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LETTER

Beyond nitrogen: phosphorus – estimating the minimum niche dimensionality for resource competition between phytoplankton

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Abstract

The niche dimensionality required for coexistence is often discussed in terms of the number of limiting resources. N and P limitation are benchmarks for studying phytoplankton interactions. However, it is generally agreed that limitation by small numbers of resources cannot explain the high phytoplankton diversity observed in nature. Here, we parameterised resource competition models using experimental data for six phytoplankton species grown in monoculture with nine potential limiting resources. We tested predicted species biomass from these models against observations in two-species experimental mixtures. Uptake rates were similar across species, following the classic Redfield ratio. Model accuracy levelled out at around three to five resources suggesting the minimum dimensionality of this system. The models included the resources Fe, Mg, Na and S. Models including only N and P always performed poorly. These results suggest that high-dimensional information about resource limitation despite stoichiometric constraints may be needed to accurately predict community assembly.

Keywords

Cyanobacteria, green algae, Hutchinson, macronutrients, microcosm, micronutrients, N:P, phytoplankton, Redfield, trace elements.

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INTRODUCTION

Redfield and resource ratios

The observation of a common stoichiometric ratio of carbon, nitrogen and phosphorus (C:N:P as 106:16:1 respectively) in marine environments and the planktonic life within it (Redfield 1934, 1958) has profoundly impacted ecological theory. The ratio of the macronutrients N and P has become a focus of phytoplankton ecology in marine (Galbraith & Martiny 2015) and freshwater (Dzialowski 2005; Poxleitner *et al.* 2016) environments. Co-limitation by N and P is common for autotrophs across systems (Elser *et al.* 2007). Internal C:N:P ratios differ from available resource ratios comparably to Redfield (Geider & La Roche 2002), as both growth and uptake are balanced by the absolute and relative availability of N and P (Klausmeier *et al.* 2004). However, resource uptake of other elements that autotrophs require is less well described (Kaspari & Powers 2016), except for certain phytoplankton species (Ho *et al.* 2003; Facey *et al.* 2019). In natural systems, coexistence of highly diverse phytoplankton communities growing on shared resources is common, despite strong competition expected due to similar resource needs of different plankton species. This 'paradox' – many species relative to the number of shared limiting resources – is described in Hutchinson (1961). Essentially, a limited number of resources constitutes a dimensionality that cannot accommodate the requirements of all species without strong overlap between their niches (Hutchinson 1957). Strong niche overlap ultimately leads to competitive exclusion, and thus contradicts the high diversity observed. Additional factors, such as temporal or spatial heterogeneity or predation, can allow for greater diversity than predicted by resource number alone (Hutchinson 1961).

Resource competition and number of resources

The resource ratio or resource competition hypothesis (Tilman 1980, 1982; Miller *et al.* 2005) states that shifts in the relative supply ratio of two resources can lead to dominance shifts between two species depending on which species can still grow at the lowest concentration of the limiting resource (R*). It has since been used in theoretical models to explain diversity patterns based on more than two resources and their absolute and relative concentrations and other environmental factors (Chase & Leibold 2003). Several authors have pointed out that empirical tests have been comparatively rare (Tilman 1980, 1982; Miller *et al.* 2005) and existing empirical studies

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have primarily considered only one or two limiting resources (Miller *et al.* 2005).

Effects of multiple resources on coexistence

For homogeneous environments, the resource ratio hypothesis predicts that a greater number of limiting resources should enable higher niche dimensionality, thereby allowing coexistence of a number of species up to the number of limiting resources. Observational studies for phytoplankton communities show that increases in the number of limiting resources from one to four (including N, P, Si and available light) linearly increased diversity as well (Interlandi & Kilham 2001). Additional abiotic factors such as temporal variability (Grover 1991), spatial heterogeneity (Lehman & Tilman 1997) or temperature (Thrane *et al.* 2017; Gerhard *et al.* 2019) or biotic factors, e.g. predators (Holt & Bonsall 2017) can allow for much higher dimensional coexistence than the number of resources can account for. Resource pulses for example as sudden spatio-temporal changes in availability modify the dimensionality (Sommer 1984). Thus, different limiting resources may vary in their effects on the structure of a phytoplankton community, and aspects of temporal and spatial heterogeneity are likely influences on successional patterns (Tilman 1982). A model indicated coexistence of up to twelve species on five limiting resources based on the varying competitive strength of each species at any combination of these resources yielding oscillatory community dynamics (Huisman & Weissing 1999). In a global study by Harpole *et al.* (2016) grassland plots lost more species when the number of limiting resources was reduced through experimental fertilisation.

Despite strong evidence for multiple resource limitation in phytoplankton communities, resources other than N, P and Si are seldom considered in empirical studies. This omission is likely driven by the difficulty of measuring concentrations of other resources (Interlandi & Kilham 2001). Fe especially is deeply involved in N uptake through nitrate reduction, photosynthetic pathways and many enzymatic reactions (Procházková *et al.* 2014; Borowitzka *et al.* 2016). Precipitation of P and Ca with Fe occurs in both fresh- and seawater, although much faster in seawater (Gunnars *et al.* 2002). Limitation by Fe on cyanobacterial growth may be lessened for species due to responsive production of siderophores in deplete conditions (Wilhelm & Trick 1994).

The novel field of *elementome* research aims to quantify multi-dimensional stoichiometric niches with regards to their environmental context (González *et al.* 2017). Vascular plants take up relatively more of secondary resources than warranted by their N and P uptake, independent of species or treatment (Ågren & Weih 2020). Other studies find differential responses of these scaling ratios to treatments for within plant concentration (Tian *et al.* 2019) and foliar elementomes of three tree species, thus improving the likelihood of their pairwise co-existence (Peñuelas *et al.* 2019). Research of multi-species phytoplankton competition in the elementome context is, however, still lacking.

