Comment on “The complex effects of ocean acidification on the prominent N₂-fixing cyanobacterium Trichodesmium”

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Hong et al. (Reports, 5 May 2017, p. 527) suggested that previous studies of the biogeochemically significant marine cyanobacterium Trichodesmium showing increased growth and nitrogen fixation at projected future high CO₂ levels suffered from ammonia or copper toxicity. They reported that these rates instead decrease at high CO₂ when contamination is alleviated. We present and discuss results of multiple published studies refuting this toxicity hypothesis.

Marine nitrogen-fixing cyanobacteria are important to the global carbon cycle and climate, as they provide vital new nitrogen supplies that allow phytoplankton to draw down atmospheric carbon dioxide (CO₂). Many experiments over the past decade have predicted that the globally distributed tropical cyanobacterium Trichodesmium spp. will grow faster and fix 30 to 60% more nitrogen under projected future doubled seawater CO₂ concentrations (1–6). Such CO₂ fertilization of marine nitrogen fixation could potentially provide a negative feedback on anthropogenic CO₂ emissions (2, 5, 6).

Hong et al. (7) argue that these often-reproduced results actually stem from chemical contamination of the widely used Trichodesmium artificial seawater culture medium YBCII. A batch of MgCl₂ reagent used in their medium preparation led to contamination of their YBCII with growth-inhibiting ammonia (~20 μmol/liter). They speculate that accidental contamination with toxic copper is also likely, although no copper measurements are presented to support this contention. As evidence, they present experiments showing that growth and nitrogen fixation rates increase when ammonia-free MgCl₂ is used to prepare YBCII, or when Trichodesmium are grown with higher levels of the trace metal chelator EDTA to bind and detoxify putative copper contamination. Crucially, they also found that the commonly observed CO₂ stimulation of Trichodesmium nitrogen fixation and growth appears to be reversed in their “uncontaminated” media. They therefore attribute the opposing results seen in nearly all prior studies to ubiquitous, previously unrecognized contamination artifacts (7).

This contamination artifact hypothesis can, however, be conclusively refuted by examining published studies. Although Hong et al. state that “all previous laboratory studies that have reported positive…effects of acidification…have been carried out with…the growth medium YBCII” (see supplementary text of (7)), in fact several Trichodesmium studies found large positive effects of high CO₂ in the same ammonia-free, trace metal-clean “Aquil-tricho” medium they advocate (5, 6, 8). Additionally, previous CO₂ experiments in both putatively “contaminated” YBCII and Aquil-tricho measured Trichodesmium nitrogen fixation rates that were as high as (or even higher than) the rates measured by Hong et al. in their “uncontaminated” medium preparations (Fig. 1). We use our own published results as examples, but most of the multiple previous studies cited by Hong et al. as being likely contaminated also documented similarly high nitrogen fixation rates. Moreover, if all these previous experiments were truly contaminated with ammonia (20 μmol/liter) as suggested, little or no nitrogen fixation would have been observed, as Trichodesmium nitrogen fixation is strongly inhibited (~50 to 100%) by ammonia concentrations of 10 to 20 μmol/liter (9–11). We have studied ammonia inhibition in nitrogen-fixing cyanobacteria (12, 13), and our measurements show that ammonia concentrations in both YBCII and Aquil-tricho are typically below detection limits (< ~0.5 μmol/liter).

Also contradicting the toxic contamination hypothesis is a study that examined seven different nitrogen-fixing cyanobacteria isolates grown across a range of CO₂ concentrations in Aquil-tricho medium (5). In every case, Trichodesmium nitrogen fixation rates closely fit a classic saturation curve model relative to CO₂ (r² = 0.95 to 1.00); one of these data sets is shown in Fig. 2, along with the corresponding Michaelis-Menten enzyme kinetics equation. This strikingly nutrient-like response to a CO₂ concentration gradient cannot be explained by invoking an inhibitory effect of contaminated growth medium.

