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Phylogenetic distance does not predict competition in green algal communities

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Abstract. Biologists have held the tenet that closely related species compete more strongly with each other than with distant relatives since 1859, when Darwin observed that close relatives seldom co-occur in nature and suggested it was because they competitively exclude one another. The expectation that close relatives experience greater competition than distant relatives has become known as the "competition-relatedness hypothesis (CRH)." The CRH is predicated on the assumption that closely related species are more likely to have similar resource requirements than distant relatives, and thus, compete more strongly for limited resources. While this assumption has been popular because it is intuitive, it has also been subject to relatively little experimentation. Over the past decade, a growing number of CRH studies have arrived at divergent conclusions showing that the strength of competitive interactions can increase, decrease, or be independent of evolutionary relatedness. Most of these studies have focused on measuring competition among species pairs as opposed to competition experienced by species when part of whole communities. We tested whether the CRH holds in communities where individual species experience interactions with a variety of other taxa, which we call the 'resident community'. We performed a laboratory mesocosm study using communities of eight species of freshwater green algae whose evolutionary relationships were quantified using a recently developed multi-gene molecular phylogeny of 59 North American green algae. We grew species alone and in various combinations in polyculture so that we could measure each species' sensitivity to competition (reduction in intrinsic growth rate when grown alone vs. with a resident community), relative yield, and competitive release (proportional change in biomass of a species when grown in a resident community missing one competitor vs. in a community with all possible competitors). While each of these metrics consistently revealed a prevalence of competitive interactions among the algal species, none were predicted by the relatedness of a species to a resident community. This suggests that the results of prior pairwise studies refuting the competition-relatedness hypothesis for green algae can be extended to larger resident communities in which more complex ecological interactions possibly occur.

Key words: community ecology; competition; competition-relatedness hypothesis; competitive release; indirect interactions; relative yield; sensitivity.

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Introduction

Ever since Darwin (1859) proposed that closely related genera tend not to coexist in the same geographic region, ecologists have embraced the idea that evolutionarily close relatives compete more strongly than distant relatives. This hypothesis, which is now commonly referred to as the competition-relatedness hypothesis (CRH; Cahill et al. 2008), stems from the presumption that closely related species are more likely to share similar morphological, physiological, and behavioral traits due to shared ancestry (Harvey and Pagel 1991, Peterson et al. 1999, Blomberg et al. 2003). The sharing of traits (many of which may influence ecological interactions) among closely related species is called "phylogenetic niche conservatism" (Wiens and Graham 2005) or "phylogenetic signal" (Losos 2008), depending on the extent of departure of species trait values from those predicted based on phylogenetic relationships. If traits determining competitive ability have more similar values for close relatives than for distant relatives, then phylogenetically grouped species would be expected to experience more competition with each other than with distant relatives due to similar ecological requirements. Stronger competition among close relatives could then result in exclusion of the inferior competitor, unless the different species diverge and evolve ecologically distinct niches (Darwin 1859, MacArthur and Levins 1967, Losos et al. 2003). The intuitive hypothesis that closely related species are more ecologically similar and compete strongly (where species that do not evolve niche differences face local extinction) has led many biologists to propose that understanding evolutionary history is critical for predicting community dynamics and the composition of species in natural communities (Brooks and McLennan 1991, Harvey and Pagel 1991, Webb et al. 2002, Cavender-Bares et al. 2009).

Over the past decade, there has been an increase in the number of studies that have directly manipulated the relatedness of species in a community and then measured the strength of competitive interactions (experiments compiled by Cahill et al. 2008, Jiang et al. 2010, Dostál 2011, Violle et al. 2011, Peay et al. 2012, Best et al. 2013, Fritschie et al. 2013, Narwani et al. 2013, Venail et al. 2014). Recent advances in genomic tools and phylogenetic construction have allowed researchers to develop more quantitative metrics for measuring species relatedness, such as phylogenetic distance (PD) that measures branch lengths between taxa on a molecular phylogeny (Faith 1992, Webb 2000). Competition in most studies has been measured as the reduction in biomass or population growth rate of a focal species when in the presence of another species relative to when the focal species is grown alone in monoculture (Gough et al. 2001, Freckleton et al. 2009). A few terrestrial plant studies have supported the CRH by showing that the presence of close relatives reduces the biomass, chance of invasion, or presence of other species for California grasses (Strauss et al. 2006) and arbuscular mycorrhizal fungi (Maherali and Klironomos 2007). Select experiments performed with microbes have similarly shown that the abundance and invasion success (i.e., positive growth of a species introduced at low density to a community at equilibrium; Chesson 2000) of a species decreases as its relatedness to the resident community increases (Jiang et al. 2010, Violle et al. 2011, Peay et al. 2012). Large PDs among co-occurring species have also been shown to coincide with decreased community stability, which was interpreted as evidence for weak competition amongst distant relatives leading to reduced compensatory dynamics (Venail et al. 2013).

While results from several studies are consistent with predictions of the CRH, an increasing number of recent studies have produced contrasting results that call into question the generality of this hypothesis and its assumptions. For example, studies using microbial communities have concluded that PD cannot predict the strength of competition or likelihood of coexistence for bacterial strains (Schoustra et al. 2012) or freshwater green algae (Fritschie et al. 2013, Narwani et al. 2013, Venail et al. 2014). A number of studies have shown no relationship between the reduction in biomass of vascular plants grown with competing species and the PD between them for wetland herbaceous species (Cahill et al. 2008), central European flowering plants (Dostál 2011), French alpine trees (Kunstler et al. 2012) and Canadian grassland species (Bennett et al. 2013). One animal field study

found that PD did not predict competition strength between North American marine amphipods (Best et al. 2013). The lack of support for the CRH is often presumed to represent a violation of the assumption that ecological traits are conserved across a phylogeny (Losos 2008, Pearman et al. 2008) and, in fact, several studies suggest that the biological traits that underlie competition can be phylogenetically labile (Losos 2008, Savage and Cavender-Bares 2012, Sternberg and Kennard 2014; A. Narwani et al., *unpublished manuscript*).

Even though competition-relatedness experiments have grown in number and breadth of study systems over the past decade, studies have largely measured competition between just two individuals or between two species' populations (but see Jiang et al. 2010, Dostál 2011, and Best et al. 2013 for exceptions). Pairwise interaction studies are the most common means to measure competition because they facilitate direct observation of competitive effects of one species on another (Cahill et al. 2008) and allow for modeling of competition coefficients (Narwani et al. 2013). However, extrapolation of pairwise competitive interaction strengths to community-wide competitive outcomes is tenuous at best (Chesson 2000, Narwani et al. 2013). This is partly because more complex forms of interaction such as indirect and intransitive interactions can mask the magnitude and even the sign (i.e., competition versus facilitation) of pairwise interactions in multi-species communities (Strauss 1991, Wootton 1994, Valiente-Banuet and Verdu 2008, Martorell and Freckleton 2014). A number of studies have empirically confirmed the presence of indirect and intransitive competition in multispecies communities (Connell 1983, Schoener 1983, Keddy and Shipley 1989, Castillo et al. 2010). This supports theoretical predictions that pairwise competition coefficients don't predict population dynamics a priori for communities of three or more competing species (May and Leonard 1975). Thus, in order to assess how PD relates to competition in multi-species communities, it may be necessary to study multi-species communities directly as opposed to inferring community-wide competitive interactions from pairwise combinations of the component species.