We investigated how uptake rates for a high-dimensional set of resources differed among species, and how a greater number of resources improved predictions of species biomass in multi-species mixtures. To this end, we parameterised monoculture uptake models for six phytoplankton species grown in experimental microcosms, based on resource uptake rates for all possible combinations of nine resources: N, P, S, Ca, Fe, K, Mg, Mn and Na, as well as two different temperatures to create species-specific differences in uptake rates. We implemented competition models to predict species-level biomass for all pairwise mixtures of these six species. These competition models took two forms: the uptake-based model, where competitive strength depends on species-specific, empirical uptake rates and the complete niche overlap model with fixed uptake rates across resources and species. Lastly, we compared model predictions to observations from separate laboratory experiments in order to determine the minimally sufficient niche dimensionality in short-term resource competition for accurate predictions in phytoplankton experiments.

MATERIALS AND METHODS

Stock cultures

We selected six pro- and eukaryotic autotrophic strains that co-occur in natural freshwater environments: the green algae *Acutodesmus obliquus* SAG 276-3a, *Desmodesmus armatus* SAG 276-4d, *Oocystis marssonii* SAG 257-1 and the cyanobacteria *Chroococcus minutus* SAG 41.79, *Microcystis aeruginosa* SAG 1450-1, *Synechocystis sp.* PCC 6803. We refer to all these species only by genus name throughout the study. All strains were obtained from the Culture Collection of Algae (SAG; University of Göttingen, Germany) and the Pasteur Culture collection of Cyanobacteria (PCC; Institut Pasteur, France). These species differ in their cellular morphology and physiology, and exhibit different growth dynamics and trait shifts across environmental conditions (Lürling *et al.* 2013; Hofmann *et al.* 2019). They contain species that are slower (*Oocystis*, *Desmodesmus*, *Chroococcus*) and faster growing (*Acutodesmus*, *Synechocystis, Microcystis*), with phycobilins (all cyanobacteria) or without (all green algae), more sensitive to warming (*Chrococcus, Desmodesmus*) or less (*Acutodemus, Synechocystis*) and high cell wall resistance (*Desmodesmus, Acutodesmus*) and low (*Oocystis*) (Dunker & Wilhelm 2018). Their stocks were maintained as semi-continuous cultures in WC-medium (see Table S1 for nutrient composition) at room temperature (*c.* 21 °C) with a 14|10 h light/dark cycle with 60 μmol photons m^{-2} s^{-1} (eco + SKY(6500K) ID15-12-13, LEDaquaristik, Hövelhof, Germany), and constantly shaken and diluted to 250 mg dry weight L^{-1} twice per week with fresh medium to maintain similar physiological states and minimise potential, bacterial contamination. Stocks were monitored with microscopy and flow cytometry.

Growth conditions

All experiments were independently replicated at 20 and 30 °C to capture a range of resource uptake kinetics. Previous experiments showed that temperature gradients were a stronger modulator of growth rates than N:P gradients (ANOVA based on data from Hofmann *et al.* 2019: $p_{temp} = 0.11 * 10^{-6}$ compared to $p_{NP} = 0.021$) in WC medium. For all species, at

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least one temperature offered maximum growth rates close to their physiological optimum (Hofmann *et al.* 2019).

Monoculture experiments

To parameterise uptake models, monoculture microcosms ran for 11 days with three replicates per treatment. Treatments were inoculated at 25 mg dry weight L^{-1} with 150 mL fresh WC medium in 250 mL Erlenmeyer flasks from semi-continuously cultured stock cultures and maintained in a climate cabinet (CU-22L, CLF PlantClimatics) at a 14|10 h light and dark cycle with 60 μmol photons m^{-2} s^{-1} (PHILIPS ALTO II F17T8/TL841 17 W). Culture flasks were manually shaken to achieve thorough mixing on sampling days, and were sampled on days 2, 4, 7 and 11 to measure biomass growth, single cellular traits and resource uptake. 12.5 mL culture volume was filtered on a polycarbonate filter with pore size 0.4 μm (LABSOLUTE®, Th. Geyer, Renningen, Germany). The filtrate was split in 5 mL for elemental analysis with an ICP-OES (iCAP 7400 Duo, Thermo Fisher Scientific) and 5 mL for colorimetric determination of nitrate (visocolor® ECO Nitrate, Macherey-Nagel, Düren, Germany). Growth was tracked based on dry weight biomass differences of filters before and after filtration. Individual-level cell traits were recorded through imaging flow cytometry with the custom order ImageStream-X Mark II (Amnis part of Luminex, Texas, Austin, USA), returning physiological traits based on red fluorescence intensity and cell-morphological traits based on picture-derived values.

Competition experiments

For competition experiments, flasks were inoculated with 12.5 mg dry weight L^{-1} concentration of each component species with 150 mL fresh WC medium in triplicates for each pairwise combination. Experimental procedure was otherwise identical to the monoculture setup.

Resource measurements

All resources were measured in culture filtrate collected for dry weight to derive indirect bulk resource uptake rates (Fig. 1). Nitrate was measured from 5 mL filtrate volume. Reagents (visocolor® ECO Nitrate, Macherey-Nagel, Düren, Germany) were added following kit protocols using three 300 μL samples transferred to 96-well plates. Following the same procedure, reagents were added to calibration standards of WC medium (0, 25, 50, 75 and 100% dilution) in ultrapure water, which were transferred to plates at the same volume and replication size. The microtitre plate was then measured with a plate-reader (Variolux Scan, ThermoFisher Scientific, Waltham, Massachusetts) at an absorption wavelength of 540 nm. NO_3 concentration was then recalculated as N (m_N/m_{NO3} = 22.58 % of NO_3).

Filtrate samples for elemental measurements were fixated 1:1 with ultrapure 4% nitric acid solution and stored at 4 °C. The ICP-OES was calibrated with mono-elemental, high-purity grade 1 g L^{-1} stock solutions of Ca, Fe, K, Mg, Mn, Na, P, S and Y for the calibration curves and an ICP Multi-Element Standard Solution in 5% HNO_3 (28 elements, 100 mg/L) (Carl Roth, Karlsruhe, Germany). An internal standard of 2 mg L^{-1} Yttrium was added to samples to compensate for matrix effects and drift correction (Table S2).

