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LETTER

Species richness and intraspecific variation interactively shape marine diatom community functioning

Patrick K. Thomas [1,2*] *Marrit Jacob* [1,3] *Esteban Acevedo-Trejos* [4,5] *Helmut Hillebrand* [1,6,7] *Maren Striebel* [1]

[1]Plankton Ecology Lab, Institute for Chemistry and Biology of the Marine Environment (ICBM), School of Mathematics and Science, Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany; [2]Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Dübendorf, Switzerland; [3]Marine Chemistry, Department of Chemistry and Biology, University of Bremen, Bremen, Germany; [4]Systems Ecology Group, Leibniz Centre for Tropical Marine Ecology (ZMT), Bremen, Germany; [5]Earth Surface Process Modelling, Helmholtz Centre Potsdam, GFZ German Research Centre for Geosciences, Potsdam, Germany; [6]Helmholtz-Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Oldenburg, Germany; [7]Alfred Wegener Institute, Helmholtz-Centre for Polar and Marine Research (AWI), Bremerhaven, Germany

Scientific Significance Statement

Emerging evidence shows that biodiversity at the within-species level can be crucial for influencing productivity, food web dynamics, and nutrient cycling, thus often rivaling the ecological significance of diversity across species for ecosystem-scale processes. However, the relative importance of within-species diversity in globally essential primary producers such as phytoplankton is currently unknown. This study provides experimental evidence that within-species diversity of phytoplankton has strong and positive effects on community biomass production, comparable to the positive effects of species diversity. Moreover, our results show species richness effects are dependent upon both the level of intraspecific diversity, the environmental context, and phenotypic trait distributions. These results emphasize the importance of integrating multiple components of aquatic biodiversity when assessing the effects of environmental change on ecosystem functioning.

Abstract

Biodiversity generally increases productivity in ecosystems; however, this is mediated by the specific functional traits that come with biodiversity loss or gain and how these traits interact with environmental conditions. Most biodiversity studies evaluate the effects of species richness alone, despite our increasing understanding that intraspecific diversity can have equally strong impacts. Here, we manipulate both species richness and intraspecific richness (i.e., number of distinct strains) in marine diatom communities to explicitly test the

*Correspondence: patrick.thomas@eawag.ch

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Additional Supporting Information may be found in the online version of this article.

relative importance of species and strain richness for biomass and trait diversity in six distinct temperature/nutrient environments. We show that species and strain richness both have significant effects on biomass and growth rates, but more importantly, they interact with each other, indicating that cross-species diversity effects depend on within-species diversity and vice versa. This intertwined relationship thus calls for more integrative approaches quantifying the relative importance of distinct biodiversity components and environmental context on ecosystem functioning.

Biodiversity, in all its forms, is vital for the functioning of ecosystems (Hooper et al. 2005; Grace et al. 2016; Duffy et al. 2017). More taxonomically diverse communities tend to foster a greater array of traits, which can help communities respond to rapid environmental change and capture limiting resources more efficiently (Ptacnik et al. 2008; Hillebrand and Matthiessen 2009). Though a majority of studies have focused on diversity at the species level, we are rapidly gaining information about how diversity at the within-species (i.e., intraspecific) level can be important for ecosystem functioning (Bolnick et al. 2011; Des Roches et al. 2018; Raffard et al. 2019).

The functional traits expressed by individual organisms in a community are a common thread linking all organizational levels of biodiversity (McGill et al. 2006; Violle et al. 2007). Therefore, theoretical frameworks based on trait distributions are relatively agnostic regarding whether traits vary at the intraspecific, species, or broader taxonomic level, as long as these traits explain how organisms respond to, and influence, their environments (Violle et al. 2012; Enquist et al. 2015; Ward et al. 2019; Zakharova et al. 2019). At the same time, quantifying the relative importance of biodiversity loss at the species vs. intraspecific levels may be important to inform conservation, especially since diversity loss at the genetic level is substantial and may be underestimated relative to species loss (Bálint et al. 2011; Ceballos et al. 2017). Therefore, holistically understanding the relationships between species diversity, intraspecific diversity, and functional trait diversity is key to understanding how ecosystems function.

The conceptual frameworks used in trait-based ecology have been previously applied to phytoplankton communities (Litchman et al. 2007; Litchman and Klausmeier 2008); however, the body of empirical data is small compared to terrestrial systems (Siefert et al. 2015; Díaz et al. 2016), and especially with respect to intraspecific trait variation (but *see*, e.g., Edwards et al. 2015). For example, the recent meta-analyses by Des Roches et al. (2018) and Raffard et al. (2019) reveal that phytoplankton are absent from their systematic literature reviews on intraspecific variation. In one case study, the dinoflagellate *Alexandrium ostenfeldii* exhibited considerable variation in key functional traits like cell size, growth rate, nutrient uptake kinetics, and toxin content (Brandenburg et al. 2018). Diatoms also harbor extensive within-species genotypic and phenotypic variation (Godhe and Rynearson 2017 and references therein); e.g., intraspecific plasticity in traits like silica shell thickness and aggregation influences food web efficiency and carbon transport (Pan et al. 1996; Turner et al. 1998; Grønning and Kiørboe 2022; Ryderheim et al. 2022). Phytoplankton cell size is a key functional trait because it varies over orders of magnitude (both across and within species) and is linked to many ecophysiological processes connecting individual variation to ecosystem function; cell size influences, for example, primary production and nutrient uptake rates, trophic transfer via grazing, and sedimentation rates (Finkel et al. 2010; Marañón et al. 2013; Marañón 2015; Malerba et al. 2016; Hillebrand et al. 2022). Phytoplankton can also alter their phenotypes significantly depending on whether they are grown alone or in a culture of their conspecifics (Collins and Schaum 2021), indicating that insights gained from single clone cultures may not represent genetically diverse natural populations. Despite clear indicators for the breadth of intraspecific variation in phytoplankton, the relative importance of intraspecific vs. species richness in these globally essential primary producers remains to be fully elucidated.

