# Competition–colonization trade-offs in a ciliate model community

Romana Limberger · Stephen A. Wickham

**Abstract** There is considerable theoretical evidence that a trade-off between competitive and colonization ability enables species coexistence. However, empirical studies testing for the presence of a competition–colonization (CC) trade-off and its importance for species coexistence have found mixed results. In a microcosm experiment, we looked for a CC trade-off in a community of six benthic ciliate species. For each species, we measured the time needed to actively disperse to and colonize an empty microcosm. By measuring dispersal rates and growth rates of the species, we were able to differentiate between these two important components of colonization ability. Competitive ability was investigated by comparing species' growth with or without a competitor in all pairwise species combinations. Species significantly differed in their colonization abilities, with good colonizers having either high growth rates or high dispersal rates or both. Although species showed a clear competitive hierarchy, competitive and colonization ability were uncorrelated. The weakest competitors were also the weakest colonizers, and the strongest competitor was an intermediate colonizer. However, some of the inferior competitors had higher colonization abilities than the strongest competitor, indicating that a CC trade-off may enable coexistence for a subset of the species. Absence of a community-wide CC trade-off may be based on the lack of strong relationships between the traits underlying competitive and colonization ability. We show that temporal effects and differential resource use are alternative mechanisms of coexistence for the species that were both slow colonizers and poor competitors.

**Keywords** Colonization · Competition · Dispersal · Growth rate · Protists

## Introduction

Understanding the mechanisms behind species coexistence is a central challenge in community ecology. In patchy environments, the competition–colonization (CC) trade-off has long been regarded as one possible mechanism enabling persistence of inferior competitors. Species coexistence through this trade-off relies on the principle that strong competitors are weak colonizers and do not reach all the available sites, leaving patches open for inferior competitors with better colonization abilities.

Modeling approaches go back to Levins and Culver (1971) who demonstrated that two species can coexist due to a trade-off in competitive and colonization ability. Tilman's (1994) multispecies model predicts that an unlimited number of species competing for a single resource can coexist in a spatially subdivided habitat, given that the inferior competitors have sufficiently higher colonization abilities than the superior competitors.

Later models relaxed some of the restrictive assumptions such as fully asymmetric competition, instantaneous competitive exclusion or global dispersal (Holmes and Wilson 1998; Pacala and Rees 1998; Higgins and Cain 2002; Levine and Rees 2002; Calcagno et al. 2006) or added demographic stochasticity (Orrock and Watling 2010). Coexistence of an unlimited number of species in a patchy environment strongly relies on the presence of a strictly asymmetric competition hierarchy (Levine and

R. Limberger (✉) · S. A. Wickham
Department of Organismic Biology, University of Salzburg,
Hellbrunnerstrasse 34, 5020 Salzburg, Austria
e-mail: romana.limberger@stud.sbg.ac.at

Rees 2002). However, without the assumption of instantaneous competitive exclusion, an inferior competitor can coexist even without a colonization advantage by exploiting the resource-rich conditions before the slower-growing superior competitor gains dominance (successional niche; Pacala and Rees 1998). Long-distance dispersal of an inferior competitor can compensate for a low colonization rate provided that the superior competitor is rare and disperses only locally (Holmes and Wilson 1998). When adding demographic stochasticity, the outcome of competition depends on community size (Orrock and Watling 2010). The importance of ecological drift in small communities benefits stronger colonizers over superior competitors with weak colonization abilities.

Empirical studies testing for a CC trade-off found mixed results (Amarasekare 2003; Kneitel and Chase 2004). Using animals, some studies confirmed the presence of a CC trade-off (Hanski and Ranta 1983; Lei and Hanski 1998; Cadotte et al. 2006; Rodríguez et al. 2007; Hunt and Bonsall 2009), while others did not (Harrison et al. 1995; Amarasekare 2000; Yu et al. 2004; Guélat et al. 2008).

For plants, there is a lot of indirect evidence for a CC trade-off based on seed size (Coomes and Grubb 2003). Small-seeded species are more fecund due to a trade-off between seed size and seed number (Turnbull et al. 1999; Coomes and Grubb 2003; McEuen and Curran 2004). Moreover, smaller seeds are dispersed over greater distances (Clark et al. 1998). Conversely, seed size is positively associated with seedling survival (Westoby et al. 1996; Coomes and Grubb 2003) and therefore higher competitive ability in the recruitment phase. Direct evidence for a CC trade-off comes from a study on wind-dispersed plants (Jakobsson and Eriksson 2003), where dispersal distance of single seeds was found to be negatively correlated with competitive ability in the recruitment phase. When, however, dispersal ability was expressed as a combination of dispersal distance of seeds and fecundity of species, competitive ability was uncorrelated with dispersal ability.