Lastly, to summarise resource uptake rates across both temperature treatments, and to account for expected correlations among resources, we conducted a PCA decomposition of monoculture uptake rates for each resource across species. The first axis of the resulting PCA explained over 80% of total variation in resource uptake rates, suggesting a relatively constrained, linear relationship among uptake rates across all species. (Fig. S1 in the supplement).

Imaging flow cytometry

Flow cytometric measurements were performed according to Dunker (2019) and Hofmann *et al.* (2019). In brief: two light paths from the flow cytometer were selected. The 488 nm excitation laser (0.15 mW intensity) yielded bright field images and fluorescence signals with bandpass 642–745 nm for red (mainly emitted by chlorophyll a) fluorescence and the 561 nm laser (80 mW intensity, neutral density filter 1.0) with bandpass 595–642 nm for orange (mainly emitted by phycobilin) fluorescence. A base template using chlorophyll a and phycobilin fluorescence was used within the firmware IDEAS 6.2 (Amnis part of Luminex, Texas, Austin, USA) to differentiate cyanobacteria and green algae. Gates were added including morphological features based on cell size depicted on the bright field images with the 'Adaptive Erode 90% mask' to distinguish between species. For differentiation between *Acutodesmus* and *Oocystis*, gates were adjusted manually to encompass the majority of uniquely identifiable single cells.

Biomass mixed culture calculation

Contrary to direct biomass measurements from filters in monocultures, cell volumes (in μm^3/cell) were calculated based on cell shape (Hillebrand *et al.* 1999) with parameter values derived from the bright field images. Selected cell shapes were: spheres for all cyanobacteria; double-cones for *Acutodesmus*; rotational ellipsoids for *Desmodesmus* and *Oocystis* (Olenina *et al.* 2006). For each treatment, the sum of individual cell volumes (in μm^3) of each species was calculated and divided by the total sum of cell volume (in μm^3) (of all species) in the sample. This fraction was then multiplied with total dry weight concentration of the sample c_{tot} to achieve species level dry weight biomass concentrations c_i per sample.

$$c_i = c_{tot} V_i / V_{tot} \quad (1)$$

Models

We used measured monoculture uptake rates to quantify species-specific biomass growth as a function of all possible combinations of the nine resources. We then fit two models of multi-species growth: an 'uptake-based' model, in which species-specific uptake rates inform competitive effects of species on each other, and a 'complete niche overlap model', where all species share a universal uptake rate for all resources.

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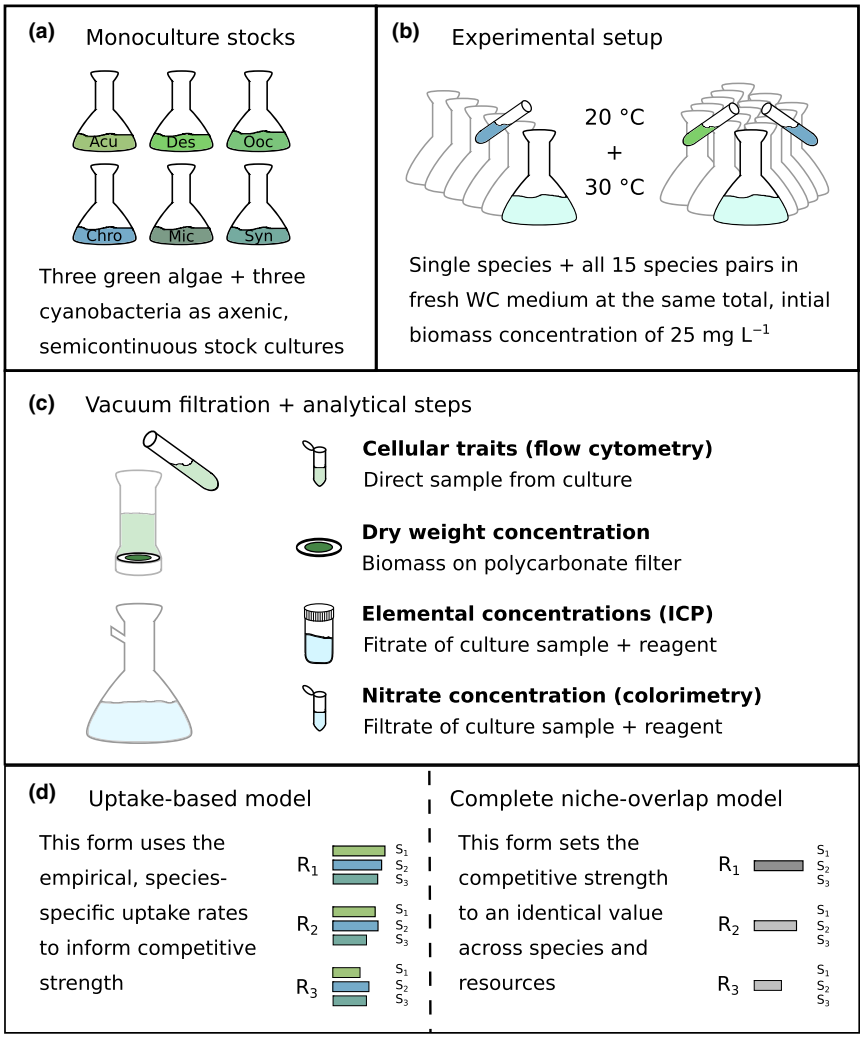

Figure 1 Conceptual flow chart detailing (a) monoculture stocks, (b) experimental setup and (c) measurement steps and (d) the two forms of the resource competition model.

