

## LETTER

# Experimental evidence that evolutionary relatedness does not affect the ecological mechanisms of coexistence in freshwater green algae

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### Abstract

The coexistence of competing species depends on the balance between their fitness differences, which determine their competitive inequalities, and their niche differences, which stabilise their competitive interactions. Darwin proposed that evolution causes species' niches to diverge, but the influence of evolution on relative fitness differences, and the importance of both niche and fitness differences in determining coexistence have not yet been studied together. We tested whether the phylogenetic distances between species of green freshwater algae determined their abilities to coexist in a microcosm experiment. We found that niche differences were more important in explaining coexistence than relative fitness differences, and that phylogenetic distance had no effect on either coexistence or on the sizes of niche and fitness differences. These results were corroborated by an analysis of the frequency of the co-occurrence of 325 pairwise combinations of algal taxa in > 1100 lakes across North America. Phylogenetic distance may not explain the coexistence of freshwater green algae.

### Keywords

biodiversity, coexistence, community phylogenetics, competition, evolutionary ecology, niche differences, phytoplankton, relative fitness differences.

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## INTRODUCTION

In 1928, Volterra introduced a dynamical model of competition that left a deep imprint on the study of ecological communities. This model and its predictions ultimately became the foundation for the 'competitive exclusion principle' (Volterra 1928; Gause 1934), which states that there should only be as many species in an ecosystem as there are 'limiting factors' (MacArthur & Levins 1967) or niches (Rescigno & Richardson 1965). This principle has inspired many hypotheses that attempt to explain why so many species-rich ecosystems appear to defy competitive exclusion. Nearly all of these hypotheses argue that diversity exists because species partition the environment into unique niches (e.g. are limited by different resources or predators) that can vary over space or time, and that this niche partitioning mitigates the negative effects of interspecific competition (Chase & Leibold 2003).

While niche partitioning has been the foundation for most explanations of the world's biodiversity, theories of species coexistence have recently undergone a major revision. In 2001, Hubbell published *The Unified Neutral Theory of Biodiversity* (Hubbell 2001), which argued that patterns of biodiversity could be explained by a simple model that did not invoke niches at all. According to this theory, species coexist because their demographic parameters are identical and the consequences of their interactions are 'neutral' (i.e. equal among species). As such, Hubbell argued that the biodiversity that we observe in nature can be explained by a series of stochastic events that allow some populations the chance to rise to dominance

while others walk towards extinction. At nearly the same time, Chesson completed a ground-breaking synthesis of coexistence theory; one that provided a road map for the integration of niche and neutral perspectives (Chesson 2000). He showed that the long-term outcome of competition between species of the same guild depends on the balance between two types of mechanisms, those that are 'stabilising' and those that are 'equalising'. Stabilising mechanisms represent various forms of traditional niche partitioning, all of which cause species to limit their own growth rates more strongly than they limit the growth of other species. In contrast, equalising mechanisms minimise relative fitness differences among species. Relative fitness differences are the differences in competitive ability among species that persist in the absence of the stabilising forces. Note that Chesson's definition of a relative fitness difference is not the same as that used by evolutionary biologists; namely, a relative fitness difference is the difference in competitive abilities between species, not individuals. Variation in several traits can influence relative fitness differences and contribute to competitive inequalities, including minimum resource requirements, resistance to consumers and demographic rates (Levine & HilleRisLambers 2009; HillerisLambers *et al.* 2012). It has since been shown that Hubbell's neutral theory represents the extreme case where niche differences and relative fitness differences are both zero, causing the outcome of competition to be approximated by a random walk to extinction (Adler *et al.* 2007).

As coexistence theory was undergoing a major revision, a separate body of research increasingly focused on how evolutionary pro-

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cesses influence species interactions that control coexistence. More than a century ago, Charles Darwin proposed two related hypotheses regarding the impact of evolution on the strength of competition and competitive coexistence (Darwin 1859). The first posited that

closely related species have more similar niches than distantly related species, and are therefore less likely to coexist. He argued that evolution should lead to divergence of species niches and the traits responsible for negative species interactions like competition (Mac-

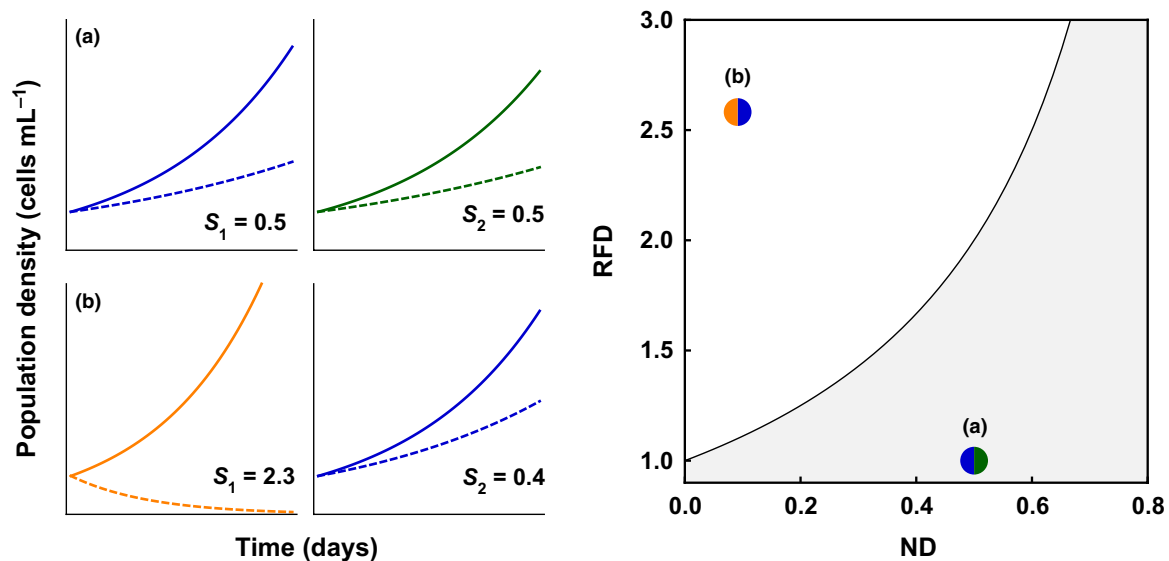
### Box 1 Quantifying niche and relative fitness differences

