

# Non-additive effects of species interactions on aquatic ecosystems responses to nutrient perturbation

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## Abstract

Nutrient perturbations are a threat to aquatic ecosystems worldwide. Interactions among important species can influence the recovery of aquatic ecosystems from nutrient perturbations, and the persistence in a clear water state for a given regime of nutrient loading. In a factorial manipulation of two foundation species (macrophytes and mussels), we measured 16 large (15000L) ponds at high resolution (15 min interval) over two years with two periods of nutrient perturbation. We found that each foundation species had strong individual effects on multiple ecosystem properties (e.g. phytoplankton densities and metabolism), largely in line with our expectations (i.e. reduction of algal biomass relative to control). However, when both species were present, we saw dramatic increases in algal biomass and other ecosystem parameters, indicating strong non-additive antagonistic effects. Overall, our results demonstrate how interactions between foundation species can cause surprisingly strong deviations from the expected responses of aquatic ecosystems to nutrient additions.

## Introduction

How organisms take up resources, grow, reproduce, or interact with competitors, pathogens, or consumers can be strongly affected by the presence of other species in an ecosystem (Stachowicz 2001; Olf *et al.* 2009; Kéfi *et al.* 2012). Interactions among species can affect the functioning of ecosystems by regulating fluxes of energy and matter, ecosystem productivity and metabolism, or by mediating the response of ecosystems to perturbation (Loreau *et al.* 2001; Harmon *et al.* 2009; Chapin *et al.* 2011). Some species interactions are more important than others for ecosystem functioning (Angelini *et al.* 2011; Falkenberg *et al.* 2012), such that modifying these interactions can have disproportionate impacts on ecosystems: so called foundation species (Dayton 1972) can define much of the structure of a community by creating locally stable conditions for other species. Disturbances can influence how foundation species can individually or interactively affect multiple ecosystem components (Ellison *et al.* 2005; Darling & Côté 2008), potentially causing surprising effects on ecosystems (Paine *et al.* 1998). Such complexity regarding the interplay between interactions of important species and environmental change makes forecasting ecosystem responses to increasing anthropogenic disturbances particularly challenging (Petchey *et al.* 2015).

Eutrophication is a threat to aquatic ecosystems worldwide (Smith *et al.* 1999; Smith 2003), and there is growing evidence that nutrient loading can cause both gradual and sudden shifts in ecosystem state, depending on the nature and strength of species interactions (Carpenter 2005). The presence or absence of different key species, including macrophytes, benthic and pelagic grazers, and phytoplankton, are thought to define ecosystem responses to nutrient perturbations through positive and negative interactions among them (Scheffer

*et al.* 1993; Kéfi *et al.* 2016). In the network of species interactions in shallow lakes (Scheffer *et al.* 1993), for example, a key interaction is the competition between macrophytes with phytoplankton communities for dissolved nutrients and light (Scheffer *et al.* 1993; Ibelings *et al.* 2007). Assemblages of macrophytes, that are considered to be important foundation species (Scheffer *et al.* 2003; Kéfi *et al.* 2016), are competitively dominant at low nutrient loading, and can persist at intermediate nutrient loading via a positive feedback between macrophyte growth and water transparency (Carpenter & Lodge 1986; Jeppesen *et al.* 1998). Compared to macrophytes, fewer models have investigated how benthic grazers can influence the dynamics of ecosystem responses to nutrient pulses. However, the mussel *Dreissena polymorpha* can directly consume large amounts of phytoplankton, (Johengen *et al.* 1995; James *et al.* 1997), and its occurrence has coincided with dramatic changes in water clarity of some lake ecosystems (Ibelings *et al.* 2007).

Beside their expected negative effect on phytoplankton biomass, foundation species like macrophytes and grazers can also affect other ecosystem properties such as dissolved organic matter (DOM) and oxygen metabolism (Scheffer *et al.* 1993; Olf *et al.* 2009; Kéfi *et al.* 2016). As such, they can mediate how external disturbances reverberate through the network of biological and abiotic interactions in aquatic ecosystems (Narwani *et al.* 2019). Such effects can culminate in changes in both the mean and variance of ecosystem parameters, which can sometimes foreshadow a sudden shifts in ecosystem state (Carpenter *et al.* 2011; Scheffer *et al.* 2012; Gsell *et al.* 2016). Studies using high resolution measurements are particularly useful for tracking the mean and variance of ecosystem metrics, for example, phytoplankton biomass and rates of ecosystem metabolism such as net primary productivity and respiration (Carpenter *et al.* 2011; Batt *et al.* 2013; Nielsen *et al.* 2013). These processes are largely driven by the autotrophic lake community, both benthic (i.e. macrophytes) and pelagic (i.e. phytoplankton), but can also be affected by DOM dynamics associated with the growth and decay of biomass (Catalán *et al.* 2014). Photosynthesis and respiration rates can be modeled with relatively high precision using repeated measurements of dissolved oxygen and water temperature (Staehr *et al.* 2010), and have been used to assess ecosystem resistance and resilience (Batt *et al.* 2013). However, such approaches, using an array of multiple high-resolution sensors (16 sondes with 5 parameters/sonde), have never been applied in factorial manipulations of foundation species in aquatic ecosystems.