Monoculture uptake model

For each species in monoculture, we used data on biomass and resource concentrations over time to parameterise resource uptake models. These draw on a long tradition of modelling resource uptake rates to predict microbe dynamics (Monod 1949; Tilman 1982), although we apply a somewhat simplified functional form. Specifically, we assume that species take up resources at a fixed rate per unit biomass growth, yielding

$$\mathrm{d}R_j/\mathrm{d}B_i = -g_{i,j} \tag{2a}$$

which we can rewrite as

$$\mathrm{d}R_j = -g_{ij}\mathrm{d}B_i \tag{2b}$$

where R_j is concentration of resource j, B_i is biomass of specieI i, and $g_{i,j}$ is the uptake rate. This implies that resource dynamics follow.

$$R_j = R_{0,j}\exp(-g_{i,j}B_i) \tag{3a}$$

which can be rewritten as

$$R_j = exp\left(log\left(R_{0,j}\right) - g_{i,j}B_i\right) \tag{3b}$$

where $R_{0,j}$ is initial resource concentration. These equations show that for every unit of growth in species i, $g_{i,j}$ units of resource j are consumed. Note that we can fit eqn (3b) to observations using ordinary least squares regression of the form `lm(log(Rj)~Bi)`, where the intercept equals log ($R_{0,j}$), and the slope equals $g_{i,j}$. This model therefore explicitly considers growth across the whole physiological spectrum, as uptake rate is maximised if nutrient concentration is high enough, and decreases only when resources are depleted.

Resource competition model

After fitting uptake rates for each species and resource following eqn (3b), we used these to predict the strength of competitive interactions between species. First, we used eqn (2) to show the effect of an increase in biomass, ΔB, on initial resource concentration R_0 as

$$R_{new} = \exp(\log(R_0) - g_i(B_0 + \Delta B)) \tag{4a}$$
$$= \exp(\log(R_0) - giB_0)\exp(-g_i\Delta B) \tag{4b}$$
$$= R_0\exp(-g_i\Delta B) \tag{4c}$$

and thus

$$\log(R_{new}/R_0)/(-g_i) = \Delta B \tag{5}$$

If we further assume that consumption vectors represent the degree to which species are limited by each resource (i.e. that the impact and requirement niches are interchangeable;

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(Chase & Leibold 2003)), then we can use eqn (5) to estimate expected changes in biomass related to a change in resource availability. We therefore used eqn (4c) to calculate R_{new}, and plugged the result into eqn (5) to calculate expected effects on species i caused by an increase in the biomass of species k as

$$\Delta B_i = \log((R_0 \exp(-g_k \Delta B_k))/R_0)/(-g_i) \quad (6a)$$
$$= \log(\exp(-g_k \Delta B_k))/(-g_i) \quad (6b)$$

For our analyses, we apply eqn (6b) to observational data using initial concentration of resources for R_0, and $\Delta B_k = 1$. The resulting statistic shows expected effects on species i of an increase of one unit biomass in species k, and can be interpreted roughly in the same manner as 'alpha' competition coefficients in the classical Lotka–Volterra competition equations, such that

$$\alpha_{ik} \approx \log((R_0 \exp(-g_k))/R_0)/(-g_i) \quad (7)$$

To combine information from across multiple resources, we calculated mean α for each species pair across resources. To estimate expected biomass of species in mixture, we used the standard formula for Lotka–Volterra competition.

$$B_i(\text{expected}) = B_i(monoculture) - \alpha_{ik} B_k(mixture) \quad (8)$$

We then used eqn (8) to generate two sets of model predictions. For the 'uptake-based model', we used empirically determined resource uptake rates from eqn (7) to generate α_{ik} values. Additionally, for the 'complete niche overlap' model, we set $\alpha_{ik} = 1$ for all species i and k (i.e. species per-capita inhibition of their own growth is equal to their per-capita inhibition of other species). For both models, α_{ik} values remained close to 1, indicating relatively simple, non-cyclical dynamics, in accordance with empirical observations.

Finally, we compared model predictions to observations of species biomass in multi-species mixtures using the E^2 criterion (Legates & McCabe 1999). This statistic is similar to R^2, but measures scatter around the 1-1 line, as

$$E^2 = 1 - \Sigma[(predicted - observed)^2]/\Sigma[(observed - mean[observed])^2] \quad (9)$$

The index ranges from 1 (perfect fit) to negative infinity (predicted values much worse than the grand mean). Values of zero indicate that predictions perform exactly as well as the grand mean, and are considered the minimum threshold for demonstrating information value in a model. Importantly, note that 'training' data (i.e. monoculture biomass) and 'testing' data (i.e. biomass in mixture) are independent. Thus, even though we use regression to calculate uptake rates, we never 'tune' models to fit observations of multi-species biomass, and there is no a priori statistical reason to expect that models that include more resources or more parameters should provide higher goodness of fit, just as is true for cross-validation, AIC and related statistics (Wenger & Olden 2012; Roberts *et al.* 2017).

RESULTS

Models that included a greater number of resources gave better predictions of biomass than models with fewer resources, and models containing Mg, S, Na K or Fe performed significantly better than those containing only N, P or N & P together. The positive relationship between goodness of fit and number of resources was therefore not simply due to an increase in dimensionality (Fig. 2); i.e. resource identity also contributed. Interestingly, the competition model based on monoculture uptake rates rarely outperformed the goodness of fit of the 'complete niche overlap' model, where the interaction coefficient $\alpha_{ik} = 1$ for all species. This indicates that the impact of one species on its competitor was to a large degree fairly independent of resource identity.

Species biomass in monocultures showed a low explanatory power (E^2) for proportional species biomass in respective mixtures 0.31 for 20 °C and 0.37 for 30 °C including all six species (Fig. 3, top row). *Acutodesmus* produced the overall highest biomass and tended to have higher biomass than expected from monoculture. Similar over-yielding was observed for *Microcystis* and *Synechocystis* in some

