LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 9999, 2019, 1–10

doi: 10.1002/lno.11298

Strong effects of elevated CO_2 on freshwater microalgae and ecosystem chemistry

Terry-René W. Brown ,* **Marc J. Lajeunesse,*** **Kathleen M. Scott**
Department of Integrative Biology, University of South Florida, Tampa, Florida

Abstract

The carbonate chemistry of freshwater systems can range from inorganic carbon-limited to supersaturated with respect to the atmosphere, and the pH of these systems can vary temporally and spatially from alkaline to acidic. Determining how these heterogeneous systems respond to increases in atmospheric CO_2 is critical to understanding global impacts of these changes. Here, we synthesize 22 studies from a variety of systems to explore the effects of elevated CO_2 on freshwater chemistry and microalgae, which form the base of autotrophic food webs. Across the variability in freshwater systems, elevated CO_2 significantly affected water chemistry by decreasing pH and increasing dissolved inorganic carbon. Microalgae were also affected by elevated CO_2 with measured increases in (1) nutrient acquisition through microalgal carbon-to-nutrient ratios, (2) photosynthetic activity, and (3) growth. While these effects were measured from controlled experiments, the results indicate a wide range of potential freshwater ecosystem effects from elevated atmospheric CO_2. Our synthesis also identified several knowledge gaps. Generally, larger sample sizes and studies of longer duration are needed for more robust analyses and conclusions. Additionally, more field experiments across a range of freshwater ecosystem types and studies involving benthic species and multiple trophic levels are needed to strengthen global predictions across the broad variability found within and among freshwater systems.

Since preindustrial times, concentrations of atmospheric carbon dioxide have increased from approximately 280 ppm to over 400 ppm and are now the highest levels measured in ice cores from the past 800,000 years (Intergovernmental Panel on Climate Change [IPCC] 2013). In addition to its role as a greenhouse gas that traps solar radiation, increasing global average land and water temperatures, the CO_2 itself impacts biotic and abiotic systems in a myriad of ways.

In terrestrial systems, CO_2 enrichment experiments using open-top chambers or free-air CO_2 fertilization have shown effects in both above- and belowground systems. A meta-analysis of elevated CO_2 effects on woody plants found increases in biomass, CO_2 assimilation, and leaf starch content in most species but no detectable impacts on belowground carbon storage (Curtis and Wang 1998). Another meta-analysis measuring elevated CO_2 effects on plants and herbivores revealed an increase in plant biomass, starch, and C:N, and a decrease in herbivore abundance (Stiling and Cornelissen 2007). In syntheses of studies involving food crops, elevated CO_2 increased yield in most species but decreased nutritional quality including decreases in nutrient concentration (e.g., in N, Zn, Fe, Ca, Mg, and P) (Broberg et al. 2017; Uddling et al. 2018). In soils, elevated CO_2 increased biomass and C:N in plant litter and roots and increased soil microbial growth and activity (Kuzyakov et al. 2019), and changed community composition of arbuscular mycorrhizal fungi (Cotton 2018).

There are also well-documented elevated CO_2 impacts in marine systems. When the oceans absorb elevated CO_2 from the atmosphere, carbonic acid (H_2CO_3) forms that rapidly deprotonates, forming HCO_3^- and releasing hydrogen ions and thereby decreasing pH (Zeebe and Wolf-Gladrow 2001). Based on IPCC greenhouse gas trajectories, by the end of the century, the pH of the ocean will have decreased by 0.06–0.32 pH units (best- and worst-case scenarios, respectively; IPCC 2014). The increase in dissolved inorganic carbon (DIC = CO_2 + HCO_3^- + CO_3^{2-}) and decrease in pH affect calcifying organisms in a variety of ways. In a meta-analysis of 107 studies that measured biological response of marine organisms including mollusks and corals to acidification and/or warming, overall, calcification, reproduction, and survival were negatively affected by acidification and were more negatively affected by the combined effects of acidification and warming (Harvey et al. 2013). In another meta-analysis of 23 studies of acidification effects on planktonic coccolithophores, calcification rates decreased overall with increasing acidity in two prolific species, but there was some variation in response depending on the species and strain studied

*Correspondence: twbrown@mail.usf.edu (TRWB) or lajeunesse@usf.edu (MJL)

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(Meyer and Riebesell 2015). Acidification thus impacts marine taxa; however, effects vary, even at the level of strain.