When facing disturbance, interactions between foundation species can cause non-additive effects on ecosystem dynamics that are difficult to anticipate (Narwani *et al.* 2019), and this may impair our ability to quantify resistance and resilience of ecosystems with a particular species configuration (Scheffer *et al.* 1993; Allgeier *et al.* 2011; Kéfi *et al.* 2016; Thompson *et al.* 2018). However, only very few studies have attempted to experimentally disentangle singular and synergistic effects of key species on ecosystem dynamics in response to changing environmental conditions (Stachowicz 2001; Angelini *et al.* 2011; Falkenberg *et al.* 2012). In shallow lake ecosystems, the presence of either macrophytes and mussels have been linked to increased capacity to maintain a clear water state with low phytoplankton abundances (Jeppesen *et al.* 1998; Bierman *et al.* 2005; Ibelings *et al.* 2007). Current theory suggests that both species may facilitate the presence of each other: macrophytes can provide habitat for *Dreissena* mussels to settle on (Ibelings *et al.* 2007; Karatayev *et al.* 2014b), and mussels can actively decrease local turbidity, thus improving environmental conditions for submerged macrophytes (Ibelings *et al.* 2007). Such facilitation is a common phenomenon in ecological communities (Stachowicz 2001; Angelini *et al.* 2011; Falkenberg *et al.* 2012), especially for foundation species, like macrophytes and mussels. However, there is also potential for antagonistic interactions between macrophytes and mussels that could unfold under nutrient perturbation scenarios: polyphenols and fatty acids produced by macrophytes to inhibit phytoplankton growth (Korner & Nicklisch 2002; Hilt & Gross 2008) may be harmful to filter feeding organisms (e.g. mussels), whereas mussels can shift the composition of phytoplankton communities to species that might be less affected by allelochemicals (Vanderploeg *et al.* 2001; Fishman *et al.* 2010).

Here, we monitored freshwater ponds in high resolution to experimentally test how a disturbance scenario characterized by multiple nutrient pulses over two years affects pond ecosystem dynamics with and without two important foundation species. We manipulated the presence and absence of the macrophyte *Myriophyllum spicatum* and the mussel *Dreissena polymorpha*, two important foundation species that are common in

freshwater ecosystems worldwide. In a factorial pond experiment, we perturbed all ecosystems by progressively increasing the input of inorganic nutrients and quantified the dynamics of several biotic and abiotic ecosystem parameters. The goal was to investigate how the presence and absence of two important foundation species affects the dynamics of a suite of ecosystem parameters during the process of pond eutrophication (our disturbance regime). Specifically, we aimed at characterizing how the nature of interactions between the two species (additive vs. non-additive, Figure 1A) was affected by nutrient perturbation. Contrary to our expectation of more stable clear water conditions under the presence of both foundation species, after both periods of nutrient perturbation (year 1, and 2), we found strong non-additive, antagonistic effects in several ecosystem parameters (see also Narwani et al. 2019, for the results from the first year of manipulation). Our results demonstrate how interactions between key species can drastically change under disturbance regimes, emphasizing the importance of understanding how species interaction networks, and how they change over time, can affect ecosystem responses to disturbance (Scheffer *et al.* 1993; Ibelings *et al.* 2007; Kéfi *et al.* 2016).

## Materials and methods

### Study design

In 20 month long experiment, we manipulated the presence and absence of two keystone species: *Myriophyllum spicatum* (Fig. 1B; hereafter *Myriophyllum*) and *Dreissena polymorpha* (Fig. 1C; hereafter *Dreissena*) in artificial pond ecosystems (15 000L). We used a fully factorial design with either both keystone species absent as a control (C), *Myriophyllum* macrophytes (M), *Dreissena* mussels (D) or *Myriophyllum* and *Dreissena* together (MD). Each factorial treatment combination of eutrophied ponds was replicated four times ( $4 \times 4 = 16$  ponds total). The ponds we used were made of fiberglass with a smooth surface (Fig. 1D), had a rounded shape with approximately four meter diameter and a shallow (0.5 m) and a deep (1.5 m) end. In the first period of perturbation, we progressively increased the pulse perturbation of inorganic nutrients to all ponds, and measured the effect of presence or absence of both keystone species on several ecosystem parameters in high frequency using automated multiparameter sondes. In the second period of perturbation, one year later, we perturbed all the ponds again with our highest pulse of nutrients, to test how the responses changed between the perturbation periods, and whether treatment effects were repeatable over two years.

### Experimental procedure

The ponds were initially set up on May 6th 2016 by adding a 5 cm thick layer of gravel (2-4 mm) and filling them with a tap-water. Afterwards the ponds were inoculated with a natural phytoplankton population (20 L, 30  $\mu\text{m}$  filtered from Lake Greifensee). The treatments were established on May 31st by distributing 100 shoots of *Myriophyllum*, each attached with a cable-tie to a small rock, among the shallow and deep ends of each pond designated to the M and MD treatment. Each pond that was designated to the D and MD treatment received 25 *Dreissena*, distributed among the shallow and deep end. We ensured prior to the distribution of plant shoots and mussels that their size distributions were similar across all ponds of the respective treatment. To ensure that all ponds started with a similar overall amount of total biomass, we added autoclaved mussels to the M ponds, autoclaved *Myriophyllum* shoots to the D ponds, and both autoclaved mussels and *Myriophyllum* shoots to the C ponds. In May 2017, we re-established our macrophyte treatment after the winter by adding the same amount of fresh and autoclaved *Myriophyllum* shoots to the respective ponds to ensure effective treatment contrasts.

