

PHYLOGENETIC DISTANCE DOES NOT PREDICT COMPETITIVE ABILITY IN
GREEN ALGAL COMMUNITIES

By

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Abstract

An assumption often made by ecologists and phylogeneticists—that closely related species possess similar traits and ecology—can be extended into the hypothesis that closely related species compete more heavily than distant relatives due to shared ecology. The intuition that related species occupy similar niches and thus compete intensely for resources, one outcome of which is competitive exclusion and local reduction of biodiversity, was formally introduced by Darwin in 1859. The past decade has seen a steady rise in tests of Darwin’s “competition-relatedness hypothesis” that experimentally manipulate relatedness, or evolutionary history represented by species in a community, and then measure interaction strengths. Despite the initial enthusiasm for using evolutionary history to predict ecology, different competition studies have arrived at different conclusions regarding whether there is a positive, negative, or no relationship between species’ evolutionary relatedness and the strength of competitive interactions between them. Furthermore, these studies have primarily measured competition for pairs of species rather than the overall competition a species experiences within a multi-species community where more complex (*e.g.*, indirect) interactions might be expected to take place.

In order to test whether the competition-relatedness hypothesis holds in communities with a species richness more representative of that found in nature, a mesocosm study was performed using communities of eight species of freshwater green algae. Species relatedness was quantified as the phylogenetic distance between species using a comprehensive multi-gene molecular phylogeny of 59 North American green algae. Three metrics of competition strength—sensitivity to competition (reduction in

intrinsic growth rate when grown from low density with competitors versus when grown in monoculture), relative yield, and competitive release (proportional change in biomass of a focal species grown with one competitor missing versus when grown with all competitors)—were not predicted by the relatedness of a species to its community. The finding that species' relatedness to their resident community was unrelated to the strength of competition they experienced concurs with previous findings from studies of interaction strength as a function of relatedness between pairs of species. This finding suggests that the results of prior studies refuting the competition-relatedness hypothesis can be extended to larger communities in which more complex ecological interactions occur.

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Table of Contents	Page Number
1. Chapter One: Introduction	1
1.1. Context	1
1.2. State of the Knowledge	2
1.3. A Novel Multi-species Competition Experiment	5
2. Chapter Two: Materials & Methods	8
2.1. Species Selection and Culture	8
2.2. Calculating Phylogenetic Distance	9
2.3. Experimental Setup and Protocol	10
2.4. Data Analysis	12
2.5. Statistical Analysis	16
3. Chapter Three: Results	18
3.1. Sensitivity and Invasion Success	18
3.2. Relative Yield	20
3.3. Competitive Release	21
4. Chapter Four: Discussion	23
4.1. Experimental Summary	23
4.2. A Defense of Negative Results	23
4.3. An Ecological Case of 1+1 Not Equaling 2	25
4.4. Caveats	27
4.5. Conclusions	28
5. Appendix 1 – Growth Curves	30
6. Appendix 2 – Supplementary Material	33
7. References	35

1.1 Context

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 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 & Pagel 1991; Peterson, Soberón & Sánchez-Cordero 1999; Blomberg, Garland & Ives 2003). The sharing of traits that potentially influence ecological interactions among closely related species is called “phylogenetic niche conservatism” (Wiens & Graham 2005) or “phylogenetic signal” (Losos 2008), depending on the extent of trait clumping among close relatives. If traits determining competitive ability are phylogenetically conserved, then phylogenetically grouped species would be expected to experience heavy competition with each other due to similar ecological requirements. Stronger competition among close relatives should then result in exclusion of the inferior competitor, unless the species evolve ecologically distinct niches (Darwin 1859; MacArthur & Levins 1967; Losos *et al.* 2003). The intuitive hypothesis that closely related species are more ecologically similar and compete strongly, and thus must evolve niche differences to coexist, has led many biologists to propose that understanding evolutionary history is critical for predicting community dynamics and the composition of species in natural communities (Harvey & Pagel 1991; Webb *et al.* 2002; Cavender-Bares *et al.* 2009).

1.2 State of the Knowledge

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, Tan & Pu 2010; Dostál 2011; Violle *et al.* 2011; Peay, Belisle & Fukami 2012; Best, Caulk & Stachowicz 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, Watkinson & Rees 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, Webb & Salamin 2006) and arbuscular mycorrhizal fungi (Maherali & 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, Tan & Pu 2010; Violle *et al.* 2011; Peay, Belisle & Fukami 2012). Large phylogenetic distances among co-occurring species also coincide with decreased temporal stability of community biomass, which has been attributed to weak competitive interactions among distant relatives (Venail *et al.*

2013) leading to reduced negative covariance between competitors' biomass within a community (Tilman, Lehman & Bristow 1998).

While some evidence is 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 phylogenetic distance cannot predict competition strength or likelihood of coexistence for bacterial strains (Schoustra *et al.* 2012), as well as for North American species of freshwater green algae (Fritschie *et al.* 2013; Narwani *et al.* 2013; Venail *et al.* 2014). Studies using vascular plants have shown no relationship between the reduction in biomass of plants grown with competing species and the phylogenetic distance between them for experiments carried out in pots with North American wetland herbaceous species (Cahill *et al.* 2008) and central European flowering plants (Dostál 2011), or carried out in field plots with French alpine trees (Kunstler *et al.* 2012) and Canadian grassland species (Bennett *et al.* 2013). One animal field study found that phylogenetic distance did not predict competition strength between North American marine amphipods (Best, Caulk & Stachowicz 2013).

Negative CRH results can be explained by several ecological and evolutionary processes resulting in a violation of the CRH assumption that ecological traits are conserved across a phylogeny (Losos 2008; Pearman *et al.* 2008). For instance, convergent evolution can lead to distant relatives possessing similar traits and, conversely, adaptive radiation can lead to close relatives possessing highly differentiated traits (Webb *et al.* 2002). For bacteria and archaea, lateral gene transfer can also produce evolutionary close relatives that are ecologically differentiated (Falkowski, Fenchel &

Delong 2008). Indeed, several studies have found that traits important for competition are phylogenetically labile and that not all traits show the same distributions across phylogenies (Blomberg, Garland & Ives 2003; Losos 2008; Narwani *et al.* 2014). Thus, the scientific community has not yet come to a consensus on whether competition can be determined from phylogenetic relatedness.

