlorophyll response. Minimum LRRs that are
negative and as large as the maximum LRRs preceding them for a given disturbance event would result
in no increase in the cumulative effect of disturbance on chlorophyll-a over the course of the
disturbance (Fig. 4b & 4c). Species manipulations had a significant effect on the maximum LRR
($F_{3,72}=3.56$, $p=0.02$), with Dunnett’s tests indicating that the Dreissena (estimate=0.50, $p=0.02$) and
Myriophyllum treatments (estimate=0.44, $p=0.05$) had higher maximum LRRs on average than the
Control, but not the MD treatment (estimate = 0.16, $p=0.73$). In contrast, there was a marginally non-
significant interactive effect of treatment and the strength of nutrient addition on the minimum LRR
($F_{3,72}= 2.27$, $p=0.09$), as well as a significant main effect of nutrient addition ($F_{1,72}= 10.51$, $p<0.001$). The
interaction effect suggests that while minimum LRR (rate of recovery) is not associated with the size of
nutrient disturbance (maximum LRR) in the M and D treatments, it tends to be positively associated
(weaker recovery) with greater disturbance in the Control and MD treatments (Fig. 4d). In short, the
largest positive responses to disturbance events in the Control and MD treatments were followed by
lower rates of recovery than when either species was present alone. This is consistent with the tendency
(though not significant) for the Control and MD treatments to have higher temporal autocorrelation of chlorophyll-a than in the M or D treatments (Fig. 4e, linear model \( F_{3,12} = 2.77, P = 0.09 \)).

**Discussion**

We found that the foundation species *Dreissena polymorpha* and *Myriophyllum spicatum* both separately reduced the standing stock of phytoplankton biomass on average over the course of the disturbance regime. Counter to our predictions however, relative rates of increase in phytoplankton biomass in response to nutrient addition events (i.e. change from the pre-disturbance value, or the inverse of resistance) were greater when individual foundation species were present than when they were absent (Control). More surprising was our finding that this effect was reversed when the two species were present together. Rates of recovery from disturbance events were greater when the species were present individually compared to when they were absent or present together, particularly as disturbance size increased. The trend towards greater temporal autocorrelation when both foundation species were present is also suggestive that these ponds had relatively low resilience and may have been approaching a critical transition or tipping point towards an alternative, turbid, stable state (44). Over the course of the disturbance regime this resulted in a lower cumulative effect of disturbance on phytoplankton biomass when the species were present alone. In contrast, when the species occurred together lower relative rates of recovery following larger disturbance events tended to cause greater cumulative effects on phytoplankton biomass over time. Responses of phytoplankton biomass, community structure and trait variation to the foundation species treatments were also associated with significant changes in biotic and abiotic ecosystem properties. A structural equations modeling approach indicated that the increases in chlorophyll-a, turbidity and dissolved oxygen, and reductions in grazer abundances that occurred when the two foundation species were present together...
were mediated by an increase in the dominance of a cyanobacteria species in the genus Synechococcus, and by an associated reduction in phenotypic trait evenness.

The species that we manipulated in this experiment had previously been demonstrated to have direct and indirect impacts on community and ecosystem functions in freshwaters. In pelagic waters, *Dreissena polymorpha* can reduce suspended particulates, increase water clarity, increase phosphorus recycling, and provide habitat structure (23, 29, 45). Dreissena also tend to reduce total phytoplankton abundance and can select for greater dominance of cyanobacteria including *Microcystis* (31, 46) and *Planktothrix* species (23), though counter examples exist (45, 46). Macrophytes can also increase water clarity by stabilizing sediments, taking up nutrients, shading phytoplankton, providing refuge for grazers, and releasing allelochemicals (7, 23, 47, 48). *Myriophyllum spicatum* in particular can cause shifts in phytoplankton community structure due differential sensitivity of particular taxa to its allelochemicals (26-28). Our results broadly support these previous findings on the impact of each species on its own: both species tended to reduce total phytoplankton abundance relative to the Control in response to nutrient additions. Contrary to our expectations however, neither species had major impacts on the phytoplankton community composition on their own. Furthermore, the interactive effects of the presence of both species non-additively reversed their individual effects, which we had not predicted a priori.

To test the hypothesis that the combined *Myriophyllum* and *Dreissena* treatment resulted in an increase in performance of the small cyanobacterium, *Synechococcus*, relative to the other taxa in the community, we ran a follow-up lab experiment. Because *Myriophyllum* did not increase greatly in biomass over the course of the experiment we hypothesized that its impacts were largely driven by allelochemicals (47). As a result, we quantified the independent and interactive effects of *Dreissena* and macrophyte “tea” (a solution containing macrophyte allelochemicals, described in Supporting Information) on the growth of the two dominant species from our pond experiment: the green alga
(Lagerheimia sp.) and the cyanobacterium (Synechococcus sp.). We compared the effects on only these two taxa because of their relatively high dominance in the different pond treatments (Synechococcus in the MD treatment and Lagerheimia in the others, ESM Fig. 5), and also because prior work suggests that green algae are less sensitive to allelochemicals than cyanobacteria (49). We found that both Synechococcus and Lagerheimia were negatively affected by the presence of Dreissena alone, but not by allelochemicals alone (ESM Fig. 6). Lagerheimia was also negatively affected by the presence of both Dreissena and allelochemicals, but Synechococcus was not (ESM Fig. 6). As a result, Synechococcus had the greatest relative growth rate advantage in the presence of both allelochemicals and Dreissena (ESM Fig. 6), confirming the results from the outdoor pond experiment. Overall, this supports the idea that the dominance of the Synechococcus in the ponds resulted due its relative insensitivity to the presence of both foundation species, while other taxa in the community experienced stronger negative effects.