In this study, we experimentally manipulated both intraspecific diversity (i.e., the richness of unique strains per species) and species richness in a community of diatoms with the goal of quantifying their independent and interactive effects on ecosystem functioning. We focus on cell size and cell shape as key functional traits that can vary both within and across species. Specifically, we use our experimental system to partition out the extent to which species and intraspecific richness effects influence function via their contributions to cell size/shape diversity vs. other traits linking biodiversity to functioning (i.e., traits not measured in our study). We use phytoplankton community biomass and growth rates as the ecosystem-level response variables. Furthermore, we conducted this experiment in six unique environments (combinations of temperature and nutrients) to test how the environment modifies diversity effects and to quantify the magnitude of diversity effects relative to the effects of temperature and nutrients. We assume that nutrients become limiting over time in the batch cultures; this temporal variation in dissolved nutrients allows niche partitioning in terms of resource-use traits (which are linked to cell size/shape) among the distinct species and strains. We tested the following hypotheses: H1: species richness and strain richness have equally positive effects on biomass and growth rates (H1a), and on functional trait diversity (H1b); H2: increasing strain richness mitigates negative effects of species loss (and vice versa); H3: greater functional trait diversity leads to greater biomass production, thereby mediating intraspecific and species diversity-functioning effects; and H4: environmental

conditions significantly influence species and strain richness effects on community biomass.

Methods

Experimental design

We employed three common marine diatoms (*Ditylum brightwellii*, *Rhizosolenia setigera*, and *Thalassionema nitzschioides*), with three unique strains per species isolated from either North Sea or Baltic Sea populations (*see* Supporting Information Methods). We used a gradient design where species richness and intraspecific richness (i.e., number of strains per species) were simultaneously manipulated to attain a gradient of 1, 2, or 3 species factorially crossed with 1, 2, or 3 strains; this resulted in 133 unique diversity combination treatments. These strain combinations were unreplicated within each environment, except for strain monocultures and full 9-strain polycultures, where replicates were included to create a more balanced design (Table S1). We grew all communities in 24-well culture plates, with 1.6 mL total volume per well, each well inoculated in a substitutive design with equal density of each constituent strain as measured by in vivo Chlorophyll *a* (Chl *a*) fluorescence (excitation/emission at 460/685 nm and output as relative fluorescence units, RFU, a unitless fluorescence measurement relative to a reference); all wells had equal total initial densities by Chl *a* fluorescence.

We created six unique environmental treatments, representing a factorial manipulation of nutrients (two levels: low, high) and temperature (three levels: 8°C, 12°C, 16°C). We used sterile-filtered North Sea water, diluted to a salinity of 25 PSU, as a growth medium for all cultures. To create the low and high nutrient treatments, we added N, P, and Si (i.e., $NaNO_3$, $NaH_2PO_4{\cdot}H_2O$, and $Na_2SiO_3{\cdot}9H_2O$) in Redfield molar proportions. The low nutrient treatment ($N = 16\ \mu mol\ L^{-1}$, $P = 1\ \mu mol\ L^{-1}$, $Si = 15\ \mu mol\ L^{-1}$) raised nutrient concentrations to levels representative of the coastal North and Baltic Seas (Topcu et al. 2011; Wohlers-Zöllner et al. 2012), while the high nutrient treatment ($N = 64\ \mu mol\ L^{-1}$, $P = 4\ \mu mol\ L^{-1}$, $Si = 60\ \mu mol\ L^{-1}$) raised nutrient concentrations to levels fourfold higher than the low nutrient treatment, to induce eutrophic conditions. Light was held constant across treatments ($125\ \mu mol\ photons\ m^{-2}\ s^{-1}$,16 : 8 light : dark).

We measured in vivo Chl *a* fluorescence daily using a Biotek Synergy H1 plate reader to track growth over time. We inspected the growth curves (Supporting Information Fig. S1) of each of the 864 experimental units daily. After a well had been in stationary phase for 3 d, it was preserved in 1% Lugol's solution for imaging (*see* Supporting Information Methods). From imaging analysis, we obtained size and shape measurements for > 41,000 cells, which were used to calculate mean, variance, and coefficient of variation (CV) in cell size and shape per experimental unit; all analyses of cell size/shape focus only on the high nutrient treatment in which cell densities were sufficient for characterizing size/shape distributions. Due to differences in growth rates among wells, experimental duration ranged from 7 to 16 d.

Statistical analyses

Analyses were performed in R version 4.3.2 (R Core Team 2020). Growth curves were fit to an exponential growth model using the R package "growthrates" (version 0.8.2; Petzoldt 2019) to estimate maximum growth rates. The growth of many communities had a poor fit with a logistic model, producing unrealistic estimates of carrying capacity. Therefore, carrying capacity (in terms of RFU) was instead calculated by taking the maximum fluorescence value per well over time.