Even though competition-relatedness experiments have grown in number and breadth of study systems over the past decade, these studies are limited in that they have largely measured competition between just two individuals or between two species' populations (but see Jiang, Tan & Pu 2010, Dostál 2011 and Best, Caulk & Stachowicz 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) as well as the modeling of competition coefficients (Narwani *et al.* 2013). But extrapolation of pairwise competitive interaction strengths to community-wide competitive outcomes is tenuous at best (Chesson 2000; Narwani *et al.* 2013), meaning it cannot be guaranteed that the conclusions from CRH studies performed using species pairs will hold in larger communities. This is partly due to the fact that in multi-species communities more complex forms of interaction, such as indirect and intransitive interactions, have potential to mask the magnitude and possibly sign of pairwise competitive interactions (Strauss 1991; Wootton 1994; Valiente-Banuet & Verdú 2008; Martorell & Freckleton 2014). A number of studies have empirically confirmed the presence of indirect and intransitive competition in multispecies communities (Connell 1983; Schoener 1983; Keddy & Shipley 1989; Castillo, Verdú & Valiente-Banuet 2010). Moreover, May & Leonard (1975) demonstrated through

population model analysis and simulation that population dynamics in communities of three or more competing species cannot be predicted *a priori* from pairwise competition coefficients. In order to assess how PD is related to competition in multi-species communities, it may therefore be necessary to study those multi-species communities directly as opposed to inferring community-wide competitive interactions from pairwise combinations of the component species.

1.3 A Novel Multi-species Competition Experiment

This study reports the results of an experiment in which the strength of competition in multi-species communities of freshwater green algae was measured and compared to species relatedness. In order to assess whether phylogenetic relatedness determines the level of competition experienced by members of a multi-species community, a laboratory mesocosm experiment was performed in which the phylogenetic distance separating eight common species of green algae was manipulated. The competitive response of each species to additions or deletions of other taxa grown in polyculture was then assessed. The relatedness of algal species comprising a community was determined using a comprehensive, new molecular phylogeny of 59 green algae species (Alexandrou *et al.* 2014). Phylogenetic distance was measured as the average PD between a focal species and each other species present in the community, both with and without species' relative abundance weighting pairwise PDs. Competition was measured in several complementary ways.

The first measure of competition, sensitivity of a focal species to competition, was measured as the change in growth rate of the focal species when introduced at near-zero

density (that is, the lowest density from which positive population growth occurs) to a community of seven other species relative to growth of the focal species when alone in monoculture (Chesson 2000; Carroll, Cardinale & Nisbet 2011). Sensitivity is thought to quantify niche partitioning (where species with completely differentiated niches should be able to grow equally well when with other species as when alone in monoculture), and is thus an indirect measure of competition (Narwani *et al.* 2013). The more reduced a species' growth rate is in polyculture relative to monoculture; the more other species hinder its growth through common use of shared resources—*i.e.*, a lack of niche differentiation. By measuring niche partitioning, sensitivity directly assesses the process by which evolutionary distance is expected to reduce competition—that is, through the reduction of ecological niche overlap of competing species (Chase & Leibold 2003). Furthermore, positive growth of a species introduced at low density to an established community indicates that it will achieve a non-zero abundance within that community within the short-term, and possibly coexist long-term (Chesson 2000). In light of the growing number of threatened and invasive species due to changing climate, habitat modification and habitat destruction; sensitivity could grow to be a useful predictor of whether a species nearing extinction would be expected to rebound from low levels or survive reintroduction (Caplat, Anand & Bauch 2010) or whether a species might invade a novel habitat (Davis, Grime & Thompson 2000). Therefore, this study also assessed whether the ability of a species to invade a community (where low sensitivities signify successful invasion and high sensitivities signify unsuccessful invasion) depends on its relatedness to resident species in that community in order to relate species relatedness to processes such as exotic species introduction and species extinction/resilience that are

important to understand for purposes of biological conservation. The competition-relatedness hypothesis predicts that species distantly related to a community should have low sensitivities to competition and easily invade that community due to their largely differentiated niches.

Two abundance-based measures of competition were used in addition to sensitivity and invasion success. Relative yield measured the biomass of a focal species grown in polyculture relative to monoculture, in which competition from other species in the polyculture is expected to depress the focal species' biomass and result in relative yields less than unity. Competitive release measured the biomass of a focal species when grown without one competitor relative to when grown with its full suite of competitors, where the absence of a competitor is expected to result in higher biomass of the focal species and a competitive release greater than unity. Assuming that competitive interactions are stronger for close relatives, it was hypothesized that competitive release should decrease as the PD between a focal species and a removed competitor increases. The concurrent analysis of relative yield and competitive release allowed for comparison between phylogenetic signal of competitive response to a whole community and competitive response to an individual species within the community. In accordance with the competition-relatedness hypothesis, it was predicted that species more distantly related to their community would experience less competition (*i.e.* higher relative yield and smaller competitive release) than species more closely related to their resident community.

2.1 Species Selection and Culture

This experiment focused on eight species of freshwater green algae from different genera within the clade containing 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. EPA NLA, 2007), all eight taxa rank among the top 50% of the most abundant freshwater green algae 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 U.S.A. (Table S1). An 8-species pool falls on the lower end of the levels of algal diversity found in natural lakes, though it is within 1-S.D. of the mean (Fig. S1). 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 data-rich, multi-gene molecular phylogeny of 59 North American freshwater green algae that provides accurate estimates of phylogenetic relatedness (Alexandrou *et al.* 2014). All species cultures were supplied from either the University of Texas Culture Collection of Algae (UTEX; Austin, USA) or the Sammlung von Algenkulturen Gottingen (SAG; Gottingen, Germany) culture collections.

2.2 Calculating Phylogenetic Distance

A robust published phylogenomic framework for green algae was used to estimate phylogenetic distances (PDs) among species (Alexandrou *et al.* 2014). 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. A multiple sequence alignment of 119 genes (totaling 19,949 amino acids for 59 species of green algae) was used to construct a Maximum Likelihood phylogeny with RAxML v 7.2.8 (Stamatakis *et al.* 2008). The phylogeny was tested for topological robustness using 100 non-parametric bootstrap replicates. Pairwise PDs (Faith 1992) were calculated using the mean branch lengths connecting each species pair (ignoring the root branch) using the custom Perl script 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 (“un-weighted” 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 experimental community were then summed to obtain the weighted average PD between that focal species and the community.

Because concurrent analysis using UPD and WPD emphasizes how conclusions are influenced by the dominance of resident species in a community, results for both measures are presented throughout this paper (Goldberg & Fleetwood 1987; Cahill *et al.* 2008). NPD should be a more accurate predictor of competition than community-averaged PDs if competition between close relatives is so strong that the nearest neighbor's effect on a focal species dominates over other competitive interactions (Castillo, Verdú & Valiente-Banuet 2010). However, because the results of analyses using NPD, UPD and WPD were qualitatively similar and the closest relative to a focal species often had very low biomass (and, as such, a small influence on the focal species' ecology); NPD is not brought up further in this paper.