Likewise, contamination does not explain the findings of an experimental-evolution study wherein growth and nitrogen fixation in six replicate Aquil-tricho–grown Trichodesmium cell lines were constitutively increased after ~850 generations of selection at high CO₂ (6). These cell lines now permanently fix nitrogen at higher rates—just as if they were growing at elevated CO₂—even when moved back to lower current CO₂ concentrations, where Hong et al. purport they should again be inhibited because of contaminants. This unique adaptive response is again wholly inconsistent with toxic inhibition.

Despite this contrary evidence strongly suggesting that culture medium toxicity is irrelevant to the results of most previous studies, we are still

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Fig. 1. Prior Trichodesmium CO₂ studies refuting the Hong et al. culture medium toxicity hypothesis. Reported Trichodesmium nitrogen fixation rates in YBCII medium (left) and Aquil-tricho medium (right) in purportedly “uncontaminated” iron-replete medium formulations from Hong et al. (open bars) and from previously published studies (hatched bars) in low CO₂ (white bars) and high CO₂ (gray bars) treatments (2, 5–7). Previous studies show no evidence for toxic inhibition of nitrogen fixation, and in fact rates are often higher than in Hong et al. Nitrogen fixation was measured and normalized the same way in all experiments shown; previously published data (2, 5, 6) were recalculated using the same 4:1 ethylene:N₂ conversion ratio used in Hong et al.

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left with the puzzling observation that Hong et al. recorded growth rates that were ~25% higher than other published rates—even though the nitrogen fixation rates supporting this rapid growth were similar to, or less than, those in previous studies (Fig. 1). However, this discrepancy is difficult to evaluate, as pertinent details are missing from their growth rate methods text. Although this is a relatively basic analysis, in the case of Trichodesmium the protocol chosen is critical. Nitrogen and carbon fixation and growth in this species follow a pronounced diel rhythm (3, 4, 6), so most cell division occurs in the afternoon. One can thus calculate anomalously elevated growth rates similar to those reported by Hong et al. simply by measuring them solely from early morning until late that afternoon (or a subsequent afternoon). These high growth rates will, however, retreat to widely published values if experiments are properly sampled over an exact 24-hour diel cycle. Unfortunately, this specific information was not provided, as it may have helped to explain why growth rates and other aspects of their study are inconsistent with previous Trichodesmium work, including shifts in diel nitrogen fixation patterns under elevated CO₂ (4–6) and trends in the abundance of many key proteins in iron-limited and high CO₂-grown cells (8, 13).

We agree with Hong et al. that iron limitation negates the positive effects of high CO₂ on nitrogen fixers. We observe similar iron-limited rates at high and low CO₂, however, rather than preferential inhibition by elevated CO₂ (8, 14). Although iron limitation indisputably constrains nitrogen fixation in much of the current ocean, increased aerosol iron supplies resulting from climate change and anthropogenic pollution may partially alleviate this limitation in the future ocean (15).

In conclusion, we certainly concur with Hong et al. that the effects of high CO₂ and attendant ocean acidification on Trichodesmium are complex, and we applaud them for alerting researchers to potential reagent contamination. It is clearly unwarranted, however, to project an unfortunate contamination problem in one laboratory onto a large, robust, and consistent body of research with important implications for changing ocean ecosystems. The reason that Hong et al. obtain results diametrically opposed to those of nearly every other similar study remains to be determined, but the evidence does not support the suggestion that this is because all other experiments are universally contaminated.

Fig. 2. Trichodesmium N₂ fixation saturation curve as a function of CO₂ concentration.

Results of a prior Trichodesmium study that measured nitrogen fixation rates across a range of CO₂ concentrations and found a highly significant fit to a nutrient-like saturation curve model (5). The corresponding Michaelis-Menten equation is shown; the solid line is the regression and dashed lines are 95% confidence intervals. This classic, well-defined positive response of nitrogen fixation rates as CO₂ limitation is relieved is fundamentally inconsistent with a toxic effect at either high or low CO₂ concentrations. Nitrogen fixation was measured and normalized in the same way (5) and recalculated using the same 4:1 ethylene:N₂ conversion ratio as in Hong et al.

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