Freshwater systems have garnered less attention than marine systems in terms of elevated CO_2 effects, and the high heterogeneity in carbonate chemistry within and among freshwater systems complicates predictions of long-term responses to elevated atmospheric CO_2 (Cole et al. 2007; Song et al. 2014). Among freshwater systems, ambient pH can range from 3.8 to 9.9 and ambient DIC ($CO_2 + HCO_3^- + CO_3^{2-}$) concentrations can vary from 0.005 to 146 mM (Cole et al. 1994). Some freshwater systems are DIC-limited or have localized or seasonal DIC limitations (e.g., Finlay 2003; Urabe et al. 2003; Hargrave et al. 2009), and other systems are rich in inorganic carbon with many supersaturated with respect to the atmosphere (e.g., Cole et al. 1994; Sobrino et al. 2009; D'Amario and Xenopoulos 2015). Pronounced daily and seasonal variation occurs as well (Maberly 1996; McDonald et al. 2013; Hasler et al. 2016), and the size of the waterbody influences DIC concentrations; for example, larger lakes typically have longer residence times and are closer to atmospheric equilibrium than smaller lakes (Hasler et al. 2016).

Biological activity also affects DIC concentrations and pH. Systems in which the primary inputs of carbon are due to inorganic carbon fixation from algal photosynthesis often have relatively low concentrations of DIC and periods of DIC limitation during peak productivity; whereas, in systems with high terrestrial inputs of inorganic and organic carbon such as from groundwater and catchment area, microbial decomposition and groundwater contribute more DIC to the system than photosynthesis can consume, and this often leads to CO_2 supersaturation relative to the atmosphere (Hasler et al. 2016). Nutrient levels (e.g., nitrogen and phosphorus) also vary within and among freshwater systems, and impacts of elevated CO_2 may be increased when nutrients are not limiting (Low-Decarie et al. 2014; Hasler et al. 2016). With such variations in freshwater DIC dynamics, it is not well understood or easily predictable how freshwater systems will respond to long-term increases in atmospheric CO_2 (Cole et al. 2007; Song et al. 2014).

Microalgae are often studied as indicators of CO_2 impacts to freshwater ecosystems, particularly those dominated by primary production. While their response to elevated CO_2 is variable, there is typically an increase in the rates of growth and primary productivity (Qiu and Gao 2002; Chinnasamy et al. 2009), and these effects can be especially pronounced when coupled with high nutrient levels (Shikano and Kawabata 2000; Low-Decarie et al. 2014; Hasler et al. 2016). Impacts to microalgae from elevated CO_2 are also expected to affect algal consumers. Responses vary, from higher consumer biomass and density resulting from increased algal primary productivity (Hargrave et al. 2009) to decreases in consumer growth rates due to lower algal nutritional quality (Urabe et al. 2003) or to increases in production of algal toxins (Van de Waal et al. 2009; Sandrini et al. 2015*a*).

To broadly assess potential impacts of elevated CO_2 on freshwater microalgae, as well as sources of heterogeneity, we used meta-analysis to synthesize the outcomes of 22 studies that elevated CO_2 up to 2000 ppm and measured its effects in four major response categories: water chemistry, as well as nutrient acquisition, photosynthesis, and growth of microalgae. We also included effects on growth in algal consumers. Furthermore, we assessed potential methodological sources of heterogeneity to determine whether experimental and culturing conditions also moderate microalgal responses to elevated CO_2.

Methods

Literature search

Our goal was to identify studies that compared the effects of elevated and ambient levels of CO_2 on freshwater microalgae. On 05 February 2016, we used Web of Science (University of South Florida Library subscription) to search the primary literature with the following search terms: (*elevat** or *enrich**); (*CO_2* or *carbon dioxide*); (*alga** or *microb** or *microo** or *microalga** or *cyano** or *diatom** or *phytoplankt**); (*aquat** or *freshwater* or *stream** or *river** or *lake** or *wetland**). This search covered journal articles up to January 2016 and generated 612 candidate papers.

Study screening and inclusion criteria

We screened the abstracts and full text of these 612 candidate studies for experiments that met the following criteria. First, elevated CO_2 level(s) must have been reported and were less than or equal to 2000 ppm. Although this CO_2 range exceeds the IPCC's worst-case scenario for the end of the century of 1000 ppm (IPCC, 2000), most freshwater systems tend to be supersaturated with respect to atmospheric CO_2 (Cole et al. 1994; Raymond et al. 2013). We chose to double this reported average to ensure that we included studies with elevated CO_2 levels that simulated scenarios resulting from the interaction of supersaturated freshwater systems with elevated atmospheric CO_2 concentrations. Our cutoff range of 2000 ppm excluded studies that simulated CO_2 conditions that far exceeded the predicted ranges of future atmospheric conditions—such as studies with levels over 20,000 ppm (e.g., Hanagata et al. 1992; Berman-Frank et al. 1994). Second, we excluded studies that did not report information necessary for the Hedges' *d* calculations (i.e., means, variances, and sample sizes) as described below. Third, studies explicitly manipulating algal competition responses were excluded since the outcomes tended to focus more on competition effects rather than CO_2 effects (e.g., Xu et al. 2010); however, if these studies reported separate experiments on responses to elevated CO_2 without competition, these were included in our meta-analysis (e.g., figs. 3a–c in Low-Decarie et al. 2011). We also excluded studies for which the data did not directly address our hypotheses such as those that measured responses in decomposers and not autotrophs (Fenner et al. 2007; Ellis et al. 2009). Based on these inclusion/exclusion criteria, 22 studies reporting microalgal responses to elevated CO_2 were identified (Table S1).