2.3 Experimental Setup and Protocol

Three treatments totaling 81 experimental units were established in an environmental chamber and grown over the course of 38 days (Fig. 1). 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 growth chamber that was kept at 20°C with a 16/8 h alternating light/dark cycle implemented using 28 W fluorescent lamps emitting $82 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ of light on average (Portable Luminaire; Underwriter Laboratories Inc.). Bottles were placed in randomly selected positions on roller racks (120 V Roller Apparatus, Wheaton®, U.S.A.) that rotated at 5 rpm, which

was fast enough to cause continuous suspension of cells and allow for even light exposure (*personal observation*). Monoculture treatments included three replicates of each of the eight species grown alone, totaling 24 bottles. Seven “invasion” treatments were established with each possible seven-species combination grown to steady state biomass, followed by invasion by the eighth species (8 treatments x 6 replicate bottles each = 48 bottles total). A full eight-species polyculture treatment included nine replicate bottles of all eight species grown together, totaling 9 bottles.

All treatments were inoculated at 800 cells/mL total density in the 1 L bottles. The inoculation density had to be low relative to final equilibrium densities in order to satisfy the assumption behind the sensitivity and invasion analyses that invaders are at essentially zero cells/mL initial density, and 800 cells/mL was the lowest density at which each species experienced positive exponential growth when grown in monoculture (*personal observation*). 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 (Cole-Parmer MasterFlex® L/S® Multichannel Pump). Two mL's of exchanged experimental media were retained for sampling after each media exchange. One mL samples of removed media were fixed with 250 μ L 10% formalin (Fisher Scientific, U.S.A.) and stored in the dark for further processing. One mL samples of removed media were directly pipetted into 48 multiwell tissue culture plates (Becton Dickinson Labware, U.S.A.) for *in-vivo* Chlorophyll-a fluorescence readings (460/685 nm excitation/emission wavelengths, measured on a Synergy H1 Hybrid Reader, Biotek) to monitor the growth

of algal communities and to determine when bottles had reached steady-state biomass. Steady-state biomass was assumed to occur once communities attained equilibrium population levels. Equilibrium conditions are necessary to compare species abundance at the same point in their growth trajectories when calculating relative yield and competitive release (Laska & Wootton 1998), as well as to satisfy assumptions behind the invasion analysis (Carroll, Cardinale & Nisbet 2011). Steady-state biomass was recognized as a saturating response in natural-log transformed fluorescence reads over time. A non-significant increase in $\ln(\text{fluorescence})$ between any two consecutive exchange days between DOE 20 and DOE 26 was considered evidence of steady-state biomass. This liberal determination of steady-state was adopted 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.

2.4 Data Analysis

Cell counts were performed to estimate species density over the course of the experiment, and density was then used to compute metrics of competition. Cells were counted and identified in 10 μL aliquots of preserved samples on a compound light microscope at 10x and 40x magnification using a hemacytometer. 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, ME, U.S.A.). Biovolumes ($\mu\text{m}^3 \cdot \text{L}^{-1}$) were

then converted to biomass ($\mu\text{g}\cdot\text{L}^{-1}$) by assuming that cells are primarily composed of water, which has a specific gravity 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 (Figs A1 & A2). Monoculture maximum intrinsic growth rates, r_{max} , and invader growth rates when rare (at inoculation density), r_{inv} , were calculated as the log ratio of density (D) on the final and first days of exponential growth divided by number of days of exponential growth (t) (equation 1).

$$r = \ln(D_{\text{final}} / D_{\text{initial}}) \cdot t^{-1} \quad \text{eqn 1}$$

The period of exponential growth was determined by maximizing the fit of linear regressions to the log-transformed growth curves of each bottle (Appendix 1).

Maximum intrinsic growth rate and growth rate when rare were used to calculate a species' sensitivity to competition as well as its invasion success. 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 (equation 2).

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

As a given species' growth rate when rare (r_{inv}) approaches its intrinsic growth rate (r_{max}), the numerator in S approaches zero, signifying low competitive pressure from the established community to which the invader is introduced. Sensitivities between zero