Here we report the results of an experiment in which we measured the strength of competition in multi-species communities of freshwater green algae. In order to assess whether PD determines the level of competition experienced by members of a multi-species community, we ran a laboratory mesocosm experiment in which we varied the PD represented by eight common species of green algae, and then assessed the competitive response of each species to additions or deletions of the other taxa grown in polyculture. We used a data-rich molecular phylogeny of 59 green algae species (Alexandrou et al. 2015) to determine the relatedness of algal species comprising each community. We measured phylogenetic distance from a focal species to the resident community as the average PD between a focal species and each other species present in the community. Average PD estimates were considered with and without weighting by the resident species' relative abundances to account for the possibly disproportionate impacts of dominant species on interactions. We additionally measured PD between the focal species and most closely related taxon and between the focal species and a specific competitor for several analyses. We measured competition in three ways: First, we calculated the 'sensitivity' of a focal species to competition as the change in growth rate of the focal species when introduced at low density to a resident community of seven other species relative to growth of the focal species when alone in monoculture (Chesson 2000). Second, we calculated the 'relative yield' of biomass of a focal species grown in polyculture relative to monoculture. Competition from other species in the polyculture depresses the focal species' biomass and results in relative yields less than unity. Finally, we calculated 'competitive release' as the biomass of a focal species when grown without one competitor relative to when grown with a suite of seven potential competitors in the full resident community. The absence of a competitor results in higher biomass of the focal species and a competitive release greater than unity. The concurrent analysis of relative yield and competitive release allowed us to search for phylogenetic signal of competitive response to a whole community alongside competitive response to each individual species removed from the community. In accordance with the CRH, we predicted that species more distantly related to members of the resident community would

experience less competition than species more closely related to the resident community. While species experienced a range from no competition to complete competitive exclusion, no measure of competition could be explained by a species' relatedness to its community.

Materials and Methods

Species selection and culture

This experiment focused on eight species of freshwater green algae from different genera within the Chlorophyta and Charophyta. The Chlorophytes included *Chlorella sorokiniana*, *Closteriopsis acicularis*, *Pandorina charkowiensis*, *Scenedesmus acuminatus*, *Selenastrum capricornutum*, and *Tetraedron minimum*. The Charophytes included the two desmids *Cosmarium turpinii* and *Staurastrum punctulatum*. According to the U.S. Environmental Protection Agency National Lake Assessment (U.S. Environmental Protection Agency 2007), all eight taxa rank among the top 50% of the most abundant freshwater green algal genera out of 429 taxa found in North American lakes (Venail et al. 2014), and all but one pair of genera (i.e., *Pandorina* and *Tetraedron*) co-occur in lakes throughout the continental USA (Appendix C: Table C1). An 8-species pool falls on the lower end of the levels of algal diversity that are found in natural lakes, though it is within 1 SD of the mean (Appendix C: Fig. C1). Aside from their ecological relevance, these eight species were selected based on their ability to be cultured in laboratory conditions using common growth media (COMBO; Kilham et al. 1998) and based on their morphological differences, which allowed for visual identification of unique species during the cell counting process. These eight taxa were also included in a new multigene molecular phylogeny of 59 North American freshwater green algae that provides estimates of phylogenetic relatedness based on an unprecedented level of genetic sampling (Alexandrou et al. 2015). This phylogeny provides a good framework for relating ecology to phylogenetic relationships because the phylogeny predominantly comprises readily available, culturable algae. All species cultures were supplied from either the University of Texas Culture Collection of Algae (UTEX; Austin, Texas, USA) or the Sammlung von Algenkulturen Gottingen (SAG; Gottingen, Germany) culture collections.

Calculating phylogenetic distance

To estimate phylogenetic distances (PDs) among species, we relied on a new phylogeny for green algae (Alexandrou et al. 2015). The phylogeny was constructed using Illumina transcriptome sequencing technology and the Osiris pipeline for phylogenetics in Galaxy (Oakley et al. 2014). This data-rich framework represents a significant step forward from previous approaches that rely on single genes for estimates of evolutionary relatedness. We used a multiple sequence alignment of 119 genes (totaling 19,949 amino acids for 59 species of green algae) to construct a maximum likelihood phylogeny with RAxML, version 7.2.8 (Stamatakis et al. 2008). The phylogeny was tested for topological robustness using 100 non-parametric bootstrap replicates. We calculated pairwise PDs (Faith 1992) using the mean branch lengths connecting each species pair (ignoring the root branch) using PD pairs as implemented in Osiris (Oakley et al. 2014).

The pairwise PDs were used to calculate three complementary metrics of relatedness between a species and a resident community: nearest-neighbor phylogenetic distance (NPD), average phylogenetic distance between a species and all members of the community that is not weighted by abundance ("unweighted" phylogenetic distance, UPD), and average phylogenetic distance between a focal species and all other species in the community weighted by the relative abundance of each other species ("weighted" phylogenetic distance, WPD). WPD between a focal species i and the community was calculated as follows: biomass values for each species were converted to a proportion of total community biomass. Pairwise PD between the focal species, i, and any other species in the community $k \neq i$, was multiplied by the biomass fraction of k. These abundance-weighted pairwise PD values between a focal species and every other species present in the community were then summed to obtain the weighted average PD between that focal species and the community.

Because concurrent analyses using UPD and WPD emphasize how conclusions are influenced by the dominance of resident species in a community (Goldberg and Fleetwood 1987, Ca-

hill et al. 2008), we present results for both throughout this paper. NPD should, in principle, more accurately predict competition than community-averaged PDs if competition between close relatives is sufficiently strong that the nearest neighbor's effect on a focal species dominates over other competitive interactions (Castillo et al. 2010). However, the same three species (*S. acuminatus*, *C. sorokiniana*, and *C. acicularis*) often dominated the community biomass irrespective of values of PD. Unless the nearest neighbor of the focal species happened to be one of those dominant species, the nearest neighbor represented a small fraction of community biomass and likely did not strongly affect the focal species' growth. Because the results of analyses using NPD were qualitatively similar to other analyses, and because NPD measures distance from competitors with vastly different biomasses; analyses using NPD are included only in Appendix B.

Experimental setup and protocol

Three treatments representing a sum 81 experimental units were set up in an environmental chamber and grown over the course of 38 days (Fig. 1). The experimental units were 1-L Pyrex glass bottles filled with 1 L modified COMBO growth medium (Kilham et al. 1998). Experimental units were all placed in a walk-in environmental growth chamber that was kept at 20°C with a 16/8 h alternating light/dark cycle implemented using 28-W fluorescent lamps (Portable Luminaire, Underwriter Laboratories, Research Triangle Park, North Carolina, USA) emitting a mean 82 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ of photosynthetically active light (measured using an Apogee Instruments Quantum light meter, Logan, Utah, USA). Bottles were placed in randomly selected positions on tissue culture roller racks (120 V Roller Apparatus, Wheaton, Millville, New Jersey, USA) that rotated at 5 rpm, which was fast enough to ensure continuous suspension of cells and allow for even light exposure. Monoculture treatments included three replicates of each of the eight species grown alone, totaling 24 bottles. Eight "invasion" treatments were set up with each possible seven-species combination grown to steady state biomass, followed by invasion by the eighth species (8 treatments $\times$ 6 replicate bottles each $=$ 48 bottles total). A full eight-species polyculture treatment included nine replicate bottles of all eight species grown together, totaling nine bottles.