We use linear models to test the interactive effects of species/strain richness, nutrients, and temperature on biomass and growth rate for the full dataset. To obtain a more integrative view of the underlying relationships among species, strain, and size/shape diversity, we also use a structural equation modeling (SEM) approach using coupled linear regressions in the "piecewiseSEM" R package (Lefcheck 2016) for the subset of units where size/shape data is available. This allows factors like size diversity to simultaneously act as both a response and a predictor variable, thereby enabling multivariate hypothesis testing that is particularly well-suited for addressing the complexity of causal pathways that drive biodiversity effects (Grace et al. 2016). To assess the relative importance of each variable in the model, raw coefficients were normalized by standard deviation (yielding standardized path coefficients), which accounts for different units and makes magnitudes of different effects directly comparable. Our selected model uses strain composition as a random effect, allowing partitioning of variance explained by fixed effects (i.e., the marginal R^2) and total variance explained by both fixed and random effects (i.e., the conditional R^2) (Nakagawa et al. 2017). Data and code are available on Zenodo (Thomas 2024).

Results

Interspecific and intraspecific variation in cell size and shape

The species used in this experiment exhibited substantial intraspecific variation in cell size and shape, which varied in magnitude among the three species (Fig. 1). In particular, the populations of *Rhizosolenia* covered approximately three orders of magnitude in cell volume. We also noted sexual reproduction in *Rhizosolenia* populations from Kiel; this is apparent in the skewed/bimodal size and shape distributions (Fig. 1; Supporting Information Fig. S2). The *Thalassionema* population from Helgoland had significantly smaller and less elongated cells than the other populations. *Ditylum*, however, had relatively little variation in size or shape distributions across strains. Plotting observations with the axes representing size × shape (Fig. 1C) further reveal clustering and separation of the strains in morphological trait space.