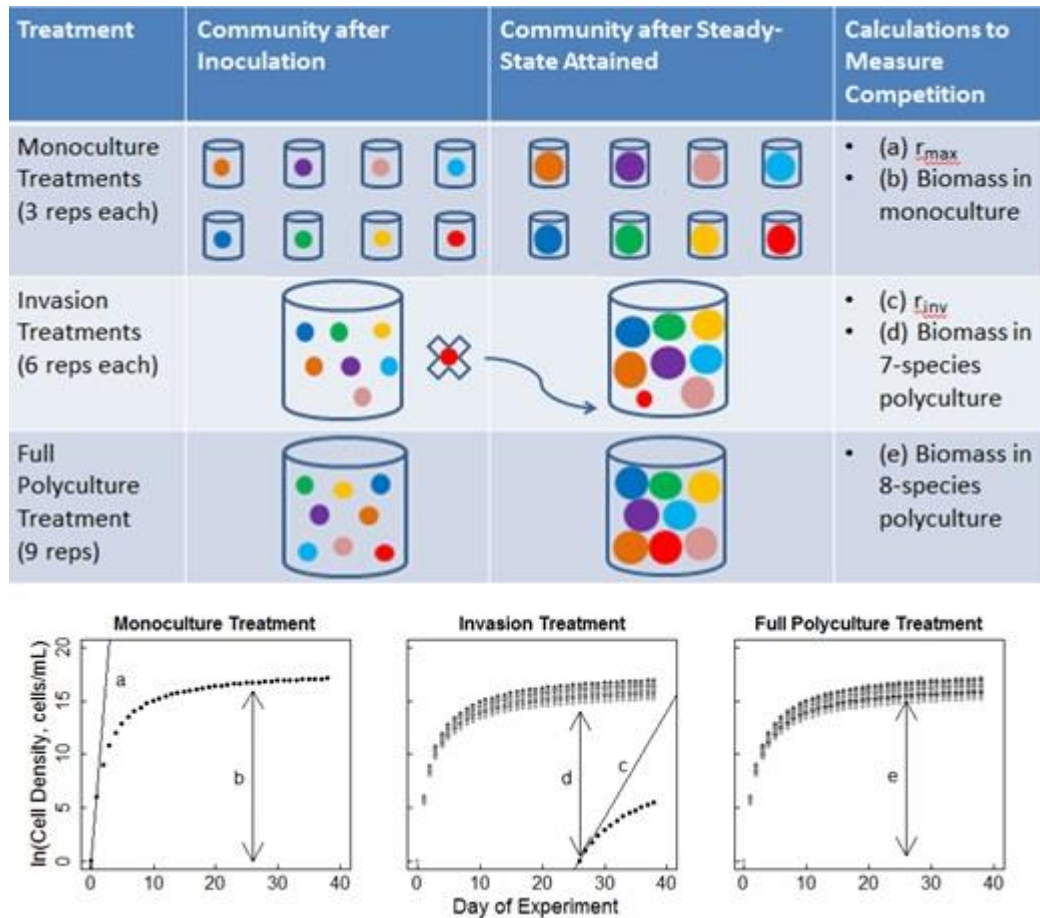


Fig. 1. Diagram of experimental setup including experimental treatments, measurements taken from each treatment and an example of the growth dynamics for each treatment over time. Each colored dot represents one of eight species and each cylinder represents a 1 L bottle. Dot size indicates cell density, where large dots indicate steady-state biomass. For the Invasion Treatments row, eight possible treatments exist for each of which one of the eight species is not included in the initial inoculation, but only one example is drawn due to limited space. The final column lists all measurements taken from algal growth curves to estimate competition, which measurements are then displayed on the example growth curves below as (a) slope = r_{max} , maximum intrinsic growth rate of a species in monoculture, (b) $M_{i,1}$, steady-state density of species i in monoculture, (c) slope = r_{inv} , maximum intrinsic growth rate of species as an invader, (d) $M_{ij,7}$, steady-state density of species $i \neq j$, where j represents the missing species, and (e) $M_{i,8}$, steady-state density of species i in full polyculture. All densities (b, d, e) were converted to biomass values for further analysis. Note: graphs are examples and do not represent experimental data.

and one signify competition but a successful invasion of the invader when rare in an established community. A sensitivity of 1 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, which is a common measure of competition (Williams & McCarthy 2001; equation 3). RY measures competitive response of a focal species to the combined competitive pressure from species 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 (equation 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 an analysis; one can learn whether phylogenetic distance effectively predicts diffuse and/or species-specific competition strength.

$$RY_i = M_{i,8} / M_{i,1} \quad \text{eqn 3}$$

$$CR_{ij} = M_{ij,7} / M_{i,8} \quad \text{eqn 4}$$

In equations 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.

2.5 Statistical Analysis

Several data analyses were performed to address whether PD predicts competitive outcomes in a multispecies community using R v. 2.15.2 (R Core Team 2012). The first analysis was a linear regression relating species' sensitivities to competition (equation 2) to phylogenetic distance, in which two separate analyses were run 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, for which every replicate of the same invader species had an identical phylogenetic distance.

The second analysis was 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 the presence of a competitor will reduce the biomass of a species (*i.e.* equation 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, equation 4). The hypothesis that competitive release decreases with PD 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 Darwin's competition-relatedness hypothesis. Because the absence of a competitor affects the relative abundance of all species in a community, it is important to

note that competitive release of a focal species probably reflects the combined impact of direct and indirect competition (as mediated by the change in biomass of other species due to the absence of a shared competitor) from the absent competitor. 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 the eight algal species (Fig. 2)), relationships between *CR* and *PD* were also examined 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.

3.1 Sensitivity and Invasion Success

Contrary to the predictions of Darwin's competition-relatedness hypothesis (CRH), no relationship was found between a species' sensitivity (S) to 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 WPD or 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). 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' relatedness to a community was not related to these metrics of competition.

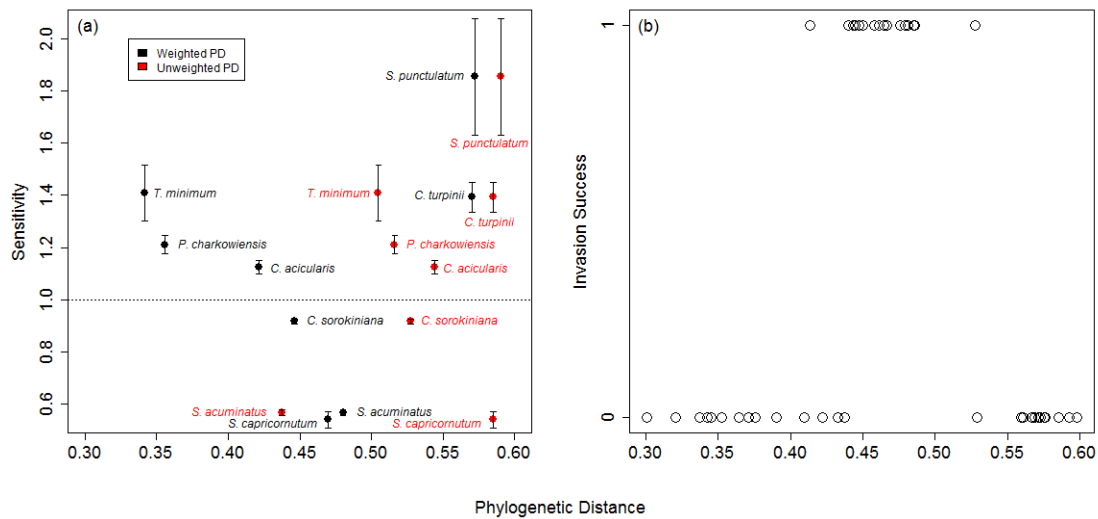


Fig. 2. (a) Invader sensitivity as a function of its phylogenetic distance to the established community. Sensitivity to competition is the reduction in intrinsic growth rate of a species introduced at low density (i.e. “invader”) to a polyculture at equilibrium relative to its intrinsic growth rate in monoculture. Sensitivity of each invading species, indicated by labelled points, was analyzed as a function of abundance-weighted average phylogenetic distance (WPD) and unweighted average phylogenetic distance (UPD) between the invading species and all other members of a polyculture community. Points below the dotted line at Sensitivity = 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 sensitivity calculated for six replicate mesocosms. Neither WPD nor UPD significantly predicted sensitivity (WPD: $n=8$, $F=0.26$, $P=0.63$; UPD: $n=8$, $F=1.39$, $P=0.28$). (b) Invasion success of species introduced at low abundance to communities at equilibrium as a function of WPD between the invading species and the polyculture community. Species with sensitivities < 1 were given an invasion success of 1 = successful, and species with sensitivities > 1 were given an invasion success of 0 = unsuccessful. PD was not able to predict invasion success ($n=48$, $Z=0.53$, $P=0.60$).

3.2 Relative Yield

Relatedness to the community was also a poor predictor of species relative yields in 8-species (full) polyculture versus in monoculture. Seven out of eight species had relative yields less than 1, which is suggestive of competition for limiting resources. However, there was no significant relationship between relative yield and WPD or UPD (Fig. 3). In contrast to the other species, *S. acuminatus* had an *RY* approximately equal to 1 (which after log-transform is 0; Fig. 3), suggesting that *S. acuminatus* either was competitively dominant or else was completely niche differentiated from 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 biomass in polyculture was similar to its biomass in monoculture. *S. capricornutum* had the lowest *S* (Fig. 2a) and *RY* (Fig. 3) recorded, making it the best and worst competitor according to each competition measure, respectively. These measures of competition suggest that initial densities and priority effects play a role in determining algal community structure (Peay, Belisle & Fukami 2012).