Stable coexistence of two species can be defined as the ability of each species to invade a steady-state population of the other from rarity ('mutual invasibility' sensu Chesson 2000). A species' sensitivity ( $S_i$ ) to competition is defined as the amount by which its per capita growth rate is reduced when invading a steady-state population of a competitor ( $\mu_{i,invading}$ ) relative to the rate achieved during exponential growth in monoculture ( $\mu_{i,alone}$ ):

$$S_i = \frac{\mu_{i,alone} - \mu_{i,invading}}{\mu_{i,alone}}$$

Any species  $S_i$  and hence its rate of invasion when rare, is jointly determined by the size of its niche difference relative to other species (what Chesson called 'stabilising' mechanisms) and the size of its relative fitness difference, or competitive inequality (the opposite of what Chesson called 'equalising' mechanisms) (Chesson 2000; Adler *et al.* 2007). Specifically, greater niche differentiation among species in a community reduces the sensitivity of both species' to competition (Adler *et al.* 2007), while greater relative fitness differences cause species to be asymmetrically affected by competition such that one species' sensitivity increases while the other's decreases (see Supporting Information). Given that niche differences will reduce both species' sensitivities, but relative fitness differences cause the sensitivities to diverge, Carroll *et al.* (2011) defined a community-level index for niche differences (ND) as one minus the geometric mean of both  $S_i$ , ( $ND = 1 - \sqrt{S_1 S_2}$ ) and similarly defined an index for relative fitness differences (RFD) as their geometric standard deviation ( $RFD = \sqrt{S_1/S_2}$ ).

Figure 1 shows how the species' sensitivities to competition reflect the magnitudes of niche and relative fitness differences that correspond to different outcomes for coexistence. In Scenario A, both species have moderate and equivalent impacts on the growth rates of one another. The low RFD reflects the low variability in their sensitivities, while the moderate ND reflects a moderate sensitivity to competition across species. Because both species are less sensitive on average (large ND) than they are variable in their sensitivities (small RFD), their invasion rates are both positive and they coexist. In Scenario B, the blue species has a low  $S_i$  while the orange species has a high  $S_i$ , indicating that orange is at a competitive disadvantage. The large variability in species'  $S_i$ s overwhelms the non-zero ND of the community and results in competitive exclusion of orange by blue.



**Figure 1** Sensitivities to competition for each species in a community reflect community-wide niche differentiation (ND) and relative fitness differences (RFD) (Scenarios A & B). Time-series panels on the left display how sensitivity to competition is estimated as the standardised reduction in a species' per capita growth rate when invading (dashed) relative to its growth rate in monoculture (solid). The average and the variability of species' sensitivities in a community (left panel) reflect the community's ND and RFD, respectively (right panel), which together describe the outcome of competition. Competitive exclusion occurs above and to the left of this curve (where  $RFD > 1/(1-ND)$ ), and coexistence occurs below and to the right.

Arthur & Levins 1967; Webb *et al.* 2002; Cavender-Bares *et al.* 2009; Hillerislambers *et al.* 2012). This gave rise to a second hypothesis, Darwin's 'naturalisation hypothesis', which states that non-native species are more likely to establish, or 'naturalise', in communities where their close relatives are absent. An increasing number of recent studies have claimed to provide evidence for Darwin's hypotheses (Strauss *et al.* 2006; Maherali & Klironomos 2007, 2012; Cahill *et al.* 2008; Cavender-Bares *et al.* 2009; Jiang *et al.* 2010; Burns & Strauss 2011; Violle *et al.* 2011; Hillerislambers *et al.* 2012; Peay *et al.* 2012), though the interpretation of numerous studies have been questioned (Losos 2008; Cavender-Bares *et al.* 2009; reviewed in Hillerislambers *et al.* 2012), and all have been challenged by counter-examples from studies that have found no evidence of a phylogenetic signal in species niches or the strength of competition (Knouft *et al.* 2006; Cahill *et al.* 2008; Best *et al.* 2013; Kunstler *et al.* 2012). Part of the reason for the disparate results to date could lie in our continued narrow focus on niche differentiation, which represents just one of the two mechanisms that moderate species coexistence. Assuming that evolution does impact ecologically relevant traits, the effects of evolution on coexistence may be positive or negative, depending on whether those traits impact niche differences or fitness differences respectively. To understand how evolution influences coexistence, we must understand how both niche and fitness differences relate to phylogenetic divergence among species (Mayfield & Levine 2010).

Here, we report the results of a laboratory experiment in which we asked whether evolutionary relatedness between algal species pairs affects their niche differences, relative fitness differences, and ultimately, their coexistence. We also asked whether niche differences or relative fitness differences were better at explaining coexistence. We studied freshwater phytoplankton because they are known to compete for limiting resources (Tilman 1981) and as a result, the coexistence of multiple species in a relatively homogeneous environment has perplexed ecologists for decades (Hutchinson 1961). We constructed a molecular phylogeny of common freshwater green algae and tested whether phylogenetic distance was a good predictor of stable coexistence, where coexistence was defined as the ability of each species in a species pair to invade an established population of the other from rarity – i.e. 'mutual invasibility' (Chesson 2000). We took a recently developed approach (Carroll *et al.* 2011) that uses species' invasion rates when rare to estimate their sensitivities to competition and, in turn, the size of their niche differences (ND) and relative fitness differences (RFD) (see Box 1 and Supporting Information). To check that our findings were not biased by the inherently simplified laboratory environment used in our experiment, and the reduced species pool required for replication, we also tested whether evolutionary relatedness among pairs of freshwater phytoplankton taxa predicted their co-occurrence in lakes across North America using the US Environmental Protection Agency's National Lakes Assessment (NLA) survey data. The NLA survey used standardised methods to identify all pelagic algae inhabiting > 1100 lakes throughout North America (see Materials and Methods).