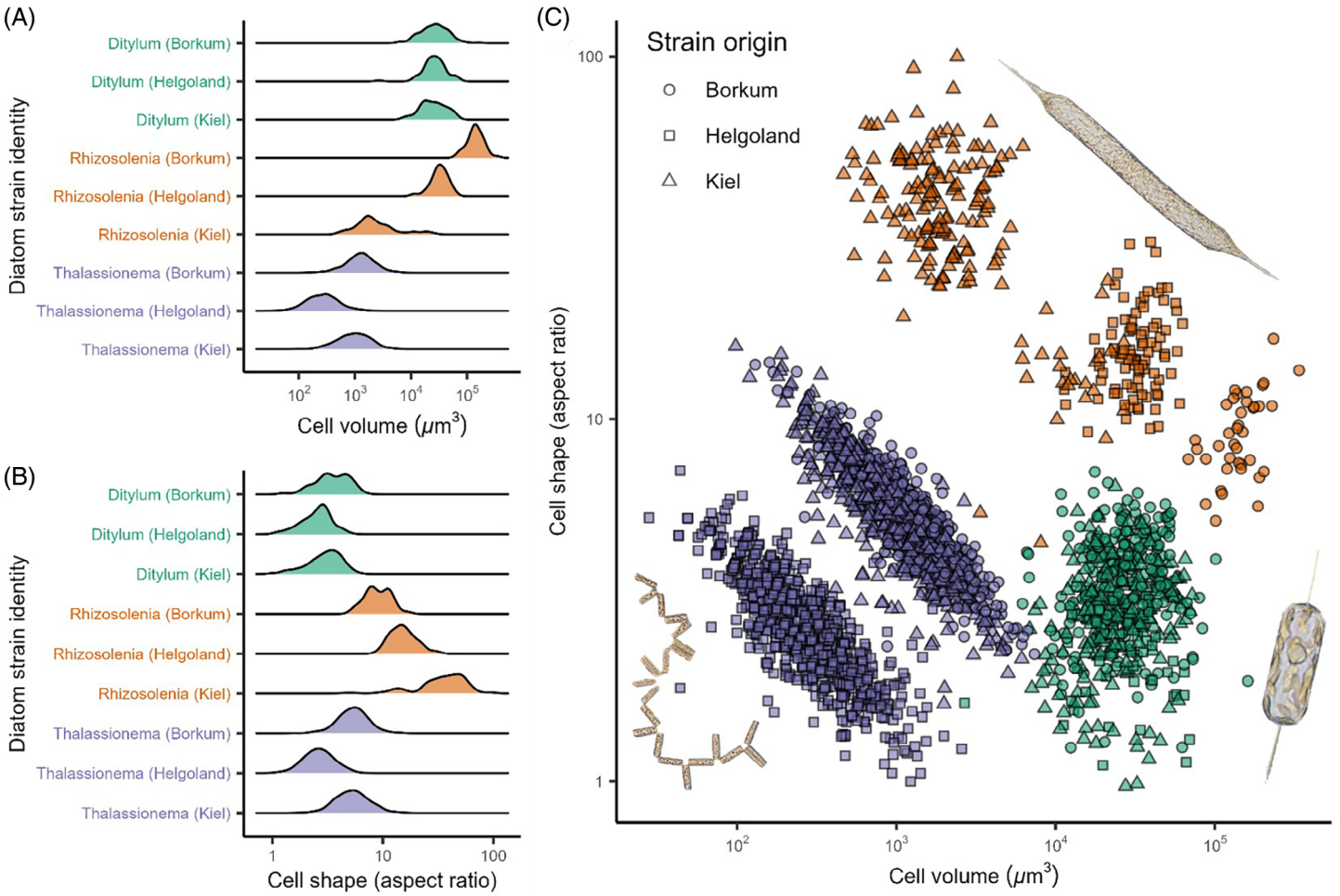

Fig. 1. The extent of variation in cell size and shape both within and across species used in this experiment when grown as strain monocultures. Density plots (**A**, **B**) show the distribution of size and shape for each strain. Each point in (**C**) represents an individual cell within each population, with color denoting species identity. Variation in cell size across populations was greatest for *Rhizosolenia* ($CV_{size} = 1.83$), followed by *Thalassionema* ($CV_{size} = 1.10$), while variation in shape was greater for *Thalassionema* ($CV_{shape} = 0.47$) than for *Rhizosolenia* ($CV_{shape} = 0.22$). *Ditylum* had the lowest variation in both size ($CV_{size} = 0.77$) and shape ($CV_{shape} = 0.15$). The data shown includes all six combinations of nutrients and temperature; *see* Supporting Information Fig. S2 for size distributions separated by each environment.

Interactive effects of species richness, strain richness, nutrients, and temperature on biomass

The maximum biomass of each experimental unit across all combinations of species and strain richness levels and environmental conditions is shown in Fig. 2 (summary data is in Supporting Information Fig. S3). Increasing strain and species richness both led to significantly greater biomass production, with effects of species richness slightly greater in magnitude than strain richness. However, a significant negative interaction term indicates that strain richness effects on biomass became weaker at higher species richness levels and vice versa (*see* Supporting Information Table S3 for interactive regression model results). These interactive diversity effects differed, however, depending upon the specific environment the communities were grown in. Most notably, when running linear models for species × strain richness interactions separately by each environment, the low nutrient treatments all had weak or insignificant diversity effects; and strong species by strain richness interaction effects only occurred at the higher nutrient level (Supporting Information Table S4).

Overall, the standardized effects of individual environmental factors like nutrients (0.38) and temperature (0.50) on biomass were greater than those of species (0.13) or strain (0.09) richness (Supporting Information Table S3). However, the model also revealed high-level interactions, including a species × strain × temperature × nutrient interactive effect, highlighting the strong interactivity among diversity and environmental conditions.

The growth rate was highly correlated with biomass (Spearman $\rho = 0.90$), and thus similar patterns emerged when assessing community-wide growth rates as the response