Here, we present the results of a microcosm experiment that tested the hypothesis of a CC trade-off in a community of benthic ciliates, occupying the same trophic level. For many groups of organisms, the measurement of dispersal is difficult, so that dispersal ability has often been quantified by use of various surrogate parameters (Kneitel and Chase 2004). In our model system, however, it is possible to measure not only colonization time of empty patches but also to partition colonization ability into its components by quantifying growth and dispersal rate of the test organisms. We measured competitive ability of the species by comparing growth in single species treatments with performance in all pairwise species combinations. Detailed quantification of ciliate abundances over the time course of

the competition experiment enabled us to detect temporal changes in competitive effects. To elucidate a potential underlying mechanism of competition, we quantified algal and bacterial resources and tested the hypothesis that the best competitor reduces the resources to the lowest level (R*-rule; Tilman 1982).

## Materials and methods

We conducted a series of microcosm experiments to quantify colonization ability, dispersal rate, growth rate and competitive ability of six benthic ciliate species. The ciliates had been isolated from freshwater habitats around the city of Salzburg, Austria, and were fed on the benthic diatom *Navicula pelliculosa* obtained from the culture collection of algae at Göttingen (SAG). Algal and ciliate cultures were non-axenic and contained a variety of bacteria.

Microcosms were small plexiglass basins ($12 \times 12 \times 8$ cm) with five holes drilled 0.7 cm above the bottom into the sides of the basins. Plexiglass fittings of 0.4 cm inner diameter were glued into the holes and served to attach silicone tubing (0.5 cm inner diameter) as dispersal corridors between basins; unused holes were blocked with silicone plugs. The basins were covered with plexiglass lids to prevent contamination.

For the simulation of a benthic system, we used ceramic tiles ($2.27 \times 2.27 \times 0.5$ cm) as artificial substrate. Four days prior to an experiment, we incubated the tiles with bacillariophycean medium and an inoculum of *Navicula pelliculosa*. After cleaning the microcosms with 10% HCl for 24 h, they were filled with 300 ml of 0.2-μm-filtered pond water and with 25 tiles covered with a biofilm of the diatom and associated bacteria. The experiments were conducted at 20°C, with a light:dark cycle of 12:12 h.

### Colonization ability

For each of the six ciliate species, colonization ability was measured as the time needed to reach an initially uncolonized patch. We therefore connected two basins with silicone tubing of 5 cm length, and stocked one of the two basins with an inoculum of 500 individuals of one of the six ciliate species, with three replicates per species. At 24-h intervals, the initially uncolonized basin was sampled by removing three tiles with a plexiglass sampler. One tile fitted tightly into the sampler, so that it was possible to remove the tile including the water column above it. The removed water volume was replaced by sterile-filtered pond water. Ciliates were rinsed from the tiles and fixed with Bouin's solution (5% final concentration), and the entire sample was counted under an inverted microscope.

During introduction of the inoculum and sampling, the connections between basins were blocked with a tube clamp to avoid creation of a current and passive dispersal of individuals. When the species was observed on two successive days after the day of arrival, the colonization was regarded as successful and the experiment was terminated. The experiment was repeated with a dispersal distance of 10 cm.

Growth rate

We introduced 500 individuals of a species into a basin and, depending on the species' growth rate ($r$), sampled the basin daily or every second day until the species had reached its carrying capacity ($K$). We fit logistic growth curves to each of three replicates and estimated $r$ and $K$ of the six species. By the time of the growth experiment, two of the species cultures had died out (Onycho, Rubri; see Table 1 for species abbreviations), their $r$ and $K$ were estimated from the single species trials of the competition experiment (see below).

Dispersal rate

Basins were stocked with 500 individuals of a species and left for 2 weeks to reach equilibrium density. Using tubing of 10 cm length, we connected each of these donor basins with an uncolonized recipient basin containing only food resources. After 5 h, recipient and donor basins were both sampled. Due to its low absolute dispersal, Frontonia was left to disperse for 48 h. The entire volumes were fixed with Bouin's solution and, depending on ciliate densities, up to the entire volume or subsamples were counted. We calculated per capita dispersal rate as the proportion of individuals dispersed from donor to recipient basin. We could measure dispersal rate for only four of the six

species, as two cultures (Onycho, Rubri) had died out by the time of this experiment. Dispersal trials were replicated five times for each of the four species.

Competitive ability

Performance of the species in all possible pairwise species combinations was compared to single species growth experiments. Unconnected basins were stocked with one or two species, respectively, for the single and the competition trials. The resulting 21 treatments were replicated three times. In order to equalize species' initial biovolumes, initial numbers of individuals ranged from 40 to 500 per basin. To guarantee similar bacterial community composition for all treatments, ciliate cultures were filtered through 5-μm filters, and the ciliate-free filtrates were added to all treatments.

Basins were sampled on days 2, 7, 14, 21, 28, 35, 42 and 56 by removing three tiles. The removed water volume was replaced by sterile-filtered pond water enriched with nutrients to ensure algal growth. For quantification of food resources, the biofilm on the tiles was scraped off with a razor blade and merged with the withdrawn water volume to a 75-ml sample. Depending on the ciliates' abundances, up to 3 ml were counted in subsamples of 0.05–0.1 ml under a dissecting microscope. Due to low abundances at the beginning of the experiment, up to 12 ml were counted during the first 2 weeks of the experiment.