The first nutrient perturbation regime began on August 12th, 2016. We progressively increased phosphate and nitrate additions of 10, 20, 30, 40 and 50  $\mu\text{g/L}$  of P (with a double Redfield ratio, N:P = 30) over eight weeks until October 10th 2016, with two week intervals between additions. Our second nutrient perturbation regime was single addition of 50  $\mu\text{g/L}$  of P on October 10th, 2017. Using multiparameter sondes (EXO2, Xylem), installed in each pond, we tracked the following four ecosystem parameters with high frequency (15 min intervals): chlorophyll-a fluorescence (hereafter chlorophyll) and phycocyanin fluorescence, DOM

fluorescence (hereafter fDOM), and dissolved oxygen. Additionally, we used the dissolved oxygen data, as well as water temperature (also measured with the sondes), light and wind data, to calculate rates ecosystem metabolism (gross primary productivity, net primary productivity, and respiration; see below). Details on sonde calibration and maintenance can be found in the Supplement.

Over the first winter period (December 1st 2016 - February 28th 2017), we could not monitor ecosystem dynamics due to ice cover in the ponds. To maintain and recalibrate the sensors, we stopped measurement from March 1st to 23rd to maintain and recalibrate all sondes (see supplement for details). A second sonde maintenance period was implemented in the fall of 2017 (September 14th - October 3rd 2017). Following this structure, we consider three phases of the experiment: Phase 1 with the first five nutrient pulses (June - December 2016), Phase 2 without nutrient pulses (March - October 2017), and Phase 3 with the final nutrient pulse (October 2017 - February 2018).

## Data treatment and analysis

*Data treatment* - We first performed an outlier analysis by excluding values higher than 3 times the median absolute deviation of all values in a sliding window (Leys *et al.* 2013) of one day window size (15 min interval = 96 data points). After aggregating four measurement points to one per hour (from 96 to 24 data points per day), we used sliding windows with a one-week window size ( $7 * 24 = 168$  data points) to calculate mean and coefficient of variation (hereafter CV) of all ecosystem parameters. We chose a seven day window size to have robust estimates of the different metrics that would not be affected by diurnal variability. Moreover, we calculated autocorrelation (hereafter AC), tailedness of the generalized extreme value distribution (hereafter GEV) and skewness, which can be used to quantify the characteristics high frequency dynamics of disturbed ecosystems are (Batt *et al.* 2013, 2017; Gsell *et al.* 2016). For example, as ecosystems are disturbed they tend to become more similar to their own past, resulting in an increase in AC (Ives 1995), whereas GEV, especially of biological variables, is expected to yield higher values (i.e. “fatter tails”) in response to perturbation (Katz *et al.* 2005; Batt *et al.* 2017). We also focused on these metrics because they are important early warning indicators for critical transitions in shallow lake ecosystems (Carpenter *et al.* 2011; Gsell *et al.* 2016), which, however, have been rarely investigated in factorial manipulation of foundation species.

*Effects of foundation species on ecosystem parameters* - Using the data derived from the sliding windows, we tested for differences between treatments using the factorial design ( $n=4$  per treatment level,  $a_D$  = main effect of *Dreissena*,  $b_M$  = main effect of *Myriophyllum*,  $C_{(D \times M)}$  = interactive effect):

$$y = a_D + b_M + C_{(D \times M)} + \text{error}$$

We used one linear model with Type III sum of squares per hour (24 models per day) to test for differences between treatments in mean and CV, AC, GEV, and skewness of each measured parameter. We report P values from linear models for mean and CV directly in Figure 2 and 3, where points below the time series colour coded by treatment indicate a significant difference of the respective treatment from the control. Because there were no systematic differences between treatments for AC, GEV, and skewness, we report results for these metrics in Supplementary Figures S1-S3. For better visual inference in all the presented figures, we further aggregated data from the sliding windows from 24 to one data point per day. In addition, we calculated the predicted additive response of *Myriophyllum* and *Dreissena* for each data-point by subtracting the control from the summed single species treatments ( $(Dreissena + Myriophyllum) - Control$ ). The interaction between the presence of *Myriophyllum* and *Dreissena* was considered non-additive, when the confidence interval of the MD-treatment did not overlap with the predicted additive response.

*Ecosystem metabolism* - We calculated gross primary productivity, net primary productivity, and respiration (hereafter GPP, NEP and R, respectively) of each pond ecosystem using the equations in Staehr *et al.* (2010) on time series of dissolved oxygen and temperature collected by the sondes, as well as wind speed at 10 m from a nearby weather station operated by Meteo Swiss (Dübendorf, Giessen). Because the ponds

were oversaturated with respect to dissolved oxygen, we included rates of change in dissolved oxygen in the formulas as the coefficient of a linear model of hourly averages of dissolved oxygen concentrations between 13:00 and 17:00 for the day and 1:00 and 5:00 for the night, where gas exchange dynamics in the ponds were considered to have equilibrated. This assumption was tested by visually inspecting the slopes for oxygen increase or decrease, which were found to be linear within these times and across all seasons. Using the metabolism data we calculated mean and CV of all three metabolism parameters by applying a sliding window with the size of 7 days. We then tested for differences between treatments with single species (M and D - main effect) and multiple species (MD - interactive effect) and control (C) using one linear model per day. We report the results from the linear models directly in Figure 4 and 5 as colour coded points that indicate significant difference in metabolic rates of M, D or MD from C. Furthermore, we calculated the predicted additive effect in the same fashion as for the other ecosystem parameters.

