

RESEARCH ARTICLE

Environmental stoichiometry mediates phytoplankton diversity effects on communities' resource use efficiency and biomass

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Abstract

1. Positive biodiversity–ecosystem functioning (BEF) relationships are predicted to increase in strength when high environmental variability allows for complementarity between resource use strategies in diverse communities. This environmental variability can be represented by spatial or temporal variation in nutrient ratios, but resource use efficiency (RUE) and therefore biomass build-up of primary producers might be restricted when nutrient ratios are highly imbalanced (i.e. limitation by one nutrient and beyond optimal ratios for growth). Although the linkages between ecosystem functioning, diversity and nutrient availability are theoretically well understood, we lack experimental evidence on how phytoplankton diversity affects resource use and biomass under variable nutrient ratios (N:P ratios).
2. Combining a mesocosm and a microcosm experiment, we tested diversity effects on ecosystem functioning by exposing a species diversity gradient generated by the loss of rare species in a natural community to different N:P ratios (uniform vs. a gradient). The N:P supply ratio gradient also allowed us to evaluate responses across balanced and imbalanced ratios.
3. We found that increased species diversity led to increased community RUE when supplied a gradient of N:P ratios; but restricted to the highest diversity level. However, diversity did not affect RUE under uniform nutrient ratios. The overall phytoplankton biomass and carbon:nutrient ratios responses to diversity reflected the patterns detected for RUE. Contrary to theoretical predictions, RUE was maintained under highest N:P supply ratios (extreme phosphorous limitation) suggesting that imbalanced N:P ratios do not necessarily decrease function. Thus, we showed that the nutrient context influences diversity effects on RUE and biomass.
4. *Synthesis.* Overall, our results suggest that the effect of rare phytoplankton species losses on community RUE and biomass can be compensated by the persistent species when nutrient ratios are uniform, but leads to decreases in ecosystem functioning under variable nutrient ratios. This work provides a first attempt for testing interactions between the nutrient context (including concentrations and

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ratios) and the diversity of (natural) communities experimentally, which is conceptually understood but poorly tested for phytoplankton.

KEYWORDS

aquatic-plant ecology, biodiversity–ecosystem functioning, co-limitation, environmental variability, N:P ratios, non-random species losses

1 | INTRODUCTION

Resource availability sets an upper limit to potential primary production, whereas autotroph diversity constrains how much of this potential is realized (Cardinale et al., 2009; Hillebrand et al., 2014; Lewandowska et al., 2016). On one side, phytoplankton biomass is constrained by nutrient concentrations and by the ratios in which limiting nutrients are available (balanced or imbalanced; Elser et al., 2007; Harpole et al., 2011). At balanced ratios, organisms are co-limited (supplied ratios match organismal needs) and the transfer into biomass is maximized (Figure 1, Cardinale et al., 2009). Traditionally, the Redfield ratio for nitrogen (N) and phosphorous (P) (N:P ratio = 16, Redfield, 1958) has been treated as balanced optimum. However, empirical and theoretical work has shown that optimal N:P ratios might vary substantially between taxa, growth rates and temperature (Elser et al., 2000; Gerhard et al., 2019; Hillebrand et al., 2013; Klausmeier et al., 2004; Thrane et al., 2017). Contrarily, imbalanced ratios decrease production efficiency as nutrient ratios do not match organismal needs for biomass build-up (Cardinale et al., 2009; Sterner & Elser, 2002).

Phytoplankton diversity on the other side is expected to increase ecosystem functioning (e.g. biomass production). This is based on the idea that diverse communities show a higher functional diversity (i.e. trait diversity; Cadotte et al., 2011; Weithoff & Beisner, 2019) and allow for complementarity effects (i.e. niche partitioning and facilitation in diverse communities generate an increased function; Loreau & Hector, 2001). Such a complementarity effect results in a higher resource use efficiency (RUE, how efficiently resources are turned into biomass production) and therefore biomass in diverse phytoplankton communities (Figure 1; Ptcnik et al., 2008; Schabhüttl et al., 2013; Striebel et al., 2009).

Using a metacommunity model, Hodapp et al. (2016) showed that complementarity in resource use enables a strong positive effect of diversity on biomass production when heterogeneity in resource supply ratios and trait diversity are high. However, the complementarity effect declines when extending the resource range involves the inclusion of highly imbalanced ratios. Contrarily, if supply ratios do not differ between sites (homogeneous resource conditions) RUE is determined by the best adapted species to the given nutrient conditions (Hodapp et al., 2016). These predictions support that biodiversity effects increase if higher biodiversity equals higher trait diversity and play out under high environmental variability, but not in uniform environments (Ptcnik et al., 2010). This is based on the idea that

environmental variability provides a wide niche spectrum that can be efficiently used by diverse communities that show complementarity in niche occupancy enhancing or maintaining ecosystem functioning (Chen et al., 2019; Norberg et al., 2001; Smith et al., 2016). Instead, homogeneous or uniform environments might select for certain traits favouring the dominance of few well-adapted species independently of the community diversity (identity effect; Lewandowska et al., 2016; Ptcnik et al., 2010). In addition to the direct effects of nutrient availability and diversity on phytoplankton primary production, resource ratios can affect species coexistence and therefore diversity effects on biomass production (resource ratio theory; Tilman, 1982). However, this aspect was not directly addressed in our work since the study was not designed to test coexistence and competitive exclusion (Figure 1).

Thus, conceptual (Cardinale et al., 2009; Hillebrand et al., 2014; Hodapp et al., 2019) and modelling (Hodapp et al., 2016) studies predict that positive biodiversity–ecosystem functioning (BEF) relationships are expected to increase in strength under variable resource availability, but the potential for biomass production is expected to be constrained by highly imbalanced resource supply ratios. However, these predictions have been poorly tested experimentally. Few experimental investigations evaluated phytoplankton RUE, biomass and stoichiometry responses to diversity and nutrients concentrations (Striebel et al., 2009), and to nutrient ratios (Frank et al., 2020; Weis et al., 2008) separately, but not combining these factors (i.e. nutrient concentrations, nutrient ratios and diversity). Additionally, phytoplankton BEF experiments have been based on random species losses, while in nature the extinction of species is driven by species-specific features (e.g. sensitivity, rarity). These artificial assemblages indirectly affect the trait diversity of the community and combine species with different environmental history or cultured in the laboratory, biasing the potential understanding of natural community assemblages (Gamfeldt et al., 2015; Srivastava & Vellend, 2005). Because rare species are considered sensitive to extinction due to their small population size (Pimm et al., 1988), in this study we used a diversity gradient generated by the loss of rare species in a natural phytoplankton community for testing BEF relationships under different nutrient scenarios.