Responses to CO_2

From each study, we extracted information on elevated CO_2 effects in four major response types:

1. Water chemistry—pH and other water chemistry parameters (DIC and its component species of CO_2, HCO_3^-, and CO_3^{2-});
2. Nutrient acquisition—Algal stoichiometry (C:N and C:P of the biomass) and nitrogen and phosphate uptake (reported as depletion from the growth media);
3. Photosynthesis—Inorganic carbon uptake and photosynthetic parameters (including oxygen evolution rate and measures of photosynthetic rates and efficiency); and
4. Growth—Algal and consumer growth (including biomass, chlorophyll *a* as a proxy for biomass, growth rate, cell density, cell volume).

Three potential sources of heterogeneity (i.e., variation in response to elevated CO_2) within each of these four major response types were explored:

1. Effects of subgroups within major response categories;
2. Effects of experimental parameters with continuous values; and
3. Effects of experimental parameters with categorical values.

To explore the effects of subgroups within major response categories, water chemistry was subdivided into pH and inorganic carbon concentration, as these are the two parameters that are affected by elevated CO_2. Nutrient acquisition was subdivided into algal biomass stoichiometry (C:N and C:P ratios, which can indicate enhanced inorganic carbon assimilation); and nitrogen and phosphorus uptake. Photosynthesis was separated into its two components: photosynthetic parameters reflecting operation of the photosynthetic electron transport chain (e.g., oxygen evolution), and carbon dioxide uptake, reflecting the activity of the Calvin cycle. Finally, growth was separated between growth of microalgae and consumers (i.e., planktonic herbivores such as *Daphnia*).

To explore the effect of experimental parameters with continuous values, effect sizes were separated into each of the four major response categories, and these subdata were explored for sources of heterogeneity (i.e., variation in response) due to experimental duration, design size, temperature, pH at start, nitrogen level, phosphorus level, and CO_2 elevation magnitude. To explore the effect of experimental parameters with categorical values, effect sizes from each of the four major categories were parsed by natural vs. cultured algal origin, closed vs. open design (e.g., flasks without replenishment vs. semicontinuous cultures in chemostats), field vs. lab setup, aeration vs. sparging CO_2 delivery, taxa (cyanobacteria, diatoms, mixed natural), benthic vs. planktonic distribution, bloom-forming or not (as reported), and light duration (14 h, 24 h, or natural daylight). If algal collection details were not reported, culture collections were consulted when identified to determine culture history and origin. Likewise, if specific culture details were provided (e.g., Bold's basal medium), growth media recipes were consulted for details on nitrogen, phosphate, and pH when not reported. Algal taxonomy was obtained from Algaebase (Guiry and Guiry 2016). When measurements were taken over time, only the maximum response values were extracted to remain consistent with studies that reported only maximum response values and because effects may dissipate with time, for example, as other culture conditions become limiting.

Data extraction, effect size calculation, and meta-analyses

When possible, the means, variances (or its surrogates), and sample sizes of all responses to elevated and ambient CO_2 were extracted from each study—these data are necessary to estimate study outcomes in terms of effect sizes. When responses were only available in figures, we used ImageJ software and the Figure Calibration Plugin (Rasband 2015) to manually extract these data. For cases where error bars in figures were ambiguous due to overlap, the larger of possible error bars were used to estimate outcome variances (e.g., fig. 2d in Sandrini et al. 2015*a*). In some studies, the raw data were available or obtainable from figures to directly calculate unreported means and variances (e.g., Collins and Bell 2006). If variances (or surrogates) were not reported in the study, responses were excluded during data extraction and data set generation, prior to meta-analyses. There is no expectation that the lack of these data would bias overall results, since they are generally assumed to be missing at random from publications (Lajeunesse 2013*b*). Not including them does decrease power to detect effects, however, due to the lower synthesis-level sample size resulting from the exclusion (Lajeunesse and Forbes 2003; Lajeunesse 2013*b*).