## Results

*Effects of foundation species on mean ecosystem parameters* - The presence and absence of *Myriophyllum* and *Dreissena* affected a wide range of ecosystem parameters. During the first nutrient addition, ponds with *Myriophyllum* or *Dreissena* alone had lower chlorophyll fluorescence, i.e. lower algal biomass, than ponds with neither species, consistent with their anticipated negative effects on the phytoplankton community (Fig. 2). However, following both disturbance periods, the co-occurrence of these species had strong non-additive antagonistic effects on algae abundance, illustrated by their positive effects on mean chlorophyll and phycocyanin fluorescence. Furthermore, after the first disturbance period, and throughout the remainder of the experiment, the presence of *Myriophyllum* increased the concentration of DOM in the ecosystems, independent of *Dreissena* presence (i.e. in M and MD treatments). The presence of *Myriophyllum* and *Dreissena*, either alone or in combination, positively affected dissolved oxygen saturation throughout most of the experiment, however, not during most of the perturbation periods: each nutrient addition dramatically increased dissolved oxygen saturation to levels (between 150 and 200 %) that were not significantly different across all species contrasts.

*Effects of foundation species on variance of ecosystem parameters* - We found only weak effects of *Myriophyllum* and *Dreissena* presence on variance patterns (CV, Fig. 3) and early warning indicators (Supplement). Overall, we found strong increases in CV across all treatment combinations and ecosystem parameters immediately after the nutrient additions, which reflects the sudden changes in the mean in response to the disturbances. Prior to the first nutrient additions, the pond ecosystems with either *Myriophyllum* or *Dreissena* alone were less variable in chlorophyll fluorescence. After the second nutrient pulse, ecosystem where both species were present variance of chlorophyll and phycocyanin fluorescence were significantly higher than when species were alone, or absent. There were almost no effects of foundation species (i.e. their presence or absence) on the variance of DOM fluorescence. There were indications of *Myriophyllum* presence affecting CV of dissolved oxygen saturation, however, only to weak effect and with variable sign. As expected, each nutrient addition led to a temporary increase in AC across all treatment contrasts and parameters (visible as spikes in the time-series, Fig. S1), but we did not identify treatment specific differences in AC. Similarly, there were no consistent treatment specific differences in GEV or Skewness in any of the ecosystem parameters (Fig. S2 and Fig. S3).

*Ecosystem metabolism* - GPP and NEP, as well as R were strongly affected by nutrient perturbation and seasonal dynamics, but less so by the presence or absence of foundation species (Fig. 4). Each nutrient addition led to correlated increases of GPP, NEP and R, which reverted within days after the maximum was reached. During each of these peaks, there were only little differences across all species contrasts and all metabolism metrics. During spring 2017, at the beginning of the second Phase, all pond ecosystems containing *Myriophyllum* or *Dreissena* had lower NEP and higher R than ecosystems devoid of foundation species. We found a similar pattern towards the end of the experiment, after the second nutrient addition in Phase 3, where both GPP and NEP were lower and R higher when foundation species were present. Overall,

there were only weak effects on variance patterns of ecosystem metabolism (Fig. 5): there was a tendency for MD ponds to have higher CV of GPP and NEP than ponds without any foundation species, especially around the second perturbation Phase. Interestingly, nutrient perturbation led to increasing CV of GPP and NEP, but not R, which had a relatively static CV of approximately 0.8 throughout the entire experiment.

## Discussion

Perturbation of the pond ecosystems with nutrients evoked strong responses in all ponds, which were dependent on the presence of foundation species and, in some cases, their co-occurrence. As expected, all nutrient pulses led to strong increases in phytoplankton abundances across all treatment combination, which, at first, was mediated by the presence of either *Myriophyllum* or mussels in the single species treatment. However, when both *Myriophyllum* and *Dreissena* were present within a pond, nutrient additions led to a contrasting pattern: phytoplankton biomass in these ponds increased stronger than in the presence of a single species or when none of the two species were present. These patterns suggest strong non-additive interactions between macrophytes and mussels that affected phytoplankton biomass during and following the disturbance periods.

Mediation of phytoplankton blooms under increased nutrient loading by either macrophytes or mussels alone was expected, and is in agreement with a large body of previous theoretical and empirical work (van Nes *et al.* 2007; Iacarella *et al.* 2018; Yamamichi *et al.* 2018). Macrophytes can keep phytoplankton biomass in the water column at lower levels compared to ecosystems that lack macrophytes. Such control of phytoplankton biomass by macrophytes is often linked to their competitive relationship with phytoplankton for nutrients and light (Scheffer *et al.* 1993) or the production of allelopathic substances that can inhibit phytoplankton growth (Korner & Nicklisch 2002; Hilt & Gross 2008), especially of some cyanobacteria (Nakai *et al.* 2001, 2012). However, these mechanisms are only effective below the “critical turbidity” threshold (Scheffer *et al.* 1993), above which light limitation prohibits macrophytes growth and can lead to macrophyte die off, which marks the transition to a turbid water state (Scheffer *et al.* 1993; van Nes *et al.* 2007; Kéfi *et al.* 2016; Yamamichi *et al.* 2018). In our experiment, macrophytes died out and did not re-establish after the final pulse of the first nutrient addition (October 10th 2016) until the following spring, which we confirmed by visual inspection of all ponds in March 2017. Therefore the observed differences between treatments with and without *Myriophyllum* can only be explained by the legacy of their prior impact throughout the summer and fall. Macrophytes also affected the dynamics of dissolved organic matter (Fig. 2): fDOM increased more rapidly and to higher levels in both M and MD treatments than in ponds without *Myriophyllum* (C and D). This was expected, as *Myriophyllum* is known to be a producer of a wide range of organic substances, including allelopathic chemicals (Catalán *et al.* 2014; Reitsema *et al.* 2018).