In a previous mesocosm study, we showed that the loss of rare species does not result in a significant decay in RUE and standing biomass as long as the common or abundant species are maintained, considering two nutrient levels (Gerhard et al., 2020). Here, we investigated the effect of the rare species loss combined with a wide N:P supply

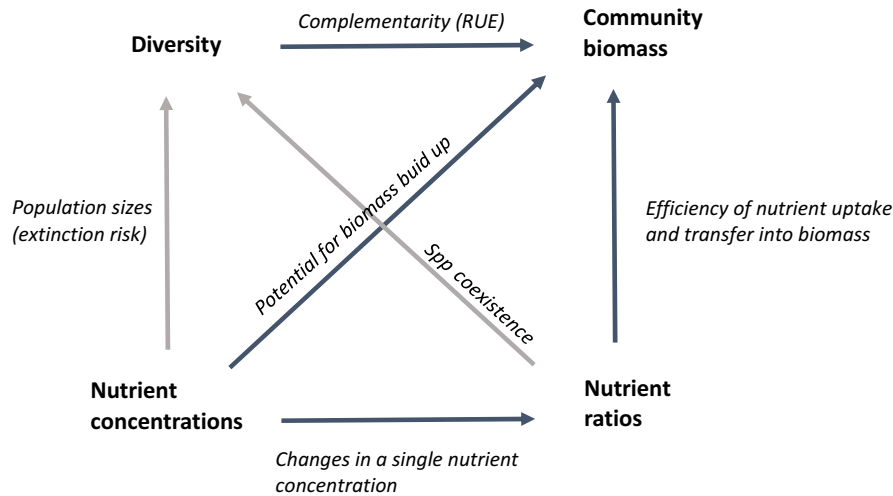


FIGURE 1 Conceptual scheme summarizing nutrients (concentrations and ratios), and diversity direct and indirect effects on primary producers' biomass. Addressed aspects in this study are highlighted in blue. RUE, resource use efficiency. Modified from Cardinale et al. (2009). While feedbacks between biomass and diversity have been supported in previous literature (Hillebrand et al., 2014), in this work the aim was to investigate diversity and nutrient effects on biomass as has been conceptualized in previous investigations (Cardinale et al., 2009) and thus feedbacks were not evaluated and not included in this scheme

ratio gradient to analyse how environmental stoichiometry affects RUE and biomass production under different diversity levels using a microcosm experiment. Results of the microcosm and mesocosm experiments were compared since they were conducted simultaneously using the same diversity gradient but different nutrient conditions. Thus, we evaluated the effect of the initial phytoplankton diversity on ecosystem functioning under different nutrient scenarios, and we hypothesized the following: (H1) Balanced N:P supply ratios (nutrient co-limitation) promote biomass production by increasing efficiency in nutrient utilization, especially in communities with high diversity since diverse communities present different nutrient strategies increasing species resource use trade-offs; (H2) The phytoplankton N:P ratios are expected to reflect the supplied ratios, but a high diversity also influences phytoplankton nutrient content by increasing complementarity in resource use of the limiting nutrient; and (H3) The increase in phytoplankton RUE with increasing species diversity becomes more important when communities are exposed to a wide N:P ratio gradient (microcosm experiment) than when exposed to different nutrient concentrations with similar ratios (mesocosm experiment), thus representing variable and uniform environments, respectively. We expect increased RUE to be mirrored in the phytoplankton standing biomass and carbon:nutrient ratios, as a result of a higher efficiency in transferring resources into biomass.

2 | MATERIALS AND METHODS

2.1 | General experimental set-up

We conducted a mesocosm experiment coupled with an additional microcosm experiment (Appendix S1: Figure S1) to evaluate the effects of the initial diversity of the phytoplankton community,

nutrient concentrations and nutrient ratios on phytoplankton community RUE, biomass and stoichiometry. Both experiments were conducted using the same initial phytoplankton community, which was collected from a lake (Grafenschaftsee, Germany, 53°33' 05"N; 7°58' 49"E) at the end of the summer (2017). After sampling, grazers were excluded by filtering the water through a 53 μm mesh. The diversity gradient (six levels, increasing in diversity from the lowest diversity at D1 to the highest at D6) was generated by dilution of the natural community (dilution factors ranged from 1:1 $\times 10^5$ to 1:1) and thus removal of rare species (Engel et al., 2017; Hammerstein et al., 2017). Each diversity level was incubated in flasks for 26 days to increase biomass prior to the start of the experiments (for methodological details, see Gerhard et al., 2020). The dilution created a diversity gradient that was maintained during the incubation until the inoculation in the mesocosm and the microcosm experiment, and therefore the diversity manipulation was based on the initial pool of species (Figure 2). We used richness and inverse Simpson diversity as diversity indices as richness better represents the loss of rare species, while inverse Simpson diversity (i.e. effective number of species) also provides information about the community evenness. Despite changes in diversity over time, the general gradient of richness remained over the duration of the mesocosm experiment (Appendix S1: Figure S2). However, no diversity data were available at the end of the microcosm experiment. Nutrient treatments were performed by manipulating N and P concentrations while other elements were supplied in excess according to WC phytoplankton growth medium (Guillard & Lorenzen, 1972). The experimental temperature was 20°C (same as lake temperature in summer) and the day-night cycle was 12:12 hr at a light intensity of about 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the mesocosms and 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the flask incubations. Although the absolute light intensity at surface was lower in the flasks, light limitation was not expected to be