All treatments were inoculated at 800 cells/mL total density in the 1-L bottles. Species in polyculture were inoculated as a replacement series at either 114 (invasion treatments) or 100 (full polyculture treatment) cells/mL. Beginning on the fourth day of the experiment (DOE 4), 10% of the media was replaced in a semi-continuous fashion at the same time every other day using peristaltic pumps (MasterFlex L/S Multichannel Pump, Cole-Parmer, Vernon Hills, Illinois, USA). Two milliliters of exchanged experimental media were retained for sampling after each media exchange. One-milliliter samples of removed media were fixed with 250 μL 10% formalin (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and stored in the dark until further processing. One mL samples of removed media were directly pipetted into 48 multiwell tissue culture plates (Becton Dickinson Labware, Franklin Lakes, New Jersey, USA) for in-vivo chlorophyll-a fluorescence readings (460/685 nm excitation/emission wavelengths, measured on a Synergy H1 Hybrid Reader [BioTek, Winooski, Vermont, USA]) to monitor the growth of algal communities and determine when bottles had reached steady-state biomass. Steady-state biomass was recognized as a saturating response in natural-log transformed fluorescence reads over time. We accepted a non-significant increase in ln(fluorescence) between any two consecutive exchange days between DOE 20 and DOE 26 as evidence of steady-state in order to inoculate invaders prior to population crashes or secondary exponential growth phases. Once all seven-species invasion treatment polycultures reached stable equilibrium (DOE 26), the eighth "invader" species was inoculated into each invasion treatment bottle at 800 cells/mL (Fig. 1). All bottles continued to receive media exchange and were sampled for twelve days post-invasion.

Data analysis

Cell counts were performed to estimate species density over the course of the experiment, which was then used to compute metrics of competition. Ten-microliter aliquots of preserved samples were counted on a compound light microscope at 100× and 400× magnification using a hemacy-

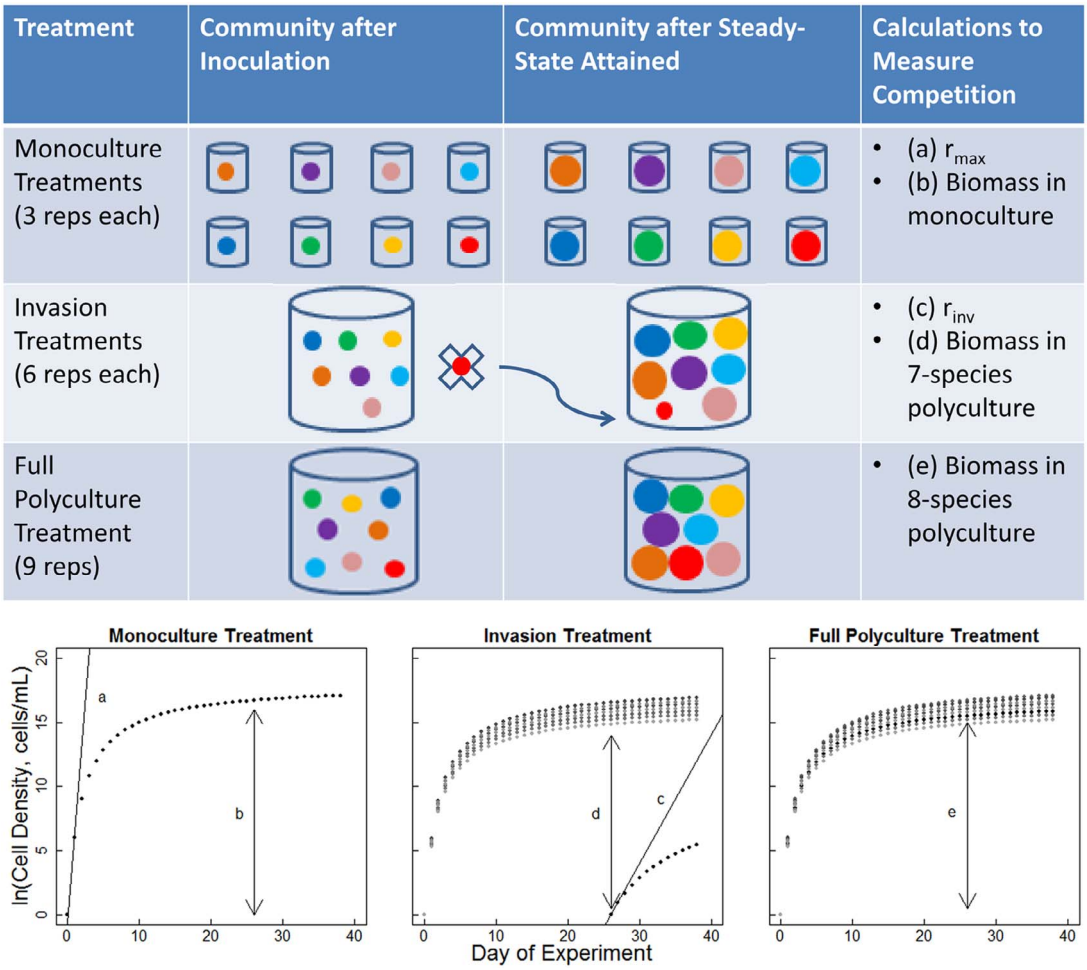

Fig. 1. Diagram of experimental setup including experimental treatments, measurements taken from each treatment and an example of the growth dynamics for each treatment over time. Each colored dot represents one of eight species and each cylinder represents a 1-L bottle. Dot size indicates cell density, where large dots represent steady-state biomass. Eight Invasion treatments were used in this experiment, but only one example is drawn in the row labelled "Invasion Treatments" due to limited space. The final column lists all measurements taken from algal growth curves to estimate competition. The lower panel shows how growth curves were used to measure (a) slope $= r_{max}$, maximum intrinsic growth rate of a species in monoculture, (b) $M_{i,1}$, steady-state density of species i in a 1-species monoculture, (c) slope $= r_{inv}$, maximum intrinsic growth rate of a species as an invader, (d) $M_{i\neq j,7}$, steady-state density of species $i \neq j$ in a 7-species resident community where j represents the absent species, and (e) $M_{i,8}$, steady-state density of species i in full polyculture of 8 species. All densities (b, d, e) were converted to biomass values for further analysis. Note: graphs are examples and do not use experimental data.

tometer. Algal biomass was approximated by multiplying cell density by species-specific cell volume, which was measured from 10 cells of each species culture used in the experiment on a Benchtop FlowCam (Fluid Imaging Technologies, Scarborough, Maine, USA). Biovolumes ($\mu m^3 \cdot L^{-1}$) were then converted to biomass ($\mu g \cdot L^{-1}$) by assuming that cells are primarily composed of water, which has a relative density of 1.

Growth curves of cell density over time were plotted for each monoculture bottle over the course of the entire experiment and for the invader species in each invasion bottle over the

twelve-day period following its introduction on DOE 26 (Appendix A: Figs. A1, A2). Monoculture maximum intrinsic growth rates, r_{max}, and invader growth rates when rare, r_{inv}, were calculated as the log ratio of density (D) on the final and initial days of exponential growth divided by number of days of exponential growth (t); Eq. 1.

$$r = \ln\left(\frac{D_{fin}}{D_{init}}\right)/t. \quad (1)$$

The period of exponential growth was determined by maximizing the fit of linear regressions to the log-transformed growth curves of each bottle (Appendix A).

Maximum intrinsic growth rate and growth rate when rare were used to calculate each species' sensitivity to competition as well as its invasion success into an established community. A given species' sensitivity to competition, S, is the reduction in its per-capita growth rate when introduced at low density to a resident community relative to its per-capita growth rate in monoculture; Eq. 2.

$$S = (r_{max} - r_{inv})/r_{max}. \quad (2)$$

As a given species' growth rate when rare approaches its maximum intrinsic growth rate, the numerator in S approaches zero, signifying low competitive pressure from the established community to which the invader is introduced. Sensitivities between zero and one signify competition even though the invader is able to establish itself in the community. A sensitivity of one indicates strong competition (complete niche overlap) from other species in a resident community. Sensitivities greater than one signify invader mortality, as r_{inv} would be negative, indicating unsuccessful invasion.