Study outcomes were quantified as the standardized mean differences between responses to elevated and ambient CO_2 using the effect size metric Hedges' *d* (Hedges 1981):

$$d = \frac{\bar{X}_E - \bar{X}_A}{\sqrt{\frac{(N_E-1)SD_E^2 + (N_A-1)SD_A^2}{N_E+N_A-2}}}\left(1 - \frac{3}{4[N_E+N_A]-9}\right)$$

where $\bar{X}_E$ and $\bar{X}_A$ are the means ($\bar{X}$) of a response under elevated (E) and ambient (A) concentrations of CO_2, and where N is the sample size and SD the standard deviation of $\bar{X}$. Hedges' *d* effect sizes quantify the magnitude and direction of experimental outcomes, where positive *d* indicate positive effects due to CO_2 elevation, and negative *d* a decrease relative to ambient treatment levels (e.g., a decrease in algal growth due to elevated CO_2). In the case of pH, we reversed the sign of the effect to maintain consistency in response. In cases where studies only reported *t*-tests, these were converted to *d* following Lajeunesse (2013*b*). Only 1% of all effect sizes were derived from these conversions (4 of 372 effect sizes). The weights used in our meta-analysis and meta-regressions were the inverse of the variance of each Hedges' *d* effect size defined as:

$$\mathrm{var}(d) = \frac{1}{N_E} + \frac{1}{N_A} + \frac{d^2}{2(N_E + N_A)}.$$

In total, 372 effect sizes were calculated from 22 papers published between 1998 and 2015 (Table S1). We combined and compared these effect sizes using mixed-effects meta-analyses (when moderators were categorical) and meta-regressions (when moderators were continuous) in *R* with the *metafor* (Viechtbauer 2010; v. 1.9.2) and *metagear* (Lajeunesse 2016; v. 0.3) packages. All analyses were based on the rm.mv function of *metafor* parameterized with maximum likelihood estimators for the between-study variance (τ^2; a requirement for random-effects models) and an additional random factor that modeled the potential overrepresentation of multiple effects derived from a single study. Between-group *Q*-tests (Q_b) were used to assess differences among moderator variables (pooled effect sizes within groups) with more than two subgroupings (similar to an *F*-test arising from an ANOVA; Hedges and Olkin 1985), Wald-type *z*-tests were used to assess whether moderator groups with two subgroups or continuous predictors were significantly nonzero (similar to a *t*-test; Lajeunesse 2013*a*), and finally *k* designates the sample size of meta-analysis (i.e., number of Hedges' *d* effect sizes) and *N* the sample size within studies (see Hedges' *d* equations earlier). The majority of studies had total *N* ranging from 6 to 10 replicates (*see* histogram Fig. S1a). This poor variation in sample sizes across studies also generates the hump-spread shape of inverse variances observed in a funnel plot (e.g., where a hump-line of effects indicates effects estimated from the same sample size; Fig. S1b). Publication bias was assessed with Egger's publication bias test (Egger et al. 1997) with the inverse standard error (*see* funnel plot in Fig. S1b). Although the test points to publication bias ($t = 9.7$, df = 370, $p < 0.0001$; tested with the *regtest* function in *metafor*), with null study outcomes being less represented in the literature, we note that Egger's test performs very poorly when sample sizes are small within studies and when there is large between study heterogeneity, as in our meta-analysis (i.e., violating two of the four criteria needed to properly assess publication bias; *see* Ioannidis and Trikalinos 2007). Finally, for additional information on meta-analysis and meta-regression, please see Koricheva et al. (2013).

Results and discussion

Water chemistry, nutrient acquisition, photosynthesis, and growth were all significantly affected by elevated CO_2 (Fig. 1a). These results encompass a broad span of elevated atmospheric CO_2 impacts to freshwater systems. The overall magnitude of these responses differed (Fig. 1a; $Q_b = 16.8$, df = 3, $p < 0.001$), with water chemistry being the most impacted by elevated CO_2 compared to both photosynthesis (Wald-type *z*-test [i.e., significantly nonzero] = −3.4, df = 1, $p < 0.001$) and growth (Wald-type *z*-test = −3.5, df = 1, $p < 0.001$), but the response in water chemistry was not significantly different from the response in nutrient acquisition (Wald-type *z*-test = −1.7, df = 1, $p = 0.082$).

Water chemistry

Elevated CO_2 had strong impacts on water chemistry (Fig. 1a), consistent with expectations of equilibrium chemistry. Decomposing chemistry components into pH and DIC concentrations did not reveal differences between the two; this result is consistent with the tight coupling of pH and DIC (Zeebe and Wolf-Gladrow 2001) (Fig. 1b; Wald-type *z*-test = −0.71, df = 1, $p = 0.475$). Higher levels of elevated CO_2 did correspond to greater chemical responses in terms of pH and DIC concentrations (Fig. 2a; Table S2). These strong effects on water chemistry were not dependent on method of CO_2 delivery; elevated CO_2 affected water chemistry whether CO_2 was directly sparged (bubbled) into the water or aerated above the water (Fig. S2). The particularly large impacts on water chemistry are consistent with predictions that chemical responses would be rapid, since they are a function of CO_2 dissolution, while biological responses could be dampened, such as by physiological mechanisms that maintain homeostasis under these manipulated conditions.