For quantification of the algal biomass, a subsample of 3.5 ml was measured fluorometrically (excitation 460 nm, emission >665 nm). Fluorescence values were transformed to abundance values after calibrating the fluorometer with samples of known algal concentration. Algal fluorescence was negatively correlated with light intensity, which was not completely homogeneous throughout the laboratory. We measured light intensity at the position of each basin to partial out the effect of light during data analysis.

**Table 1** Biovolume ($n = 15$), $r$ (growth rate, $n = 3$), $K$ (carrying capacity, $n = 3$), $d$ (dispersal rate, $n = 5$), arrival time ($n = 6$, averaged over 5 and 10 cm dispersal distance), bootstrap estimates of colonization and competitive rank with 2.5 and 97.5 percentiles in brackets

| Species | Biovolume ($10^3$ μm$^3$) | $r$ (ind ind$^{-1}$ day$^{-1}$) | $K$ (ind cm$^{-2}$) | $d$ (ind ind$^{-1}$ day$^{-1}$) | Arrival time (days) | Colonization rank (2.5, 97.5 percentiles) | Competitive rank (2.5, 97.5 percentiles) |
|---|---|---|---|---|---|---|---|
| Tachy | 17 | 1.75 | 1,501 | 0.001 | 2.8 | 4.1 (3, 5) | 2.3 (1, 4) |
| Stylo | 31 | 1.47 | 455 | 0.014 | 1.7 | 5.5 (4.5, 6) | 4.6 (2, 6) |
| Onycho | 57 | 0.28 | 905 | | 4.5 | 2.6 (2, 3.5) | 5.9 (5, 6) |
| Rubri | 65 | 0.59 | 110 | | 4.8 | 2.5 (1, 4) | 3.5 (2, 5) |
| Fronto | 89 | 0.35 | 137 | 0.003 | 9.2 | 1.1 (1, 2) | 1.2 (1, 3) |
| Para | 216 | 0.62 | 167 | 0.083 | 1.8 | 5.3 (4.5, 6) | 3.4 (1, 5) |

Ranks are from worst to best: high rank means short arrival time and high competitive ability, respectively

Tachy *Tachysoma pellionellum*, Stylo *Stylonychia pustulata*, Onycho *Onychodromopsis flexilis*, Rubri *Rubrioxytricha ferruginea*, Fronto *Frontonia angusta*, Para *Paramecium caudatum*; *ind* individuals

For enumeration of bacteria, a subsample of 1.6 ml was fixed with glutardialdehyde (4% final concentration), and then sonicated to disaggregate clumps of algae and bacteria. The samples were shock-frozen in liquid nitrogen and stored at −70°C until quantification by cytometry. Following Marie et al. (2005), samples were stained with SYBR Green I (Molecular Probes) and measured on a FacsCanto II flow cytometer (Becton–Dickinson) equipped with an argon laser (488 nm). To discriminate between small and medium-sized bacteria, cyanobacteria and diatoms, the cells' forward scatter, side scatter, SYBR Green-induced green fluorescence, phycoerythrin-induced orange fluorescence and chlorophyll-induced red fluorescence were measured. Data acquisition and analysis was performed with FACSDiva Software (Becton–Dickinson).

As some of the basins became contaminated with microflagellates, we quantified flagellates in all our samples. A subsample of 10 ml was fixed with glutardialdehyde (2% final concentration), and then gently sonicated. The sample was stained with DAPI (2.5 µg ml$^{-1}$ final concentration) and filtered onto a black polycarbonate membrane filter (0.8 µm pore size; Nuclepore). Flagellates and bacteria too large to be measured by cytometry (>10 µm length) were counted by epifluorescence microscopy in 50–100 randomly selected fields at ×1,000 magnification.

Data analysis

We used a two-way ANOVA to test whether arrival time differed between the six species and the two dispersal distances (5 and 10 cm). Arrival day was $\log_{10}$ transformed to homogenize variances. As the ANOVA showed no effect of dispersal distance (see "Results"), arrival times were averaged over the two dispersal distances for further analyses. To estimate colonization ranks of the six species, we used a bootstrap procedure. In each of 10,000 simulations, one of six replicates was chosen randomly for each species and species were then ranked by their arrival time. Mean ranks and 2.5 and 97.5 percentiles were calculated from this bootstrap procedure. To detect correlations between arrival time, $r$, $K$, dispersal rate and species' cell biovolume, Spearman's correlation coefficients were calculated.