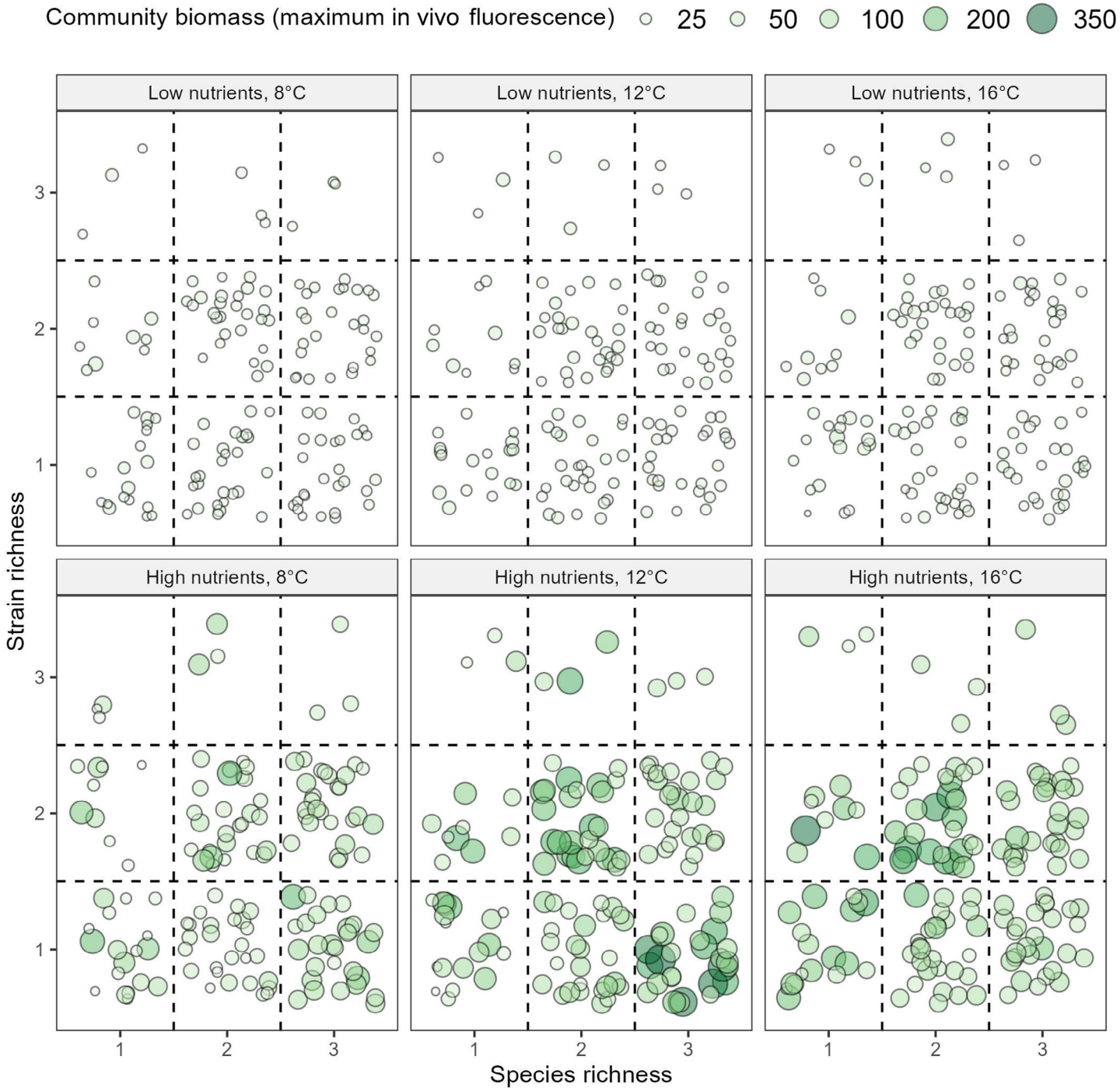

Fig. 2. Effects of species and strain richness on biomass production in six distinct environments. Each point represents one experimental unit. Biomass (in terms of relative fluorescence units, RFU) is shown by both color and point size. In high nutrient treatments, greater species richness (*x*-axis) led to increased biomass; however, the strength of these effects depended on the strain richness (*y*-axis). Under low nutrients, weakly negative effects of increasing species and strain richness were observed.

variable (Fig. 3; Supporting Information Fig. S4; Supporting Information Table S5); specifically, growth rates were also driven interactively by environment and species/strain richness. A model evaluating the presence/absence of certain strains showed that the presence of *Ditylum* strains had positive effects on biomass and growth rates, while *Rhizosolenia* and *Thalassionema* were linked to reduced growth (Supporting Information Table S6).

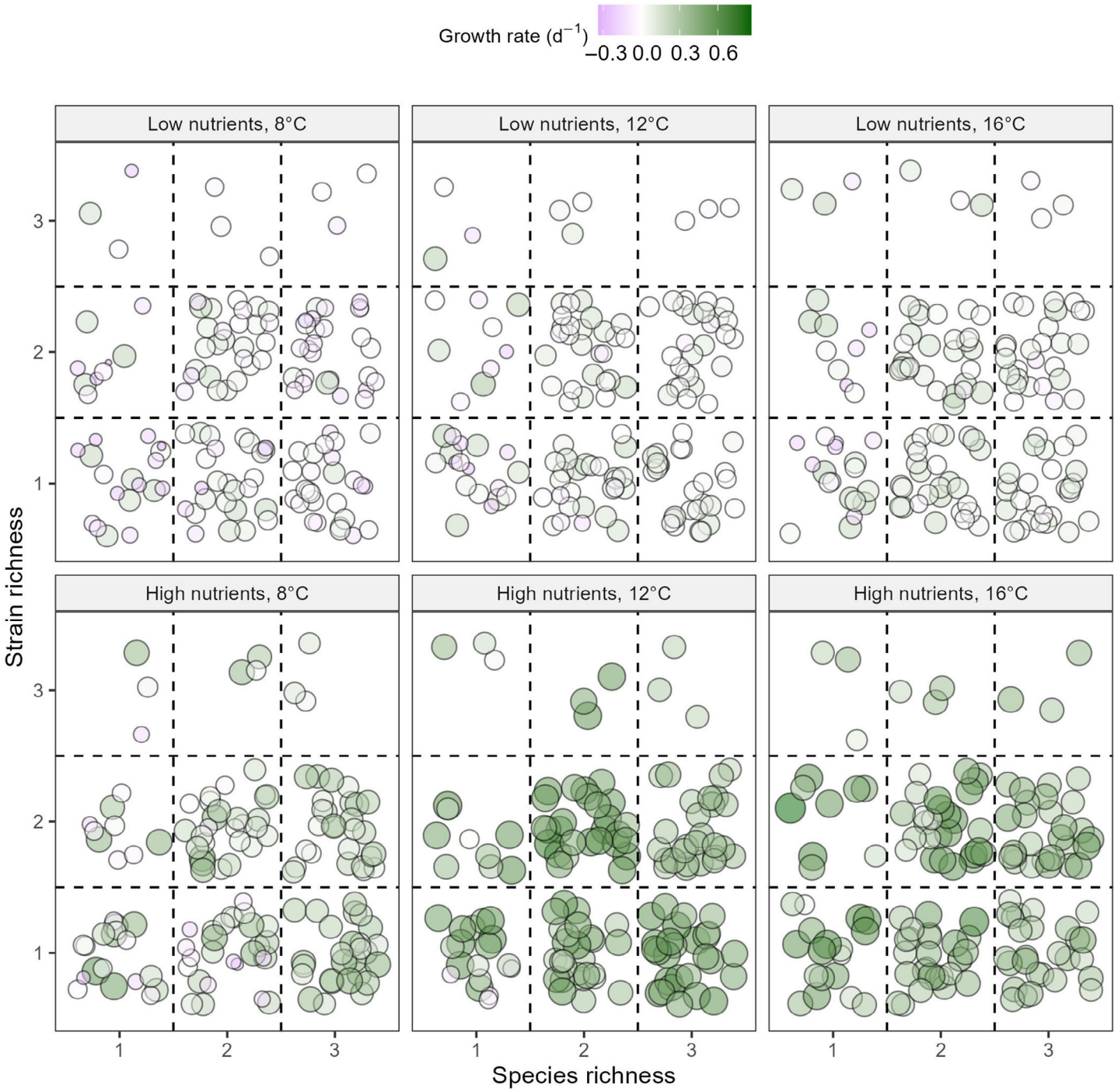

Fig. 3. Effects of species and strain richness on community-wide growth rate in six distinct environments. Each point represents one experimental unit. The growth rate is shown by both color and point size. As in the effects of diversity on total biomass (Fig. 2), species richness effects depend on the strain richness, and vice versa.