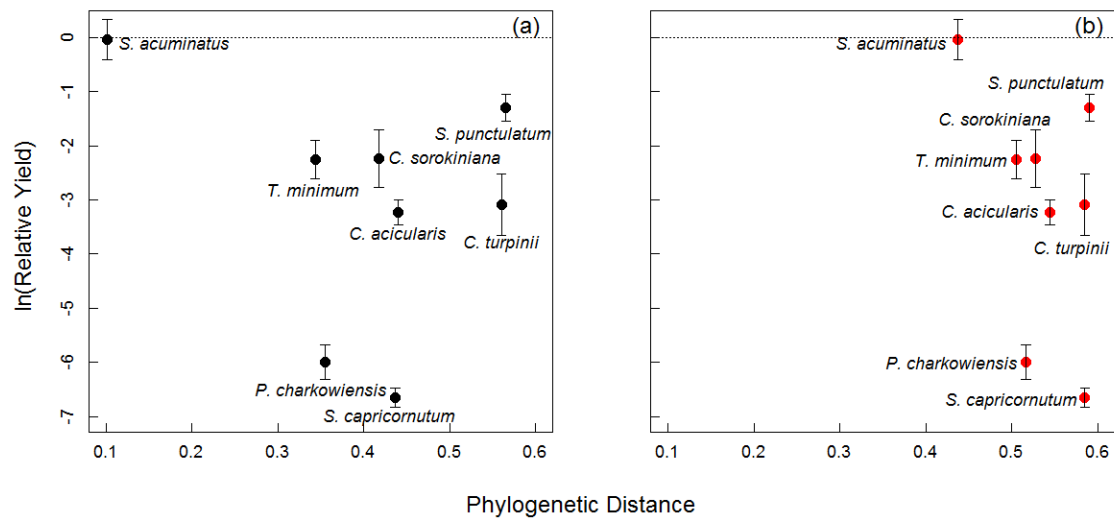


Fig. 3. Relative yield (RY) of a focal species in polyculture versus monoculture as a function of abundance-weighted phylogenetic distance (WPD, (a)), and un-weighted phylogenetic distance (UPD, (b)), between the focal species and all other taxa in the polyculture. Points are labelled with focal species names. RY values are natural log-transformed and standard errors approximated as in Hedges, Gurevitch & Curtis (1999). The dotted line at $\ln(\text{RY}) = 0$ 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: $N=8$, $F=1.40$, $p=0.28$).

3.3 Competitive Release

Phylogenetic distance 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 competitive release in several focal species (Fig. 4a, b, g), even though no single species greatly impacted the biomass of *S. acuminatus* (Fig. 4e). These findings corroborate *S. acuminatus* being 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 instances probably represent facilitation by the absent species (Fritschie *et al.* 2013).

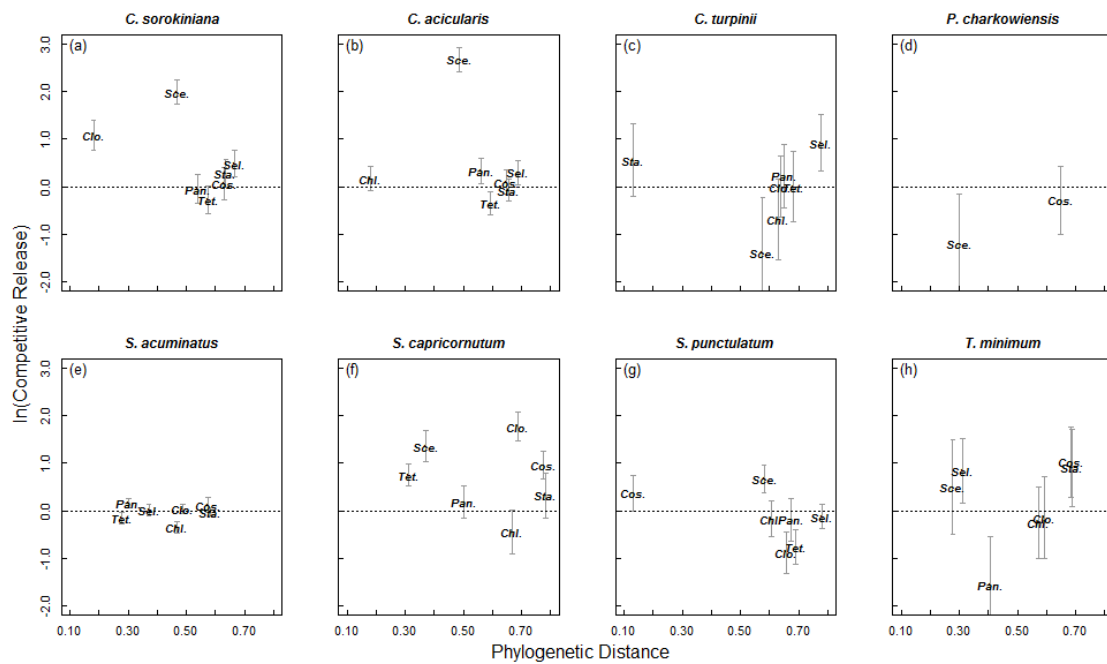


Fig. 4. Competitive release (CR) as a function of phylogenetic distance between a focal species and the missing competitor. CR is the yield of a focal species (each point) in a 7-species polyculture that is missing one competitor relative to the yield of that same focal species in a full 8-species polyculture. Subplot labels refer to focal species and points within each subplot are the first three letters of the genus of the missing competitor. Each subplot, a-h, corresponds to the relationship between PD and competitive release experienced by *C. sorokiniana*, *C. acicularis*, *C. turpinii*, *P. charkowiensis*, *S. acuminatus*, *S. capricornutum*, *S. punctulatum*, and *T. minimum*, respectively. CR values are natural log transformed and error bars represent standard error calculated according to Hedges, Gurevitch & Curtis (1999). Points were jittered to improve visualization, but they retain their relative positions. The horizontal dashed line at $\ln(\text{CR}) = 0.0$ corresponds to $\text{CR} = 1$ after transformation. 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).

4.1 Experimental Summary

Within this species-rich green algal community, the relatedness of a particular species to its community did not correspond to the strength of competition it experienced, calling into question the validity of the assumptions behind and ecological applications of the competition-relatedness hypothesis. Four measures of competition (sensitivity, *S*; invasion success; relative yield, *RY* and competitive release, *CR*) were unrelated to phylogenetic distance (*PD*) between a focal species and its community, regardless of whether *PD* was weighted by competitors' relative abundance within the community. Rather than any general relationship of competition to phylogenetic relatedness, particular species (*i.e.* *S. acuminatus*) appeared to drive competition strengths across the community. This study therefore extends the generality of past CRH results from green algae systems (*e.g.* Fritschie *et al.* 2013; Narwani *et al.* 2013; Venail *et al.* 2014)—which predominantly measured pair-wise interactions—to multi-species communities in which interactions are more complex and not readily predicted from pair-wise interaction strengths. Contradiction of the competition-relatedness hypothesis in this more realistic multispecies community demands that ecologists, phylogeneticists and conservation biologists revisit the validity of analytical and conservation practices based on the presumption that evolutionary history and ecology are universally linked (Vane-Wright, Humphries & Williams 1991; Faith 1992; Harvey & Pagel 1991; Cavender-Bares *et al.* 2009; Losos 2011).