Biomass of each species was determined for each monoculture, invasion (7-species) and full polyculture (8-species) bottle at stable equilibrium (DOE 26) for use in competition calculations (Fig. 1). Biomass of species in 8-species (full) polyculture was compared with their biomass in monoculture and in 7-species polyculture to calculate relative yield and competitive release, respectively. Relative yield, or *RY*, is the biomass of a species grown in polyculture relative to its biomass in monoculture; Eq. 3. *RY* measures competitive response of a focal species to the combined competitive pressure from all species present in its community. In contrast, competitive release, or *CR*, compares the biomass of a species grown in a community missing one member versus in the full polyculture; Eq. 4. *CR* assesses the extent to which competition experienced by a focal species within an eight-species community depends on specific pairwise competitive interactions. Thus, by including both *RY* and *CR* in our analysis, we can learn whether PD effectively predicts diffuse and/or species-specific competition strength.

$$RY_i = M_{i,8}/M_{i,1} \quad (3)$$

$$CR_{ij} = M_{i\neq j,7}/M_{i,8}. \quad (4)$$

In Eqs. 3 and 4, M is the biomass of a focal species i on DOE 26. Subscript j refers to the species missing from the seven-species polycultures prior to invasion, ranging from 1 to 8 but excluding $j = i$. Subscripts 8, 1 and 7 refer to 8-species polyculture, monoculture and 7-species polyculture, respectively.

Statistical analysis

Several data analyses were performed to address whether PD predicts competitive outcomes in a multispecies community using R, version 2.15.2 (R Development Core Team 2012). First, we used linear regression to relate species' sensitivities to competition, Eq. 2, to phylogenetic distance, where we ran two separate analyses using WPD and UPD as the independent variable. Sensitivities were also analyzed using a logistic regression to ask whether the likelihood of invasion (1 = successful, 0 = unsuccessful) increases with PD between a community and an introduced species. WPD was the only PD metric used for the logistic regression because it allowed each replication to be used as an independent data point as opposed to UPD or NPD, for which every replicate of the same invader species had an identical phylogenetic distance.

We then performed a linear regression of *RY* against WPD and UPD to assess whether phylogeny predicts how competition affects equilibrium yields of species in a community. *RY* values were natural log transformed to normalize residuals. *RY* values were expected to increase towards unity with increasing phylogenetic distance. In addition to the expectation that

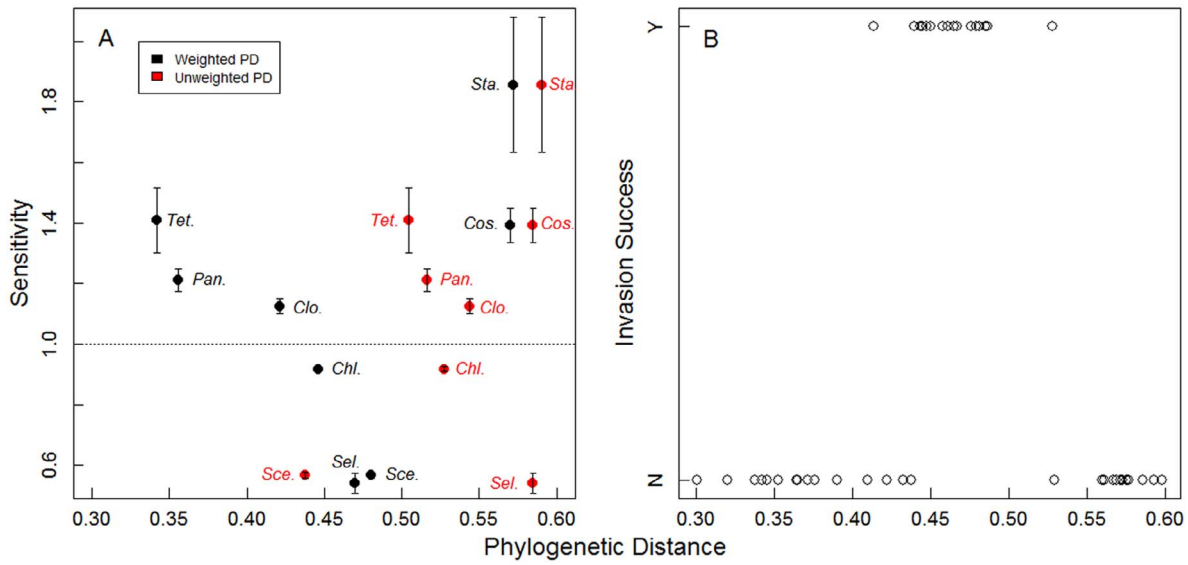

Fig. 2. Invader sensitivity (S) as a function of its phylogenetic distance to the resident community (A) and invasion success of species introduced at low abundance to communities at equilibrium as a function of their phylogenetic distance to the resident community (B). Sensitivity to competition is the reduction in intrinsic growth rate of a species introduced at low density (i.e., "invader") to a polyculture at equilibrium relative to its intrinsic growth rate in monoculture. S of each invading species, indicated by points labelled with the species' abbreviated genus name, was analyzed as a function of abundance-weighted PD (WPD) and unweighted average PD (UPD) between the invading species and all other members of a polyculture community. Points below the dotted line at $S = 1.0$ indicate species with positive growth-when-rare and points above the dotted line indicate species with negative growth-when-rare. Error bars show standard error of S calculated for six replicate mesocosms. Neither WPD nor UPD significantly predicted S (WPD: $n = 8$, $F = 0.26$, $P = 0.63$; UPD: $n = 8$, $F = 1.39$, $P = 0.28$). Species with $S < 1$ were given an invasion success of 1 = successful, and species with $S > 1$ were given an invasion success of 0 = unsuccessful (B). WPD between the invading species and the polyculture community was not able to predict invasion success ($n = 48$, $Z = 0.53$, $P = 0.60$).

the presence of a competitor will reduce the biomass of a species (i.e., Eq. 3), the reverse should also be true: the removal of a competitor from a community should result in the release of competition and hence a relatively larger biomass of any species left behind (i.e., competitive release, Eq. 4). Assuming that competitive interactions are stronger for close relatives, we hypothesized that competitive release should decrease as the PD between the focal species and its removed competitor increases. This hypothesis was assessed by linear regression of *CR* of a focal species versus PD between the focal and missing species, where a negative slope would support the CRH. Though the regression of *CR* against PD for each species might be significant, the relationship for each species could have a unique intercept and slope that when analyzed compositely would produce no significant trend. To account for species' unique responses to competitors, (which was shown by the broad range of sensitivities of our eight algal species, Fig. 2), we additionally looked for relationships between *CR* and PD for each species individually. Because *P. charkowiensis* did not appear in any replicate for five invasion treatments (probably due to competitive exclusion), nothing could be said about its competitive release from these five species and only two points appear in Fig. 4D.