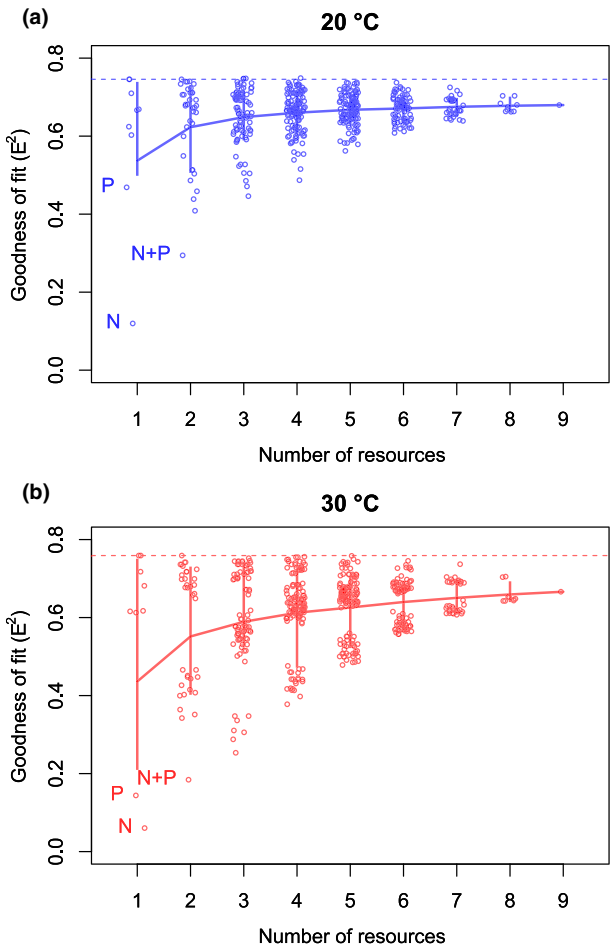

Figure 2 Goodness of fit for predictions of species biomass in mixture as a function of the number of resources used to calculate competition coefficients. Each point is for a single combination of resources. Line shows mean trend with confidence interval. For comparative purposes, models that include only N and P are labelled. The dashed lines show the mean prediction of 'complete niche overlap' model (i.e. interaction coefficients set to 1) at each temperature. Note that goodness of fit tends to increase with the number of resources, but rarely out-performs predictions from the model with complete niche overlap.

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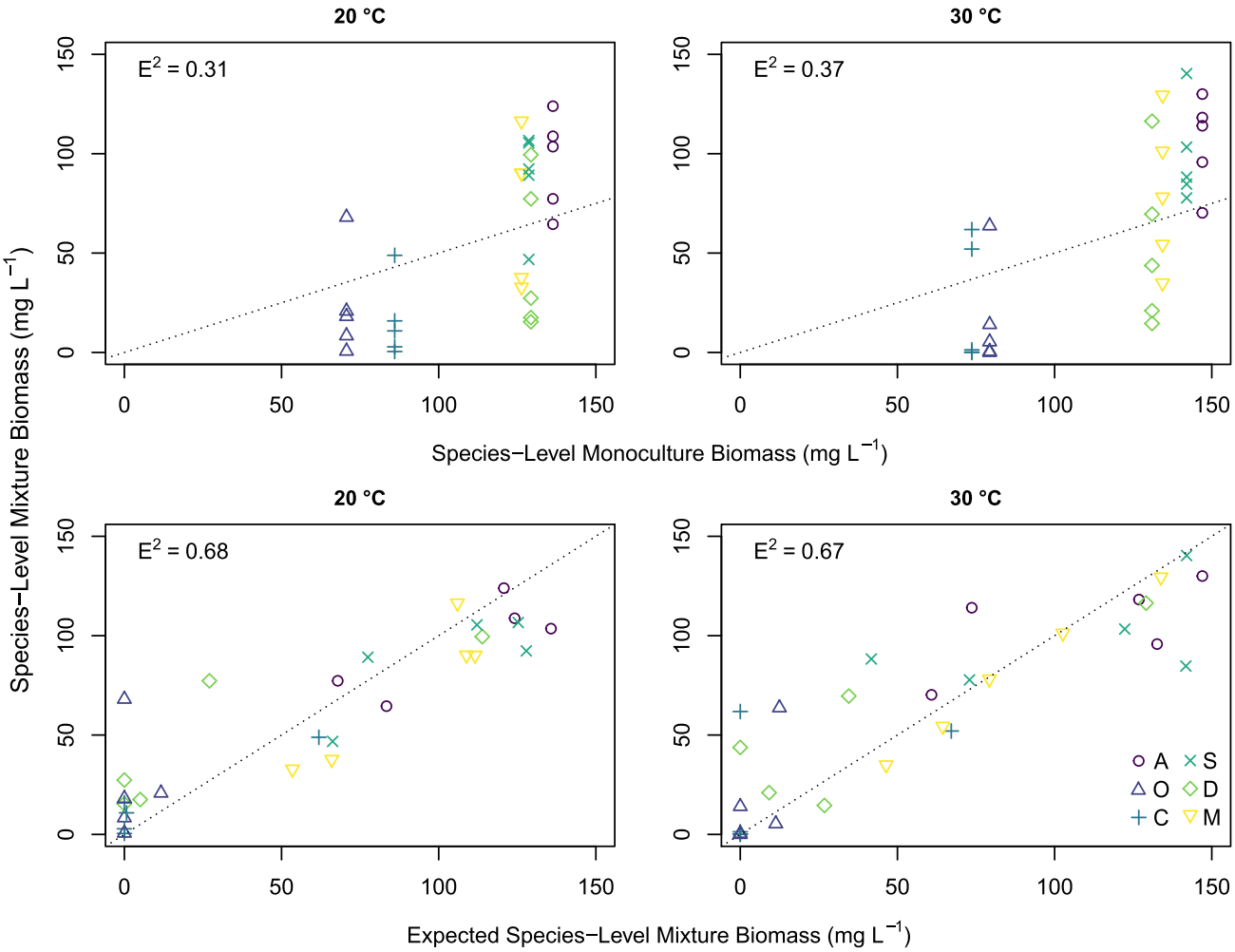

Figure 3 Example of observed vs. expected yields for 20 °C and 30 °C experiments for models that use information from all nine resources. Top row shows the relationship between observed monoculture and mixture biomass, with a dashed line showing a slope of one half. Bottom row shows relationship between expected and observed mixture biomass for a model including all nine limiting resources. Note that monoculture biomass alone is not sufficient to explain species biomass in the mixture, and that including information about resource competition leads to substantial improvements in model fit. Symbols are as follows: A*: Acutodesmus obliquus,* C*: Chroococcus minutus,* D*: Desmodesmus armatus,* M*: Microcystis aeruginosa,* O*: Oocystis marssonii*, S*: Synechocystis* sp.

