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# Growth responses of a green alga to multiple environmental drivers

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**One feature of global change is that biota must respond not to single, but to multiple environmental drivers. By growing a model photosynthetic microbe in environments containing between one and eight different drivers, including changes in $CO_2$, temperature, and pH, in different combinations, we show that the number as well as the identities of drivers explain shifts in population growth rates. This is because the biotic response to multiple environmental drivers depends on the response to the single dominant driver, and the chance of a driver of large effect being present increases with the number of drivers. Interactions between drivers slightly counteract the expected drop in growth. Our results demonstrate that population growth declines in a predictable way with the number of environmental drivers, and provide an empirically supported model for scaling up from studies on organismal responses to single drivers to predict responses to large numbers of environmental drivers.**

A major challenge facing freshwater and marine biologists is to quantify how aquatic biota will respond to our changing climate. One of the hallmarks of global change is that it is complex; changes in temperature, pH, light levels, carbon dioxide and oxygen concentrations, nutrient availability, salinity and other environmental variables can occur together[1,2]. Predicting the action of multiple environmental drivers (MEDs) on population growth is required for understanding how aquatic biota, at all levels from individual genotypes to communities, respond to global change[3]. Studies in freshwater[3,4] and marine systems[5,6] have historically focused on understanding organismal responses to key environmental drivers alone, such as changing temperature, $CO_2$ levels, or light levels, or investigated MEDs by using pairs or trios of drivers[1]. This has shown that interactions between the effects of environmental drivers vary with the drivers and the organisms being tested[6], but use a small number of environmental drivers relative to the number of drivers in most natural environments[2]. This leaves open the possibility that when the number of environmental drivers is larger, the effects of interactions between individual drivers may become less important in determining overall organismal responses. The goal of our study is to determine if knowing the interactions between specific environmental drivers at the organismal level is necessary when the number of environmental drivers is large, or whether patterns emerge that allow us to predict organismal responses without knowing particular driver interactions.

Studies on MEDs until now are mainly concerned with understanding interactions between the effects of individual drivers (see ref. 3 for definitions). Driver effects can either be additive, where the response to MEDs is equal to the sum of their individual effects, or multiplicative, where the response exceeds the sum of their individual effects. Interactions that are additive or multiplicative can be further synergistic (having a positive feedback) or antagonistic (having a negative feedback). Antagonistic interactions can thus lead to outcomes where responses to MEDs are less than the sum or product of their individual effects. These definitions must be contextualized in terms of the level of organization they affect, such as cellular processes or community composition. Driver interactions can be studied mechanistically, where the interactions are between drivers themselves (for example, the chemistry that links pH and $CO_2$ levels), or be outcome-based and describe effects on organisms. Here, we use an outcome-based definition of drivers and driver interactions. We focus on the effects of drivers and interactions as population-level organismal responses.

Building an outcome-based prediction of biotic responses to MEDs by understanding specific interactions between key drivers requires that key drivers be identified and the interactions between them be measured. This approach is fruitful when the number of drivers is small. For example, high $CO_2$ and low pH enhance the detrimental effects of ultraviolet irradiation on a key pelagic calcifier, *Emiliania huxleyi*[7], and although many diatom assemblages do not respond to $CO_2$ enrichment alone, $CO_2$ and high light levels interact synergistically to reduce their growth rates[8]. These experiments can investigate the interactions between drivers, but are difficult to scale up, because measuring interactions between all drivers becomes impossible as the number of drivers increases. This is problematic, because these and similar studies on natural phytoplankton assemblages[4], *E. huxleyi*[7,9–11], *Phaeodactylum tricornutum*[12], and the freshwater alga *Chlamydomonas reinhardtii*[13], suggest that interactions among drivers are not easily predicted even if they can be explained once observed. If this is the case, then one cannot use studies of pairs or trios of drivers to predict responses to those same pairs when many other drivers are also present (for example, if pH, $CO_2$ and ultraviolet levels change alongside temperature, oxygen levels, and micronutrient levels). One way to reduce the size of experiments is to measure responses to groups of MEDs using combinations of drivers that are likely to change in concert[2]. This requires knowing how drivers group, and how these groups change on relevant geographic and temporal scales.

Alternatively, it may be possible to make reasonable outcome-based predictions of responses to MEDs based on the number of environmental drivers. Our general reasoning can be explained using environmental tolerance curves (Fig. 1), which usually show the relationship between some aspect of organismal function (for example, growth) and an environmental value (for example, temperature). Here, we consider a tolerance curve showing the relationship between organismal function and the total environment

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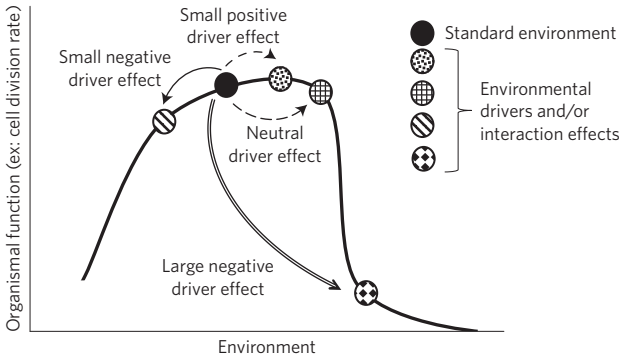