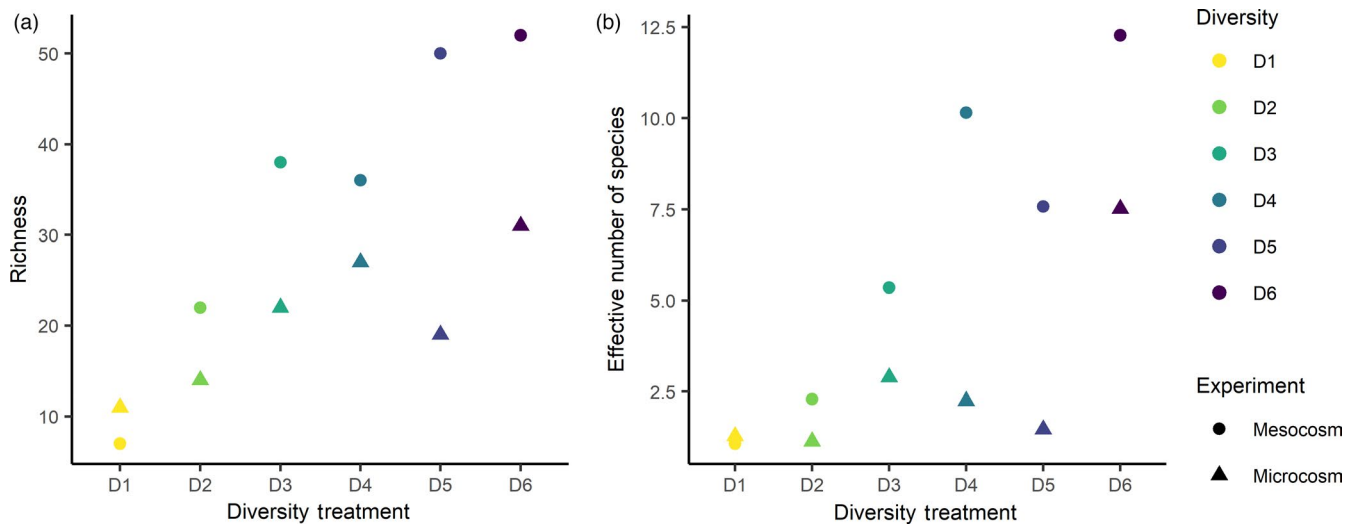


FIGURE 2 Realized species diversity in the dilution treatments at the beginning of the mesocosm and microcosm experiment represented by (a) Richness and (b) Effective number of species (inverse Simpson diversity). The beginning of the mesocosm experiment was at day 0 (inoculum) and the beginning of the microcosm experiment was at day 7 (low nutrient treatments in the mesocosm experiment). Diversity levels (increasing from D1 to D6) are indicated by colours and the experiment by shape. Richness showed a clearer relationship with the dilution treatment and was better maintained over time than the effective number of species. Diversity decreased over time and was therefore lower at the beginning of the microcosm than the mesocosm experiment. These patterns are expected since the presence of rare species is better represented by richness while the effective number of species reflects also the community evenness; and the loss of species over time cannot be replaced because closed experiments lack the possibility of recolonization. Species diversity data have been published in Gerhard et al. (2020)

higher than in the mesocosms as, given the Lambert–Beer law, the low water depth in the flasks had little potential for self-shading. Changes in biomass were monitored photometrically by daily optical density measurements (wavelength 440–450 nm) using a custom-tailored device (Frank et al., 2020).

2.2 | Mesocosm experimental set-up

The six diversity treatments were inoculated under two nutrient levels (low and high) in 600 L indoor mesocosms comprising 12 experimental units (Appendix S1: Figure S1; Gall et al., 2017). The phytoplankton inoculum (corresponding to the six diversity levels) was added in small volumes (between 80 and 150 ml) to the mesocosms assuring the same initial biomass amount in all treatments. The richness of the inoculated communities ranged from 8 to 52 (from D1 to D6; Figure 2). For the low nutrient treatments, 0.65 $\mu\text{mol/L}$ of P and 14.2 $\mu\text{mol/L}$ of N were added, mimicking the lake nutrient conditions (concentrations and ratio measured before the start of the experiments). To simulate nutrient enrichment, but maintaining the same N:P ratios, 1.9 $\mu\text{mol/L}$ of P and 42.6 $\mu\text{mol/L}$ of N were added in the high nutrient treatments. However, the total nutrient concentrations and ratios measured during the experiment showed deviations from the nutrients initially added in the treatments, but maintaining two levels and a narrow N:P ratio range (Appendix S1: Figure S3). The mesocosms were sampled every 4 days from day 7 to day 27 comprising a total of six samplings. The experiment was continued until the phytoplankton communities reached the stationary growth

phase. In every sampling event, 10% (60 L) of water was replaced with water containing the design nutrient concentrations. From the replaced volume of water, 20 L were filtered through a 105 μm mesh to control the presence of large zooplankton (small zooplankton was controlled when processing phytoplankton samples). Experimental details of the diversity gradient generation and mesocosm experiment are described in Gerhard et al. (2020).

2.3 | Microcosm experimental set-up

The microcosm experiment was carried out using a factorial design where each of the six diversity levels was incubated under 25 different nutrient conditions leading to a total of 150 experimental units. For that, cell culture flasks (250 ml polystyrene, Sarstedt Ltd.) were filled with 200 ml water taken from the six diversity treatments of the mesocosms (from the low nutrient treatment) on day 7 (see Appendix S1: Figure S1). The richness of the inoculated communities in the microcosm experiment ranged from 11 to 31 (from D1 to D6; Figure 2). The nutrient conditions were based on combinations of 0.1, 0.7, 1.3, 1.9 and 2.6 $\mu\text{mol P/L}$ and 3, 19, 35, 48 and 62 $\mu\text{mol N/L}$ and added in each diversity level treatment. This factorial design generated treatments including intermediate ‘balanced’ ratios with different concentrations, and extreme ‘imbalanced’ ratios. However, the nutrient ratios were used as a gradient treatment (not classified as balanced or imbalanced) for considering flexibility in optimal ratios among communities. Because the water from the mesocosms had a slightly different

base of nutrient concentrations, the final nutrient conditions in the flasks showed small variations among diversity treatments, but in all cases a wide N:P supply ratio gradient was established (Appendix S2). To prevent sinking losses and differences in light availability, the flasks were manually shaken and randomly rearranged every day. Samples were taken when the stationary growth phase was reached. The treatments corresponding to the communities with diversity levels from two to six (D2–D6) reached the stationary phase after 24 days, communities of the lowest diversity treatment (D1) after 38 days.