4.2 A Defense of Negative Results

Several explanations have emerged from previous laboratory experiments and community phylogenetics reviews for the lack of signal between competitive ability and

evolutionary history (Cavender-Bares *et al.* 2009; Vamosi *et al.* 2009; Mayfield & Levine 2010). One possibility is phenotypic plasticity, where organisms can modify phenotypic expression depending on biotic and abiotic components of their environment (Agrawal 2001). If the ability of individuals of a species to vary ecologically relevant phenotypes is on par with or exceeds variation in these phenotypes across species in a community, then that species could become niche differentiated to the extent that relatedness does not predict its ecology (Sinervo & Adolph 1994; Miner *et al.* 2005). However, such strong phenotypic plasticity is believed not to occur or to be very rare for green algal traits related to competition: for instance, stoichiometry and resource acquisition ability (Klausmeier *et al.* 2008; Litchman & Klausmeier 2008; Schwaderer *et al.* 2011). Similarly, rapid evolution of ecological characters can abolish the relationship between ecology and relatedness (Schluter 2000; Rheindt, Grafe & Abouheif 2004; Losos 2011). Lineages that have experienced adaptive radiation in traits important to competition at a rate faster than baseline speciation or extinction would result in close relatives that do not compete strongly, where use of species from these lineages to experimentally test the CRH would refute it (Revell, Harmon & Collar 2008). Finally, convergent evolution can produce distantly related species that compete strongly, which pattern opposes the CRH (Cavender-Bares *et al.* 2009; Mayfield & Levine 2010).

Processes such as adaptive radiation and convergent evolution would not be expected to significantly occur within the timeframe of a single-season experiment; rather, it is the evolutionary *history* of the species chosen for a given experiment that impacts whether the experimental species pool would be expected to exhibit phylogenetic signal in traits important to competition. (As an aside—because evolutionary history is quantified using

molecular phylogenies, evolutionary history might not accurately predict competitive ability if traits important for competition evolved separately from those used to construct the tree—*i.e.*, for trees constructed from spacer regions. Because this study calculated phylogenetic distance from a phylogeny constructed using 25 chloroplast and 94 nuclear gene orthologs, it is unlikely that the phylogeny is not based upon genes important to competition.) While the traits determining competitive outcomes for the green algae species used in this experiment have yet to be identified, 13 out of 17 traits related to nutrient uptake, stoichiometry and cell morphology appear to lack phylogenetic signal across a phylogeny of 48 species inclusive of the eight used in this experiment (Narwani *et al.* 2014). Several of the algal traits related to nitrogen uptake and cell morphology have recently diverged while others related to stoichiometry diverged anciently (Narwani *et al.* 2014), both of which evolutionary histories could result in competitive abilities that do not vary proportionally with species relatedness (Losos 2011). Thus, several distinct evolutionary and ecological scenarios could explain results that do not support the competition-relatedness hypothesis.

4.3 An Ecological Case of 1 + 1 Not Equaling 2

While the overall conclusion that phylogenies cannot be used to predict competition within a community was shared between competition studies carried out with species pairs and in this 8-species community; comparison of competition strengths between the pairwise studies and this study suggested that competitive interactions in multi-species communities differ from interactions documented for the same species grown in biculture. In brief, averaging pairwise sensitivities from a companion study using green algae species taken from the same experimental cultures (Venail *et al.* 2014) did not

always predict the competition experienced by a species when grown with all seven species together. In particular, the abundant and competitively dominant *S. acuminatus* appeared to mediate disconnects between interactions within species pairs and between a species and a community. The competition experienced by two species (*C. acicularis* and *S. capricornutum*) in the 8-species community was not predicted from pairwise sensitivities (Fig. 4 of Venail *et al.* 2014). *CR* values suggest that this was due to *S. acuminatus* indirectly releasing competition on *S. capricornutum* by depressing the growth of its strong competitor, *C. acicularis* (Fig. 4b,f), in polyculture communities. While *CR* values do not give insight into the mechanism behind competitive interactions, they depicted *C. acicularis* experiencing competition almost exclusively from the dominant *S. acuminatus* (Fig. 4b, point “*Sce*” significantly above $\ln(CR) = 0$) and *S. capricornutum* experiencing the strongest competition from *C. acicularis* and *S. acuminatus* (Fig. 4f, points “*Clo*” and “*Sce*”). Therefore, one could conclude that *S. acuminatus* alone contributed to *C. acicularis*’ reduced competitive ability in multispecies polyculture relative to when averaged across each biculture combination and that the presence of *S. acuminatus* in multispecies polyculture competitively inhibited this strong competitor of *S. capricornutum* sufficiently for *S. capricornutum* to experience low sensitivity and successful invasion (Fig. 2a,b) despite its strong competitive interactions with many other species in polyculture (Fig. 4f, many points above $\ln(CR) = 0$).

Similarly, the relative competitive ability of the next-to most dominant species in full polyculture, *C. sorokiniana* and *C. acicularis*, appeared to be higher for the former according to *S* and *RY* (Figs 2a & 3) but the latter according to *CR* (Fig. 4a,b). It is

possible that the very strong competition between *S. acuminatus* and *C. acicularis* (Fig. 4b) either depressed *C. acicularis* biomass or forced it to adopt a unique niche, allowing *C. sorokiniana* to rise to second most-dominant in the full 8-species community through indirect competition. In summary, several instances were found for which competitive ability of species in bicultures did not predict their competitive ability in polyculture. This discovery highlights the fact that in any competition study, pairwise interaction strengths cannot be assumed to accurately depict competitive interactions in a community composed of all the same species grown together (May & Leonard 1975; Strauss 1991). It is therefore necessary to perform competition studies—for instance, those testing the competition-relatedness hypothesis—in experimental communities comprising the full diversity of a system of interest rather than subsets of that system's species.