We show that species coexistence was more heavily influenced by the size of species' NDs than their RFDs, that neither coexistence in the laboratory experiments nor co-occurrence in natural lakes could be explained by the phylogenetic distances (PD) among taxa. These results suggest that Darwin's competition-relatedness hypothesis is not supported for freshwater green algae.

## MATERIALS AND METHODS

### Species selection for the laboratory experiment

Our laboratory experiment focused on eight species of freshwater green algae: *Chlorella sorokiniana*, *Coelastrum microporum*, *Cosmarium turpinii*, *Elakatothrix viridis*, *Scenedesmus acuminatus*, *Selenastrum capricornutum*, *Staurastrum punctulatum* and *Tetraedron minimum*. Cultures were obtained from collections at the University of Texas at Austin or the University of Göttingen (Germany). The US Environmental Protection Agency's 2007 NLA survey showed that these species rank among the 48 most common genera (of 282) in over 1100 lakes in the United States, and that each occurred in at least 16% of the EPA lakes. In addition, each pair-wise combination of these eight species was found together in anywhere from 3 to 55% of all sites, indicating a wide range of co-occurrence (Table S1).

These species also met a number of criteria that were crucial for our experimental design. It was necessary that all eight species could grow in a common culture medium (COMBO (Kilham *et al.* 1998)) and could be distinguished under a microscope. We also aimed to achieve a wide and uniform distribution of PDs between species pairs, ranging from small PDs on the order of those observed for congeneric species to those approaching the deep split between Chlorophyta and Charophyta (Hall *et al.* 2008). This ensured that the species we selected were representative of the range of the phylogenetic diversity of freshwater green algae and that their PD distribution met the assumptions of our statistical tests. Only 37 green algal species identified in the US EPA's NLA survey, were both available as cultures from culture sources (UTEX and SAG culture collections), and had gene sequence data on GenBank. After using the gene sequence data to construct the molecular phylogeny (described next), we selected the focal eight species pool to meet the previously mentioned criteria.

### Phylogeny construction and the calculation of phylogenetic distance (PD)

We constructed a phylogeny using partial 18S ribosomal RNA and *rbcl* sequences for 37 species of freshwater phytoplankton that were available on GenBank. We then extended taxon sampling outside of this species pool, by including three representative species from the Chlorophyta and Charophyta, so as to place the pool within a broader phylogenetic framework. These three species were identified as useful outgroups in previous work (Rodríguez-Ezpeleta *et al.* 2005). We used the 18S and *rbcl* sequences because they provided the most complete data for the original species pool used in this experiment. We constructed alignments independently for each gene using Muscle v 3.8.31 (Edgar 2004), and selected a nucleotide substitution model for each gene using the Akaike Information Criterion as implemented in jModelTest v 0.1.1 (Posada 2008). We then constructed an unsmoothed Maximum Likelihood phylogeny using RAxML v 7.2.8 (Stamatakis *et al.* 2008). The analysis was partitioned by gene using a mixed partition model. It used random starting trees for each independent tree search, and it tested for topological robustness using 100 non-parametric bootstrap replicates. The resulting branch lengths are 'unsmoothed' because they represent the number of mutational changes present in the alignment and not time. In addition, we reconstructed a rate smoothed Bayesian phylogeny, estimated using Beast v1.6.2 (45), assuming a relaxed uncorrelated lognormal clock with all other parameters on default. We

conducted the relaxed clock analysis to estimate relative divergence times, thereby converting branch length values from substitutions per site to an estimate of time since divergence from a common ancestor. The Bayesian MCMC chain ran for 10 millions generations, sampled every 1000 generations. Stationarity and effective sample sizes (ESS > 200) were examined using Tracer v 1.5 (Rambaut & Drummond 2004), discarding all trees under the asymptote as burn-in. We constructed a consensus tree with mean node heights from the posterior distribution using TreeAnnotator v1.6.2 (Drummond & Rambaut 2007). We calculated the phylogenetic diversity (PD (Faith 1992)) of bicultures using the mean branch lengths connecting each species pair (ignoring the root branch) using a custom Bioperl script (Stajich *et al.* 2002). In one case, sequences were not available for our experimental species (*C. turpinii*) and we used distances for the genus rather than the species by including two representative species per genus and calculating distances from the genus.

### Test of pairwise coexistence using mutual invasibility

With eight taxa there are 28 possible pairwise species combinations. For each combination, we conducted replicate competition experiments in which we (1) measured the growth rate of each species when grown alone in monoculture ( $\mu_{\text{alone}}$ ), and (2) measured the growth of each species when introduced at low density to each of the other species already at steady state ( $\mu_{\text{invading}}$ ). We documented species pairs as coexisting when both species had positive growth rates as invaders ( $\mu_{\text{invading}} > 0$ ). The overall design included 8 focal species  $\times$  7 invaders  $\times$  3 replicates per invasion. Each of the eight species was inoculated at 100 cells mL<sup>-1</sup> into 21 replicate wells (3 wells in each of seven 6-well plates). Each well contained 8 mLs of autoclave-sterilised COMBO medium. Well plates were covered with Breathe-Easy Membranes™ (Diversified Biotech, Dedham, MA, USA) containing a 1 cm diameter hole in the centre. This minimised evaporation and contamination but allowed sampling and media exchange.