2.4 | Laboratory analysis

Water samples for particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) were filtered onto acid-washed pre-combusted glass-fibre filters (Whatman GF/C). Filters for POC and PON were measured using an elemental analyser (Flash EA 1112, Thermo Scientific). Water samples for dissolved nitrogen fractions (NO_x^- and NH_4^+) were filtered and determined following the method described by Schnetger and Lehnert (2014) for NO_x^- (NO_3^- and NO_2^-), and a modified version of Benesch and Mangelsdorf (1972) method for ammonium (NH_4^+). POP and dissolved phosphorus (PO_4^{3-}) were measured by molybdate reaction of filtered water samples for the dissolved fraction and after digestion with potassium peroxydisulphate ($\text{K}_2\text{S}_2\text{O}_8$) solution for the particulate fraction (Wetzel & Likens, 2013). Phytoplankton samples corresponding to the initial communities of each experiment and the end of the mesocosm experiment were preserved in Lugol solution until counted and identified under an inverted microscope based on Utermöhl's method (Utermöhl, 1958). Sedimentation volume was adjusted according to the samples biomass assuring a consistent amount of biomass was counted. Phytoplankton was identified to the species level and morphospecies were used when clear assignment of a species name was not possible.

2.5 | Statistical analysis

In both experiments, we analysed the treatment effects at the phytoplankton community level for the standing biomass, RUE and stoichiometry (carbon:nutrient and N:P ratios). The standing biomass was measured as POC, and RUE was calculated as unit of biomass per unit of total nutrient in the system ($\text{RUE}_P = \text{POC}/\text{total P}$ and $\text{RUE}_N = \text{POC}/\text{total N}$; Ptacnik et al., 2008), where total nutrients represent the sum of dissolved and particulate N or P, respectively. Because we have previously shown that N was the limiting nutrient in all treatments in the mesocosm experiment (Gerhard et al., 2020), we only report results for RUE_N in the main paper (but see Appendix S1: Table S1 and Figure S4 for RUE_P). The nutrient ratios were calculated using molar dissolved N:P ratios for supply and particulate nutrients ratios for phytoplankton. Thus, we considered phytoplankton RUE as the molar ratio of biomass produced per total nutrient, while the molar particulate carbon:nutrient ratios represent the stoichiometry of carbon

assimilation over nutrient uptake. For both datasets, the initial diversity gradient was included as a numeric explanatory variable in the models where numbers from 1 to 6 were assigned to the levels (corresponding to the dilution sequence, D1–D6). The argument of using the dilution treatment instead of the realized diversity is that determination of diversity indices include uncertainty, especially expected for complex natural phytoplankton identification and counting. Thus, this violates the model assumption that independent variables are measured without error. However, models including the realized initial richness as explanatory variable were performed for comparison and showed the same results as those obtained by including the dilution treatments as explanatory variable (Appendix S1: Table S2 and Table S3).

In the microcosm experiment, data from the end of the incubations ($n = 150$) were analysed including the diversity gradient and the N:P supply ratio gradient as interactive continuous explanatory variables in a linear model. The response variables and the N:P supply ratios were ln-transformed for the statistical analysis to obtain linear relationships. For data visualization in the figures, non-linear curves were fitted using the local polynomial regression (loess) method for RUE_P and POC, while RUE_N curves were fitted using linear models for each diversity treatment across the N:P supply ratio gradient. Alternative models including N and P concentrations as predictors (instead of N:P supply ratios) are included in the Supplementary Information (Appendix S1: Table S4). The data of the mesocosm experiment were analysed using linear mixed models. The diversity gradient, nutrient level (high and low), and time were included as fixed effects ($n = 72$). The random component was defined by comparing the random intercept of the experimental units (mesocosms), random slopes of experimental units over time and both together. We accounted for the non-independence of the data by testing for autocorrelation (autocorrelation function and residual analyses) and including the AR-1 autocorrelation structure in the models. Model selection was done using Akaike information criterion following the protocol recommended by Zuur (2009). The mixed models were performed using lme function (NLMLE package). The marginal R^2 (R_m^2) and the conditional R^2 (R_c^2) (Nakagawa & Schielzeth, 2013) were estimated for each model (r.squaredGLMM function in the MuMIn package). The POC and RUE_N data of the mesocosm experiment are shown in Gerhard et al. (2020), but also included here for better comparison with the microcosm experiment and in order to answer different scientific questions.

All statistical analyses and figures were performed in R version 3.6.1 (R Development Core Team, 2019) and a level of $\alpha = 0.05$ was considered for statistical significance in the analyses. *F*-tests and *p* values were calculated to identify significant effects and model validation was performed by residual analyses.

3 | RESULTS

3.1 | Microcosm experiment

In the microcosm experiment, phytoplankton RUE_N , RUE_P and biomass (POC) showed significant responses to the phytoplankton

diversity gradient, the N:P supply ratio gradient, and their interaction (Table 1). Species diversity had a positive effect on RUE, which was driven by the extreme (highest and lowest) diversity levels: RUE_N and RUE_P were consistently highest for D6 and lowest for D1, while intermediate levels of diversity led to intermediate values of RUE but did not mirrored the diversity gradient (Figure 3). The diversity effect on RUE (i.e. the difference in RUE between extreme diversity levels) increased with higher N:P supply ratios (interactive effect of diversity and N:P supply ratios, Table 1). RUE_N linearly declined with increasing N:P supply ratios in all diversity levels, reflecting the high N availability (Figure 3a). RUE_P increased with increasing N:P supply ratios reflecting the low P availability, but the response was saturating rather than linear (Figure 3b). Here, the lowest diversity treatment D1 reached saturation at the lowest N:P supply ratio (~30), whereas D6 remained most linear.

According to RUE, the phytoplankton biomass showed a positive diversity effects that was driven by the extreme diversity levels (D1 and D6), and the difference between the lowest and highest diversity level became more evident at high N:P supply ratios (Table 1; Figure 4a). The phytoplankton biomass showed saturation curves with increasing N:P supply ratios in diversity treatments from D2 to D6 (Figure 4a), indicating that high standing biomass was maintained under N-rich imbalanced ratios. Only in D1 the biomass peaked at intermediate N:P ratios and decreased at high N:P supply ratios. Thus, the lowest diversity treatment was not able to use P efficiently at high N:P supply ratios (showed the lowest RUE_P); and given the reduction in RUE_N , D1 was not able to maintain high biomass production at high N:P supply ratios (Figures 3 and 4a).

The phytoplankton N:P ratios reflected the N:P supply ratios increasing with N and decreasing with P concentrations (Table 1; Figure 4b; Appendix S1: Table S4 and Figure S5). The diversity gradient had a negative effect on phytoplankton N:P ratios showing that more diverse phytoplankton communities maintained higher relative P content than less diverse communities. The relationship between the phytoplankton and the supply N:P ratios was close to 1:1 up to an N:P supply ratio around 30, but above that phytoplankton N:P increased less rapidly than N:P supply ratios (Figure 4b). The phytoplankton carbon:nutrient ratios in the microcosm experiment reflected the RUE patterns (Appendix S1: Table S5 and Figure S6).