As a measure of competitive strength, we used an index proposed by Fox (2002). We calculated the competitive response $C_{ijk}$ of species $i$ when grown with competitor $j$ in replicate $k$ as

$$C_{ijk} = (K_i - N_{ijk})/K_i$$

where $K_i$ is the abundance of species $i$ in the single species trial, averaged over the three replicates, and $N_{ijk}$ is the abundance of species $i$ when grown together with competitor $j$ in replicate $k$. Abundances were averaged over the last two sampling dates (days 42 and 56). This index measures the competitive response of species $i$ to competitor $j$ as its decrease in abundance relative to its equilibrium density in the single species trial. Likewise, it quantifies the competitive effect of species $j$ as its ability to reduce the abundance of species $i$ below its equilibrium density in the single species trial. An index of 1 means competitive exclusion, while values below 0 indicate facilitative effects. The index we used measures the total effect of one species on another and not the per capita interaction strength as proposed elsewhere (Laska and Wootton 1998; Haddad et al. 2008).

For calculation of species' competitive abilities, we took into account both species' responses and species' effects (Haddad et al. 2008). Competitive ability of species $i$ is the difference between its mean effect and its mean response, each averaged over the five competitors. To calculate ranks and confidence intervals, we used a bootstrap procedure with 10,000 simulations. For each species, one of the three competition trials was drawn randomly and competitive response, effect and ability of species were calculated. The species were ranked from lowest to highest competitive ability, and mean ranks and 2.5 and 97.5 percentiles were calculated over the 10,000 random draws. We correlated competitive response, effect and rank with $r$, $K$, cell biovolume and colonization rank using Spearman's correlation coefficients.

To test the hypothesis that strong competitors reduce the limiting resource to lower levels than weak competitors (R*-rule; Tilman 1982), we compared resource abundances (flagellates, small, medium and large bacteria) in the single species trials using ANOVA. Algal abundances were compared with an ANCOVA, using light intensity as covariate. All resource abundances were log-transformed and averaged over the last two sampling dates prior to analyses. Furthermore, we calculated correlations between competitive rank and algal and bacterial abundances, respectively, using Pearson's correlation coefficients.

Bootstrap procedures were calculated with R 2.10.0, all other analyses were conducted with SPSS 16.0 for Windows.

## Results

Colonization ability

The six ciliate species differed significantly in their arrival times, whereas dispersal distance of 5 and 10 cm, respectively, had no effect (two-way ANOVA; distance: $P = 0.157$; species: $P < 0.001$; distance × species: $P = 0.827$). Averaged over the two dispersal distances, arrival times ranged from 1.7 to 9.2 days (Table 1).

**Fig. 1** Relationships of arrival time (averaged over 5 and 10 cm dispersal distance) of six benthic ciliate species with **a** growth rate and **b** cell biovolume, respectively. Values are means ± SE; $n = 3$ for growth rate, $n = 6$ for arrival time, and $n = 15$ for cell biovolume
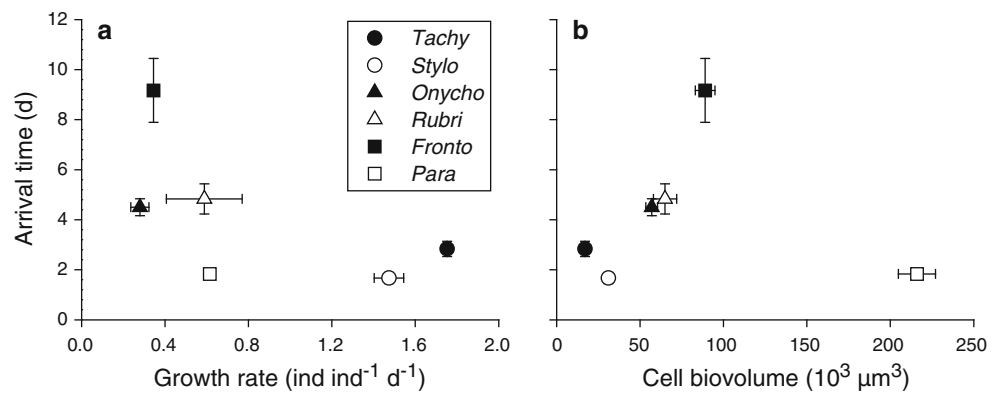


Tukey's post-hoc test differentiated between four groups of species ($Stylo = Para \leq Tachy \leq Onycho = Rubri < Fronto$; see Table 1 for species abbreviations). No significant correlations between arrival time and $r$, $K$, biovolume or dispersal rate were found, though some non-significant trends could be observed. Species with high growth rates tended to have short arrival times (Fig. 1a; Spearman's $r = -0.657$, $P = 0.156$), while the early arrival day of *Para* was due to is comparatively high dispersal rate (Table 1). The relationship between arrival time and cell biovolume showed a unimodal trend (Fig. 1b): species with the shortest arrival times were either small (*Tachy*, *Stylo*) or very large (*Para*).