On the fourth day after inoculation, we began sampling of 10% of the volume (800  $\mu$ L) daily and replacing sampled volume with new, sterile medium. Samples were preserved with 34- $\mu$ Ls of 25% Gluteraldehyde (final concentration of 1%) and later used to estimate cell densities. In addition to collecting density samples, we monitored the accumulation of biomass of each monoculture daily by reading *in-vivo* fluorescence of chlorophyll-a in each well on a Biotek® H1 Hybrid plate reader (BioTek Instruments Inc., Winoski, VT, USA). Chlorophyll-a is a widely used proxy of algal biomass (Clesceri *et al.* 1998).

By day 36 of the experiment, all species monocultures had achieved steady state biomass (zero population growth) based on visual inspection of the fluorescence curves. On this date, we inoculated each monoculture with one of the other seven species (invaders), replicating each species' invasion 3  $\times$  for a total of 168 experimental wells (8 species  $\times$  7 invaders  $\times$  3 replicates). To ensure we could measure both positive and negative growth rates, invaders were inoculated at a density of 1000 cells mL<sup>-1</sup>. This density was an order of magnitude lower than the steady state density of all of the focal species in monoculture, except for *C. turpinii*. This ensured that species were inoculated below the inflection point of their population growth curves (i.e. half of the steady state density). For *C. turpinii* alone, invasion cultures were inoculated at 100 cells mL<sup>-1</sup>.

We measured the growth rate of each species alone ( $\mu_{\text{alone}}$ ) and as an invader ( $\mu_{\text{invader}}$ ) over a 6 day period (T) as:

$$\mu = \frac{1}{T} \cdot \ln \frac{D_T}{D_0} \quad (1)$$

where  $D_0$  and  $D_T$  are cell densities at day 2 and 8 for monocultures, or day 38 and 44 for invaders in biculture. In cases where the invader density was < 100 cells per mL on day 44 of the experiment, we used the samples from the next latest time point with detectable densities to measure growth rates. We measured growth rates for these 6-day time periods after inoculation because, based on monoculture growth curves, we estimated that this time period maximised the likelihood of detecting growth for slow-growing species while minimising the potential for density-dependent feedbacks for fast-growing species. All estimates of growth rate were obtained by counting preserved algal samples at 100  $\times$  magnification on a 100- $\mu$ m flowcell using a Benchtop FlowCam® (Fluid Imaging Technologies Inc., Yarmouth, ME, USA). For a select few species combinations, we were unable to accurately distinguish low densities of the invader from the resident species or cellular debris, and it was necessary to count these on a haemocytometer under 400x using a microscope. We verified that the counting method did not influence density estimates by cross-validating methodologies with standard curves (Table S2).

### Measuring the mechanisms of coexistence

We averaged the growth rates of the three replicates of each species treatment to obtain mean values of each species' sensitivity to competition, and to calculate the niche differences (ND) and relative fitness differences (RFD) for each species combination. Sensitivities ( $S_i$ ) were calculated as in eqn 1 (Box 1). The ND was calculated as one minus the geometric mean of the  $S_i$ , which for any two species combination is given in the following:

$$ND = 1 - \sqrt{S_1 S_2} \quad (2)$$

Relative fitness differences is then the geometric standard deviation of the  $S_i$ . For two species indexed such that  $S_j \geq S_2$ , the RFD is given in the following:

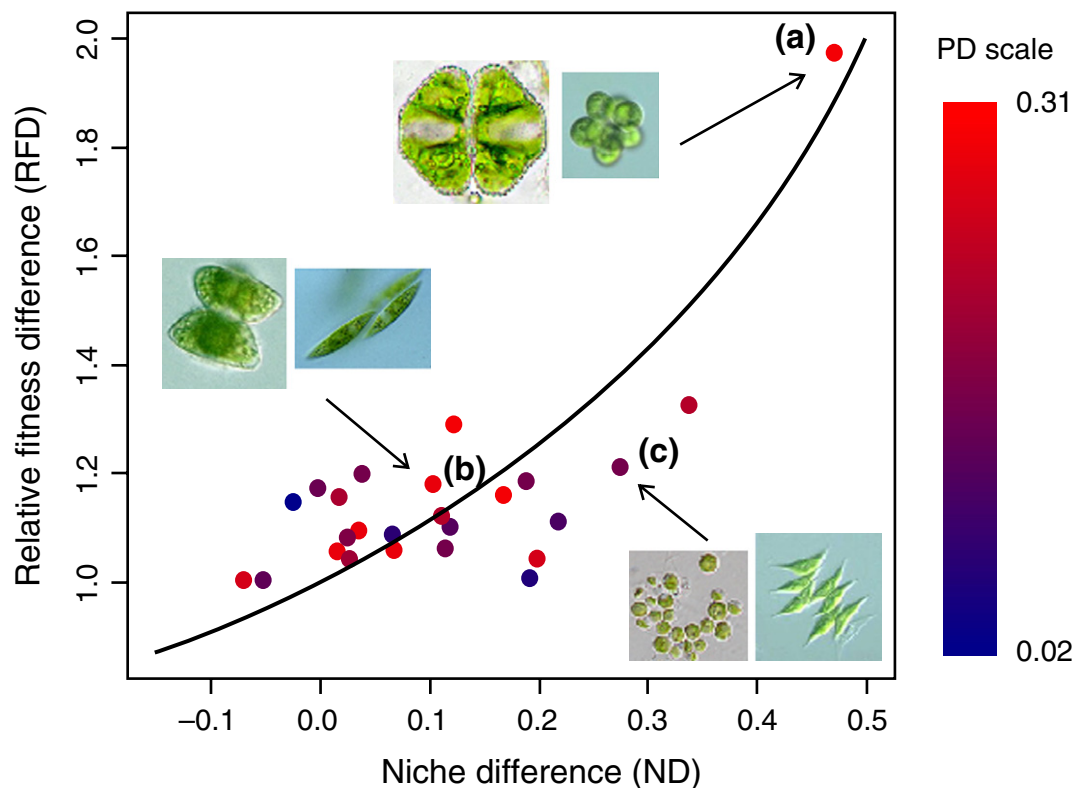
$$RFD = \sqrt{S_1/S_2} \quad (3)$$

Mutual invasibility requires that both species show positive growth rates as invaders, which translates to both  $S_i$ 's < 1. With  $S_2 < S_1$  as above, only one additional requirement is needed to guarantee mutual invasibility:  $S_j < 1$ . Note that dividing both sides of this inequality by  $\sqrt{S_1 S_2}$  gives  $\sqrt{S_1/S_2} < 1/\sqrt{S_1 S_2}$ . When the definition of ND and RFD from eqns 2 and 3 are substituted into this inequality, we obtain the following:

$$RFD < (1 - ND)^{-1} \quad (4)$$

Equation 4 gives the criterion for mutual invasibility expressed in terms of niche and relative fitness differences among species. This line separates the regions of coexistence and competitive exclusion plotted in Figs. 1 and 2.