Results

Contrary to predictions of the competition-relatedness hypothesis (CRH), we found no relationship between a species' sensitivity (S) to

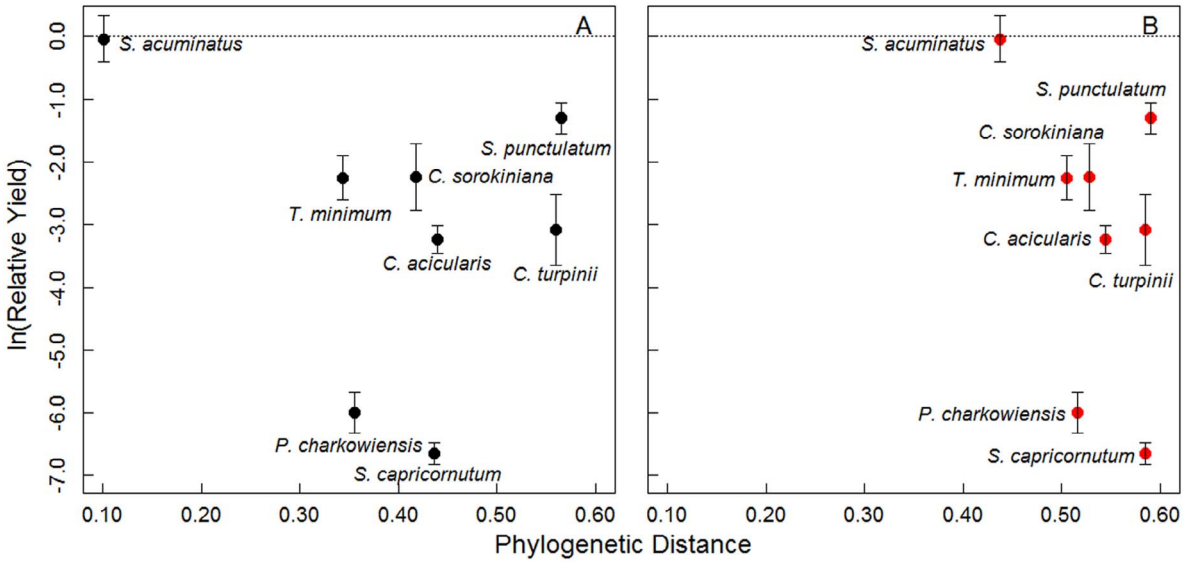

Fig. 3. Relative yield (RY) of a focal species in polyculture versus monoculture as a function of abundance-weighted PD (WPD, (A)), and unweighted PD (UPD, (B)), between the focal species and all other taxa in the polyculture. Points are labelled with focal species names. RY values are natural log-transformed and standard errors approximated as in Hedges et al. (1999). The dotted line at $\ln(RY) = 0$ marks a relative yield of 1 after transformation, which indicates equivalence of focal species biomass in polyculture and in monoculture. No significant relationship was found (WPD: $n = 8$, $F = 0.52$, $P = 0.50$; UPD: $n = 8$, $F = 1.40$, $P = 0.28$).

interspecific competition and its relatedness to other resident members comprising an algal community. No significant trends were observed in a linear regression of sensitivity versus average abundance-weighted phylogenetic distance (WPD) or average unweighted phylogenetic distance (UPD; Fig. 2A). Using $S > 1$ as an indicator of an unsuccessful invasion and $S < 1$ as an indicator of a successful invasion, phylogenetic distance also did not predict whether a species introduced at low density could successfully invade a community at equilibrium in a logistic regression of invasion success (positive growth-when-rare) against WPD (Fig. 2B). The lack of a significant relationship between PD and invasion success was partly driven by the two charophyte species (*C. turpinii* and *S. punctulatum*), which had the highest values of PD (the species are more distantly related to the chlorophytes than chlorophytes are to each other), but which were rarely able to invade resident communities (invasion success = 0). These results indicate that whether sensitivity is interpreted as a continuous metric of competition strength or converted to a binary of successful/unsuccessful invasion, species' PD to a community was not related to these metrics of competition.

Relatedness to the community was also a poor predictor of species relative yields (*RY*s) in 8-species (full) polyculture versus in monoculture. Seven out of eight species had *RY*s less than 1, which suggests competition for limiting resources. However, there was no significant relationship between RY and WPD or UPD (Fig. 3). In contrast to the other species, *S. acuminatus* had an RY approximately equal to 1 (which after log-transformation is 0; Fig. 3), suggesting that *S. acuminatus* was competitively dominant over the other seven species used in this experiment. Surprisingly, several species that had high RY values (i.e., experienced low competition in polyculture) also had high S values (i.e., were highly sensitive to competition), and vice-versa. For instance, *S. punctulatum* had the highest S (Fig. 2A), meaning its growth rate was most depressed by the presence of the other species, but also the second-highest RY (Fig. 3), meaning its steady-state biomass was largely unaffected by the presence of other species relative to its biomass in monoculture. *S. capricornutum* had the

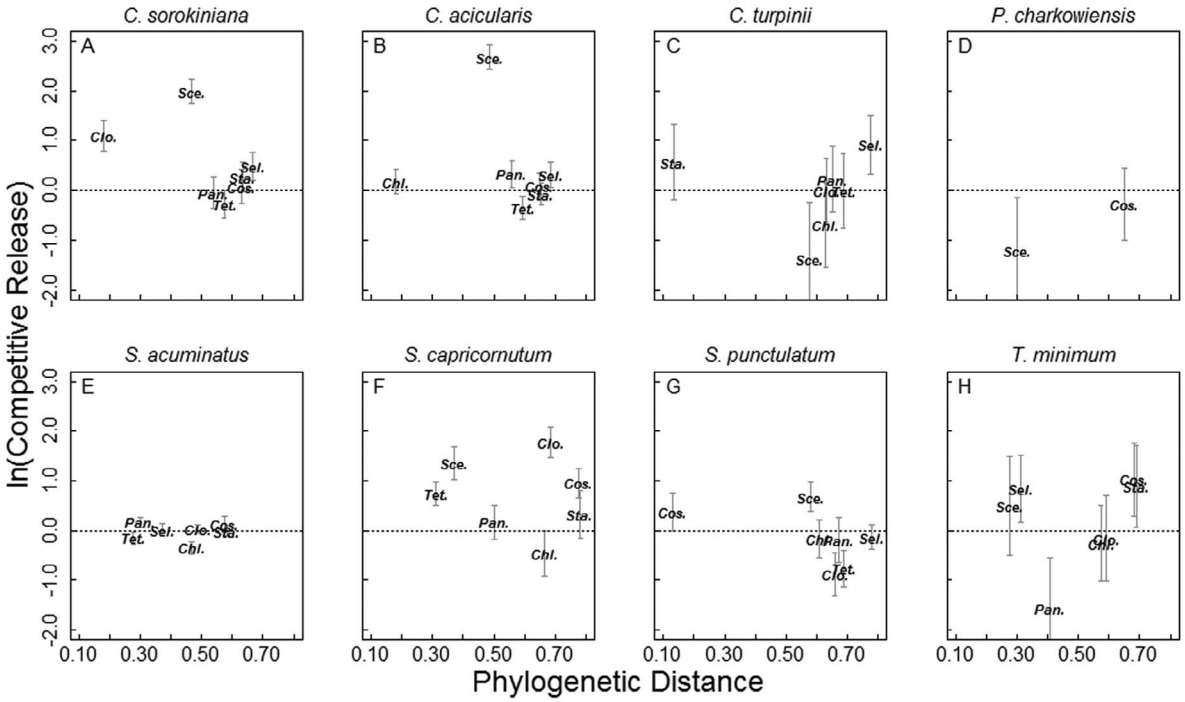

Fig. 4. Competitive release (*CR*) as a function of phylogenetic distance between a focal species and the missing competitor. *CR* is the yield of a focal species in a 7-species polyculture that is missing one competitor relative to the yield of that same focal species in a full 8-species polyculture. Subplot labels refer to focal species and points within each subplot are the first three letters of the genus of the missing competitor. For all linear regressions (except that of *P. charkowiensis*, for which too few data points were recovered for linear regression), there was no significant relationship ($n = 7$, $P > 0.24$ for all). Each subplot, (A–H), corresponds to the relationship between PD and competitive release experienced by *C. sorokiniana* ($P = 0.24$), *C. acicularis* ($P = 0.71$), *C. turpinii* ($P = 0.86$), *P. charkowiensis*, *S. acuminatus* ($P = 0.94$), *S. capricornutum* ($P = 0.76$), *S. punctulatum* ($P = 0.25$), and *T. minimum* ($P = 0.66$), respectively. *CR* values are natural log transformed and error bars represent standard error calculated as in Fig. 3. Points were jittered to improve visualization, but they retain their relative positions. The horizontal dashed line at $\ln(CR) = 0.0$ corresponds to $CR = 1$ after transformation.

lowest *S* (Fig. 2A) and *RY* (Fig. 3) recorded, making it the best and worst competitor according to each competition measure, respectively. The difference between these two measures of competition suggests that initial densities and priority effects may have played a role in determining algal community structure (Peay et al. 2012).