4.4 Caveats

As with any laboratory experiment, this study system represented an oversimplification of natural communities and, as such, there are obligatory caveats that may limit the applicability of these results to natural algal communities. For instance, relatively static environmental conditions such as semi-continuous supply of nutrients, mixed (homogeneous) media, continuous light exposure and lack of disturbance other than media exchanges may have reduced spatial and temporal niche opportunities (Hutchinson 1961; Connell 1978) and resulted in enhanced competition. Additionally, natural processes such as dispersal, immigration and herbivory were not included in this experiment. If the species used here experience such neutral and trophic dynamics in the nature, then the competitive outcomes observed in the contained, 1-L, predation-free mesocosms likely would not be reproducible in natural algal communities (Vamosi *et al.*

2009; Cavender-Bares *et al.* 2009). For instance, grazing pressure might affect vertical distribution patterns of algae due to preferential grazing (for instance, preference for large prey cells; Lampert & Taylor 1985), cause algae to invest more heavily in defensive tactics rather than on resource acquisition (Yoshida, Nelson & Hairston 2004), or select for species with high intrinsic growth rates (Reichwaldt, Wolf & Stibor 2004). These processes would all change the composition of algal competitors with whom one species of algae interacts and minimize the strength of competition by creating more spatial and temporal niches (Hutchinson 1961). This experiment's results could also be contested based on the common criticism of all tests of the CRH: namely, that the species pool did not encompass the correct phylogenetic scale (Cavender-Bares, Keen & Miles 2006; Silvertown *et al.* 2006; Swenson *et al.* 2006; Losos 2011). However, there is evidence that this species pool encompassed the correct scale of phylogenetic distance for testing the CRH, which is the scale at which interactions between species occur (Vamosi *et al.* 2009). All species in the experiment except for *S. acuminatus* experienced competition (Figs. 2-4). Competition strengths ranged from very weak to very strong interactions with one (*e.g.* *C. acicularis*) or many (*e.g.* *S. capricornutum*) other species. Furthermore, the experimental simplification of spatial, temporal and trophic niche opportunities should make this a relatively conservative test of the competition-relatedness hypothesis by artificially augmenting competitive interactions.

4.5 Conclusions

In summary, this study explored the strength of algal competitive interactions as a function of phylogenetic distance in multi-species freshwater green algae communities. The experiment allowed for direct manipulation of PD and subsequent measurement of

competition experienced by each of eight species within a community, providing a novel fully reciprocal polyculture test of the CRH. Because certain species (*i.e.*, *S. acuminatus*) dominated trends in competitive interaction strength, it appears that understanding the traits important to competition for a given species pool and how those traits are distributed on a phylogeny may be a better means of predicting competitive interactions within natural communities than phylogenetic distance alone. In other words, measures taken to account for the presumed relationship between phylogeny and ecological similarity should not be used without first ensuring that the community displays phylogenetic signal in the traits of interest. For instance, ecologists should not automatically correct for phylogenetic relatedness in trait studies and biodiversity management should not necessarily be based on maximizing phylogenetic distance. It is also important to note that competition strength of species in 8-species polyculture was not always predicted by their competition strength measured in bicultures with species from the same experimental species pool. Thus, if PD continues to be used as a conservation tool and estimator of species' ecology, (*i.e.* for communities in which competitive traits show phylogenetic signal), then algorithms will be needed to incorporate the outcome of indirect and intransitive interactions into ecological predictions made based on PD. While more work is clearly needed to incorporate realism into experimental tests of the CRH, this and other experiments have amassed sufficient evidence over the past several years to justify a re-evaluation of the universality and applicability of Darwin's competition-relatedness hypothesis.

Monoculture treatments (Fig. A1): Exponential growth phase was determined to occur over the linear portion of the log-transformed growth curve. Linear portions of log-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 generally taken to signify best fit, visual determination of final day of exponential growth was used in preference to R^2 values in cases where 1) the best linear fit included less than three data points, and 2) 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 equation 1 and appear in Fig. A1 as the slope of the mean of the best least-squares fits to the log-transformed growth curves of the three replicate bottles over points pertaining to exponential growth phase.

Invasion treatments (Fig. A2): Exponential growth phase was determined to occur over the linear portion of the log-transformed growth curve. Linear portions of log-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 equation 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-when-rare (r_{inv}) for all species except *S. capricornutum*, in which case the 6th day after introduction was considered its final day of exponential growth.

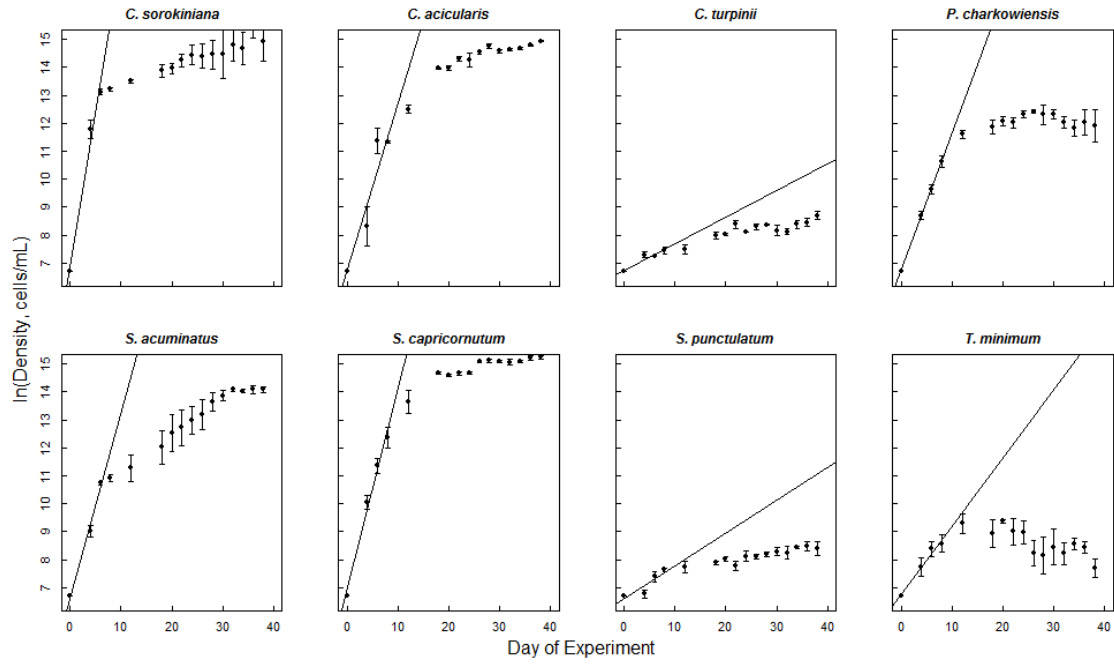


Fig. A1: Growth curves for monoculture treatments. Each subplot shows the mean density of the three replicate bottles for the species labelled above the plot, where the error bars represent standard error of the three replicates. Lines represent the maximum intrinsic growth rate, r_{max} for each species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