## Competitive ability

During the final period of the experiment, one of the six species was clearly the strongest competitor: *Onycho* decreased the abundances of its competitors by 64–90% relative to their single treatment abundances (Table 2), whereas itself showed low response to competition. This resulted in a high competitive rank and a narrow confidence interval (Table 1). When only final abundances were taken into account instead of averaging over days 42 and 56, *Onycho*'s effect was even more pronounced (80–97% reduction of its competitor's abundances). *Fronto* and *Tachy* were weak competitors, being strongly affected by the other species and having low influence on the equilibrium abundances of their competitors (Table 2). *Stylo*, *Rubri* and *Para* had intermediate competitive ranks and large confidence intervals (Table 1), with strong effects mainly on the two weaker competitors.

Competitive effects and responses changed over the time course of the experiment (Fig. 2). During the initial phase of the experiment, *Tachy* negatively affected the abundance of *Stylo* (Fig. 2b), with some weak negative effects also on initial growth of *Onycho* (Fig. 2c). *Fronto*, though the weakest competitor in the end of the experiment, strongly decreased the abundance of *Stylo* during the

**Table 2** Competitive effect (column) and response (row) based on abundances averaged over days 42 and 56; e.g. the effect of *Stylo* on *Tachy* is a 53% decrease in *Tachy* abundance relative to its equilibrium density without *Stylo*; the response of *Stylo* to *Tachy* is a 30% decrease in *Stylo* abundance relative to its equilibrium density without *Tachy*

|  | *Tachy* | *Stylo* | *Onycho* | *Rubri* | *Fronto* | *Para* |
|---|---|---|---|---|---|---|
| *Tachy* |  | **0.53** | **0.82** | **0.61** | 0.30 | **0.54** |
| *Stylo* | 0.30 |  | **0.64** | −0.58 | 0.23 | 0.16 |
| *Onycho* | 0.20 | 0.18 |  | 0.25 | −0.07 | 0.23 |
| *Rubri* | −0.18 | −0.36 | **0.90** |  | **0.55** | **0.57** |
| *Fronto* | **0.76** | **0.59** | **0.89** | **0.82** |  | 0.42 |
| *Para* | 0.50 | **0.71** | **0.71** | 0.09 | 0.27 |  |

Effect and response >0.5 in bold

intermediate period of the experiment (Fig. 2b), and had some negative effects also on *Tachy* (Fig. 2a). The strong effect of *Onycho* manifested itself only during the final period of the experiment, when *Onycho* reached high abundance in all treatments (Fig. 2c).

Competitive exclusions were found in only 4 out of 45 competition trials. *Rubri* was excluded by *Onycho* in one of three replicates, and *Fronto* went extinct in one of three replicates when grown together with either *Onycho*, *Stylo* or *Tachy*.

Only one of the correlations between measures of competitive strength and species' traits was significant. Competitive effect was positively correlated with species' carrying capacities, but only when expressed as biovolume (Spearman's $r = 0.943$, $P = 0.005$; Fig. 3a). The species that had by far the highest competitive effect (*Onycho*) had a very high $K$ in terms of biovolume. However, its $K$ showed large variation between the three replicates. Competitive rank was unrelated to colonization rank (Spearman's $r = 0.371$, $P = 0.468$; Fig. 3b). The weakest competitor was also the slowest colonizer, the strongest competitor had an intermediate colonization ability and the best colonizers had intermediate competitive abilities.