Nutrient acquisition

Elevated CO_2 resulted in significant increases in microalgal nutrient acquisition (Fig. 1a). However, when nutrient acquisition was parsed into biomass stoichiometry (C:N and C:P) and nitrogen and phosphate uptake from the growth media, only biomass stoichiometry was significantly affected by elevated CO_2 (Fig. 1b). This impact on C:N and C:P ratios, but not on N or P uptake, could be driven by the lower levels of N and P in the studies measuring stoichiometry compared to nutrient uptake (stoichiometry median $N = 120\ \mu M$; median $P = 4\ \mu M$; nutrient uptake median N = 4, 464 μM; nutrient uptake median P = 100 μM). Elevated C:N and C:P ratios have been noted in other studies in which CO_2 concentrations were increased under low N and P conditions, presumably due to limitations on N and P assimilation, and unbalanced growth, under these conditions (Verschoor et al. 2013). Additionally, N or P uptake may be by heterotrophic microbes (e.g., bacterioplankton) that are less affected by CO_2 levels.

Nutrient acquisition responses to elevated CO_2 were positively affected by CO_2 elevation magnitude but negatively affected by duration of the experiments (Fig. 2b,c; Table S2). For natural populations, this may reflect changes in the species composition of the samples taken over the course of the experiment. For monocultures, this diminished response over time may reflect either physiological or genetic changes. Long-term cultivation of microalgae under elevated CO_2 conditions can result in diminishment of carbon-concentrating mechanism (CCM) activity (Collins and Bell 2004, 2006; Collins et al. 2006). If CCMs were initially active in these cultures, they could have resulted in elevated C:N and C:P ratios under elevated CO_2 conditions, due to increased inorganic carbon transport and fixation by the cell, and accumulation of starch or lipids. Over time, loss of the CCM would result in lower C:N and C:P ratios. However, this effect is dependent on CCMs being expressed, initially, under elevated CO_2 conditions. More typically, CCMs are