competition experiments, whereas *Oocystis* and *Chroococcus* tended to show underyielding. Both *Oocystis* and *Chrooccus* yielded the lowest biomass during monoculture growth. The best model predictions for community yield, including resource uptake by the species for all nine resources measured, matched observed values across all treatments better (Fig. 3 bottom row) with an explanatory power of *c*. 0.67. This correspondence was not due to a trivial relationship between biomass yield in monocultures and mixtures (Fig. 3 top row). Species positions along the first PC axis based on our decomposition of uptake rates, which explained about 90% of the total variation, were almost identical for the low- vs. high-temperature treatments, suggesting that species relative resource uptake rates did not change significantly between the two treatments (Fig. S1). For both temperature treatments, the first PC axis showed positive correlations among all but three resources – P, Mn and Fe – suggesting that species with high uptake rates for these resources tended to have lower uptake rates for others (Fig. S3).

The model predictions for species biomass in the competition cultures of the best models are shown in the bottom row of Fig. 3. Models for each combination of resources were calculated and the respective goodness of fit was plotted in Fig. 2 against the respective number of resources, included in the models. Models including more resources gave significantly better predictions (Table 1), and these improvements were stronger in the 30 °C treatment. Although predictive strength in the 30 °C treatment was significantly lower (Fig. 2). Goodness of fit of monoculture uptake-based predictions for species biomass in mixtures showed higher variability and lower mean at the 30 °C level (Fig. 2). Models using only N or P and their combination achieved the lowest goodness of fit across treatments. The uptake-based model predictions never reached the goodness of fit of the complete niche overlap model predictions.

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Table 1 Log-transformed goodness of fit as a function of log-transformed number of resources, and the temperature treatments. Regressions are done using mean E^2 values for each diversity level, meaning that autocorrelation due to repeated sampling of resource mixtures is largely controlled for, but R^2 value for regression is inflated (as it tracks only deviation of mean values)

	Estimate	Std. Error	*t*-value	*P*-value
Intercept 20 °C	−0.573	0.0267	−21.299	4.58e-12***
Resource number	0.097	0.017	5.681	5.67e-5***
Temperature (30 °C)	−0.193	0.038	−5.076	1.69e-4***
Resource number * temperature	0.081	0.024	3.364	0.005**

Residual standard error: 0.0350.08028 on 1018 14 degrees of freedom. Multiple *R*-squared: 0.9210.09892, Adjusted *R*-squared: 0.9040.09627, *F*-statistic: 54.5737.25 on 3 and 1018 14 DF, *P*-value: 5.71e-08s.

The minimally sufficient dimensionality ranged from three to five resources in the best performing models. Within these models, the resources with the most consistent contribution (> 8% of all models) were magnesium (Mg), sulphur (S), sodium (Na), potassium (K) and iron (Fe), whereas phosphorus (P), manganese (Mn) and nitrogen (N) contributed least often to these models (<6% of all models) – this finding was also consistent across temperatures (Fig. 4).

Average resource uptake from the medium for all resources and for all species was analysed and plotted per resource in Fig. 5 (see Fig. S3 for comparison of uptake vs. initial resource concentrations). As references, elemental ratios were derived from (Redfield 1934) and (Moore *et al.* 2013). The mean value is thus in the range of other studies, besides larger variations with respect to Fe uptake. There is neither general phylogenetic difference between green algae and cyanobacteria elemental ratios and nor major difference between temperatures. The monovalent (K^+, Na^+) to divalent (Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, S^{2+}) ratio on the final day in our monoculture media also did not differ between phylogenetic groups, but varied more for green algae than for cyanobacteria (Table S4).