**Figure 1 | Cartoon of the effects of multiple drivers on organismal function using an environmental tolerance curve.** Some aspect of organismal function, such as growth, is plotted as a function of the environment experienced by the organism, with the value of 'Environment' being determined by multiple environmental drivers. Initially, organismal function is high (solid black circle). When single drivers change, organismal function changes (patterned filled circles). Although the effect of each driver may be unknown, as an increasing number of drivers occur, the likelihood of at least one driver or driver interaction having a large detrimental effect on organismal function increases. This thought experiment does not require that the population be in its optimal environment, just that, among the environments sampled, the control environment be one where organismal function is high. Figure 2a shows that this is the case here, as the control environment is among the 'best' environments available in this experiment. Note that this cartoon is meant to illustrate our thought process, and not to indicate the quantitative effects of the specific environments used in this experiment. Please refer to Fig. 2 for quantitative data.

experienced by the organism in a multidriver environment. We assume that organismal function is initially somewhere on the plateau. Changes to one or more randomly chosen drivers will affect organismal function in some unknown way, either as a direct result of one driver, or as a result of interactions among drivers. If a subset of drivers or their interactions have large enough effects to push organismal function off the plateau, but the effects of most drivers are unlikely to be severe (as organisms are generally tolerant of some environmental variability), the chances of at least one of these large-effect drivers occurring grows as the number of drivers and interactions increases. This is analogous to altering organismal function through genetic mutations, where most mutations have small effects on organismal function, but mutations of large effect will eventually occur if enough mutations are sampled[14]. Here, we instead approach the idea that organismal phenotype is a result of interactions between genotype and environment using environmental 'mutations' rather than genetic ones.

We carried out a large experiment to disentangle the effects of the number of environmental drivers from the identities of the drivers in determining population growth responses to MEDs. We show that for small numbers of drivers, interactions between drivers determine growth responses, but as the number of drivers increases, growth responses can be predicted from the number of drivers (if the composition of the environment is unknown), or the single driver with the largest effect alone (if the composition of the environment is known). Populations of the model microalga *C. reinhardtii* were grown in 96 different test environments that differed from a standard laboratory environment by between one and eight of the following drivers: high $CO_2$, low pH, high temperature, low light, ultraviolet irradiation, phosphate starvation, general nutrient depletion, and herbicide (Supplementary Table 1). These drivers were chosen because they are generally studied as single environmental changes[15,16], occur in many aquatic ecosystems[17], and are 'drivers' (*sensu* Boyd and Hutchins, 2012) in that they elicit a response in *C. reinhardtii* and many other microbes[13,18–39]. Because a laboratory strain of *C. reinhardtii* was used for this study, and our hypothesis is based on how reactions to changes in the environment affect organismal growth, drivers are environmental values that differ from the usual laboratory environment of the particular population used to start this experiment. The control environment is thus not arbitrary, even though it may differ from the optimal environment for other strains of *C. reinhardtii* that have been maintained under different conditions. Whenever possible, control and test environments reflect anticipated changes in the natural world. For example, the control environment uses 430 ppm $CO_2$, whereas the test environments containing high $CO_2$ use 2,000 ppm $CO_2$, in line with IPCC predictions[40]. In other cases, the usual laboratory environment for this strain required that we choose the test environment value using pilot studies. This experiment requires that the test environments be different from the environment usually experienced by this particular strain at the beginning of the study, not that the control environment be the average or optimal one for this species over many studies. See online methods for a detailed discussion of each test environment. In each test environment, we measured population growth, a trait commonly used to predict how populations will fare under environmental change[41], including whether they are likely to persist[42]. See Methods and Supplementary Information for test environments and experimental design.

## The number of drivers can explain population growth

Population growth rate declines as the number of drivers in test environments increases (Fig. 2). We see that the number of drivers is the strongest predictor of population growth, explaining approximately 37% of the decrease in growth rate independently of the particular combination of environmental drivers involved, which is in line with our hypothesis that knowing the number of environmental drivers alone is informative ($F_{1,93} = 11.1766$, $P = 0.001$, Fig. 2a; see Supplementary Methods). Regime (the particular drivers in any unique test environment) explains some (32%) of the decrease in population growth rate in test environments, and the overlap in the environmental drivers between regimes also explains some (about 10%) of the variation in growth ($F_{1,93} = 3.877$, $P = 0.052$, Fig. 2a). As expected, extinction is more likely in test environments with a greater number of drivers ($F_{1,93} = 3.310$, $P = 0.072$, Supplementary Fig. 1).

Because regime explains some of the variation in population growth, we further investigate whether this is due to interactions between drivers, or to the actions of single drivers within regimes. We find that the drop in population growth rate can be explained by the single driver in a regime that has the largest effect on growth when it is experienced alone ($r^2 = 0.43$; $P < 0.0001$, Fig. 2b). This is consistent with population growth rates being largely determined by one overriding driver, rather than by interactions between them, at least with the drivers investigated here. The relationship is thus best described by a simple comparative model (Supplementary Table 4). Antagonistic interactions occur, where the effect of the most detrimental driver is often mitigated if other drivers are present. Because of this, the realized average population growth rate is higher than predicted by the comparative model. Here, high $CO_2$ (Fig. 3), which increases population growth in *C. reinhardtii* and many other chlorophytes[27,43–46], counteracts the growth effects of detrimental drivers and gives rise to antagonistic interactions. When $CO_2$-enriched test environments are removed from our data set, populations in the remaining test environments have lower average growth rates, and fit the predictions of the comparative model without antagonistic interactions ($r^2 = 0.58$; $P < 0.0001$, Supplementary Fig. 2).