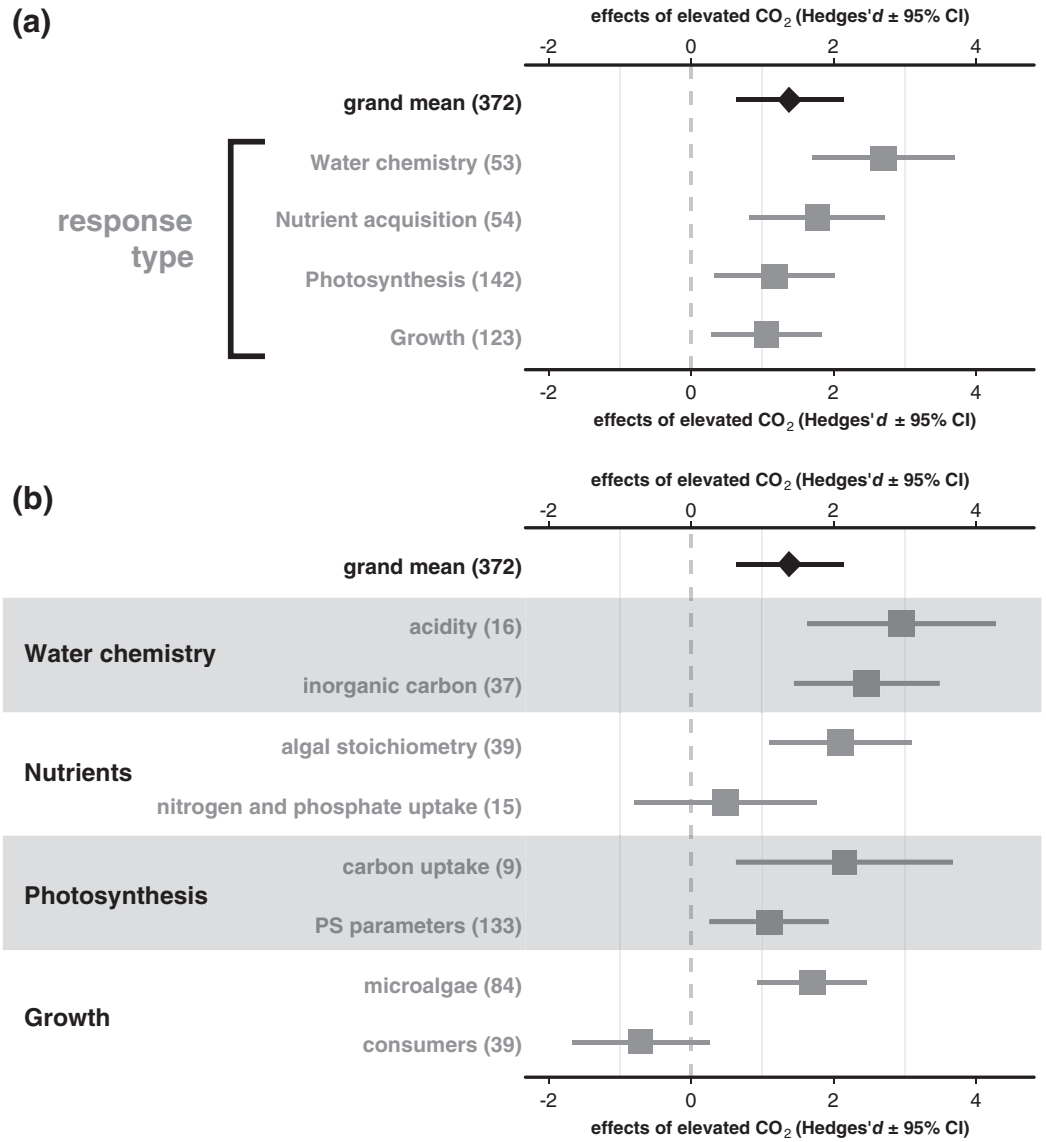

Fig. 1. Forest plot of mixed-effects meta-analyses across major classes of microalgae responses to elevated CO_2. Pooled responses (Hedges' *d*) are significant when confidence intervals do not overlap zero, and (*k*) number of effect sizes per group are in parentheses. Emphasized in black is the grand mean of the pooled effect across all studies, and in gray are subgroupings of these effects. (**a**) Pooled effect sizes among the four major response types. (**b**) Major response types further decomposed among classes of measured responses (these pooled effects were based on separate mixed-effects meta-analyses parsed by these response types). PS parameters include oxygen evolution, photosynthetic rate, and photosynthetic efficiency.

induced under low CO_2 conditions, though organisms can express them at low levels under elevated CO_2 conditions (Price et al. 2008).

Nutrient acquisition response varied by algal type with mixed manipulated cultures, diatoms, and green algae positively affected while cyanobacteria, protists, and mixed natural cultures (i.e., mixed cultures collected from the field) were not significantly affected (Fig. S3). Given that the response of nutrient acquisition to CO_2 was largely due to changes in biomass stoichiometry (see above), this effect is likely due to changes in stoichiometry among these different types of organisms. Heterogeneity has been noted in biomass stoichiometry, even within taxonomic groups of algae (Finkel et al. 2010). Too few studies have been done to determine whether taxonomic groups of algae have distinctive responses in biomass stoichiometry in response to CO_2 concentrations. The differences noted in our study suggest that this may be the case, but the mechanisms for this taxonomic effect (possibly different mechanisms for nutrient uptake and assimilation) remain to be determined.

Photosynthesis

Elevated CO_2 had an overall significant and positive impact on photosynthesis (Fig. 1a). There were no differences between inorganic carbon uptake and other photosynthetic parameters