3.2 | Mesocosm experiment

The mesocosm experiment did not show any significant response of phytoplankton RUE_N , biomass and N:P ratios to diversity or its interaction with the nutrient level and time (Table 2; Figure 5). The RUE_N was significantly higher in the low, compared to the high, nutrient level, and both, RUE_N and biomass (POC) increased over time reflecting the phytoplankton growth during the experiment (Table 2; Figure 5). Despite the lack of significant diversity effects based on the diversity gradient, the diversity treatments responded differently based on the nutrient level: at high nutrients RUE_N and POC tended to increase in the highest diversity level (D6); at low nutrients, the diversity level D2 had the highest RUE_N and POC during the experiment (Figure 5). Interestingly, the diversity treatment D2 also showed high RUE and biomass in the microcosm experiment. However, in the microcosm experiment, RUE and biomass were lower in D2 than in the highest diversity level (D6) in all nutrient treatments.

The phytoplankton N:P ratios showed an interactive effect of the nutrient level and time (Table 2; Figure 5). However, the R_m^2 indicated that the fixed effects explained a low proportion of the phytoplankton N:P ratio variance. Deviations from the supplied nutrient concentrations and consequently N:P supply ratios in the mesocosm experiment (see Methods) were reflected in the phytoplankton N:P ratios, likely driving the detected patterns (Appendix S1: Figure S3). The phytoplankton carbon:nutrient ratios were also not significantly affected by diversity and D2 showed the highest values for C:N and C:P ratios. The C:N ratios increased under low nutrient conditions towards the end of the experiment (Appendix S1: Table S1 and Figure S4).

4 | DISCUSSION

Overall, our data showed that diversity increased RUE, standing biomass and carbon:nutrient ratios when phytoplankton was exposed to a wide N:P supply ratio gradient, while ecosystem functioning was not affected by diversity under uniform N:P supply ratios (according to H3). Hence, we highlight the importance of phytoplankton rare species on ecosystem functioning when a wide range of nutrient scenarios is considered, contrasting empirical

	POC	RUE_N	RUE_P	N:P
R_{adj}^2	0.50	0.55	0.92	0.92
lnNP	96.5 (<0.001)	37.1 (<0.001)	1,487.6 (<0.001)	1,808.7 (<0.001)
Div	53.0 (<0.001)	139.8 (<0.001)	159.6 (<0.001)	25.7 (<0.001)
lnNP × Div	4.5 (0.040)	5.3 (0.023)	14.9 (<0.001)	0.6 (0.445)

TABLE 1 Statistical results for the microcosm experiment. The explained variance (R_{adj}^2), $F_{1,146}$ values and p values (between brackets) are given for each model. Significant effects ($p < 0.05$) are highlighted in bold. lnNP, N:P supply ratios (ln-transformed); Div, diversity; POC, particulate organic carbon; RUE_N , resource use efficiency based on nitrogen availability; RUE_P , resource use efficiency based on phosphorus availability. All response variables were ln-transformed

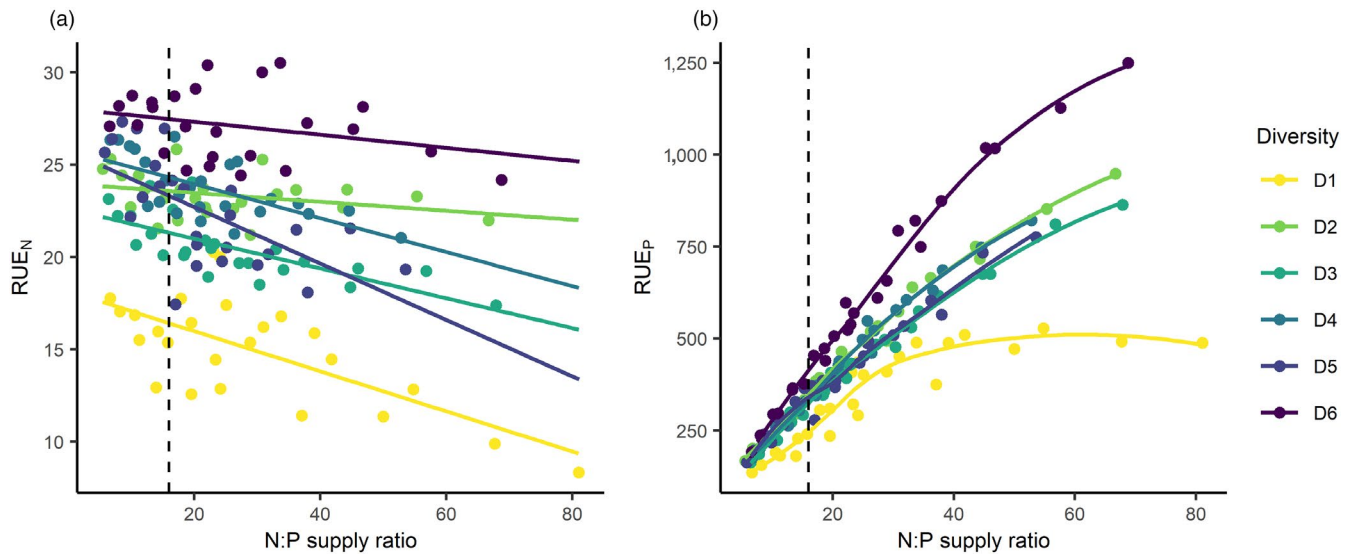


FIGURE 3 Phytoplankton resource use efficiency (RUE) across the N:P supply ratio gradient in the microcosm experiment. (a) RUE based on nitrogen availability (RUE_N) and (b) RUE based on phosphorus availability (RUE_P). Diversity levels (increasing from D1 to D6) are indicated by colours. The vertical dashed lines represent the Redfield ratio (i.e. N:P of 16). RUE_P non-linear curves were fitted using the loess method. The RUE_N and RUE_P were not maximized at balanced N:P supply ratios (contrary to H1) and showed a positive effect of diversity (supporting H3)