Our key finding is that the number of environmental drivers can be used to predict growth in the test environments even without knowing which drivers make up each test environment. We propose

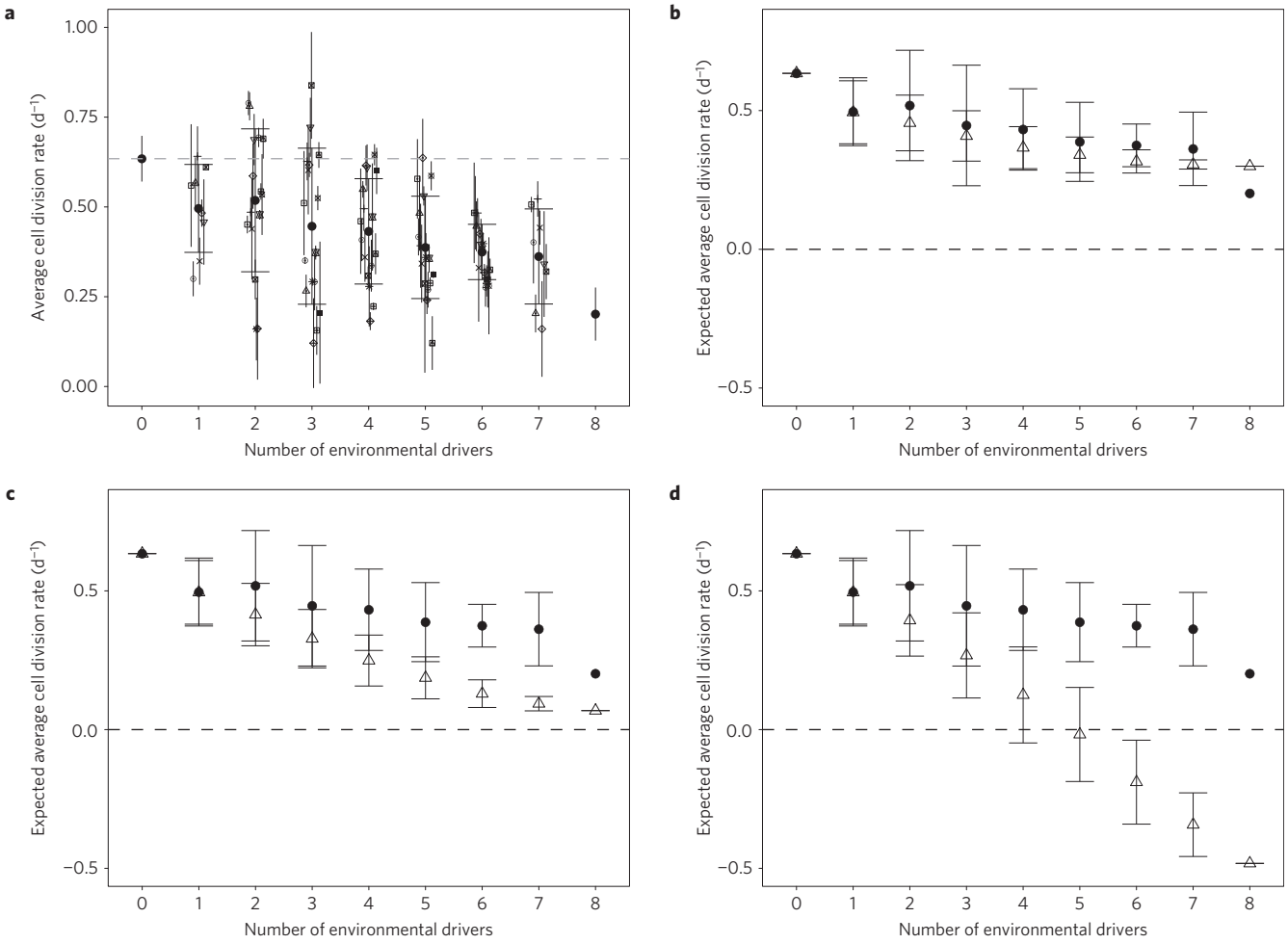


**Figure 2 | Population growth rate of *C. reinhardtii* under zero to eight environmental drivers. a**, Black data points and bars represent means and standard deviation between regimes for each NED. See Supplementary Table 3 for regimes. Different shapes within each NED indicate individual regimes. Dashed line in **a** indicates growth in the control environment. **b**–**d**, Population growth rates (mean and standard deviation) predicted by a model (white triangles) alongside measured values (black circles), followed by goodness-of-fit, for three models: comparative model ($r^2 = 0.43$, $P < 0.0001$) (**b**), multiplicative model ($r^2 = 0.33$, $P < 0.0001$) (**c**) and additive model ($r^2 = 0.25$, $P < 0.0001$) (**d**); extinction (indicated by dashed line in panels **b**–**d**) is predicted in environments with >5 changes.