Integrating species richness, strain richness, and functional trait diversity effects using SEM

SEM was used to integrate direct and indirect effects of species/strain richness on biomass, including mean cell size and cell size diversity as intermediary factors. Figure 4 (and Supporting Information Table S7) shows the full SEM pathway analysis for the best-fitting model, while Fig. 5 shows partial effects of select variables from the fixed effects version of this

model. The SEM approach found the interaction term between species and strain richness to be among the strongest effects even after accounting for other variables (including strain composition), reaffirming that the effects of species richness in this experiment can only be interpreted when accounting for the effects of strain richness, and vice versa. This is also evident in the mutual dependency of species and strain richness effect slopes on one another (Fig. 5A,B). Temperature effects on biomass were positive but smaller in magnitude relative to species richness. Species richness and temperature had moderate positive effects on cell size variance (Figs. 4, 5C), while strain richness and the species/strain interaction had no effect (Figs. 4, 5D). Increased cell size diversity had a negative effect on biomass (Figs. 4, 5E); thus, increased cell size diversity per se was not responsible for linking species/strain diversity to positive effects on ecosystem function. Greater mean cell size, however, was associated with higher biomass (Figs. 4, 5F). Strain composition explained the majority of the variance in cell size diversity (54%) and mean cell size (75%); explanatory power by the fixed effects was low for these responses (Fig. 4). However, the fixed effects had higher explanatory power for biomass (29%) than for cell size. Additional formulations of the SEM, including cell shape and absence of random effects, had poorer fit in terms of AIC; however, their conclusions are consistent with those of the model shown here (*see* Supporting Information Tables S8, S9).

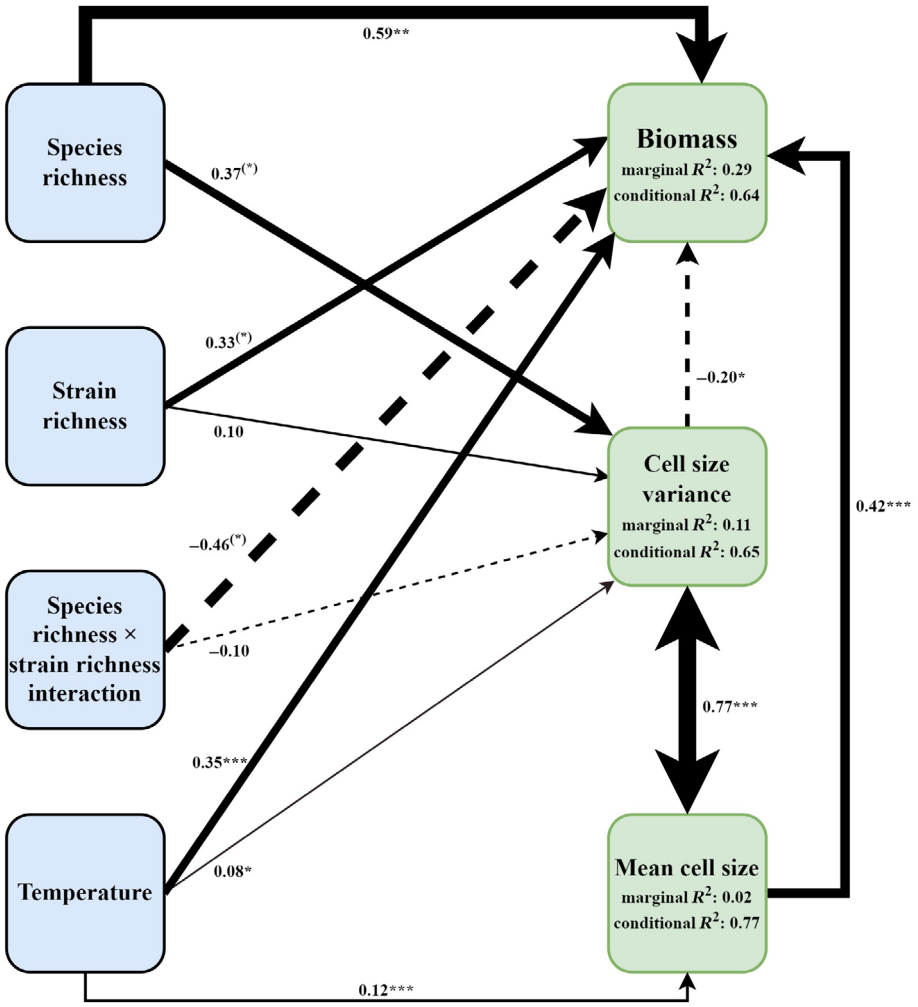

Fig. 4. Structural equation model results showing effects of species/strain richness and temperature on biomass production and cell size variance of diatom communities. Line thickness and numbers adjacent to lines both show the standardized path coefficients, a measure of the relative magnitude of each variable's impact (i.e., normalized by standard deviation to account for units of different magnitudes). Solid lines indicate positive effects; dashed lines indicate negative effects; significance codes are: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; $^{(*)}p < 0.1$. Single-headed arrows denote hypothesized unidirectional effects while double-headed arrows denote correlations. Marginal R^2 corresponds to the variance explained by fixed effects; conditional R^2 corresponds to the total variance explained by the random effect (i.e., strain composition) and the fixed effects. This SEM path analysis shows species and strain richness both have significant direct effects on biomass production, as well as indirect effects mediated by size diversity.