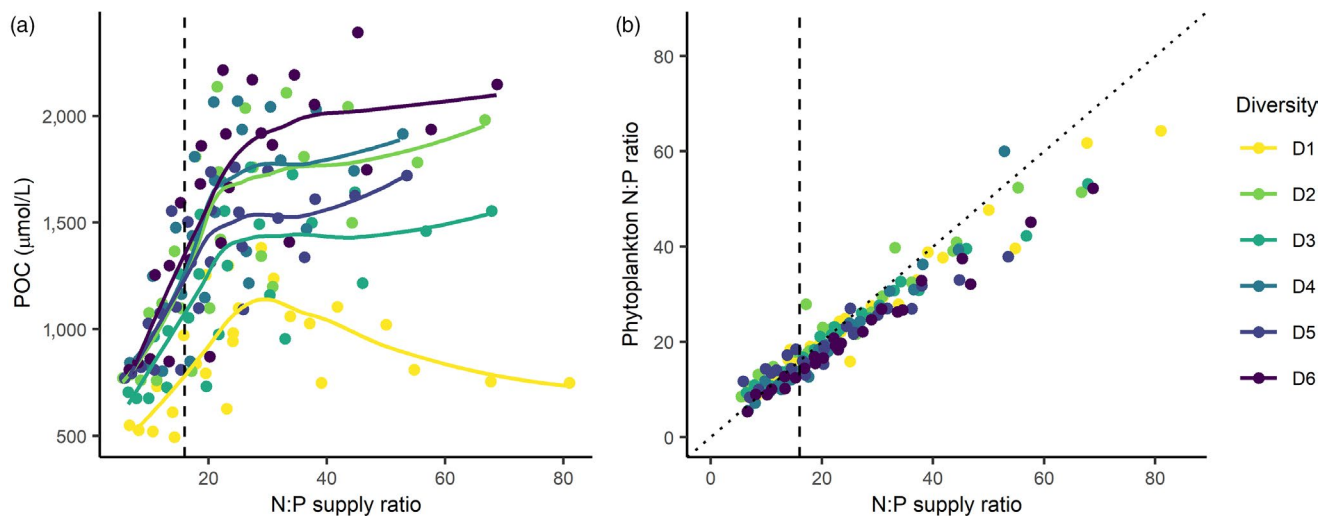


FIGURE 4 Phytoplankton responses to the N:P supply ratio gradient in the microcosm experiment. (a) Biomass as particulate organic carbon (POC) and (b) N:P ratios. Diversity levels (increasing from D1 to D6) are indicated by colours. The vertical dashed lines represent the Redfield ratio (i.e. N:P of 16), and the dotted line in (b) represents the 1:1 line. POC non-linear curves were fitted using the loess method. The POC was not maximized at balanced N:P supply ratios (contrary to H1) but increased with diversity (supporting H3). The phytoplankton N:P ratios reflected the supplied ratios and were affected by diversity (supporting H2)

results found under more uniform nutrient conditions (Gerhard et al., 2020), and results for other biological communities like terrestrial plants (Smith & Knapp, 2003; Yoshihara et al., 2019) and bacteria (Roger et al., 2016), where rare species losses did not affect ecosystem functioning. These results support the idea that positive biodiversity–ecosystem functioning (BEF) relationships are detected when combining variable environmental conditions and high diversity, since environmental variability promotes functional diversity to play out as suggested in conceptual (Hillebrand

& Matthiessen, 2009; Ptacnik et al., 2010) and modelling (Chen et al., 2019; Hodapp et al., 2016; Smith et al., 2016) studies. However, the strongest diversity effect was not detected under balanced nutrient ratios, and balanced N:P supply ratios resulted in a biomass peak only for the lowest diversity treatment in the microcosm experiment (rejecting H1). The phytoplankton stoichiometry reflected the supplied nutrients and showed that highest diversity allowed communities to incorporate more P under extreme high N:P ratios (supporting H2).

TABLE 2 Statistical results for the mesocosm experiment. $F_{1,8}$ values for effects between subjects and $F_{1,56}$ values for effects within subjects are presented. p values (between brackets) are shown and significant effects ($p < 0.05$) are highlighted in bold. Marginal (R_m^2) and conditional (R_c^2) explained variance are presented for each model. Div, diversity; Nut, nutrient level; Time, experimental day; POC, particulate organic carbon; RUE_N , resource use efficiency based on nitrogen availability; N:P, phytoplankton N:P ratios

	POC	RUE_N	N:P
R_m^2	0.72	0.87	0.23
R_c^2	0.87	0.90	0.28
Div	0.1 (0.763)	0.6 (0.474)	1.4 (0.266)
Nut	0.5 (0.511)	28.9 (<0.001)	0.0 (0.860)
Div × Nut	0.5 (0.487)	0.8 (0.393)	2.9 (0.125)
Time	164.9 (<0.001)	279.6 (<0.001)	1.7 (0.200)
Time × Div	0.8 (0.987)	1.3 (0.257)	0.0 (0.9)
Time × Nut	3.4 (0.067)	21.8 (<0.001)	4.9 (0.03)
Time × Div × Nut	2.1 (0.153)	1.2 (0.272)	0.4 (0.522)

4.1 | Effect of balanced and imbalanced N:P supply ratios on community RUE and biomass (H1)

For multiple resource limitation, standing biomass is expected to increase when nutrient ratios are balanced (co-limitation) because nutrient uptake is more efficient, and decrease as resources become imbalanced (Cardinale et al., 2009; Hillebrand et al., 2014; Hodapp et al., 2016, 2019). However, our empirical results (from the microcosm experiment) showed that imbalanced nutrient ratios do not necessarily decrease RUE and biomass (contrary to H1). This is consistent with the idea that RUE depends not only on the availability of the limiting nutrient, but also on the stoichiometric context- and species-specific characteristics (Frank et al., 2020). Furthermore, increasing differences in community RUE and standing biomass between the extreme diversity levels were detected when N:P supply ratios were highest. This suggests that diverse communities' performance increased under P limiting ratios in comparison to low diversity communities (contrary to H1). Generally, N limited the overall biomass in most treatments (Appendix S1: Table S4 and Figure S5) since communities increased RUE_p at limiting P buffering the biomass decay, and P limitation was only detected for the lowest diversity (D1). Thus, losing rare species reduced RUE and biomass in communities that grew under highly P-limiting conditions.

4.2 | Nutrient context and diversity effects on community stoichiometry (H2)

The phytoplankton N:P ratios strongly reflected the N and P availability, but were also influenced by diversity (supporting H2).