Fig. 2 Mean abundances
($n = 3$) of a *Tachy*, b *Stylo*,
c *Onycho*, d *Rubri*, e *Fronto* and
f *Para* when grown alone
(*dashed line*) or in competition
with *Tachy* (*filled circle*), *Stylo*
(*open circle*), *Onycho* (*filled
triangle*), *Rubri* (*open triangle*),
*Fronto* (*filled square*) and *Para*
(*open square*), respectively.
Note log-scale of *y* axes
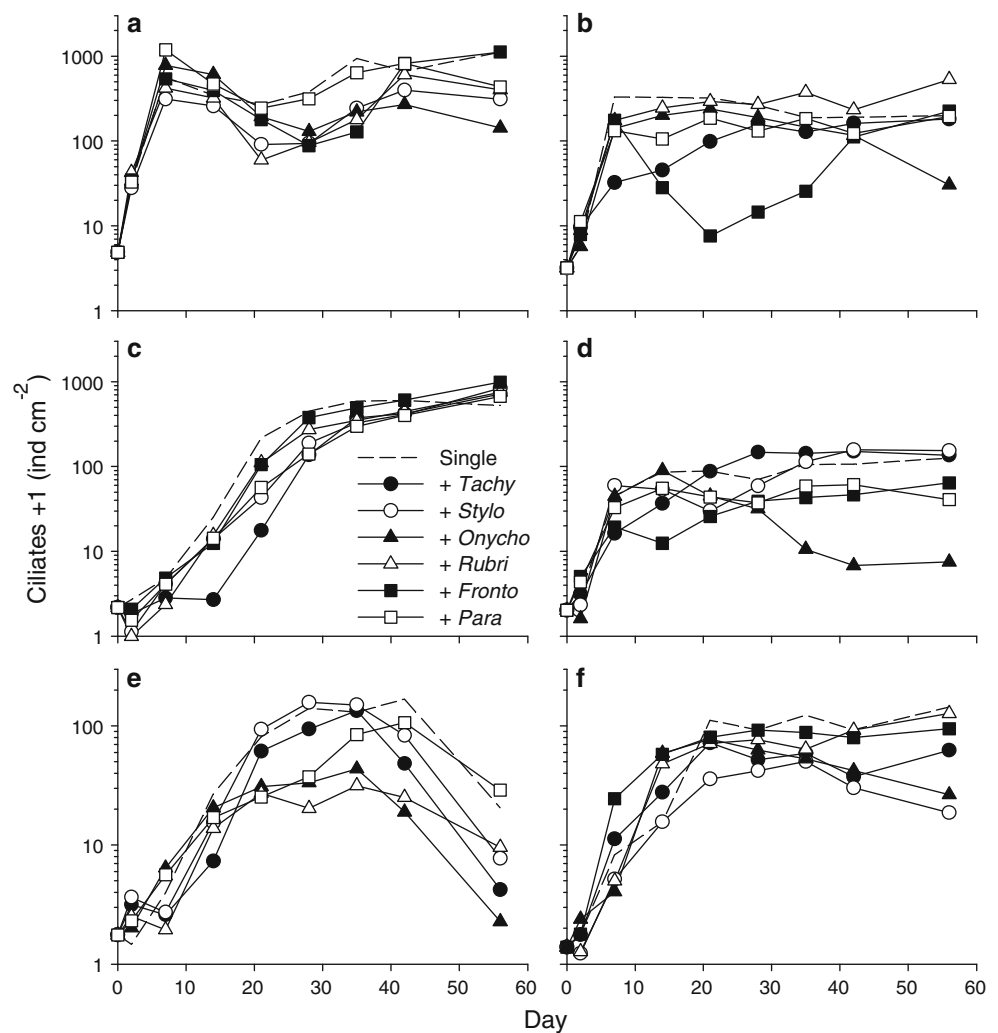


## Resources

Equilibrium abundances of small bacteria in the single species treatments significantly differed between the six ciliate species (one-way ANOVA: $P = 0.022$; Tukey's post-hoc test: *Onycho* < *Fronto*, $P = 0.02$), whereas abundances of flagellates, medium and large bacteria showed no differences between the species treatments. Equilibrium algal abundances were significantly influenced by light and species treatment (ANCOVA; light: $P = 0.006$; species: $P = 0.026$; Sidak's post-hoc test: *Tachy* < *Stylo*, $P = 0.032$, *Tachy* < *Onycho*, $P = 0.042$), while the interaction between light and species was not significant ($P = 0.174$).

Final bacterial abundances were significantly negatively correlated with species' competitive ranks (Pearson's $r = -0.885$, $P = 0.019$; Fig. 3c), while final algal abundances showed no significant correlation with competitive ranks (Pearson's $r = 0.602$, $P = 0.206$; Fig. 3d). Prior to analysis, algal abundances had been adjusted for the effect of light using ANCOVA.

## Discussion

While the ciliates used in our study showed a clear competitive hierarchy, there was no simple trade-off between competitive and colonization ability (Fig. 3b). Only within a subset of species was a trade-off observable: The strongest competitor had intermediate colonization abilities, while some of the inferior competitors were better colonizers (*Stylo*, *Para*), implying that a CC trade-off may be a mechanism of coexistence for this subset of species. Most other studies failing to find a CC trade-off did so because the species did not differ in their colonization abilities (Harrison et al. 1995; Amarasekare 2000; Guélat et al. 2008). In contrast, our test organisms did differ in their colonization abilities, but nevertheless we did not find a negative correlation between competitive and colonization ability. A probable reason is that the underlying organismal traits are unrelated or related only in complex ways.

To shed light on possible reasons for the absence or presence of a CC trade-off, it is important to take a look at the organismal traits behind competitive and colonization