that this is because test environments with a greater number of drivers are more likely to contain at least one severely detrimental driver or driver interaction and that, once a severely detrimental driver is present, the addition of other drivers is unlikely to depress growth much more, barring extinction. This can be seen in Fig. 2a, where the lowest fitness at NED = 2 is about 0.16 divisions/day (regimes for the two lowest points are pH + phosphate starvation and phosphate starvation + low light, both have the same average growth rate), but at higher NED this minimum does not decrease, indicating that once a very stressful driver or driver interaction is present, further drivers or driver interactions do not, on average, depress growth more. However, interactions do matter for low NED; the populations with the lowest growth rates at NED 2 (lowest average growth rate for a regime is 0.16 divisions/day) do far worse than those with the lowest growth rates at NED 1 (lowest average growth rate for a regime is 0.30 divisions/day). Interestingly, this shows that if the goal of empirical studies is to predict population responses to MEDs when many drivers are present, the most useful course of action when only a limited number of populations can be observed is to determine which single drivers affect growth most, or even determining how many drivers are likely to co-occur. In contrast, focusing on interactions between a few specific drivers may produce results dominated by interactions that sum nearly to zero when more realistic scenarios of environmental change are considered.

The goal of this study was to disentangle the role of the number of environmental drivers from the specific drivers present in test environments. Each driver is used only at a single intensity in our study (see Methods for explanations of the choice of intensities of particular drivers), and the rank order of the driver effects are probably due to both intensity and identity. In our data set, the most detrimental drivers are herbicide presence and phosphate starvation. Presumably, neither would be as detrimental if we had used lower concentrations of herbicide and higher concentrations of phosphate, respectively. However, it is reasonable to suppose that in most natural environments, drivers will vary in intensity as well as identity. Our interpretation of our data hinges on the growth effects of drivers, not their identities per se, and we expect that if this experiment were repeated with different drivers, or a different organism, the qualitative results would be the same—the drivers with the largest effect on growth would determine responses even when populations experienced them together with numerous other drivers. Although the size of our study precluded multiplying it to measure the relative contributions of identity and intensity of drivers to organismal responses, this indicates a direction for future experiments.

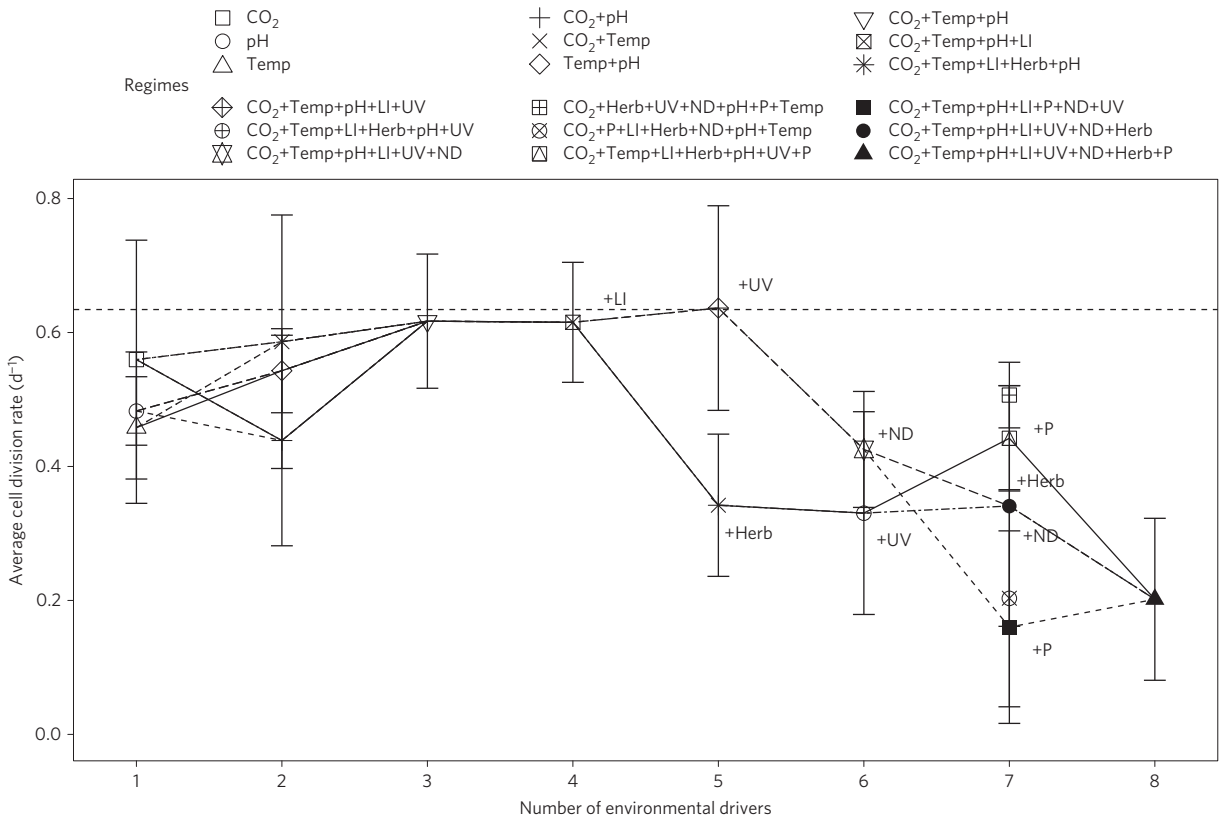


**Figure 3 | Population growth rates of *C. reinhardtii* in test environments containing high $CO_2$, low pH, and high temperature.** Each point shows mean and standard deviation for three replicate populations. The identity of regimes is indicated by the shape of each point. The shape of each line shows the unique patterns of increasing the number of drivers in test environments. In general, population growth drops when environmental changes of larger effect than previously present (herbicide, nutrient depletion (ND) and phosphate starvation) are added.