The greater total primary producer biomass in the Myriophyllum and Dreissena treatment in our study were associated with changes in species dominance and a reduction in trait evenness. The structural equation modeling approach also suggested that the reduction in trait evenness was a causal variable determining the strength of the response of chlorophyll-a to the foundation species. This is in line with work suggesting that functional and trait variation among species is important for ensuring the stability of ecosystem functions (8, 16, 50-52). In our case, the combined effect of nutrient additions and both engineers was to reduce taxonomic and trait evenness, such that subsequent responses to disturbance were dominated by the response of a single, unstable taxon (see also 53). Given that greater dominance will cause ecosystem functions and their stability to be governed by the extent of dominance and the relevant traits of the dominant taxon (54, 55), other outcomes are also possible. For example, in an experimental manipulation of seagrass genotypic diversity, polycultures showed greater resistance and recovery of shoots following high levels of disturbance, but this positive effect was partly due to the beneficial impacts of the dominance of a particularly stable genotype (52). Similarly,
resilience in an algal microcosm experiment was positively affected by the dominance of particular taxa (56). Predicting whether shifts in dominance promote or hinder stability at the ecosystem-level will depend on the ecological dynamics and feedbacks established by the traits of the dominant species (7, 54, 55, 57). Though we cannot be sure of the mechanism that led Synechococcus to become dominant in the combined Myriophyllum and Dreissena treatment, we expect that it involves traits conferring relative insensitivity to allelochemicals and filter-feeding, combined with a rapid growth response in the presence of nutrient additions.

In conclusion, we have demonstrated that presence of foundation species can have significant, strong and repeatable impacts on ecosystem functioning and stability even in relatively complex, semi-natural experimental systems. We demonstrated that the co-occurrence of such species can culminate in unanticipated effects, both in magnitude and direction, which are mediated via changes in community composition. So, while important taxa may have individual effects on communities and ecosystems that are relatively well-known, their interactive effects can be surprisingly unpredictable. We recommend the use of caution when considering the use of such taxa in management or restoration efforts, particularly when little is known about the potential for interactive effects on community structure and ecosystem functioning.
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Figure Legends

Figure 1. Phytoplankton-based responses to nutrient additions and foundation species. Panels represent effects of foundation species on variables that are determined by phytoplankton over time: a) chlorophyll-a, b) cyanobacterial dominance of total community biovolume, c) phytoplankton trait evenness, d) carbon to nitrogen molar ratio (C:N), and e) carbon to phosphorus molar ratio (C:P). Grey vertical lines indicate the timing of successive nutrient additions, from 10 µg/L up to 50 µg/L of phosphorus. Error bars indicate standard errors (n=4). Treatment colors are: orange=Control (nutrient additions, but no foundation species), blue=Dreissena, green=Myriophyllum, pink=Myriophyllum and Dreissena, and grey = Oligotrophic (no nutrients and no foundation species).

Figure 2. Ecosystem-level responses to nutrient additions and foundation species. Panels represent effects of foundation species treatments on non-phytoplankton based community and ecosystem-response variables over time: a) dissolved nitrate nitrogen, b) dissolved phosphate phosphorus, c) turbidity, d) dissolved oxygen, and e) pH. Colors, vertical lines and error bars are as in Figure 1.

Figure 3. Piecewise SEM – the best fit structural equations model. Arrows indicate significant causal paths, yellow indicating a positive relationship and blue negative. Arrow widths are proportional to the path coefficients. For clarity we have excluded non-significant effects, correlations, and effects without any paths leading back to the experimental treatments (see Table 1 for all paths). Experimental treatments are shown in black boxes, biotic variables in green boxes, and ecosystem properties in white boxes. Coefficients are standardized by standard deviations and vary between -1 and 1. R² values for endogenous variables reflect the marginal variance explained by the model. The piecewise SEM model, containing Pond as a random effect and including temporal autocorrelations of the 1st order (corAR1), has a Fisher’s C value of 179.47, p=0.50 and df=180, suggesting that the model provides a good fit to the data.
Figure 4. Chlorophyll-a dynamics and stability. a) The effects of foundation species on chlorophyll-a after standardizing for seasonal trends by subtracting the mean of chlorophyll-a in the Oligotrophic ponds (n=4) at each time point. b), Cumulative chlorophyll-a production over time. This is calculated by summing the area under the curve for each pond from panel a. c), Log response ratios (LRRs) of chlorophyll-a over time and in response to nutrient additions. These are calculated only during the period of nutrient addition, indicated by the horizontal dashed lines in panels a and b. d), The minimum LRR as a function of the maximum LRR of chlorophyll-a for each pond, taken over the 5 nutrient addition windows in panel c. e) Temporal autocorrelation of chlorophyll-a, calculated at a lag of 1. Colors, vertical lines and error bars are as in Figure 1.
Figure 1

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Figure 2

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Figure 3

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