PD between a focal species and a competitor species was unrelated to the yield of the focal species grown in a 7-species polyculture (without the competitor) relative to in full 8-species polyculture (with the competitor). There was no significant relationship between competitive release (*CR*) and phylogenetic distance between a focal species and the missing competitor ($N = 51$, $F = 0.32$, $P = 0.57$). In addition, there was no relationship between *CR* and PD to the missing species for any of the eight taxa when examined individually (Fig. 4). Individual competitors appeared to greatly impact the biomass of focal species. In particular, the absence of *S. acuminatus* led to a large *CR* in several focal species (Fig. 4A, B, G), while no single species greatly impacted the biomass of *S. acuminatus* (Fig. 4E). These findings corroborate results in Fig. 3 that suggest *S. acuminatus* was a superior competitor. Several species showed *CR* values less than 1 (or less than 0 after log transformation, Fig. 4), meaning their biomass decreased when one competitor was absent from the community. These cases probably represent instances of facilitation by the

absent species (Fritschie et al. 2013).

Discussion

This experiment adds to a growing body of literature that assesses whether closely related species compete more strongly than distantly related species—an idea now known as the competition-relatedness hypothesis (CRH; Cahill et al. 2008). Of the studies that have directly measured competition strength, several have supported the CRH (Valiente-Banuet and Verdu 2007, Jiang et al. 2010, Burns and Strauss 2011, Violle et al. 2011, Peay et al. 2012). However, an increasing number of recently published studies have found that measures of competition at different life stages lead to differing conclusions about the validity of the CRH (Castillo et al. 2010) or that there is no evidence for stronger competition amongst close relatives (Cahill et al. 2008, Dostál 2011, Best et al. 2013, Fritschie et al. 2013, Narwani et al. 2013, Venail et al. 2014). Thus, support for the CRH to date has been mixed.

The competition experiments cited above have trended towards comparing competition as a function of phylogenetic distance among species pairs. While there are numerous benefits of pair-wise competition studies (Cahill et al. 2008), interactions measured in pair-wise combinations may or may not predict how relatedness affects competition in more complex, multi-species communities (Castillo et al. 2010). There is, in fact, some reason to believe that the species used in this study interact differently when they are in multi-species communities. Evidence for this comes from a comparison of our results to those of a companion experiment in which Venail et al. (2014) measured the sensitivity of species to competition (S, just as in Eq. 2) for all pair-wise interactions of the same eight species used here (see Fig. 4, x-axis; Venail et al. 2014). When we compared the values of S measured in our study to those measured in Venail et al. (2014), we found no significant correlation between the two sets of measurements ($R = 0.51$, $N = 8$, $P = 0.20$). Of course, the lack of correlation could be due to differences in methodologies among the two experiments. But it could also indicate that the strength of competition measured in pair-wise interactions does not predict the strength of competition for the same species interacting in multi-species communities. Regardless, we still reached the same conclusion as previous experiments showing that no measure of competition was significantly influenced by species relatedness. Rather than any general relationship of competition to phylogenetic relatedness, particular species appeared to drive competition strength across the community. Our study thus extends the generality of past CRH results to multi-species communities in which interactions may be more complex and not readily predicted from pair-wise interaction strengths.

The lack of any relationship between competition and PD suggests that the biological traits that underlie competition in algae do not show phylogenetic signal. While the traits determining competitive outcomes for the green algal species used in this experiment have yet to be identified, a recent study showed that 13 out of 17 traits related to nutrient uptake, stoichiometry and cell morphology lack phylogenetic signal across a phylogeny of 48 species inclusive of the eight used in our experiment (A. Narwani et al., *unpublished manuscript*). Numerous other studies have also found a lack of phylogenetic signal for traits thought to underlie competitive interactions for other groups of organisms (Rheindt et al. 2004, Cavender-Bares et al. 2006, Silvertown et al. 2006*b*, Anderson et al. 2011, Savage and Cavender-Bares 2012, Best and Stachowicz 2013).

Several hypotheses have already been proposed to explain the lack of signal between competitive ability and phylogenetic distance in prior studies (Cavender-Bares et al. 2009, Vamosi et al. 2009, Mayfield and Levine 2010). One possibility is phenotypic plasticity, where organisms can modify phenotypic expression depending on biotic and abiotic components of their environment (Agrawal 2001). Species with highly plastic ecological traits (i.e., intraspecific trait variability is greater than interspecific variability) can adopt new niches over time and reduce interspecific competition (Miner et al. 2005). Phenotypic plasticity could explain the lack of phylogenetic signal found in our study since certain types of algal traits are labile (Litchman et al. 2012); but in contrast to this hypothesis, algal trait averages often differ more strongly between species than within species (Edwards et al. 2012). Alternatively, rapid evolution of ecological char-

acters can negate any relationship between ecology and phylogenetic distance (Schluter 2000, Rheindt et al. 2004, Losos 2011). Rapid trait evolution within a lineage—for instance, through adaptive radiation—in traits important to competition can result in close relatives that do not compete strongly (Revell et al. 2008). Recent evolution of competitive traits could account for our finding no phylogenetic signal for competition. Several traits important for competition, including those related to nitrogen uptake and cell morphology, have evolved relatively recently on the green algae phylogeny (A. Narwani et al., *unpublished manuscript*), suggesting rapid trait evolution may be responsible for low phylogenetic signal amongst the algal species in this study. Lastly, convergent evolution can produce distantly related species that are ecologically similar and potentially compete strongly—a pattern that would oppose the CRH (Cavender-Bares et al. 2009, Mayfield and Levine 2010). The key point here is that several distinct evolutionary and ecological scenarios could explain why our results do not support the CRH.

As with any laboratory experiment, our study system represents an oversimplification of nature and, as such, certain caveats limit the extension of these results to natural algal communities. The relatively static environmental conditions that included a semi-continuous supply of nutrients, mixed (homogeneous) media, continuous light exposure and lack of disturbances (other than media exchanges) reduce spatial and temporal heterogeneity relative to natural conditions. This, in turn, may reduce niche opportunities and the expression of different biological traits, forcing competition to be more prominent in our system. Alternatively, one could imagine competition being more exaggerated in natural waters than in the laboratory mesocosms for the same reasons mentioned above—i.e., minimization of variability in a given resource's supply. For instance, shallow moving waters may dissolve gases more effectively than in our roller bottles and thus remove the opportunity for species to differentiate based on CO_2 or O_2 availability. Additionally, natural processes such as dispersal and herbivory—which are known to influence species interactions (Cyr and Face 1993, Amarasekare et al. 2004)—were not included in our experiment, and may limit the reproducibility of our results in more natural algal communities. Therefore, we caution against extrapolating results from our study to natural lake ecosystems without confirmation from field experiments.