The presence of *Dreissena* alone lead to the expected mediation of phytoplankton biomass, relative to the control without foundation species during parts of the first, and, by tendency, also throughout the second period of nutrient addition. Filter feeding organisms like *Dreissena* can remove large quantities of algae and suspended materials from the water column, which can help stabilizing aquatic ecosystems in a clear water state, even when the nutrient input is high (Gulati *et al.* 2008; McLaughlan & Aldridge 2013). In this context, *Dreissena* have higher persistence than *Myriophyllum*, because they are not limited by increasing turbidity like macrophytes. It has been shown that population growth of mussels can be very high in eutrophic lakes (Karatayev *et al.* 2014a; Strayer *et al.* 2019), if sufficient amounts of hard substrate are available (Ibelings *et al.* 2007; Fishman *et al.* 2010). In such cases, *Dreissena* can not only affect water clarity and nutrient cycling, but also directly lead to shifts in the composition of the phytoplankton community towards a higher proportion, in some cases dominance, of cyanobacteria like *Microcystis* (Vanderploeg *et al.* 2001; Bierman *et al.* 2005; Fishman *et al.* 2010). *Dreissena* can selectively reject particles as pseudofeces that bypass the digestive tract, thus releasing less palatable particles like cyanobacteria back to the environment (Vanderploeg *et al.* 2001). If this loosely consolidated substrate contains viable cyanobacteria, these cells can resuspended in the water column while other phytoplankton species are absorbed by the mussel.

The observed non-additive antagonistic effect of *Myriophyllum* and *Dreissena* coincided with a dramatic

shift towards cyanobacteria that occurred when both macrophytes and mussels were present (Fig. 2). As found by Narwani et al. (2019), who determined phytoplankton community composition from pond water samples taken at regular intervals in the first year of the study, the small cyanobacterium *Synechococcus* was dominant when both *Myriophyllum* and *Dreissena* were present in a pond. In a parallel laboratory experiment, Narwani et al. (2019) tested how the presence of allelochemicals (“*Myriophyllum* -tea”) or *Dreissena*, alone and in combination, affected the relative concentration of two species of microalgae that were most dominant in the pond ecosystems (*Lagerheimia* sp. and *Synechococcus* sp.). Similar to the dynamics observed in the pond experiment, *Synechococcus* increased in abundance relative to the green algae *Lagerheimia* when both *Dreissena* and allelochemicals were present. This suggests that a relative advantage of cyanobacteria in the presence of both foundation species, while other taxa in the community experienced stronger negative effects, may have contributed to the shift of phytoplankton communities toward cyanobacteria, resulting in an overall increase in phytoplankton biomass (Narwani et al. 2019).

Throughout the study, we found strong effects of the nutrient disturbances on the dynamics of ecosystem metabolism, which varied among treatment combinations of the foundation species. In both periods of disturbance (i.e. Figure 4, Phase 1 and 3), the dynamics of ecosystem metabolism such strong evidence of non-additivity, whereas in the intervening period (Figure 4, Phase 2) the differences among treatments were more subtle, and the overall patterns were driven by seasonality. For example, all metabolic rates increased over the spring until the middle of June, and then decreased until the final nutrient addition at the beginning of Phase 3 in October. Moreover, in the MD treatment the CV of GPP was often higher than the other treatments during the period when seasonality and weather events likely dominated the dynamics. CV is a commonly used metric for early warning sign for shifts in ecosystem state (just like AC, GEV, and skewness - see supplement), and the increase towards the end of Phase 2 hints that the ecosystems might respond differently to the impending pulse disturbance in Phase 3 (Figure 4). This suggests that high frequency time series might provide insight into how ecosystems will respond to disturbance. Following the final nutrient addition, all ecosystems containing foundation species (D, M and MD) showed significantly lower GPP and NEP, but higher R. This could be because chlorophyll concentration in the control ponds without foundation species continued to increase throughout the winter 2017/2018, whereas DOM concentration in all other ponds decreased (Fig. 2). As a consequence, higher productivity from phytoplankton in the control ponds and higher respiration from DOM breakdown in all other ponds may be responsible for the observed divergence in metabolic patterns towards the end of the experiment.

Multiple lines of evidence suggest that non-additive interactions between *Myriophyllum* and *Dreissena* strongly affected ecosystem dynamics in ponds experiencing progressive nutrient perturbations. This was especially visible in the phytoplankton communities: the presence of both *Myriophyllum* and *Dreissena* led to a higher algae biomass relative to control, instead of a decrease when only one species was present in the ponds. This demonstrates how a non-additive, antagonistic interaction between two foundation species can have dramatic effects on the ecosystem, by providing an opportunity for a third species, in this case cyanobacteria, to dominate the community. Ecological synergies following ecosystem perturbation are a known, but not well researched phenomenon (Suttle *et al.* 2007; Darling & Côté 2008; Thompson *et al.* 2018). In some cases it may be difficult to uncover the effects that non-additive species interactions have on ecosystems, particularly when they are only expressed under disturbance conditions: in our experiment, the phytoplankton biomass decreased again after we ceased the nutrient additions. Nevertheless, the ecological mechanisms underlying these effects might persist over time, even though the dynamics are not evident during times of no disturbance (e.g. Phase 2). In our study, even after perturbing the ecosystems a year later with a single strong pulse of nutrients, the effect was stronger than during the first addition, indicating that the non-additive effects of species interactions can persist over time in a repeatable way.