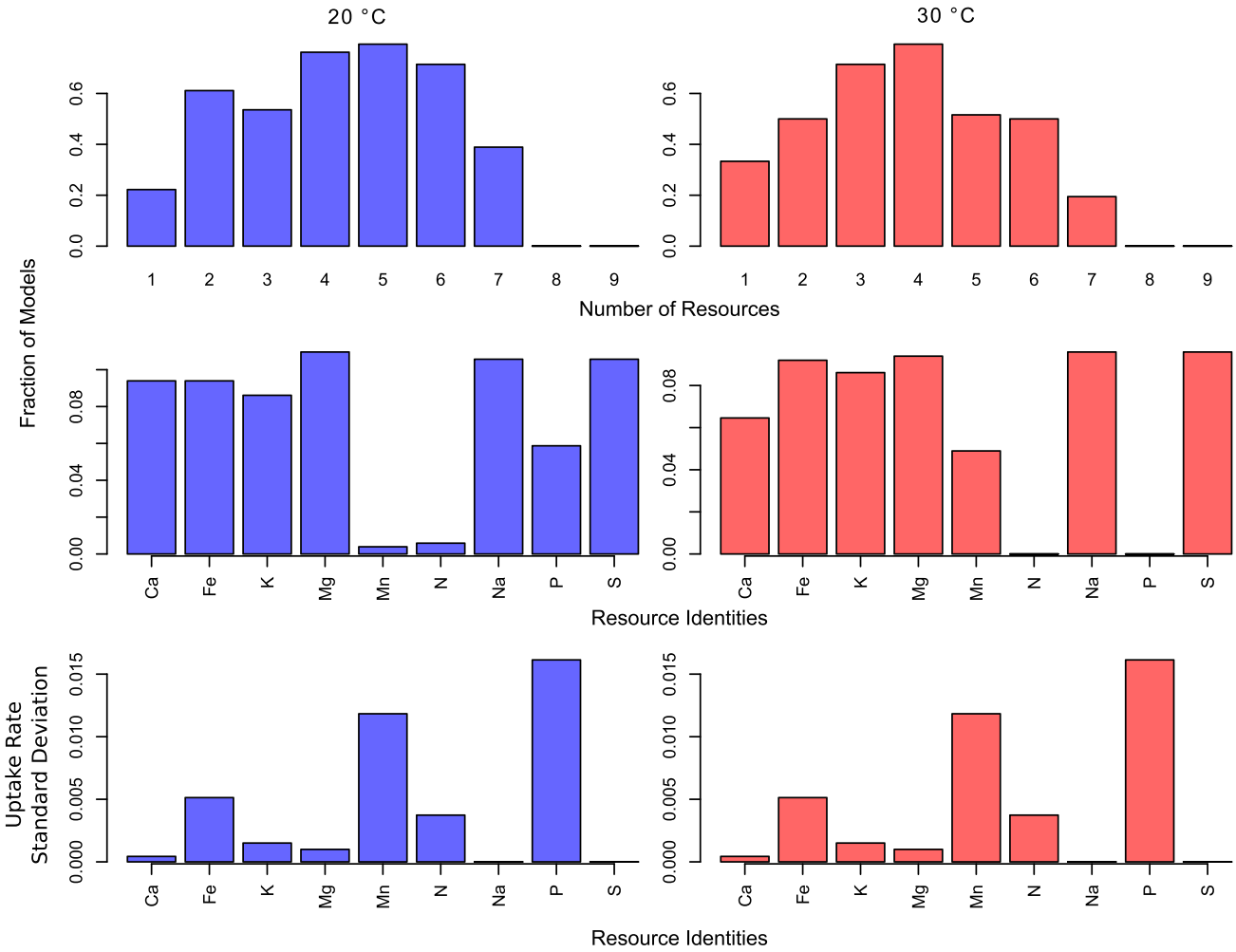

Figure 4 Summary statistics for the best performing models – i.e. those that performed at least one standard deviation above the mean goodness of fit observed across all models. Blue and red indicate the 20 °C and 30 °C treatments respectively. Top row shows the fraction of models for each resource level that were included in the best performing subset. Note that models with between 3 and 5 limiting resources most consistently provided good predictions. Mid row shows the relative frequency with which each limiting resource appeared in the best performing models. Note that micronutrients tend to be more represented in these models than are N and P. Bottom row shows the standard deviation of uptake rates for each resource across species. Note that both resources with both high and low interspecific variability in uptake rates are represented among the best fitting models.

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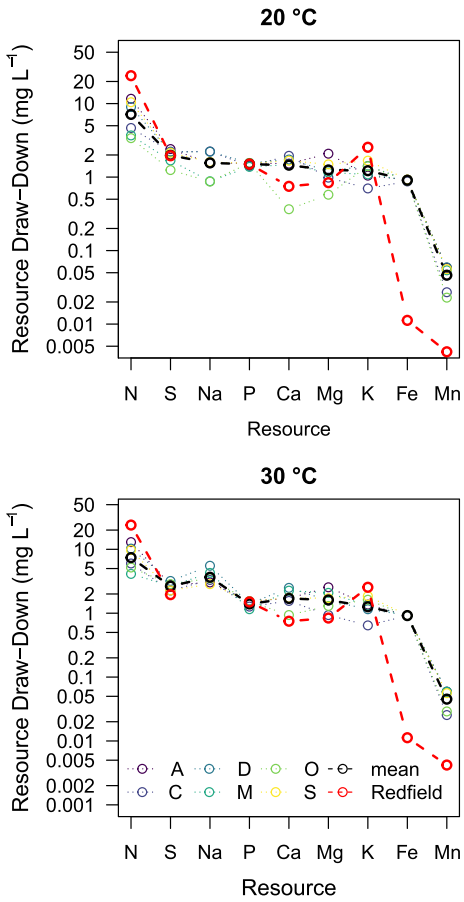

Figure 5 Individual and mean monoculture resource concentration draw - down for each species, measured over the course of the experiment. Dashed black line shows the mean trend observed across all species. Dashed red line shows the expected stoichiometric relationship, based on elemental resource quota in marine phytoplankton as reported by Redfield (1958) and Moore *et al.* (2013), normalised to match observed draw-down rates at 20 °C for P. Symbols are same as in Fig. 1.

DISCUSSION

More resources give better predictions than few

Since models with three to five resources tended to fit best, it is suggested that lower dimensional models do not suffice to capture resource competition between species – and in particular N and P on their own or together. The overall low explanatory power of N and P in our study is remarkable though since phytoplankton studies commonly focus on both to predict growth (Dzialowski 2005; Elser *et al.* 2007; Poxleitner *et al.* 2016). This should be a point of concern as, regardless of whether methodological or biological reasons caused this low goodness of fit in our models, they appear to be less reliable predictors than all other resources measured in this study. In other words, N and P should not be assumed to be the 'main' or 'primary' limiting elements driving resource competition. The differences in structural or storage allocation optima of N:P ratios to maximise growth according to growth phase (8.2:1 in exponential phase) or limitation by N (37.4:1), P (45:1) or light (35.8:1) might also factor in here (Klausmeier *et al.* 2004). Elemental composition further depends on the abiotic and biotic environment as well as interactive effects between resources (e.g. nitrate reduction requires Fe). In line with our findings we therefore recommend to include at least one additional resource in future experiments and models to sufficiently mirror the physiological complexity of phytoplankton species. Potentially because of the strong relationship among uptake rates observed across resources, the number of resources included in our models appears to have been more important than the precise identity of those resources (Fig. 3). If this finding holds in other systems, then it might suggest that a focus on the dimensionality of species resource needs, rather than on the specific nature of their needs for individual resources, may be an efficient strategy for parameterising predictive models.