Sample sizes for estimates of species' growth rates, sensitivities to competition and coexistence were reduced from 28 species pairs to 27 in the laboratory portion of the experiment because one species pair (*C. sorokiniana* and *C. microporum*) could not be distinguished under the microscope, even at 400  $\times$  magnification, and samples



**Figure 2** The effects of niche differences (ND) and relative fitness differences (RFD) on coexistence freshwater green algae. Species coexistence was measured for 25 combinations of freshwater green algae using the mutual invasibility criterion. The curve represents the condition for competitive exclusion:  $RFD = 1/(1-ND)$ . Competitive exclusion occurs above and to the left of this curve, and coexistence occurs below and to the right (see Materials and Methods for further detail). The colour-coding of the dots indicates the level of phylogenetic distance (PD) between the species in the species pair, with bright red indicating high PD and dark blue indicating low PD. Images of select algal combinations, which are discussed in the main text, are labelled a-c. These images are not to scale. See Table S5 for photo credits.

for this combination were discarded. Samples sizes for estimates of the niche and relative fitness differences were further reduced from 27 to 25 combinations because in two species combinations one species (*C. microporum*) showed higher growth rates on average when it was an invader than when it was in monoculture, suggesting facilitation occurred in the presence of *E. viridis* and *T. minimum*. We omitted these species combinations because the mathematical analysis of niche and relative fitness differences (eqns 2–4) was not designed to include positive species interactions.

We performed logistic regressions to test for the abilities of ND and RFD to individually explain coexistence (i.e. mutual invasibility). We also performed a logistic regression to test for an effect of PD on the likelihood of coexistence. Linear regressions and Mantel tests were then used to test for effects of PD on ND and RFD separately. We tested the assumption of normality of residuals for the linear regression. Residuals of the regression of RFD on PD were non-normal, and as a result we re-ran the analysis without one outlier ( $RFD = 1.97$ ), for which residuals were normal. The regression was still non-significant ( $F_{1,22} = 3.77$ ,  $P = 0.07$ ). We used unsmoothed values of PD for the statistical analyses presented in the Results section, but in order to ensure that our findings were not biased by our method of estimating phylogenetic distances, we also performed the analyses using smoothed estimates of PD. These results were not qualitatively different from those using unsmoothed PDs (Table S3).

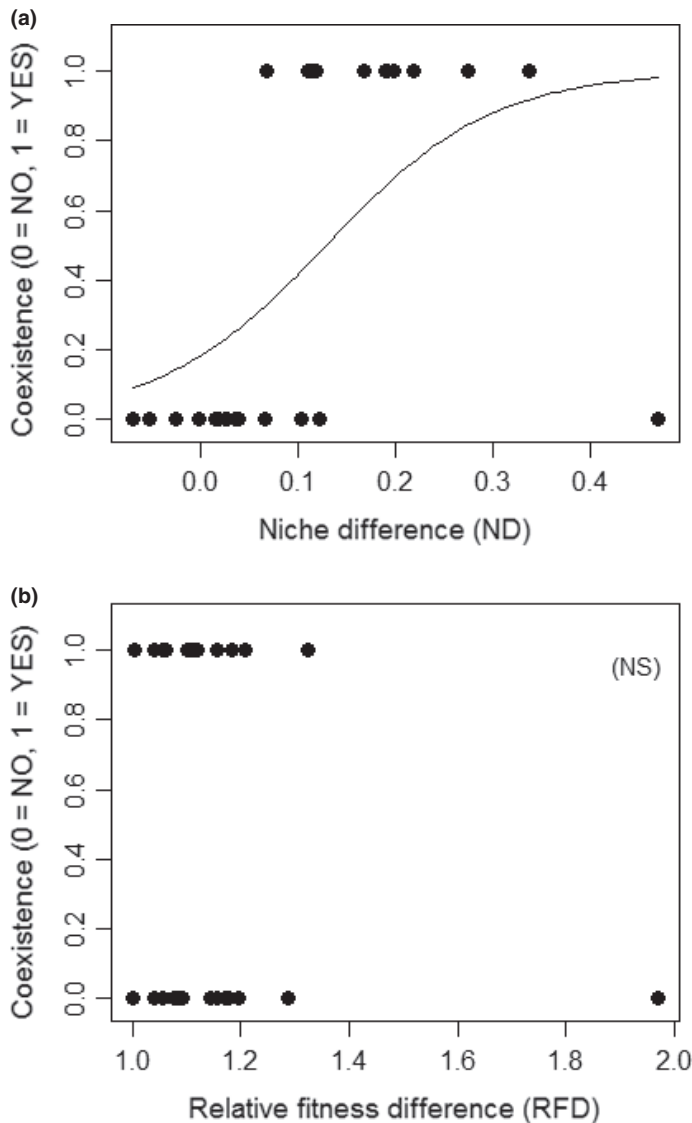
All statistical analyses were performed in R v. 2.13.1 (R 2012) except for power analyses, which were performed using G\*Power v.3.1.5 (Faul *et al.* 2012).