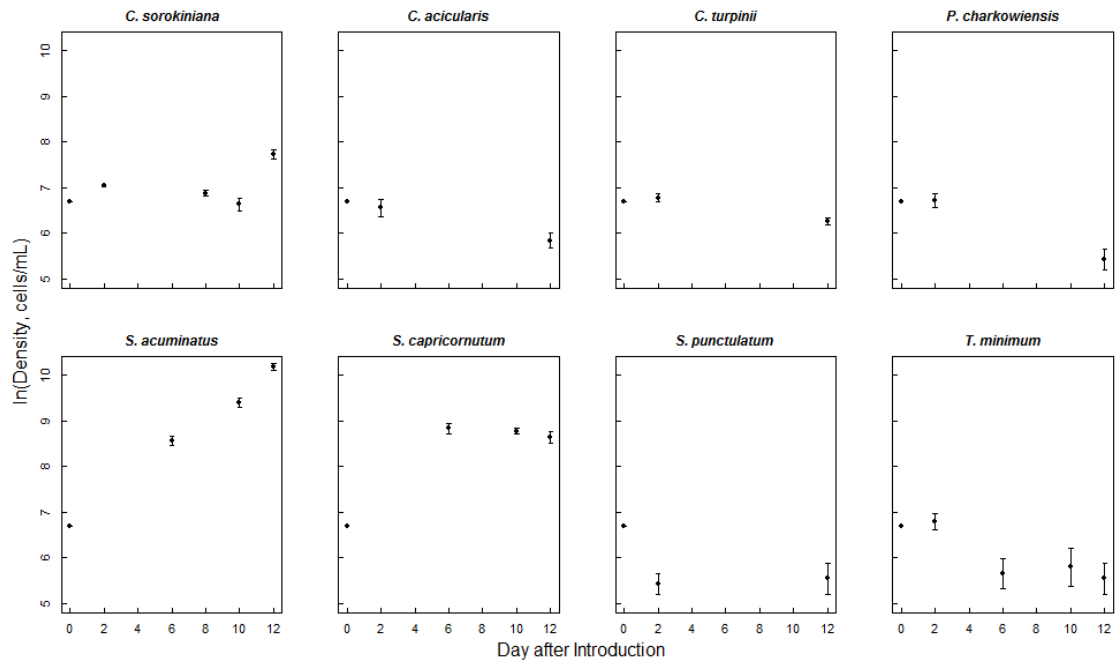


Fig. A2: Growth curves of the “invader” species in the invasion treatments. Each subplot shows the density of the invader, indicated by the subplot label, averaged over six replicate invader bottles. Error bars represent standard error of the six replicates.

<i>T. minimum</i>	<i>S. punctulatum</i>	<i>S. capricornutum</i>	<i>S. acuminatus</i>	<i>P. charkowiensis</i>	<i>C. turpinii</i>	<i>C. acicularis</i>	<i>C. sorokiniana</i>	
							0	<i>C. sorokiniana</i>
						0	1.64	<i>C. acicularis</i>
					0	4.32	15.38	<i>C. turpinii</i>
				0	1.56	0.52	0.95	<i>P. charkowiensis</i>
			0	2.25	28.69	4.84	19.62	<i>S. acuminatus</i>
		0	12.88	1.38	10.20	2.85	3.03	<i>S. capricornutum</i>
	0	8.12	20.74	1.04	17.46	3.98	8.90	<i>S. punctulatum</i>
0	9.08	3.28	16.42	0.00	11.32	2.51	9.85	<i>T. minimum</i>

Table S1. Co-occurrence matrix of each pairwise combination of genera used in this experiment in continental U.S. lakes compiled from the U.S.A. EPA National Lakes Assessment “Phytoplankton Soft Algae Count Data (CSV)” dataset. Numbers inside the 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.

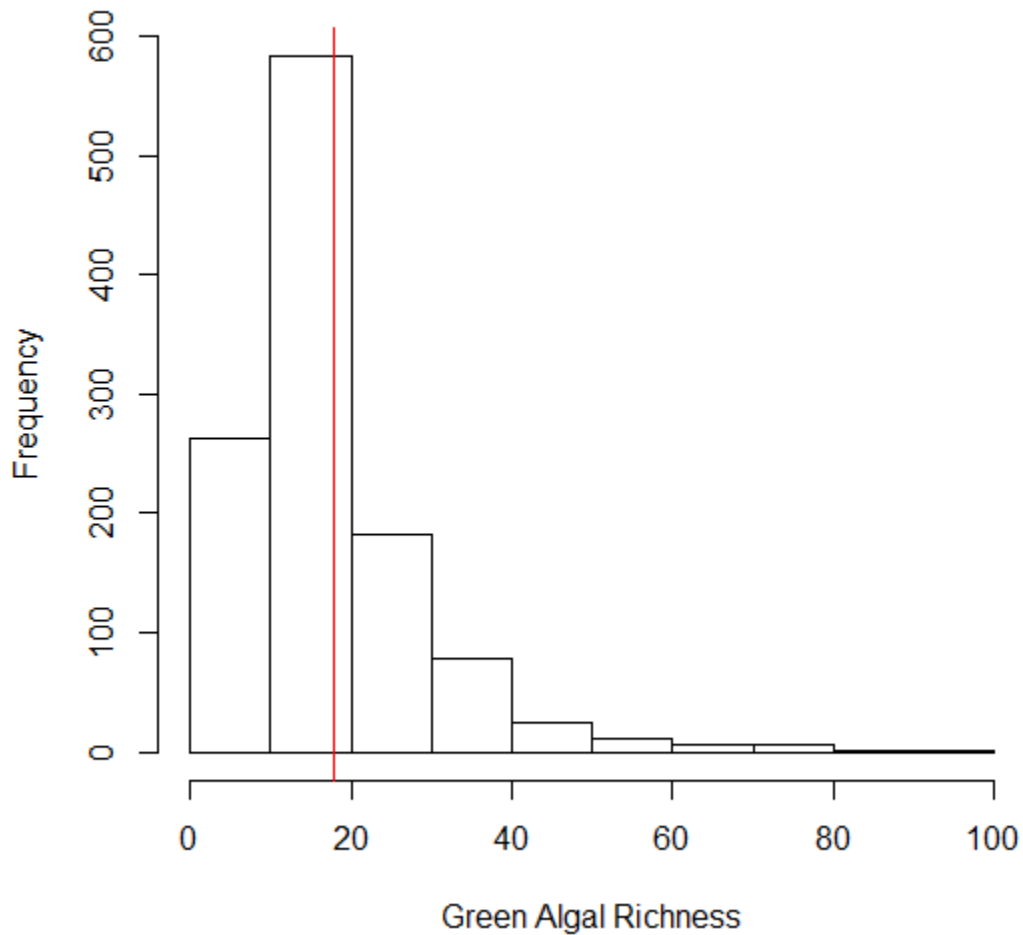


Fig. S1. Frequency histogram of the number of lakes having a given green algal richness. Data was taken from the U.S.A. EPA National Lakes Assessment “Phytoplankton Soft Algae Count Data (CSV)” dataset. The red line at Richness = 17.84 represents the mean number of green algae species found across 1157 lakes in the continental U.S., where each lake was visited twice. The number of species used in this study, 8, falls within one standard deviation of the mean (standard deviation = 11.51).

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