While phytoplankton N:P ratios decreased with diversity in the microcosms, the mesocosm experiment did not show a significant response of phytoplankton N:P ratios to diversity, but a slight tendency to decrease with diversity over time could be observed. A smaller effect of the treatments on phytoplankton N:P ratios in the mesocosm than in the microcosm experiment is expected considering that total N:P ratios were maintained within a narrow range decreasing potential for variation. Schabhüttl et al. (2013) found an increase in phytoplankton P content with richness, suggesting that nutrient uptake might be influenced by complementarity processes. In our study, despite the diversity effect on phytoplankton N:P was significant in the microcosm experiment suggesting complementarity in P uptake, phytoplankton N:P ratios strongly reflected supplied nutrients as expected in the stationary growth phase (Klausmeier et al., 2008). Deviations of phytoplankton N:P ratios from the supplied ratios at high N:P values suggest the presence of physiological limits (P-limitation) with increasing ratios (Hall et al., 2005). This pattern has been previously shown in phytoplankton natural communities (Gerhard et al., 2019), but the saturation curve (i.e. P limitation) was weaker in the present study mirroring the high capacity of the communities to cope with P limitation indicated by the RUE_p patterns (except for D1 that showed a low capacity for dealing with P limitation).

4.3 | Diversity effects on community RUE and biomass under uniform and variable environmental stoichiometry (H3)

Ecosystem functioning showed different responses to diversity in the mesocosm (neutral) and microcosm (positive) experiment, sustaining that the nutrient context influenced the role of diversity on phytoplankton performance (supporting H3). The mesocosm experiment showed that a potential decrease in RUE and biomass caused by the loss of rare species was generally compensated by commonly preserved species according to previous terrestrial plant studies (Smith & Knapp, 2003; Yoshihara et al., 2019). This was supported by the lack of diversity effects on RUE and biomass in the mesocosm experiment, where ecosystem functioning tended to decline only when most phytoplankton species were extirpated (in D1). The biomass production in systems with one limiting nutrient is expected to be determined by species with the highest RUE for the limiting nutrient (Hillebrand et al., 2014). In this case, greater species diversity does not necessarily enhance ecosystem functioning if the dominant species is a highly productive species (species identity effect; Lewandowska et al., 2016; Vaughn, 2010). This is partially supported by our mesocosm experiment, as no significant diversity effects on RUE, biomass and carbon:nutrient ratios were detected when nutrient concentrations changed maintaining N limiting conditions. Additionally, the second diversity treatment in the gradient (D2) represented almost a *Monoraphidium contortum* monoculture (Appendix S1: Figure S7) and showed the highest RUE_N and biomass under low nutrient concentrations, but also the highest

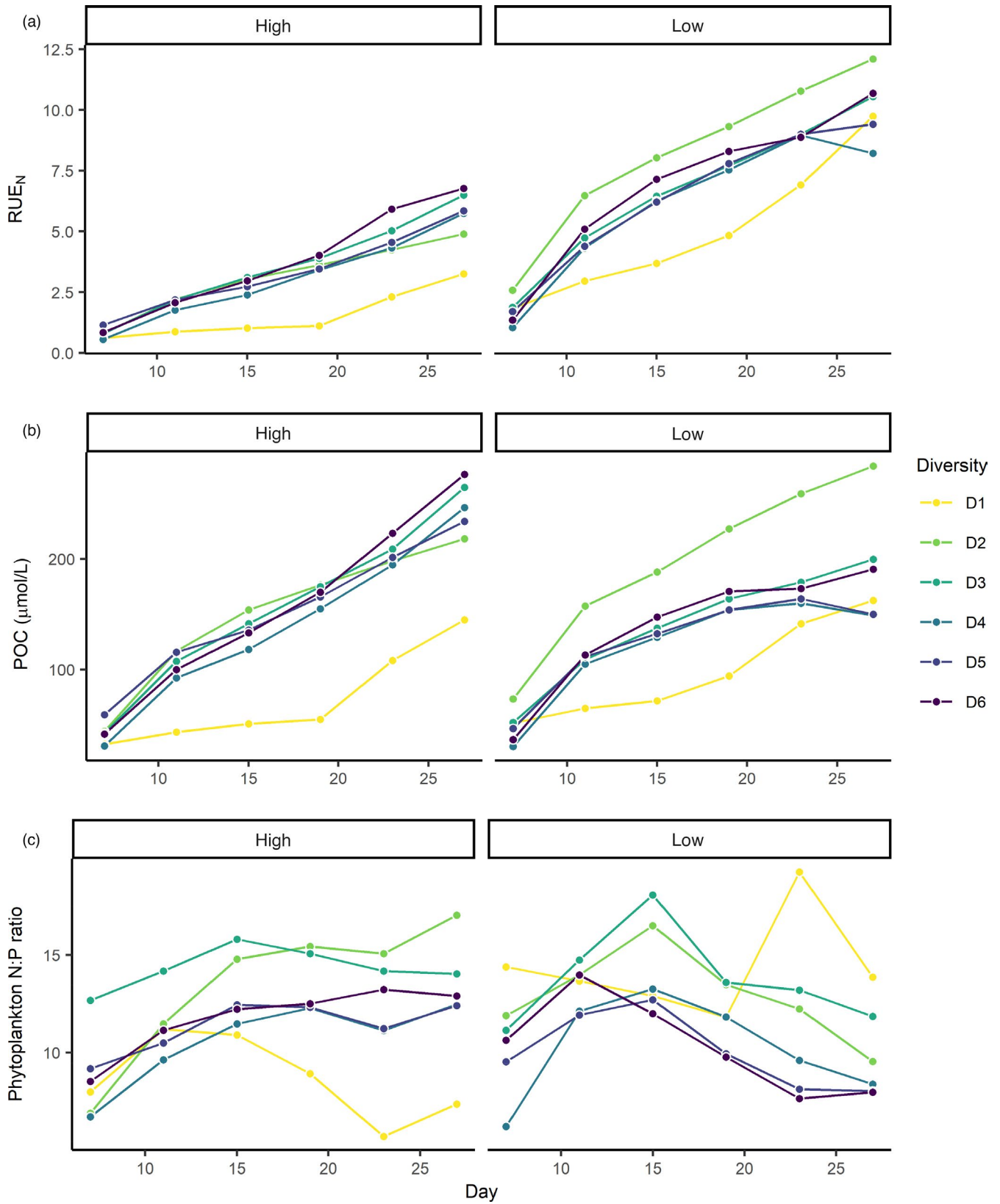


FIGURE 5 Phytoplankton responses to treatments in the mesocosm experiment. (a) Resource use efficiency based on nitrogen availability (RUE_N), (b) Biomass as particulate organic carbon (POC) and (c) Phytoplankton N:P ratios. Diversity levels (increasing from D1 to D6) are indicated by colours and nutrient levels are shown in the right (low) and left (high) panels. None of these variables was significantly affected by diversity (supporting H3). The POC and RUE_N data for the mesocosm experiment are shown in Gerhard et al. (2020)