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## Data availability

In case of acceptance of this manuscript we will make all raw data and code leading to the presented results available at the Dryad data repository, and provide the corresponding DOI at the end of the article.

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## Figure legends

**Figure 1 - A)** Schematic depiction of how the absence (Control=blue line) and the presence of foundation species (*Dreissena polymorpha* = orange line, *Myriophyllum spicatum*= green line) is hypothesized to affect a generic ecosystem variable (e.g. chlorophyll-a concentration). From these three time series, we can generate an expectation for what would be a perfectly additive effect on the ecosystem variable (black line = (*Myriophyllum* + *Dreissena*) - Control). If the recorded time series from ecosystems with both foundation species present is lower than the expected additive effect, both species would interact synergistically; if it is higher, both species would interact in an antagonistic fashion. **B)** *Dreissena polymorpha*, “Zebra mussel” (Photo credit: N. Sloth). **C)** *Myriophyllum spicatum*, “Eurasian water millfoil” (Photo credit: P. Dynowski). **D)** Schematic of the experimental ponds: the ponds are approximately 4 m in diameter and have a deep (1.5 m) and a shallow end (0.5m), where we planted macrophytes and mussels. In the middle of each pond we placed a multiparameter sonde at 1 m depth to monitor ecosystems dynamics.

Figure 2 - Effect of foundation species on mean of ecosystem parameters in Phase 1, Phase 2 and Phase 3 (left, middle, and right panel, respectively). The lines indicate the respective average of all four ponds per treatment per hour (mean  $\pm$  SE), the gray shading indicates the disturbance phases, and the coloured bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour: (orange=*Dreissena* main effect [D treatment], green=*Myriophyllum* main effect [M treatment], purple=interactive effect [MD treatment]). The data stem from a seven day long sliding window (168 data points). The black line indicates the predicted additive response based on the sum of the separate macrophyte or mussels treatment with the control subtracted (e.g. (Macrophyte *Chl* + *Dreissena Chl*) - Control *Chl* = predicted additive response).

Figure 3 - Effect of foundation species on variance (Coefficient of variation: CV) of ecosystem parameters in Phase 1, Phase 2 and Phase 3 (left, middle, and right panel, respectively). The lines indicate the respective average of all four ponds per treatment per hour (mean  $\pm$  SE), the gray shading indicates the disturbance phases, and the coloured bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour: (orange=*Dreissena* main effect [D treatment], green=*Myriophyllum* main effect [M treatment], purple=interactive effect [MD treatment]). The data stem from a seven day long sliding window (168 data points).

Figure 4 - Effect of foundation species on mean metabolic rates of the ecosystems in Phase 1, Phase 2 and Phase 3 (left, middle, and right panel, respectively). The lines indicate the respective average of all four ponds per treatment per hour (mean  $\pm$  SE), the gray shading indicates the disturbance phases, and the coloured bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour: (orange=*Dreissena* main effect [D treatment], green=*Myriophyllum* main effect [M treatment], purple=interactive effect [MD treatment]). All rates were calculated using Odum's diel oxygen technique (Staehr *et al.*2010). The black line indicates the predicted additive response based on the sum of the separate macrophyte or mussels treatment with the control subtracted.

Figure 5 - Effect of foundation species on variance (Coefficient of variation: CV) of ecosystem metabolism in Phase 1, Phase 2 and Phase 3 (left, middle, and right panel, respectively). The lines indicate the respective average of all four ponds per treatment per hour (mean  $\pm$  SE), the gray shading indicates the disturbance phases, and the coloured bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour: (orange=*Dreissena* main effect [D treatment], green=*Myriophyllum* main effect [M treatment], purple=interactive effect [MD treatment]). The data stem from a seven day long sliding window (168 data points).