Another potential criticism of our study is that our species pool did not encompass the appropriate phylogenetic scale (Cavender-Bares et al. 2006, Silvertown et al. 2006*a*, Swenson et al. 2006, Losos 2011). This criticism is commonly levied on empirical tests of the CRH that fail to find significant results, as it is often argued that significant results might appear if the focal species were more closely (e.g., Cavender-Bares et al. 2006) or more distantly (e.g., considering an entire genus' niche for climate tolerance; Wiens and Graham 2005) related. However, we do not believe this criticism applies to our work. The appropriate scale for testing the CRH is a scale at which species interactions like competition are prominent (Vamosi et al. 2009). Indeed, "scale" needs to be defined on the basis of the ecological interaction one is trying to explain, and the question is whether or not phylogenetic relationships are predictive of species interactions for the species for which interactions occur. Competition was prominent among the species used in our study, with interaction strengths that ranged from near zero (e.g., *S. acuminatus*; Fig. 3A) to nearly complete competitive exclusion (e.g., *P. charkowiensis*). These interactions spanned nearly the entire range of what is possible for negative interactions, and we would argue that this continuum represents an appropriate template to determine whether phylogenetic relationships can explain variation in interaction strengths.

Researchers from many disciplines have been attracted by the promise of a predictive relationship between evolutionary history and species' ecology. For example, ecologists have used phylogenies to infer patterns of community assembly and the mechanisms that lead to species co-occurrence (Webb et al. 2002). Conservation biologists have promoted using PD to maximize the adaptability, resilience, and ecological function of ecosystems (Vane-Wright et al. 1991, Faith 1992, Strauss et al. 2006, Forest et al. 2007, Cadotte et al. 2008, Cadotte et al. 2009). The fact that competitive ability is often unrelated to phylogenetic distance for communities of microorganisms, plants and animals implies that the traits underpinning species' ecology are often not

conserved. It may, therefore, be incorrect and (in the case of biodiversity conservation) risky to develop ecological principles, and extend these to management recommendations, based on the assumption that distant relatives are ecologically distinct. Unless scientists can develop a deterministic model for predicting which sets of species meet the CRH assumption of phylogenetic signal of ecologically relevant traits, it may ultimately prove unjustifiable to make ecological inferences and conservation decisions based solely on the relatedness of species within a community.

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Supplemental Material

Appendix A

Monoculture and invader growth curves and growth rate estimation

The exponential growth phase for monoculture treatments was determined to occur over the linear portion of the natural log-transformed time-series of population cell densities (Fig. A1). Linear portions of ln-transformed monoculture growth curves were assessed visually, then confirmed via the least-squares regression coefficient (multiple R^2) for the linear fit to the data points thought to represent exponential growth phase. While the highest multiple R^2 value was taken to signify best fit, visual determination of final day of exponential growth was used in preference to R^2 values in cases: (1), when the best linear fit included less than three data points, and (2), when data points giving better R^2 values due to inclusion or exclusion of spurious points did not represent the intrinsic growth rate over what appeared to be the full exponential growth phase. Maximum intrinsic growth rates (r_{max}) were calculated according to Eq. 1 and appear in Fig. A1 as the slope of the mean of the best least-squares fits to the log-transformed growth curves for the three replicate bottles over points pertaining to exponential growth phase.

The exponential growth phase of invaders in the invasion treatments was determined to occur over the linear portion of the invader's natural log-transformed growth curve (Fig. A2). Linear portions of ln-transformed invader growth curves were assessed visually. If no clear exponential phase existed (i.e., for all species except *S. capricornutum*), the invader species were assumed to still be in exponential growth (or decline) at the end of the experiment. According to Eq. 1, the log ratio of cell density between invader inoculation and the final day after introduction (12 days later) was used to calculate invader growth-

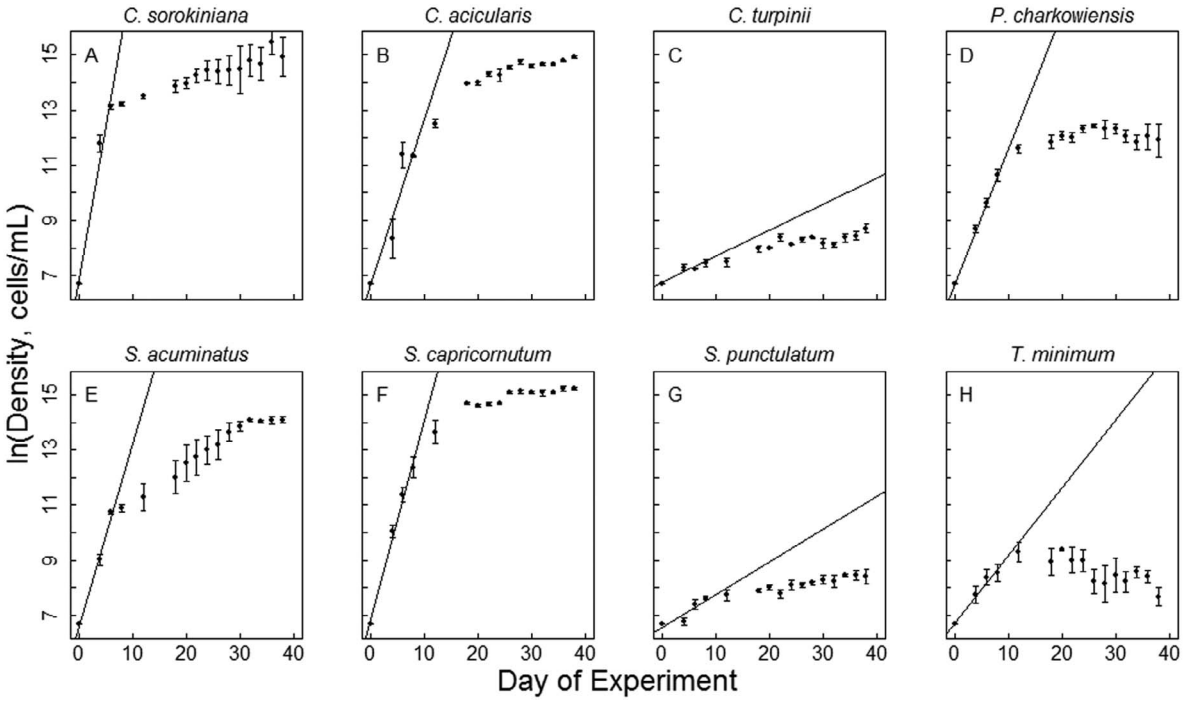

Fig. A1. Growth curves for monoculture treatments. Each subplot shows the mean density of the three replicate bottles for the species labelled above the plot, where the error bars represent standard error of the three replicates. Slopes of the lines represent the maximum intrinsic growth rate, r_{max}, for each species when grown in monoculture.