## Environmental similarity

In our experiment, test environments become more similar as the number of drivers increases, although this similarity explains less than 11% of the variation in growth. If increases in environmental similarity were driving our results, we would expect that variation among regimes drop as the number of drivers within regimes increases, but this is not the case (correlation between the number of drivers and variance among regimes with the same number of drivers; *post hoc* fit $r^2 = 0.06$, $P = 0.53$). Increasing environmental similarity with an increasing number of drivers per test environment is a limit of performing an experiment with a finite number of drivers. To understand how increasing environmental similarity affects our data, we simulated the same experiment using infinite environments with the same distribution of effects on growth for single environmental changes as in our experiment. We found that using a finite number of possible environmental changes in our experiment slightly underestimates growth rates in regimes with many drivers, but the effect is small (Supplementary Fig. 3), confirming that the increase in similarity between regimes with an increasing number of drivers does not explain the overall pattern of our data.

## Case study involving temperature, $CO_2$ and pH

To understand how interactions between focal drivers change when additional drivers are present, we measured the effects on population growth of increased $CO_2$, increased temperature and decreased pH—either alone, in pairs, all together, or all together in the presence of other drivers (Fig. 3). When these focal drivers occur singly, populations grow fastest under $CO_2$ enrichment, slower under low pH, and slowest under high temperature. In pairs, the effect of $CO_2$ enrichment counteracts that of high temperature so that these populations have higher growth rates than those under high temperature alone, whereas the combined effects of $CO_2$ enrichment and low pH reduces growth. Populations grown in low pH and high temperature grow faster than those subjected to either driver alone, and populations subjected to all three drivers together grow faster than any of the paired or single cases. In these test environments, containing between one and three drivers, specific interactions between responses to drivers determine growth effects, and the most informative way to explain changes in growth is by investigating the physiological mechanisms involved[47].

In contrast, when elevated $CO_2$, low pH, or high temperature co-occur with other drivers, changes in population growth are predictable from the effects of single drivers. This prediction is more robust when a greater number of drivers are present in the test environments. For example, if $CO_2$, pH and temperature change, decreasing light intensity does not affect growth further, as expected from the small effect of light intensity on growth alone relative to the effect of other drivers already present in the regime. In contrast, the presence of herbicide, which has a drastic effect alone, reduces growth when it is added to a test environment that already contains several other drivers. The addition of nutrient depletion has very little effect on growth and is masked by the dominant effects of herbicide.

These interactions are all expected under the simple comparative model. Interestingly, at high NED, phosphate limitation has an antagonistic interaction when herbicide is present. This is surprising, as both herbicide and phosphate are dominant environmental drivers. The herbicide used here is atrazine, which directly blocks the photosynthetic electron transport chain, reducing photosynthetic efficiency[13]. Phosphate is a limiting factor in many natural environments, yet it is a necessary

macronutrient that photosynthetic organisms such as *C. reinhardtii* require in large amounts[35]. Previous work[13] suggests protection mechanisms such as nonphotochemical quenching of excess light energy and adjustment of the photosystem stoichiometry to explain the antagonistic interaction observed between atrazine and very high light in *C. reinhardtii* and arrested growth with no loss in viability in low light conditions. Similar protection mechanisms may be in place here to protect populations from the lethal effects of atrazine under limited resources at high NED. Antagonistic interactions between phosphate depletion and other environmental changes have also been found in a nitrogen-fixing species (*Trichodesmium*[48]), where phosphate-limited populations are also $CO_2$-limited, so that high $CO_2$ can increase population growth rate when phosphate is limiting. Our case study broadly supports the observation that elevated $CO_2$ can partly mitigate the drop in growth in test environments with MEDs, including phosphate-limited environments. However, we also find some exceptions where growth is not increased by high $CO_2$, such as when nutrients and phosphate are co-limiting.

Although interactions between drivers increase variation in the reduced data set that excludes high-$CO_2$ test environments (Supplementary Fig. 2) relative to the full data set shown in Fig. 2, the overall relationship between population growth and the number of drivers is the same. When many drivers co-occur, the effects of individual drivers, in particular of the driver with the single largest effect alone, are reasonable predictors of population growth. Our data also show that even if the individual effects of drivers on growth are unknown, the number of drivers offers a good estimate of the expected growth rate when large numbers of drivers co-occur. As with the full data set, this is due to test environments with a greater number of drivers having a higher chance of containing at least one severely detrimental driver so that, generally, growth decreases as the number of drivers increases.