## Figures

Figure 1

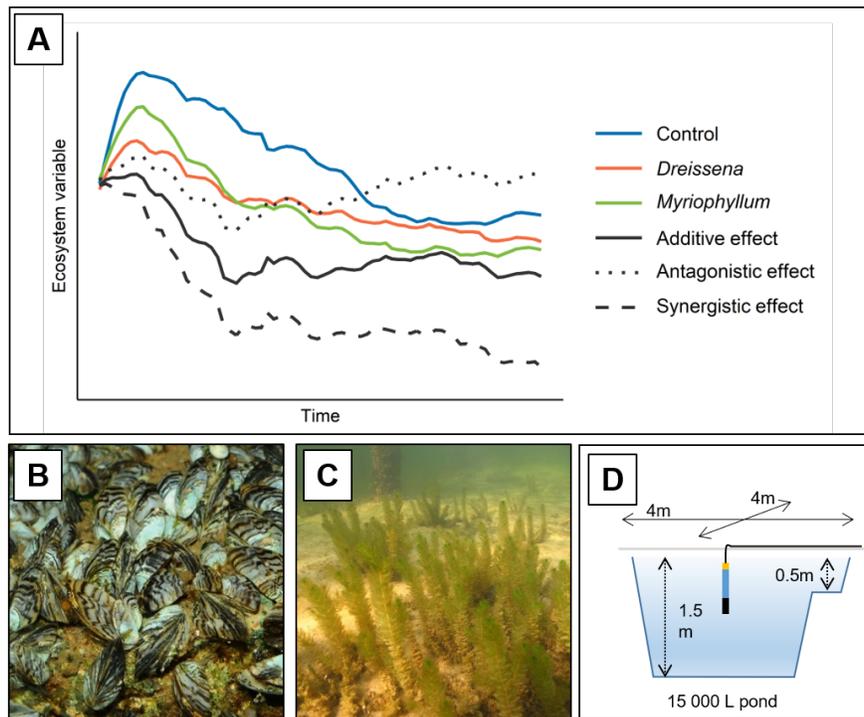


Figure 2

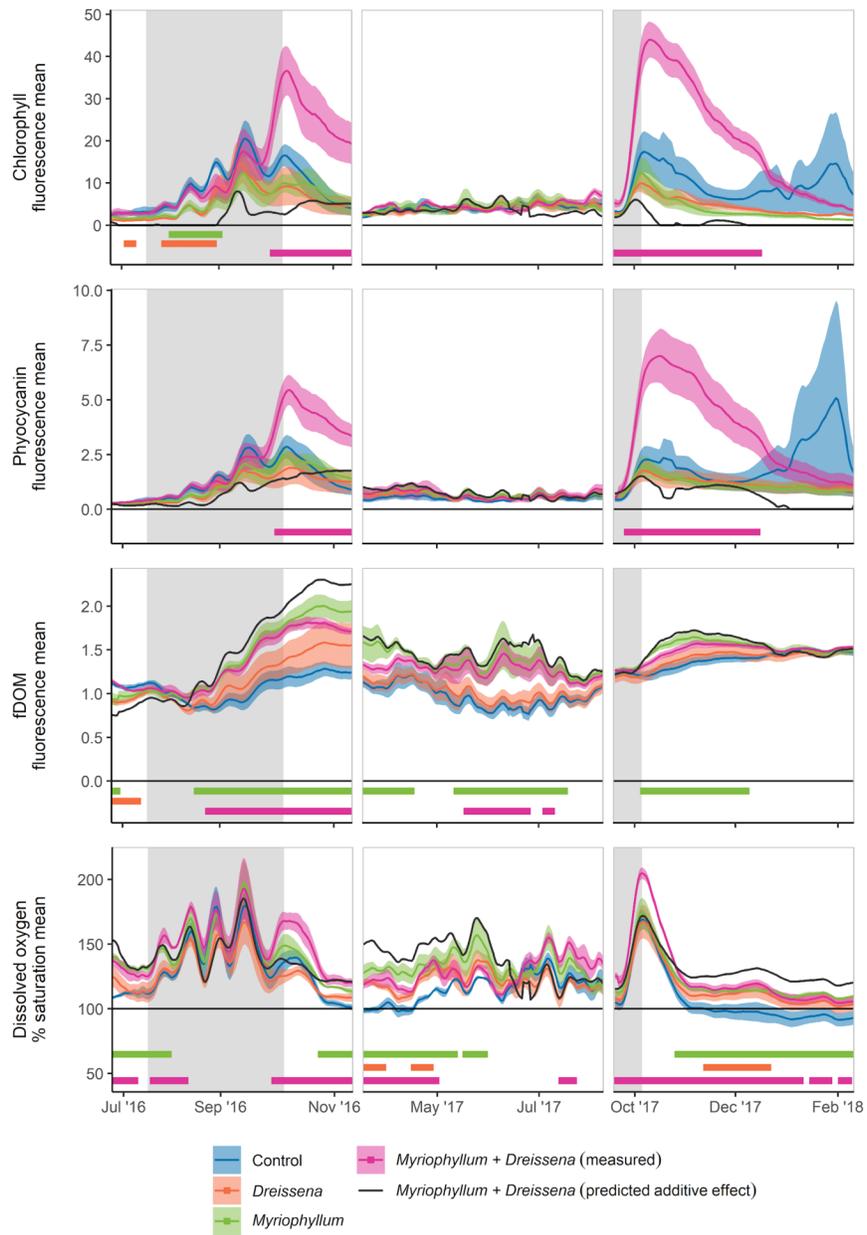