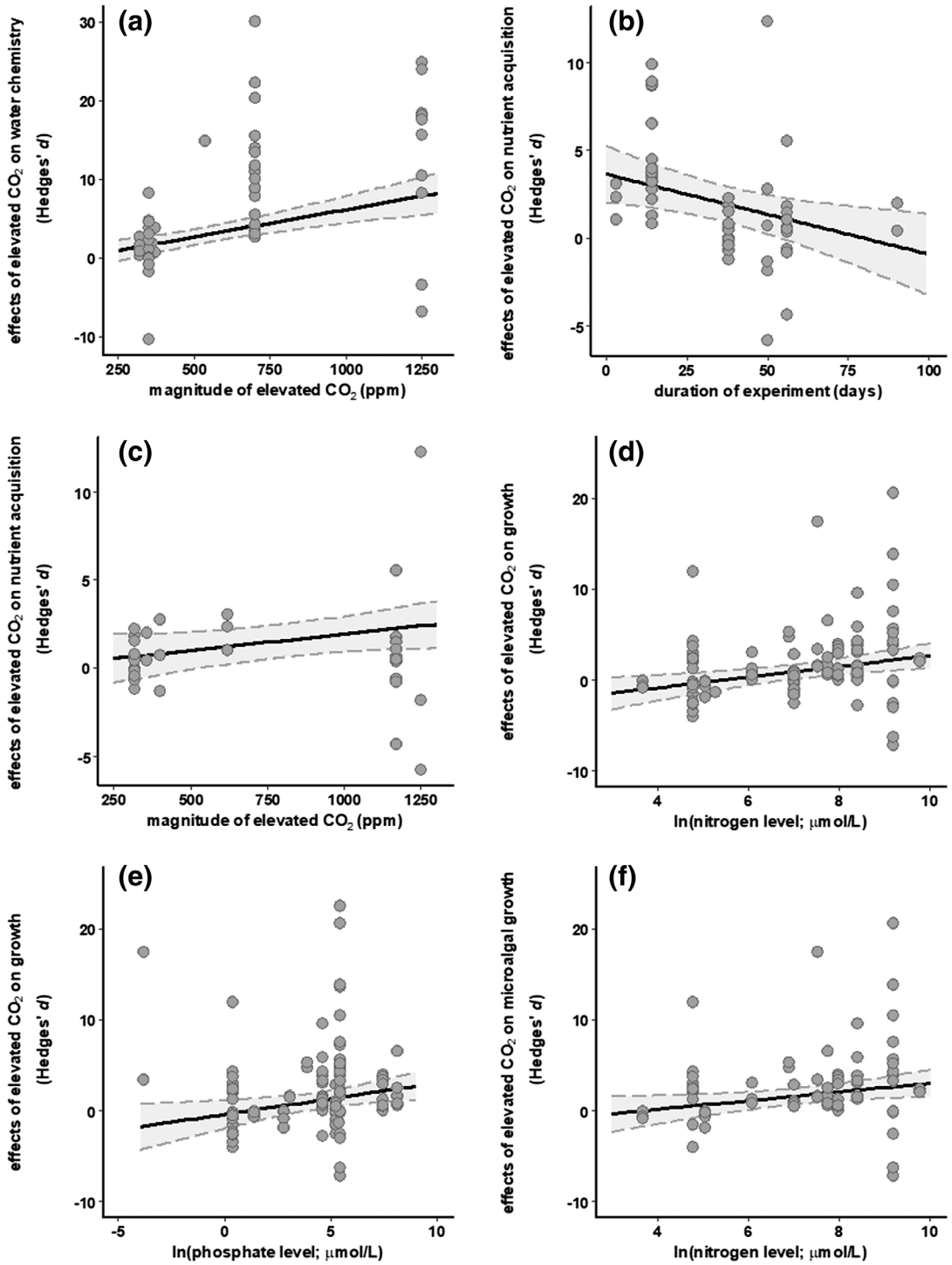

Fig. 2. (**a**) Predictors of effects of elevated CO_2 on water chemistry, (**b**,**c**) nutrient acquisition, and (**d**–**f**) growth based on mixed-effects meta-regressions with 95% confidence intervals for the fitted slope. Predictors include the magnitude of elevated CO_2 (e.g., the total amount of enrichment in ppm relative to ambient levels), duration of experiments, and levels of nitrogen and phosphorus. Note differences in units on x- and y-axes across figures. Only significant relationships are shown here; *see* Table S2 for the full set of results. (f) represents growth in microalgae only (herbivore consumers were removed from analysis).

(Wald-type z-test = −1.32, df = 1, $p = 0.19$; Fig. 1b). Photosynthesis responses to CO_2 were not affected by experimental design parameters with continuous values (Table S2). Categorical experimental conditions affected overall photosynthetic response (e.g., grand mean in Fig. S4). Photosynthesis effects were also significant for planktonic, but not benthic algae (Fig. S4), which may also reflect

taxonomic differences between organisms with these lifestyles. Differences in significance are apparent in response of photosynthesis by algae collected from the field ("natural" in Fig. S4) vs. cultured, which may reflect differences in taxonomy of the organisms tested.

The effect of taxonomy on response of photosynthesis to CO_2 concentrations is supported when these results are parsed by algal type. Elevated CO_2 had a positive effect on photosynthesis for cyanobacteria, diatoms, and mixed natural samples, with similar values of Hedges' *d* (Fig. S4). The effects were statistically distinguishable from zero only for cyanobacteria, which may reflect the much larger number of cyanobacterial effect sizes (k = 108) vs. other groups (k = 8 and 14). Elevated CO_2 had a small negative effect on photosynthesis for green algae that could not be distinguished from zero. The stimulatory effect of elevated CO_2 on photosynthesis by cyanobacteria and other algae is likely due to the presence of CCMs in these organisms (reviewed in Badger and Price 2003). Elevated CO_2 concentrations result in downregulation of these CCMs, less energy allocated toward DIC uptake, and can result in increased photosynthetic rates. Given that green algae also have CCMs (Giordano et al. 2005), it is unclear why elevated CO_2 did not have a positive effect on photosynthesis across the studies used for this meta-analysis.