Fig. 3 Relationships between
measures of competitive
strength and **a** species' carrying
capacities $K$, **b** colonization
rank, **c** equilibrium bacterial
density and **d** equilibrium algal
density. Algal abundances had
been adjusted for the effect of
light by an ANCOVA. **c**, **d** Note
that untransformed data are
presented on a log-scale but the
correlations and ANCOVA
presented in the text were
calculated with log-transformed
data. Values are means ± SE
for carrying capacity ($n = 3$),
competitive effect ($n = 15$),
bacterial and algal abundance
($n = 3$). Colonization and
competitive ranks are based on
10,000 Monte Carlo
simulations, and the *error bars*
represent the 95% confidence
intervals. High rank means high
colonization and high
competitive ability, respectively
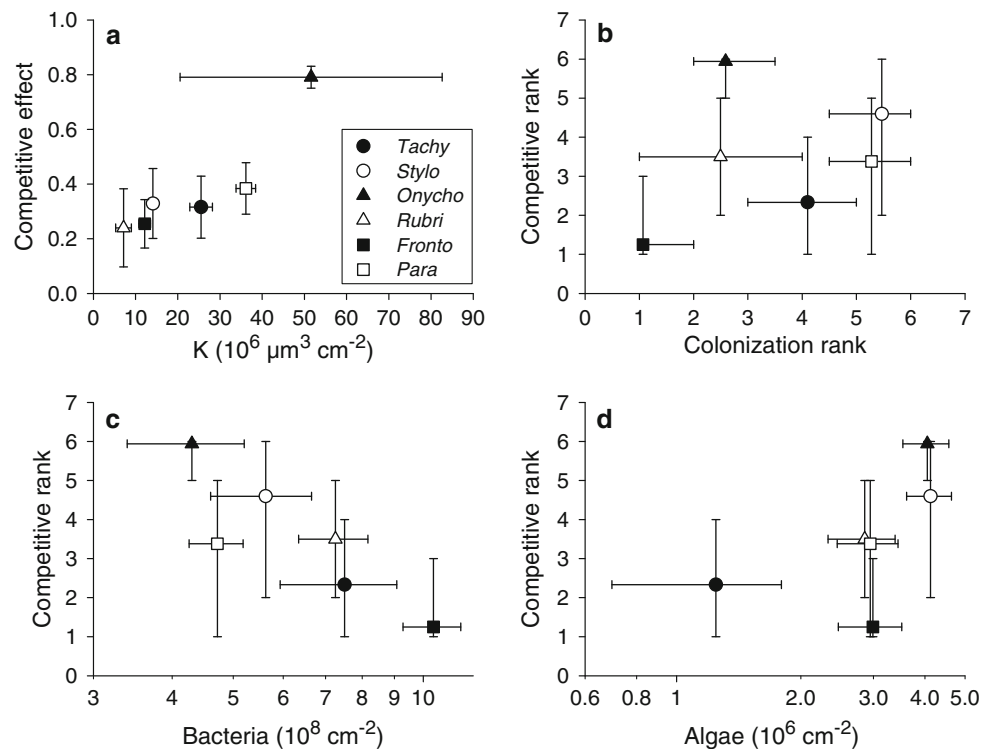


ability and at the relationships between these traits (Suding et al. 2003). A community-wide CC trade-off can be expected with high probability when the underlying organismal traits are strictly negatively correlated due to physiological or genetic constraints. An example is the trade-off between allocation to root and allocation to reproduction found among grassland plants (Tilman and Wedin 1991). This trade-off between organismal traits translates into a trade-off between ability to compete for soil nutrients and ability to colonize abandonned fields (Tilman 1994). Similarly, when competitive ability and colonization ability are both determined by the same organismal trait but in an opposing way (e.g., large body or seed size leading to high competitive ability but low colonization ability), then a CC trade-off is unavoidable. When, however, the organismal traits behind competitive and colonization ability are weakly or complexly related, then a community-wide CC trade-off cannot a priori be expected.

When taking a closer look at the organismal traits potentially underlying competitive and colonization ability in our ciliate community, we found at best weak or complex correlations. Colonization ability was uncorrelated with any of the measured traits; however, some trends were observable. The three species with the highest colonization abilities (*Stylo*, *Para*, *Tachy*) had high growth rates (*Tachy*) or high dispersal rates (*Para*) or both (*Stylo*). These results indicate that species with

low dispersal rates can nevertheless be good colonizers if they have sufficiently high growth rates. Conversely, low growth rates of larger-sized species can be compensated for by high dispersal rates. This might often be the case in communities of actively dispersing animals where larger-sized species compensate for low fecundity by moving over larger distances (Yu et al. 2004). Moreover, dispersal ability of actively moving organisms is often complicated by behavior. Even in our community of comparatively simple organisms, dispersal rate was mostly determined by the behavior of the organisms, with a species spending most of the time in the water column having a much higher dispersal rate than those crawling on the substrate. The dependence of colonization ability on growth and dispersal rate led to a complex relationship between body size and colonization ability (Fig. 1b), with high colonization abilities of the two smallest, fastest-growing species and of the largest, fastest-moving species.

Similar to colonization ability, competitive ability in our ciliate community was uncorrelated with body size, biovolume and most of the other measured traits. The only significant correlation was that between competitive effect and $K$ in terms of biovolume, probably expressing the efficiency of resource use. Another study with protists also found competitive ability to be uncorrelated with cell size, $r$, $K$ and dispersal rank (Haddad et al. 2008). Conversely, using 13 protozoan and rotifer species, Cadotte et al.

(2006) not only found a positive correlation between competitive ability and cell mass but also a trade-off between competitive and colonization ability. However, while they used a larger set of species, their community contained some species with different resource niches (bacterivory, omnivory, mixotrophy), which possibly influenced the observed competitive hierarchy.