carbon:nutrient ratios. This pattern is in concordance with previous studies where *M. contortum* was identified as a high productive species in monocultures (Bogen et al., 2013) and dominating natural communities (Ferragut & de Campos Bicudo, 2012) under N limitation. However, D2 did not show the highest RUE_N and biomass under high nutrients suggesting that nutrient concentration influenced responses to the diversity treatments (this was also supported by RUE_P results that showed a positive diversity effect under high nutrient treatments, see Appendix S1: Table S1 and Figure S4).

Contrasting the one limiting nutrient scenario, communities with high species diversity (including rare species) played an important role in increasing RUE and biomass when exposed to a gradient of nutrient ratios in the microcosm experiment (supporting H3). Interestingly, previous experiments that evaluated monocultures and artificial mixtures (of 4 and 5 species) under a stoichiometric gradient did not find an effect of the number of species on RUE and biomass (Frank et al., 2020; Weis et al., 2008). In our experiment, the presence of a high number of species (close to natural conditions) in D6 increased RUE and biomass production in the microcosm experiment suggesting that the highest diversity level might have presented a variety of strategies in resource use that allowed the community to be more efficient under different nutrient conditions. This was supported by trends detected in the phytoplankton size diversity, which increased with taxonomic diversity (Appendix S1: Figure S8). Cell size is related to nutrient uptake strategies and therefore, a high size diversity is expected to reflect a high variety in the use of resources increasing phytoplankton RUE and biomass (Acevedo-Trejos et al., 2018; Litchman et al., 2010; Marañón, 2015). Size diversity was positively related to taxonomic diversity in both experiments. While a higher size diversity was not translated into higher RUE and biomass under uniform environmental conditions (mesocosm experiment), it might have influenced the positive BEF relationship showed under variable conditions (microcosm experiment). Hence, our results are in concordance with the idea that high trait diversity combined with high variability in nutrient availability favours complementarity effects of resource use (Hodapp et al., 2016). However, it is important to note that our analysis is based on the effect of the initial diversity generated by dilution and changes in species composition and diversity over time were conditioned by this initial manipulation (i.e. species richness and pool, changes in competition).

Although the microcosm experiment showed a diversity effect on RUE and biomass, this was driven by the extreme diversity levels of the rarity gradient and responses at intermediate diversity might be determined by other aspects (e.g. community composition, evenness; Bonachela et al., 2016; Dickman et al., 2006; Lewandowska et al., 2016). Thus, even if the treatments were generated using the same initial community (same history), non-random species loss can develop in different community assemblages as a consequence of biotic interactions and have a strong influence on RUE and standing biomass. For example, in diversity level D2 *M. contortum* dominated the community composition generating high-biomass treatments, but the contribution to biomass of *M. contortum* decreased when

competitive pressure was higher in communities with more species (Appendix S1: Figure S7).

4.4 | Experimental considerations

In this study we manipulated the diversity of a natural community to minimize common experimental limitations of artificial assemblages: we included a high number of species which co-occur in nature and present similar environmental histories. We did not manipulate trait distribution by arbitrary selection of species, but we simulated non-random species losses. However, other experimental limitations present in this work need to be considered. Using natural phytoplankton communities' increases the complexity of sample handling (i.e. count and identification), what led us to lack of diversity and composition data at the end of the microcosm experiment and therefore limited the mechanistic understanding of the detected patterns. Microscopy approaches for species identification can lead to an underestimation of the total number of species in the communities since only a subsample is counted. In our study, a potential underestimation might be expected in the highest diversity levels where the proportion of rare species is higher. Thus, such bias can affect the total richness detected in the treatments, but it is not expected to change the general diversity gradient. In addition, our experimental systems were closed and only one nutrient combination (N and P concentration) was supplied to each experimental unit. Thus, recolonization of species as expected in natural environments was not possible, and each experimental unit only experienced a unique nutrient scenario (i.e. one nutrient pulse without fluctuations). A step further for generating controlled nutrient supply variability scenarios is the use of meta-community approaches, but these experimental set-ups also need to deal with limitations (e.g. manipulation of dispersal). Finally, we prioritized the inclusion of a wide gradient of treatments instead of replication, which has a higher risk of detecting stochastic effects. Beyond these limitations, we consider that it is worth generating novel experimental set-ups enabling theoretical predictions to be tested under complex scenarios (closer to natural than simple experiments).

5 | CONCLUSIONS

In this study, we investigated how the loss of rare species in a natural phytoplankton community affects ecosystem functioning under different resource supply scenarios. We compared phytoplankton community responses in a diversity gradient under uniform nutrient ratios and under a N:P supply ratio gradient, as well as analysed patterns across the N:P supply ratio gradient. We found that rare species loss might not affect ecosystem functioning under uniform nutrient ratios, but can decrease ecosystem functioning when N:P supply ratios are variable. Thus, our results suggest that the nutrient context can influence community processes (e.g. identity effect, complementarity) resulting in different BEF relationships. However,

contrary to expected, we found that community RUE and biomass do not necessarily decline under highly imbalanced N:P ratios. Most diversity levels coped with P limitation suggesting that community features (e.g. composition, diversity, trait distribution) might buffer expected decay in biomass under nutrient limitation. In general, our study showed that environmental stoichiometry modulates phytoplankton community performance and highlights the importance of including multiple nutrient scenarios when addressing diversity effects on ecosystems functioning. Because natural systems are exposed to environmental variability, it is crucial to investigate how this variation interacts with community features for understanding ecosystem responses to change.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

AUTHORS' CONTRIBUTIONS

M.G., M.S. and H.H. conceived the ideas and designed the methodology; M.G., M.S. and A.S. collected and analysed the data; M.G. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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DATA AVAILABILITY STATEMENT

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