## Conclusions

Global change involves many environmental drivers, but biotic responses are often studied using few environmental drivers, so it is vital that we explore if and how studies using few environmental changes inform predictions of biotic responses to higher numbers of drivers. Mechanistically understanding all interactions between the relevant drivers in aquatic systems[47] cannot be tackled experimentally—with current methods, full factorial experiments are simply too large to carry out. That being said, we can make a tradeoff between a mechanistic understanding of interactions between specific drivers and predicting overall biotic reactions to MEDs. One well-established way to do this is by using scenarios[2] where suites of environmental variables are changed in concert and organismal responses measured. Here, we propose a complementary method suitable for situations where a larger number of drivers is considered, based on data showing that average changes in population growth in a model microalga are largely predictable from either the number of environmental drivers, or the effect of the single most detrimental driver, in cases where a large number of environmental drivers occur together. As with scenarios, our approach trades mechanistic understanding for predictive power. Although the ideal solution to understanding organismal responses to MEDs may be to replace 'black box' approaches such as ours with a mechanism-based understanding that allows prediction, this may not be realistic given current knowledge. Our approach is appropriate when constructing scenarios of environmental change carries significant uncertainty, because of uncertainty in predicting the intensities of individual drivers, of correlations between changes in drivers, or even in the identity of the particular drivers involved at the relevant geographical and temporal scales for focal organisms. It is also useful when data on responses to drivers or scenarios cannot be gathered for all organisms where it is needed. Another use of our method is in making between-species or between-genotype comparisons by uncovering differences in sensitivities to particular drivers. If the effect of many individual drivers is measured on different species or genotypes, then studies can be used to both understand differences in responses between species or genotypes, and to predict the likely range of responses to MEDs within communities containing many species or genotypes.

We show that specific interactions between drivers determine growth responses when only a few drivers change, but these interactions do not need to be taken into account to predict average growth responses when many drivers change. This provides hopeful evidence that continuing to build our understanding of how single drivers affect population growth is indeed informative for understanding population-level responses to MEDs.

## Methods

Methods and any associated references are available in the online version of the paper.

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## Author contributions

G.B. and S.C. designed the experiment, G.B. performed experiments and S.C. supervised laboratory work. Both authors contributed to statistical analysis and writing the article.

## Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.C.

## Competing financial interests

The authors declare no competing financial interests.

## Methods

**Experimental design.** All populations were founded from a single cell of *C. reinhardtii* (CC-2931, mt-; Chlamydomonas Resource Center, University of Minnesota), grown in sterile Sueoka's high salt medium, buffered with Tris-HCl (HSMT; ref. 39), under continuous rotation (50 r.p.m.) at 25 °C and constant light at 32 μmol $m^{-2}$ $s^{-1}$ photon flux density (Fisher Scientific Traceable Dual-range Light Meter), at 420 ppm $CO_2$ (Supplementary Tables 1 and 2). These variables were controlled using incubators (Infors AG CH-4103). This strain of *C. reinhardtii* is from a culture collection, and has been grown in our lab for over seven years—this medium, temperature and light levels represent the usual benign growth conditions for this strain.

**Experimental environments.** Experimental populations were grown for approximately three generations in replicate test environments that differed from the benign control environment (430 ppm $CO_2$, pH 7.2, temperature 25 °C, full light and nutrients, no herbicide and no ultraviolet), by between one to eight of the following parameters: increased $CO_2$ to 2,000 ppm, temperature to 26 °C, decreased pH to 6.5, light levels to 18 μmol $m^{-2}$ $s^{-1}$, reduced phosphate to 1.69 mM, general nutrient depletion by 75%, and added 0.5 μM of the herbicide atrazine. In addition, test environments with ultraviolet were exposed to a dose 8.1 kJ $m^{-2}$ ultraviolet radiation once a week as part of the batch culture protocol (Supplementary Tables 1 and 2). There are 96 test environments in total in this study and 288 populations (3 independent replicate populations per test environment ×96 test environments, Supplementary Table 3). The large size of this experiment motivates using *C. reinhardtii* as the model alga, as it grows easily in small volumes in media that it is already adapted to that have sufficient buffering capacity to control pH when $CO_2$ is varied, has a wealth of information available on responses to the individual drivers used in our study, and is a common model system in algal physiology and evolution. Cultures were grown in 48-well plates containing 1.6 ml of culture media. Each population was acclimated to its test environment for seven days (three generations), and then transferred to fresh test environment medium for each regime.

Details of how individual drivers were manipulated and our reasoning behind specific manipulations are below. In general, driver intensities were kept in line with future climate change scenarios where possible[17,49,50], but modified to accommodate logistics, the starting point of the benign lab environment, the need that each driver affect growth, and avoiding rapid extinction in environments that contained only one driver. Although extinction is one possible outcome of populations being exposed to changes in environments, the goal of our study was to learn how responses to one environmental driver predicted responses to multiple environmental drivers; this requires meaningful measures of growth in the single-driver environments. With the exception of $CO_2$/pH, we did not attempt to control chemical interactions between drivers; these interactions may contribute to organismal responses and to subsequent patterns of how response scales with the number of drivers. Because this study aimed to understand average biotic responses with increasing numbers of drivers, we had more power to detect a pattern by including a greater number of drivers rather than focusing on specific chemical interactions among drivers.