### Analysis of species co-occurrence in real lakes

To assess patterns of species co-occurrence in lakes, we used the 2007 US Environmental Protection Agency's National Lakes Assessment database for soft algal phytoplankton ([http://water.epa.gov/type/lakes/NLA\\_data.cfm](http://water.epa.gov/type/lakes/NLA_data.cfm)). This database contains algal counts for 1157 lakes, all of which were sampled at least once (95 lakes were sampled twice) in the summer of 2007 using standard methods. We used this database to create a community matrix representing the presence or absence of each of the 27 genera found on our phylogeny at each lake visit. One genus (*Arthrodesmus*) was found on our phylogeny but not in the NLA dataset, leaving only 26 genera. We calculated the PDs of all 325 genus combinations, and when more than one species of a genus was present on our phylogeny, we used the average of their PDs. We calculated pairwise species co-occurrence across lakes using Schoener's index ( $C_{ij}$ ) (Schoener 1970):

$$C_{ij} = 1 - 0.5 \cdot \sum_b |p_{ib} - p_{jb}| \quad (5)$$

where  $C_{ij}$  is the co-occurrence of species  $i$  and  $j$  and  $p_{ib}$  is the proportion of occurrences of the  $i^{\text{th}}$  species in the  $b^{\text{th}}$  lake visit. We



**Figure 3** The effects of niche differences (ND) and relative fitness differences (RFD) on the likelihood of coexistence. (a) ND had a positive impact on the coexistence of species pairs ( $\chi^2 = 7.47$ ,  $P < 0.01$ ), whereas (b) RFD had no significant impact on coexistence ( $\chi^2 = 0.52$ ,  $P = 0.47$ ,  $N = 25$ ).

tested for a correlation between species co-occurrence and PD using the `comm.phylo.cor` command in the `picante` package in R. The significance of the correlation was tested against a null model in which genus placements were randomised across the phylogeny 999 times, creating a distribution of random expectations for correlation coefficients (Cavender-Bares *et al.* 2004). We also used a *t*-test to determine whether genus pairs that co-existed in the laboratory co-occurred in a greater number of lakes.

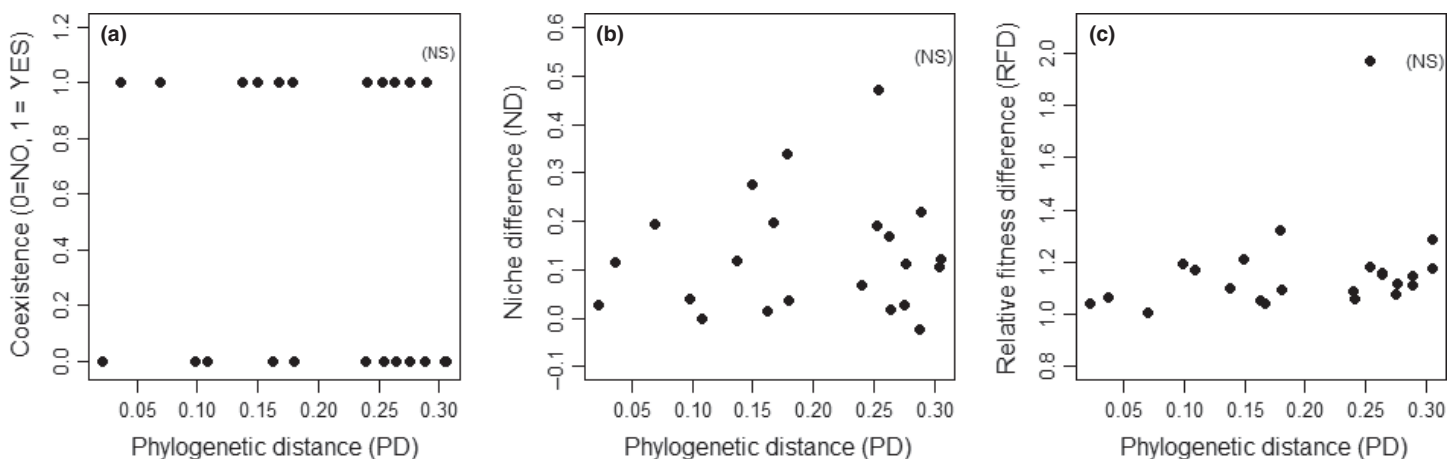
## RESULTS

Together, the influence of species pairs' NDs and RFDs resulted in the coexistence of 11 out of 25 species pairs, where coexistence was measured as mutual invasibility ( $\mu_{i,invading} > 0$  when species *i* & *j* are introduced into established populations of one another) (Fig. 2). The other 14 species pairs displayed competitive exclusion. While