Figure 3

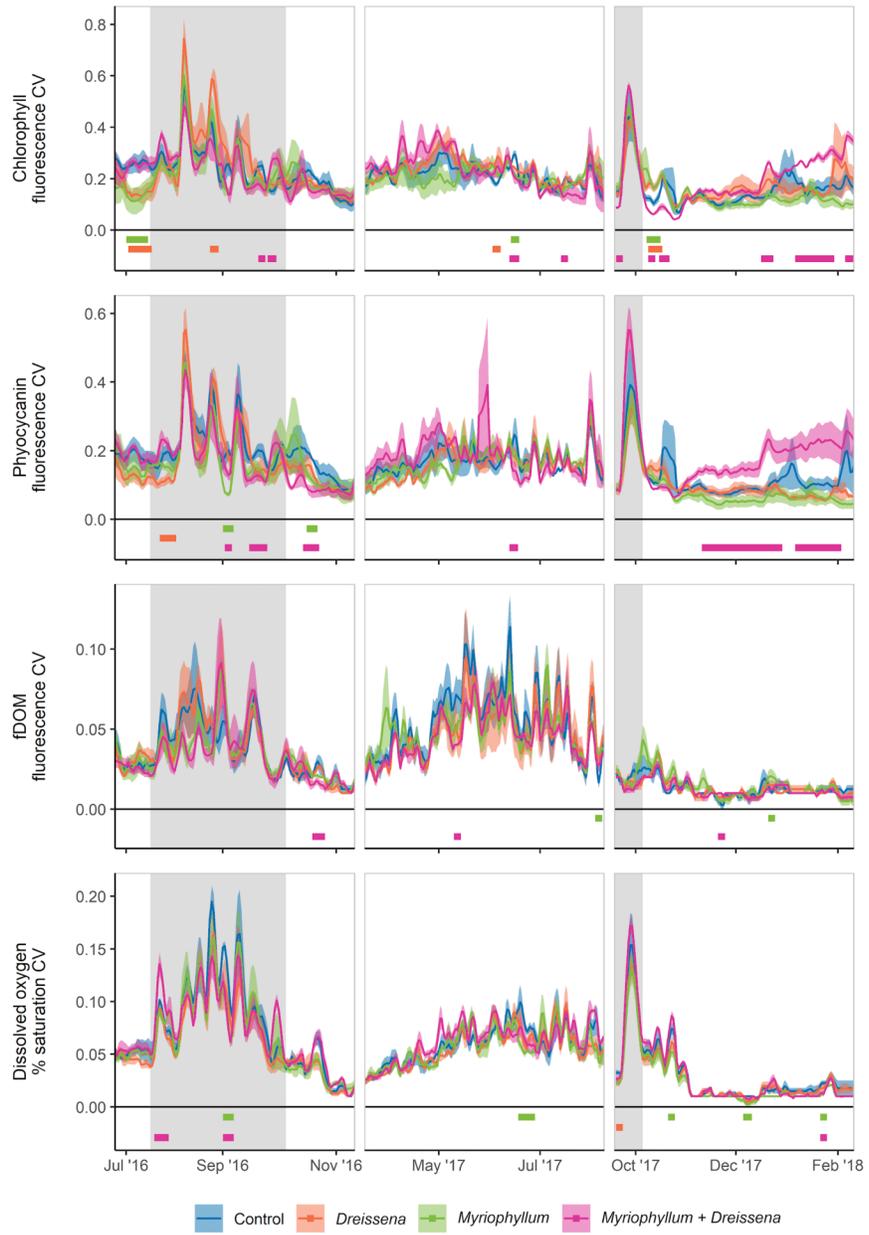


Figure 4

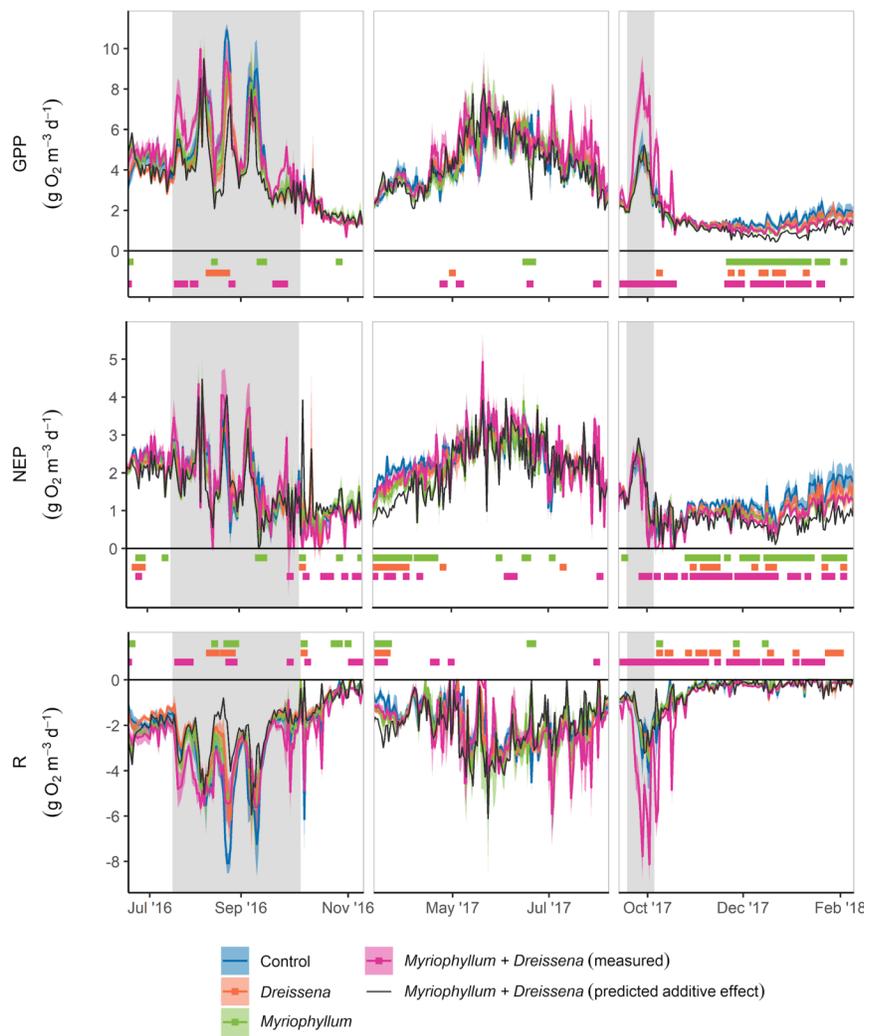


Figure 5