**Temperature.** A conductive heat mat (Exo Terra Heat Wave substrate heat mat) was placed under experimental plates to increase the temperature of the culture media to 26 °C. This did not affect the control temperature set within the incubator and was controlled using a thermostat (Rootit Heat Mat Thermostat). Our reasoning is that a 1 °C rise in temperature could be produced without affecting the overall temperature of the incubator or causing condensation on the culture vessel lid, falls within the range of predicted temperature rises for aquatic ecosystems[49] and produces a change in growth rate in *C. reinhardtii*—and can thus act as a driver—but does not cause mortality (we wanted to avoid large numbers of extinctions during the experiment).

**$CO_2$.** Sterile breathable films (AeraSeal breathable sealing film) were used instead of the of the 48-well plate lids that came with the plates. This allows increased $CO_2$ diffusion into the media. Although we did not quantify the precise level of $CO_2$ in the media, growth in the high-$CO_2$ conditions was stimulated, indicating that it was acting as a driver, which is all that was needed for the purpose of this study. $CO_2$ levels in the test environments were chosen based on projected $CO_2$ levels, and are in line with other experiments investigating microalgal responses to $CO_2$ enrichment.

**pH.** The pH of the culture media was altered by adding 2% HCl. This required one to two drops per litre of HSMT, so the concentration of nutrients was not altered by changes in volume. The pH was measure with a pH meter (Thermo Orion Star A121 pH Portable Meter) and buffered by adding Tris-HCl. Even though this drop in pH (0.7 units) is large relative to changes expected in marine ecosystems[49] it is well within those experienced in freshwater systems[39]. On the basis of pilot work, this drop reliably affects growth in the *C. reinhardtii* in our laboratory cultures.

**Ultraviolet.** A ultraviolet lamp (UVM-57) was used to provide a dose of ultraviolet radiation at acclimation and at T0 (Supplementary Fig. 5). The breathable films were removed from the culture plates under sterile conditions during ultraviolet irradiation. The lamp was mounted 5.1 cm from the surface of the culture plates, providing an irradiative exposure of 33.75 W $cm^{-2}$. Populations were irradiated for 4 min, which corresponds to a ultraviolet dose of 8.1 kJ $m^{-2}$.

**Light intensity.** Overall light intensity was reduced by approximately 40% using a neutral density light filter (0.15 optical density), designed to reduce the light intensity across all wavelengths equally and attenuate light by absorption with minimal reflection. The filter was secured to the top of the experimental plates, allowing sufficient room for $CO_2$ to circulate. Our rationale for decreasing light was pragmatic; it is possible to put a filter on some of the culture vessels, but difficult to selectively increase light levels reliably for only a few populations during an experiment of this size. Furthermore, increasing light levels for *C. reinhardtii* often lead to bleaching and mortality[51,52]. We found that with this strain the light intensity used was high enough for growth, but limited the amount of bleaching in populations.

The strain we used (CC-2391) has been used by other experiments where light levels were 60 μE $m^{-2}$ $s^{-1}$, equivalent to 60 μmol $m^{-2}$ $s^{-1}$ (ref. 53), and 50 μmol $m^{-2}$ $s^{-1}$ (ref. 54). These light intensities are lower than the 'low light' intensity reported in ref. 13, although a different strain of *C. reinhardtii* (CC-125) was used. However, previous experiments used cultures that differed from ours in terms of volume, as well as other details, and so should not be directly compared. We have been growing this strain in the laboratory for several years under the light levels used in this experiment (32 μmol $m^{-2}$ $s^{-1}$), and as the experiment depends on using environmental change (that is, change relative to a control environment that the organism usually experiences), deviation from the light levels usually experienced is needed. We verified that the light levels in the control environment allow faster growth than the light levels in the test environments (see Supplementary Fig. 9). Neutral density filters were used to decrease light levels, and we show that the filter used in our test environments (0.15 optical intensity) significantly reduced growth relative to the control light levels, and that the control light levels are not low down on the growth curve.

**Herbicide.** Atrazine was used at a concentration of 0.5 μM in HSMT. Atrazine was then added to the culture media used for this treatment freshly whenever populations were transferred into fresh media. On the basis of pilot work, this concentration of atrazine reliably affects growth in the *C. reinhardtii* genotype used.

**Nutrients.** All nutrients within Hutner's trace elements (HTE) were reduced equally to a concentration factor of 0.25 relative to the control concentration (see Supplementary Table 2 for concentration of each nutrient within HTE). As laboratory strains are used to growing in rich media such as HSMT, increasing trace nutrients has no measurable effect on growth. The reduction in nutrients needed to act as a driver in this experiment was determined empirically during pilot studies.

**Phosphate.** Phosphate was reduced to a concentration factor of 0.125, relative to the control concentration[39]. Salts lost by the removal of dipotassium phosphate ($K_2HPO_4$) and monopotassium phosphate ($KH_2PO_4$) were replaced with potassium chloride (KCl). The level of phosphate needed to act as a driver was based on pilot work and previous studies[55].