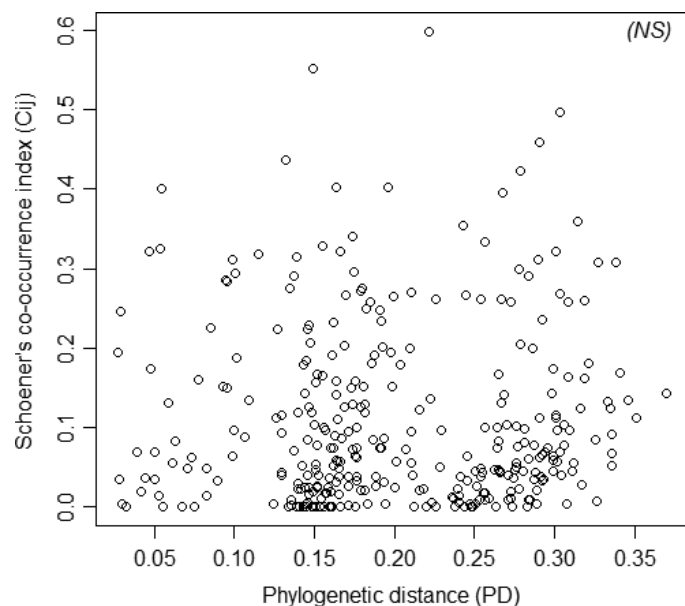
ND and RFD jointly determine whether species coexist, only ND was a significant explanatory variable when considered alone (Fig. 3). Pairs of algae with larger NDs had a higher probability of coexistence (Fig. 3a,  $\chi^2 = 7.47$ ,  $P < 0.01$ ,  $N = 25$ ). In contrast, community RFDs alone did not explain significant variation in species' coexistence (Fig. 3b,  $\chi^2 = 0.52$ ,  $P = 0.47$ ,  $N = 25$ ). Nevertheless, the effect of NDs on coexistence clearly depended on the size of the RFDs between species. As an example, *C. turpinii* had a sensitivity to the presence of *C. microporum* that exceeded one, while *C. microporum* had a low sensitivity to the presence of *C. turpinii*. As a result, this species pair had a large RFD (i.e. large variation in sensitivity to the presence of the other species). The variability was so great that despite a rather large ND, coexistence did not occur, resulting in competitive exclusion by *C. microporum* (Fig. 2 point a). On the other hand, small RFDs also did not guarantee coexistence because NDs may simultaneously be too small to prevent competitive exclusion, as was the case for *S. punctulatum* and *E. viridis* (Fig. 2 point b). Only when NDs were large enough to overcome RFDs did coexistence occur, as was observed for *S. acuminatus* competing against *C. sorokiniana* (Fig. 2 point c).

There was no impact of phylogenetic distance on the ability of species pairs to coexist (Figs. 2 & 4a,  $\chi^2 = 0.02$ ,  $P = 0.89$ ,  $N = 27$ ). Furthermore, neither mechanism of coexistence was significantly related to PD (Fig. 4b, ND,  $F_{1,23} = 0.56$ ,  $P = 0.46$ ; Fig. 4c, RFD,  $F_{1,23} = 2.23$ ,  $P = 0.15$  with outlier,  $F_{1,22} = 3.77$ ,  $P = 0.07$  without outlier). This absence of an effect of PD on ND and RFD was confirmed by non-significant Mantel tests, which quantify the correlation between two distance matrices, each containing pairwise species distances (PD vs. ND Mantel statistic: 0.15,  $P = 0.27$ ; PD vs. RFD Mantel statistic: 0.30,  $P = 0.07$ ). For two species combinations used in our experiment (*E. viridis*/*T. minimum*, and *S. acuminatus*/*S. capricornutum*), both species were unable to invade one another. For these combinations, both species showed positive growth rates in monoculture but displayed negative growth rates as invaders. While we included these combinations in our statistical analyses, the mutually negative invasion rates suggest these species exhibit priority effects (Alford & Wilbur 1985). Removing these two species combinations had no impact on the outcome of statistical analyses (effect of PD on coexistence:  $\chi^2 = 0.35$ ,  $P = 0.55$ ; effect of PD on  $\log(\text{ND}+1)$ :  $F_{1,21} = 0.14$ ,  $P = 0.71$ ; effect of PD on  $\log(\text{RFD})$ , no outlier:  $F_{1,21} = 2.90$ ,  $P = 0.10$ ; effect of ND on coexistence:  $\chi^2 = 5.48$ ,  $P = 0.02$ ; effect of RFD on coexistence:  $\chi^2 = 1.24$ ,  $P = 0.27$ ).

When we examined patterns of co-occurrence for 325 genus pairs from our phylogeny for > 1100 lakes sampled during the 2007 EPA NLA survey, we found that the number of visits on which two genera co-occurred in the NLA dataset was significantly greater for species pairs that coexisted in the laboratory than species pairs that did not coexist ( $t_{\text{one-sided}} = 4.21$ , d.f. = 23,  $P = 0.05$ ). Thus, stable coexistence observed in our laboratory experiment was a good predictor of the frequency of co-occurrence in nature. We failed to detect a significant correlation between PD and co-occurrence in the EPA lakes data set (Fig. 5,  $r = 0.05$ ,  $P = 0.68$ ). Therefore, neither our laboratory experiments nor analyses of the field data provided evidence that evolutionary divergence promotes coexistence or co-occurrence in freshwater green algae. While the failure to detect statistically significant effects may have been the result of low statistical power in some instances, this was not the case for our main finding that phylogenetic distance had no effect on the probability of coexistence (Table S4).



**Figure 4** The effect of phylogenetic distance (PD) on coexistence. (a) The effect of PD on coexistence as measured by mutual invasibility. If both species showed positive growth rates as invaders, then they were able to coexist (1), but if one or both species showed zero growth or a negative growth rate as an invader, then coexistence was not possible (0).  $N = 27$  (one combination excluded, see Materials and Methods). (b) The effect of PD on the strength of stabilising forces via niche differentiation between pairs of species of green freshwater phytoplankton.  $N = 25$  (three combinations excluded, see Materials and Methods). (c) The effect of phylogenetic distance on the size of relative fitness differences between pairs of species of green freshwater phytoplankton.  $N = 25$  (three combinations were excluded, see Materials and Methods). ‘NS’ indicates that the regression was not significant at  $P \leq 0.05$ . There was no significant effect of PD on coexistence or the mechanisms of coexistence (see Results).



**Figure 5** Schoener's index of co-occurrence of genus pairs across all EPA National Lakes Assessment lake visits for 325 pairwise combinations of genera across our phylogeny as a function of their phylogenetic distance. The correlation between species PD and co-occurrence is not significant ( $r = 0.05$ ,  $P = 0.68$ ).

## DISCUSSION

While niche and relative fitness differences jointly determine long-term, stable coexistence, we found that overall, niche differences were more important than fitness differences in explaining the coexistence of the pairs of freshwater green algae that we examined in this laboratory experiment. Counter to the competition relatedness

hypothesis, phylogenetic distance between species pairs did not predict the likelihood of coexistence in either the laboratory-based experiments or the > 1100 lakes sampled during the 2007 EPA NLA survey. Co-occurrence in natural lakes may respond to a great variety of abiotic and biotic factors such as climate variability or natural enemy density, among others, while coexistence in the relatively homogenous laboratory environment likely resulted from competition for inorganic resources (e.g. nutrients and light). However, the general agreement between laboratory and field-based findings suggests that our findings are robust.