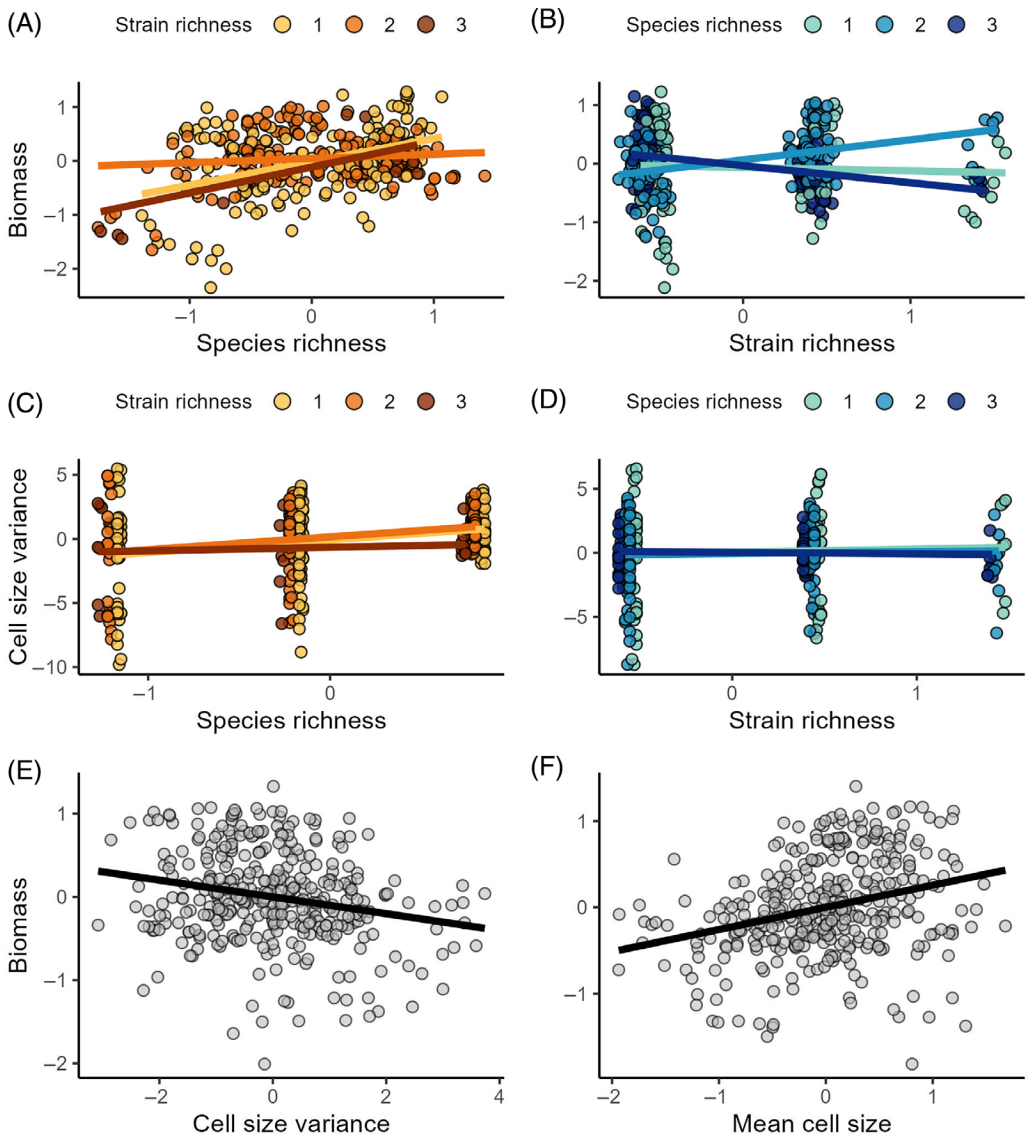

Fig. 5. Partial effects of species richness and strain richness on biomass (**A**, **B**); partial effects of species richness and strain richness on cell size variance (**C**, **D**); and partial effects of cell size variance (**E**) and mean cell size (**F**) on biomass. Specifically, the slopes of the lines show the magnitude of the effect of one variable after all other variables are accounted for. Panels **A–D** show the degree of interactions between species and strain richness by showing that the slope of the species richness effects varies depending upon the strain richness level (and vice versa). Effects of species richness (**C**) and strain richness (**D**) on cell size diversity, however, were additive, with a predominance of species richness effects. Partial effects of size diversity (**E**) and mean size (**F**) on biomass were negative and positive, respectively. All panels show data for the high nutrient conditions only; units are standardized partial residuals from the fixed effects version of the multivariate SEM model in Fig. 4 (*see* Supporting Information Table S9), produced with the "partialResid" function in the "piecewiseSEM" package.

Discussion

Interactive effects of intraspecific and species richness

Our results demonstrate the importance of intraspecific variation in diatom communities by showing that species and intraspecific diversity effects on biomass are nearly equivalent in magnitude (thus supporting H1a). Supporting H2, our results reveal how intraspecific diversity can mitigate the effects of species loss on ecosystem processes. The data do not support H1b, as species but not strain richness significantly influenced functional trait diversity.