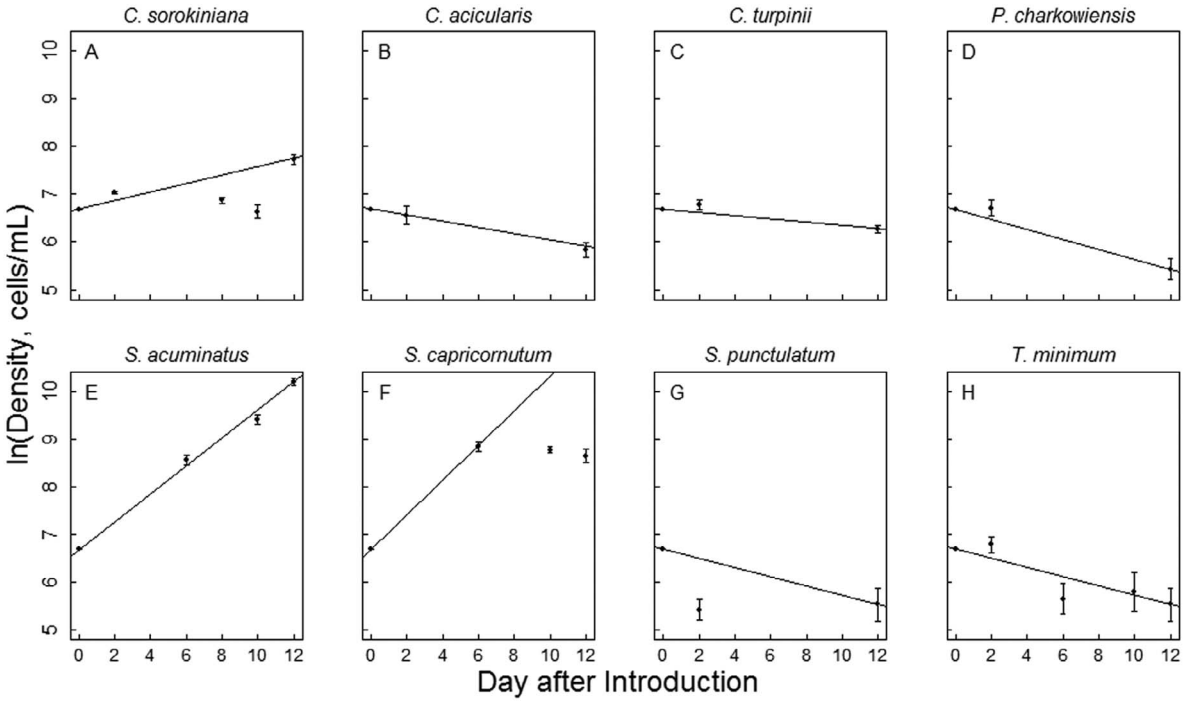

Fig. A2. Growth curves of the "invader" species in the invasion treatments. Each subplot shows the density of the invader, indicated by the subplot label, averaged over six replicate invader bottles. Error bars represent standard error of the six replicates. Slopes of the lines represent invader maximum growth rate, r_{inv}, after being introduced to steady-state resident communities.

when-rare (r_{inv}) for all species except *S. capricornutum*, in which case the sixth day after introduction was considered its final day of exponential growth.

Appendix B

Nearest neighbor phylogenetic distance (NPD) analyses

No measure of competition was significantly related to a species' phylogenetic distance to its closest relative in a community. Sensitivity (S) versus NPD gave a statistically non-significant negative trend (Fig. B1A). Relative yield (RY) also showed a non-significant negative trend with NPD (Fig. B1B). Thus, both analyses performed using NPD (the only measure of PD used in this study based on statistically independent comparisons) do not support the CRH.

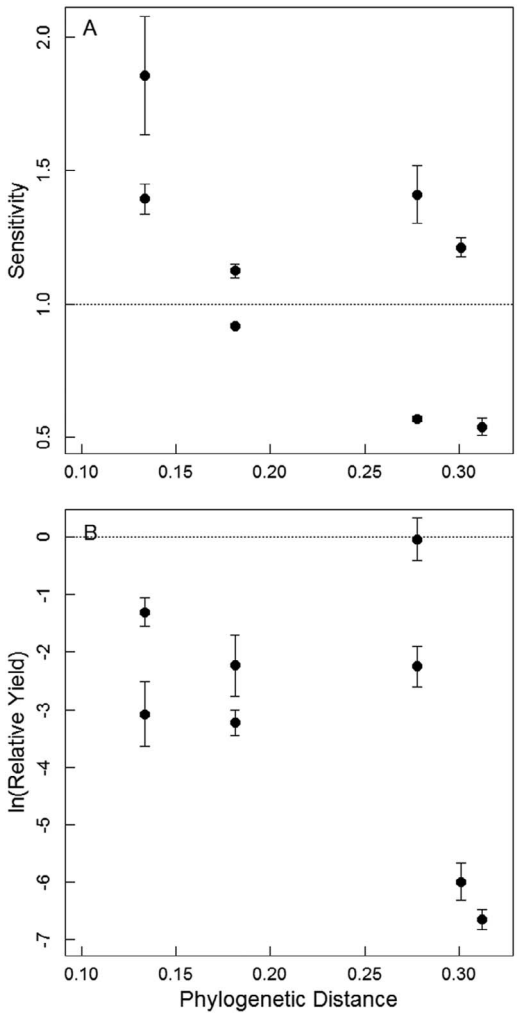

Fig. B1. Sensitivity, or S, (A) and relative yield, or RY, (B) of a focal species versus the phylogenetic distance between it and its closest relative in the community ("nearest neighbor phylogenetic distance," NPD). There was no significant relationship between S and NPD (A; $n = 8$, $F = 3.41$, $P = 0.11$) or between RY and NPD (B; $n = 8$, $F = 1.46$, $P = 0.27$). The dotted line in panel (A) at $S = 1.0$ and error bars are the same as in Fig. 2A. In panel (B) RY values are natural log-transformed, standard errors approximated as in Hedges et al. (1999), and the dotted line at $\ln(RY) = 0$ is the same as in Fig. 3.

APPENDIX C

Data summaries from the U.S. Environmental Protection Agency's National Lake Assessment (2007)

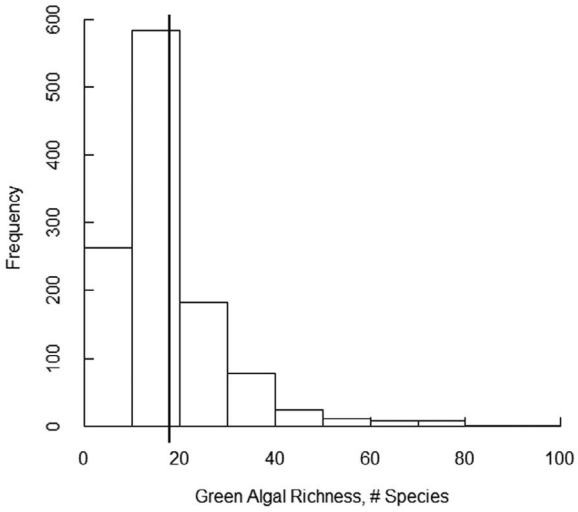

Fig. C1. Frequency histogram of the number of lakes having a given green algal richness out of 1157 lakes in the continental USA (U.S. Environmental Protection Agency 2007). The vertical line at richness = 17.84 represents the mean number of green algae species per lake. The number of species used in this study, 8, falls within one standard deviation of the mean (standard deviation = 11.51).

Table C1. Co-occurrence matrix of each pairwise combination of genera used in this experiment in continental U.S. lakes (U.S. Environmental Protection Agency 2007). Numbers inside cells are the percentage of lakes out of 1157 in which the genera were observed together, where each lake was visited twice. Analysis was done using Microsoft Access and Excel.

	Percent (%) co-occurrence of listed genus with focal genus							
Focal genus	*Chlor.*	*Clos.*	*Cos.*	*Pand.*	*Scen.*	*Sel.*	*Staur.*	*Tet.*
Chlorella	NA	1.64	15.38	0.95	19.62	3.03	8.90	9.85
Closteriopsis		NA	4.32	0.52	4.84	2.85	3.98	2.51
Cosmarium			NA	1.56	28.69	10.20	17.46	11.32
Pandorina				NA	2.25	1.38	1.04	0.00
Scenedesmus					NA	12.88	20.74	16.42
Selenastrum						NA	8.12	3.28
Staurastrum							NA	9.08
Tetraedron								NA

Notes: Empty cells are a mirror image of filled cells. "NA" denotes the "non-applicable" co-occurrence of a genus with itself. Column subheadings are abbreviated genus names as listed fully under "Focal genus."

SUPPLEMENT

Data files used in the main text (*Ecological Archives*, http://dx.doi.org/10.1890/ES14-00502.1.sm).