Nevertheless, goodness of fit was the highest for the simplest model that we tested, which assumed complete niche overlap models among all species (Fig. 3). This result accords with observed resource uptake patterns, which were generally similar among species, and closely followed expectations from the Redfield ratio observed in marine phytoplankton (Moore *et al.* 2013) (Fig. 5). Taking both findings into account emphasises how selected strains exert overall similar impacts on their environment through overall similar bulk resource uptake rates. Generally, temperature is expected to increase competition strength due to faster enzymatic reaction kinetics according to van 't Hoff equation and metabolic rates. For Na, S and Ca uptake was higher at 30 °C compared to the 20 °C treatment (Fig. S3) and most pronounced for Na. Higher alkalinity at 30 °C may increase the replacement of protons by Na^+ affecting the sodium-phosphate co-transport (Mohleji & Verhoff 1980; Ullrich & Glaser 1982). Furthermore, we saw a higher variation in the goodness of fit in the 30 °C experiment, which could allude to deviating forms of competition like interference (Dunker *et al.* 2017) or luxury uptake (Sommer 1985). These competition forms, much like facilitation, as well as conditions, where dominant exploitation strategies can affect competition outcomes, are not considered within the scope of the model, but represent opportunities to gauge the strength of these effects in relation to exploitation competition by comparing the model prediction with experimental results. The findings indicate that in mixed cultures no strong contribution of biotic interference mechanisms can be concluded and further points to the overlap in uptake rates for individual resources between species (Fig. S1).

The deviations from the reported marine quota (Redfield 1958; Moore *et al.* 2013) occurred mainly for N, K, P and Fe, which could illustrate general differences between freshwater and marine environments. The remaining 10% of variation in uptake rates not covered by the first PC axis (Fig. S1) may at least partially explain the noise in our low dimensional estimates. Interestingly, the differences in uptake rates between species were also highest for Mn and P (and to a lesser extent Fe) for both temperatures (Fig. 4).

Implications

Although goodness of fit was consistently high for the model with complete niche overlap, which assumed that species had identical uptake rates for each resource, it was not for models that considered only individual resources. In other words,

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although it was possible to predict species-level biomass in mixtures, based on the mechanistic hypothesis that species have similar resource requirements (i.e. following the Redfield ratio), it could not be assumed that these similarities allowed accurate predictions to be drawn from a small number of resources. There are several potential explanations for this difference. First, measurements of species uptake rates could simply be sufficiently noisy that many rates must be measured before their average effect can be accurately measured. Alternatively, it could be that species uptake rates are only on average similar, but still differ for individual resources. This second explanation appears to be better supported by our data, as we find that even resources with high variability in uptake rates among species were often included in the best performing models (Fig. 3). This explanation also accords with theoretical expectations that interspecific trade-offs should prevent any single species from being the best competitor under all possible conditions during resource limitation (Tilman 2011).

Considering the role micronutrients (such as Mg, Mn, Ca, Fe) play as essential cofactors for metallo-enzymes (relevant for light capture, photo oxidation, cellular energy budget, etc.) within phytoplankton cells (Reynolds 2006), their potential as limiting growth factors in the ocean has been recognised (Sunda 1989). Importantly, colimitation by them can naturally occur due to varying degrees of their availability (Saito *et al.* 2008). To date, fertilisation experiments in the field still yield contradictory results for limitation by micronutrients, showing limitation, co-limitation or no limitation depending on the trophic status and focal organisms (Paerl *et al.* 2001; Reynolds 2006). The ratio of monovalent to divalent cations $(Na^{+} + K^{+})/(Ca^{2+} + Mg^{2+})$ in lakes has been linked to the ratio of Desmidiacae to diatoms (Pearsall 1922) or Cyanobacteria, Chlorococcales, centric diatoms and Euglenophyta (Shoesmith & Brook 1983), although the authors suggested this ratio represents a proxy for, e.g. concentration of bicarbonate ions rather than causal mechanisms. The ratio thus likely condenses too much information contained in the resource uptake rates. While we did not observe phylogenetic differences groups for the valency ratio, models including the mono- to divalent resources (with the exception of Mn) gave better predictions likely due to impact on osmotic balance and their role as 'exchange currency'. Based on the physiological meaning of ion valency, it would be important to consider it, when applying our framework to other studies.

More than the expected minimum number of limiting resources equal to the species number (Hutchinson 1957) was required to improve model accuracy for pairwise mixtures in this study. We would expect that in more complex systems, even higher resource dimensionality might be integral for competition (especially in the absence of heterogeneity or predation). Building on the ecological aspects of stoichiometry (Sterner & Elser 2002), the emergent field of elementomes argues for a more comprehensive inclusion of the elemental resources and their ratios: Revealing the distinct elementomes of primary producers, invertebrates and vertebrates across aquatic and terrestrial systems (González *et al.* 2017) or forest type according to their climate (Peñuelas *et al.* 2019). Diverse systems may indeed be sustained through a high number of resources depending on the spatial structure of the system (Passy & Larson 2019) and interplay of resource availability across scales augmenting effects of multiple resource limitation (Kaspari & Powers 2016).

CONCLUSION

The minimum niche dimensionality required for our resource competition models was greater than the minimum expectation of two resources for coexistence of two species (Tilman 1982). Therefore, it seems imperative to include resource treatments and measurements beyond N and P as resource uptake of competing species was high dimensional. Aside from the importance of other elemental macro- and micro-nutrients for cell physiology and photosynthesis, we show that considering additional limiting resources opens avenues for more reliable resource competition models that can capture the minimum niche dimensionality and account for stoichiometric constraints of the competing species.

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AUTHORSHIP

Peter Hofmann (PH), Adam Clark (ATC), Antonis Chatzinotas (AC), W. Stanley Harpole (WSH) and Susanne Dunker (SD) worked closely together to conceive and develop the ideas of this work. PH and SD jointly designed the experiment, Petra H developed the nutrient measurement protocols, PH and Petra H performed the measurements, PH analysed the data, ATC and PH collaborated for the development of the model together, ATC did the statistical analysis, AC and WSH substantially contributed to the discussion of the results and all authors worked on the manuscript together.

DATA ACCESSIBILITY STATEMENT

We hereby confirm that – upon Acceptance of this manuscript – we will upload all necessary data and supporting information to a permanent, DOI-linked scientific repository such as Dryad (https://doi.org/10.5061/dryad.h70rxwdhd).

PEER REVIEW

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