Our results are congruent with recent syntheses highlighting that the effects of intraspecific diversity on ecosystem functioning are often equivalent to the effects of species diversity (Des Roches et al. 2018; Raffard et al. 2019). However, our data also provide novel information regarding the interactivity and substitutability of species and strain richness. As one illustrative example, starting with species richness = 3 and

strain richness = 1, biomass is relatively high (Fig. 2; *see* the 12° and high nutrient treatment). Removing one species causes an average reduction in biomass. However, if strain richness is simultaneously increased (i.e., species richness = 2 and strain richness = 2), then biomass again reaches a relatively high level.

However, increasing strain richness led to reduced biomass in some cases, particularly when increasing strain diversity to three strains. Such underyielding effects have been previously reported for species richness effects (Schmidtke et al. 2010). While mechanisms for intraspecific underyielding are less clear, potential explanations include growth reduction linked to chemical cues (Collins and Schaum 2021), dominant strains being less productive (Schmidtke et al. 2010), or antagonism by diatom-associated microbial symbionts (Sison-Mangus et al. 2014). Although we chose diatoms as representative marine phytoplankton, other taxa with distinct packages of functional traits could have idiosyncratic diversity-functioning relationships differing from our observations.

The role of functional trait diversity

The results from our experiment reject H3 because cell size diversity had a negative relationship with biomass after accounting for other variables. One possible explanation is that the optimum cell size converged to a single value, making variation around the mean detrimental to biomass production. This is indeed one prediction of Trait Driver Theory: while shifting environmental conditions should induce changes in trait distributions, a static environment should select for one optimum trait value with little to no trait variance. This means that increased variance of any key trait linked to productivity should have a negative effect on productivity under fixed environmental conditions (Enquist et al. 2015). In our study, larger mean cell size was associated with higher biomass, indicating that larger cells may have been more optimal under the high-nutrient treatments and that deviations from this could reduce biomass. Thus, our results from marine diatoms are analogous to illustrative examples in terrestrial trait-based ecology, where greater mean specific leaf area correlates positively with productivity, while greater variance in specific leaf area correlates negatively with productivity (Enquist et al. 2015). Our results are also consistent with modeling work showing phytoplankton size diversity may have weak negative effects on productivity (Chen et al. 2019).

In addition, the level of abiotic heterogeneity in this high-throughput lab experiment was relatively low. Theory predicts that trait diversity-functioning relationships should be strongest when high environmental variability matches high trait diversity (Hodapp et al. 2016), and that temporally variable environments should have greater trait variance than static ones (Enquist et al. 2015). Previous observations over broad biogeographic scales indeed show that phytoplankton cell size diversity and primary production are greater in temperate than tropical systems, which may be linked to greater variability in temperate oceans (Acevedo-Trejos et al. 2018). Complementary work in the future should thus assess the role of intraspecific trait variation in phytoplankton in fluctuating environments (Gerhard et al. 2019, 2022). As with any simplified lab environment, important natural processes like losses to grazing, parasitism, and sinking were also excluded. Incorporating these factors could create additional trade-offs between traits and alter the relationship between size diversity and functioning (Kiørboe 1993; Litchman and Klausmeier 2008; Smith et al. 2011).

The fact that cell size alone does not fully explain how functional traits mediate diversity-functioning relationships also points toward the complexity of functional traits in phytoplankton. Although cell size is generally considered a master trait (Litchman and Klausmeier 2008; Marañón 2015), as it is strongly linked to resource uptake, grazing rates, sinking rates, growth rates, and so forth, it does not necessarily scale perfectly with other processes, especially when considering size-specific scaling (Hillebrand et al. 2022). This means information on other traits (e.g., thermal performance, secondary metabolites, colony formation, mixotrophy, nutrient requirements/uptake rates/storage, and many others) can provide substantial information in addition to size (Litchman and Klausmeier 2008). Quantifying many distinct traits poses a substantial challenge for empiricists as it requires greater methodological complexity. However, we suggest that working towards characterizing the entire multivariate trait-based fitness landscape ("trait-scape") of phytoplankton (Argyle et al. 2021; Walworth et al. 2021) is a promising approach for future biodiversity-functioning studies.

Implications for biodiversity and ecosystem functioning research

In support of H4, diversity-functioning relationships were contingent on the environmental context. The effect of nutrients on diversity-functioning relationships was strongest, as diversity effects were weakly negative in low nutrients and mostly positive with higher nutrients. This contrasts with recent work showing no interaction between phytoplankton diversity and nutrient supply on biomass (Gerhard et al. 2020). We expected diversity effects to be stronger with low nutrients due greater importance of resource-use complementarity; however, our results suggest the potential importance of non-resource based interactions. Temperature also influenced diversity effects, which were greatest at 12°C. This corresponds closely to the sea surface temperature upon collecting and maintaining each strain (i.e., the temperature strains were most acclimated to). Our results thus highlight the importance of considering environmental contingency in diversity-functioning research, for both past and future studies.

Our analysis stresses how multivariate hypotheses can be used to better understand the nature of relationships between diversity, traits, and ecosystem processes. The fact that strain

richness significantly modified species richness effects points to a clear need to integrate intraspecific variation into future research on biodiversity change. In particular, we hope our results will stimulate further research on the importance of intraspecific variation in other phytoplankton taxa, both freshwater and marine, where data is currently lacking. In summary, this experiment provides an example of how biodiversity-ecosystem-functioning research can incorporate both intraspecific variation and environmental heterogeneity to better explain the role of biodiversity in aquatic ecosystems.

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