There are a number of possible explanations for the absence of an effect of phylogenetic distance on coexistence in this experiment. First, the traits responsible for niche and relative fitness differences may be evolutionarily labile on this phylogenetic scale. Species may have experienced strong divergent or convergent selection throughout their histories, causing ecological trait differences to become decoupled from genetic differentiation (Webb *et al.* 2002; Knouft *et al.* 2006; Losos 2008; Revell *et al.* 2008; Cavender-Bares *et al.* 2009; Best *et al.* 2013). Second, it is possible that evolution has had antagonistic impacts on the multiple genes or traits that determine competitive ability and niche partitioning, with the cumulative impacts cancelling out on average. Last, it is possible that the phylogenetic scale of our investigation was too small to detect a signal of evolution on ecological traits in freshwater algae (Losos 2008; Cavender-Bares *et al.* 2009). For example, Schwaderer *et al.* (2011) found that resource-use traits in freshwater phytoplankton were conserved at higher taxonomic levels of organisation, but were labile at the levels of genus and species. Our species selection included some of the greatest possible divergence times among green phytoplankton, so a greater phylogenetic scale was not possible without considering other types of algae (e.g. diatoms, dinoflagellates)—a potential avenue for future study.

The lack of an evolutionary signal on ecological mechanisms of coexistence contradicts the common assumption that phylogenetic

distance reflects ecological divergence among species (Hilleris-lambers *et al.* 2012). While this assumption is actively being cited to promote the use of phylogenetic information in studies of community assembly, conservation biology and restoration ecology (Faith 1992; Wiens *et al.* 2010; Verdu *et al.* 2012), the evidence supporting this assumption is mixed. Certainly, there have been some prominent studies that have demonstrated a positive effect of phylogenetic distance on biodiversity (Maherali & Klironomos 2007) and on ecosystem functioning (Cadotte *et al.* 2008; Flynn *et al.* 2011), and negative effects of phylogenetic diversity on the frequency of competitive exclusion (Violle *et al.* 2011) and the size of competitive priority effects (Peay *et al.* 2012). However, there have also been studies demonstrating that phylogenetic distance has no impact on the strength of competition (Cahill *et al.* 2008; Kunstler *et al.* 2012) or biodiversity (Best *et al.* 2013). Furthermore, in order for ecological interactions and their outcomes to display a phylogenetic signal, the species' traits that generate these interactions must also display a signal (Mayfield & Levine 2010), but there are plenty of examples of ecological traits that do not (Losos 2008).

We have used a relatively new method for measuring niche and relative fitness differences for simple, two-species communities of algae. We believe that this method will be useful as empiricists continue to elucidate the ecological mechanisms that maintain biodiversity for two reasons. First, the method does not rely on the fit of data to any particular model of competition, or the concomitant, somewhat subjective, identification of parameters thought to reflect the influences of inter- and intraspecific competition for a given model, as is required by other methods (see Supporting Information, Adler *et al.* 2007; Levine & HilleRisLambers 2009; Hilleris-lambers *et al.* 2012). As a result, it may enable comparisons across ecosystems, community types and modes of competition; e.g. how do the strengths of niche and relative fitness differences in communities of algae compare to those in grassland or tropical forest communities? Second, it requires far less data than methods relying on fitting competition models to community time series data (Hilleris-lambers *et al.* 2012). Nevertheless, this method has drawbacks. For example, it may not work when species display competitive intransitivities or facilitative interactions, although to our knowledge no method to date has incorporated such factors. Also, the utility of the method for more species rich communities has yet to be confirmed, primarily because niche overlap has historically been defined for pairwise competition (Chase & Leibold 2003). The few models of multispecies competition with existing measures of niche overlap show that the qualitative effect of niche overlap is a reduction in the average low-frequency growth rate, and as such ND should reflect this reduction (see Supporting Information). Carroll *et al.* (2011) showed that the definitions of ND and RFD correspond to measures of niche overlap and fitness differences in a MacArthur model of competition, but how they relate to parameters in other models of competition has not yet been determined. Finally, these measures do not elucidate the mechanism of competition or which species traits are responsible for the measured niche and relative fitness differences. Where a detailed understanding of the mechanism of competition is desired, additional data and model fitting are preferable.

In summary, we have shown that coexistence among pairs of freshwater green algae is more strongly influenced by species' niche differences than by their relative fitness differences. Our data do not support Darwin's competition-relatedness hypothesis, as phyloge-

netic distance had no impact on species coexistence or the mechanisms of coexistence. These findings reflect the maturation of a very old question in ecology (Mayfield & Levine 2010). For many years, the question of how species coexist had been answered with incomplete (in hindsight) theories and empirical observations that focused only on niche differentiation. Today, Chesson's theory of coexistence has spawned the generation of operational measures of the mechanisms of coexistence, including both niche and relative fitness differences among species, that can completely explain the outcome of competition (e.g. Fig. 2). This maturation is key for ecologists to be able to address many ecological dilemmas presently facing humanity, including the causes and consequences of biodiversity loss, and the impacts of species invasions, range shifts resulting from climate change, and land-use changes and homogenisation. All of these questions are fundamentally tied to the question of how species coexist and how adaptable their mechanisms of coexistence are. The further investigation of the influence of evolution on both niche and neutral mechanisms of coexistence will help us to make more effective management and conservation decisions aimed at protecting biodiversity into the future.

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#### AUTHORSHIP

AN and BJC designed the research, AN performed the research, MAA and THO completed phylogenetic analyses, ITC contributed mathematical interpretation of theory, AN analysed data, and AN, ITC and BJC wrote the manuscript.

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