Growth

Elevated CO_2 differentially affected the growth of microalgae and their herbivore consumers (Fig. 1b; Wald-type z-test = 5.33, df = 1, $p < 0.001$), so meta-analyses were performed on microalgae only as consumers had too few measurements for separate analyses. Microalgal growth increased under elevated CO_2 conditions, which is consistent with predictions (Xia and Gao 2003; Chinnasamy et al. 2009; Low-Decarie et al. 2011). Growth response to elevated CO_2 was positively affected by nitrogen levels and by phosphate levels (Fig. 2d–f; Table S2). Increase in growth was significant in cultures of cyanobacteria or green algae, but not in cultures of protists or in mixed cultures originally collected from the field (Figs. S5 and S6). Microalgal growth response included stimulations to bloom-forming species when consumers were excluded (Fig. S6), with observed increases in biomass, cell abundance, population densities, and chlorophyll *a* in bloom-forming *Microcystis aeruginosa* (Qiu and Gao 2002; Sandrini et al. 2015*a*, *b*), and increases in cell division rate, biovolume, growth rate, and chlorophyll *a* in bloom-forming *Cylindrospermopsis raciborskii* under elevated CO_2 conditions (Wu et al. 2012; Pierangelini et al. 2014). Elevated CO_2 may thus worsen the impacts of algal blooms, although it is difficult to extend the results from the lab to the field. DIC drawdown by blooms may result in localized carbon limitations, which may be alleviated by having an additional source of elevated atmospheric CO_2; this may worsen (i.e., expand and/or extend) the blooms.

Data limitations and knowledge gaps

Some aspects of experimental design may limit predictions. Experiments exceeding 60 d in duration are scarce (2 of 22 studies: Hargrave et al. 2009; Low-Decarie et al. 2011), and such longer-term experiments may be needed to address adaptation and other longer term effects from elevated CO_2. Within-study sample sizes (N) also tended to be small with the majority ranging from 6 to 10 replicates (Fig. S1a), thus the statistical power and resulting conclusions of many studies are limited. Additionally, of the 22 studies included in the meta-analysis, only 4 were conducted in the field (Table S1). This is in contrast to the many field studies conducted in terrestrial systems, recognizing that this may be, at least in part, because field studies are often logistically more challenging in water than on land. Nevertheless, in inland waters where CO_2 is generally more variable and frequently higher than what most land plants experience, field experiments are needed to inform predictions and to understand how results gained from controlled, short-term experiments are applicable to natural systems. The lack of freshwater field data adds to the considerable difficulty of predicting algal response to elevated CO_2 in inherently variable systems.

Our meta-analysis also draws attention to several knowledge gaps. Studies determining and comparing elevated CO_2 effects in specific systems such as between lentic and lotic, tropic and temperate, and blackwater and clearwater river systems are needed. Firm conclusions on differences in response by planktonic vs. benthic algae were also not possible; only 2 of 22 studies focused on benthic algae, 1 on algae isolated from soil, and all remaining studies tested effects on planktonic algae (Table S1). Similarly, responses in cyanobacteria are relatively well studied but more emphasis is needed on other algal taxonomic groups. When algae were cultivated under elevated CO_2 conditions, growth in herbivore consumers was overall negative but the trend was not significant (Fig. 1b); this emphasizes the need for additional studies at higher trophic levels and highlights current limitations on the ability to make broad and robust predictions of potential cascading effects of elevated CO_2 on freshwater communities and ecosystems. These gaps considerably limit broad global predictions on the effect of elevated CO_2 on freshwater systems.

Conclusions

Through a meta-analysis of freshwater algal responses to elevated CO_2, we found strong effects in all four major response classes tested, including water chemistry, biomass stoichiometry, and microalgal photosynthesis and growth. In systems that experience localized CO_2 drawdown due to seasonally high productivity and those with heavy nitrogen and phosphate loading, ecosystem-scale effects by elevated atmospheric CO_2 affecting microalgal growth and stoichiometry may occur. While we provide some of the fundamental predictions of the extent of the effects and highlight areas of particular vulnerability, a greater diversity of studies, especially in

the field, are urgently needed to improve the scope of predictions of elevated CO_2 effects on the vast global heterogeneity of freshwater systems.

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Acknowledgments

The authors thank Susan S. Bell for helpful suggestions throughout the course of the project. The authors also thank the Integrative Biology Department at the University of South Florida for project support and funding. M.J.L. was supported by National Science Foundation grants DBI-1262545 and DEB-1451031.

Conflict of Interest

None declared.

Submitted 20 January 2019
Revised 01 June 2019
Accepted 04 July 2019

Associate editor: John Downing