**Population growth.** Cells were counted by flow cytometry every 24 h for a total of 120 h using a BD FACSCanto II (BD Biosciences) flow cytometer calibrated with Cytometer Setup and Tracking (CS&T) beads. The data were acquired with the BD FACSDiva v6 software. Each culture was counted twice. The cell counts were transformed into cells per millimetre and the number of divisions per day per starting cell was calculated using equation (1):

$$\text{Rate of division}\left(\text{day}^{-1}\right) = \frac{(\log_2(N_t/N_o))}{(t_f - t_0)} \quad (1)$$

where $N_t$ is the cell density (cells $ml^{-1}$) at time $t_f$ (hours) and $N_o$ is the cell density at time $t_0$ (hours). This calculation was used because different environments produced different shaped growth curves (Supplementary Fig. 6), and the usual metric of maximum growth rate was not useful, whereas this measures the average number of divisions per day per founder cell in a transfer cycle, and allows comparison of populations with different growth strategies[41]. In particular, this measure gives the average number of divisions per day that have taken place per founder cell in the population, where cells divide by binary fission, as is the case here. It is also a metric that is not affected by $N_0$, which is required because the population size reached during the acclimation period differs between environments—this is to be expected given that the environments were chosen to have a range of effects on growth. Here, even though many of the curves seen in Supplementary Fig. 6 do not appear exponential, an exponential process (binary fission) underlies them, and thus justifies the use of equation (1). There are several

reasons why an exponential process may fail to produce a full exponential growth curve, such as the rate of cell division not being constant over the entire time period measured, or a low number of division events occurring within the time window of interest. In different environments, maximum cell division rates, the tempo of cell division events over the transfer cycle, the presence and length of lag phases, and carrying capacities, in any combination, may differ. The metric of the average number of reproductive events per unit time over the time window of interest is a general one, although a different equation would have to be used in the case where the organism being studied did not reproduce by binary fission. In our experiment, populations in the control environment were never nutrient-limited (cultures never reached carrying capacity). For comparison, we also used a more conventional measure of population growth (see Supplementary Methods) that simply measures the slope of the growth curve, which is the average number of cell divisions per day in the entire culture, rather than per cell in the starting population. In this case, it is possible to have a larger number of cell divisions simply by having a higher population density at the end of the acclimation period, leading to a larger value of $N_o$, so the measure of slope is sensitive to small differences in the initial population size. The two methods reach the same conclusions, probably because starting population sizes were similar over different populations in our experiment.

**Statistical analysis.** The effect of the identity and NED on growth was analysed using a mixed model in R (ref. 56), using the packages lme4 and lmerTest. Number of environmental drivers (0–8; referred to as NED) is a fixed factor, as is overlap between regimes within each level of NED (measured as the average number of shared drivers between different test environments for a given NED—see below). Regime and replicates within each regime are random factors. To directly compare the contributions of fixed and random factors to variance, the percentage contribution of fixed factors (Supplementary Table 5) was estimated by using equation (2).

$$\text{Percentage of fixed effect variance} = \left(\frac{\sigma_F^2 \times (b^2 - se^2)}{\sigma_X^2}\right) \times 100 \quad (2)$$

where $\sigma_F^2$ is the variance of the fixed effect, $b$ is the slope of the fixed effect estimated by the mixed effects model, se is the standard error of the fixed effect as estimated by the mixed effects model and $\sigma_X^2$ is the variance of the response variable.

***Post hoc* analysis.** A *post hoc* mixed model was used to detect effects of particular drivers (for example, of $CO_2$ or pH) where the identities of each driver were nested within NED were added to the random part of the model in place of regime and overlap, as described above.

**Overlap of NED between regimes.** Each regime is unique—however, because regimes become more similar as the number of environmental drivers increases, overlap between regimes for a given NED was calculated as an average pairwise difference between regimes, where each environmental driver is coded as a binary variable (present or absent). Average overlap for each NED is calculated as 1 − (average pairwise distance). The overlap for test environments with 0, 8 and 1 environmental drivers is zero as there is only one control regime, one regime with all eight environmental drivers and in test environments with 1 driver, all eight changes were assayed alone. The analysis was performed the same way for each subset of the presented data (including the case study and full data set less $CO_2$). The effect of sampling from a finite number of possible environmental drivers was explored using a simulation written in R (Supplementary Information).

**Models.** Expected numbers of division ($N_{exp}$) for each regime were calculated for each of three models (simple comparative, multiplicative and additive), using the observed number of divisions ($N_{obs}$) measured for NED = 1, where each driver is experienced alone. For the simple comparative model, $N_{exp}$ is equal to the most dominant individual environmental driver relative to the control ($1 - N_{obs}$). For example, if herbicide is found to elicit the largest change in population growth, any other driver present within that regime would have no additional effect. For the additive model, $N_{exp}$ is calculated as the sum effects of all individual drivers present in the regime when experienced alone (at NED = 1). For the multiplicative model, $N_{exp}$ is the product of $N_{obs}$ for each of the drivers present in the regime when they are experienced alone (at NED = 1). Model fits were compared using the $r^2$ values. The expected number of divisions for each model (simple comparative, multiplicative and additive) was fitted against the observed fitness using a linear model. This was completed in R using the lm function within the R basic stats package.

**Data.** All data and R scripts are available from Datadryad (http://dx.doi.org/10.5061/dryad.jt1fb).

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