One possible reason for the differing results of our experiment and that of Cadotte et al. (2006) could be the smaller number of species in our community. We used those benthic ciliate species which were able to grow on our resource community and which survived in single species cultures for at least 8 weeks. As a further criterion, species had to be easy to distinguish under a dissecting microscope. However, if a larger pool of species with similar resource niches could have been found, our results would have certainly been more robust. A further difference between our study and that of Cadotte et al. (2006) is the way competitive ability was measured. Cadotte et al. (2006) used the number of competitive extinctions caused by the target species and the number of its survivals in competition trials as competitive effect and response, respectively. As there were hardly any competitive exclusions in our experiment, we compared abundances in competition and single species trials, a method often used in studies measuring the competitive ability of protists (Fox 2002; Haddad et al. 2008). Weaker competitive interactions could be the reason for the lower number of competitive exclusions in our community compared to that of Cadotte et al. (2006) and may explain our nonsignificant result.

The rareness of competitive exclusions in our ciliate model community implies that some mechanism of coexistence enabled persistence of the weaker competitor. The inferior competitor was excluded in less than 10% of the competition trials, and this number might even be overestimated, given the small subsamples counted. Possible reasons for coexistence might be differential use of the two resources (algae, bacteria) or differential use of space. One of the six species (*Para*) spent most of the time swimming in the water column, whereas the other species were mostly crawling on the substrate. However, in an experiment testing explicitly for possible explanations for coexistence of two competing ciliate species, none of the tested hypotheses (differential resource use, differential space use, chemically mediated interference) was a sufficient explanation for coexistence (Fox and Barreto 2006). Although the two species differed in resource use, this resource partitioning could not explain their coexistence, with the two species coexisting even when grown on bacterial monocultures. These results show that the mechanisms of coexistence in protistan communities are far from being understood.

Although there were only few competitive exclusions in our protist community, one species was clearly the strongest competitor. The mechanism behind the competitive outcome was probably competition for bacteria, as species with high competitive ranks reduced bacterial abundances to lower levels than inferior competitors (Fig. 3c). This result is in accordance with the R*-rule predicting that the species able to reduce the limiting resource to the lowest level excludes its competitors (Tilman 1982). Most tests of the R*-rule used phytoplankton species as test organisms, with most tests supporting the hypothesis (Grover 1997). Experiments working with bacterivorous protists found mixed results, some confirming the R*-rule (Cochran-Stafira and von Ende 1998; Fox 2002), others finding no differences in bacterial R*-values between competitors (Steiner 2005; Liess and Diehl 2006) or even results opposite to the R*-rule (Balčiūnas and Lawler 1995).

When having demonstrated presence or absence of a CC trade-off, the next question is its role in species coexistence or, alternatively, the importance of other mechanisms of coexistence. The mere presence of a CC trade-off does not necessarily imply that it is important for the coexistence of the species in the community. In assemblages of annual grassland plants, a CC trade-off based on seed size was found to be insufficient for maintaining diversity in these systems (Turnbull et al. 1999; Levine and Rees 2002). Competition between species was not asymmetric enough so as to explain coexistence by a CC trade-off alone, but rather environmental heterogeneity and species-specific niches appeared to be important for the persistence of inferior competitors. Conversely, our results show that absence of a community-wide CC trade-off does not necessarily imply that this mechanism is completely unimportant for the maintenance of diversity. It may enable coexistence at least for a subset of species. In our community, some of the inferior competitors (*Stylo*, *Para*) had better colonization abilities than the superior competitor, implying that a CC trade-off may enable persistence of this subset of species. A prerequisite for this mechanism to operate in nature is the presence of a spatially patchy environment. When patches of similar environmental conditions are linked to a metacommunity, coexistence is possible, given a CC trade-off (patch dynamics perspective; Leibold et al. 2004).

Three of our test organisms (*Tachy*, *Rubri*, *Fronto*) were not only poor competitors but at the same time had colonization abilities similar to or even weaker than the strongest competitor. However, these weak competitors are common in freshwater habitats, with *Fronto* and *Tachy* even reaching high abundances, whereas *Onycho*, the strongest competitor in the experiment, is rare in nature (Foissner et al. 1991, 1994; Berger 1999). Clearly our experiments did not and could not measure all potential

mechanisms of species coexistence. While we measured competitive rankings only for one type of resource community, natural metacommunities often contain patches with differing environmental conditions and resources, thus leading to spatial variation of competitive rankings of species. When species show trade-offs in their performances under these varying conditions, then they are able to coexist regionally, and with sufficient dispersal even local coexistence is possible via source–sink dynamics (species sorting and mass effects perspective, respectively; Leibold et al. 2004). As an example, temporal changes in competitive ability of *Fronto* suggest importance of resource community composition for its persistence. While this species was the weakest competitor at the end of the experiment, it had strong negative effects on *Stylo* and *Tachy* during the intermediate period of the experiment. However, towards the end of the experiment, *Fronto* density declined in all treatments including the single species treatment (Fig. 2e). Changes in the resource community are a likely explanation for this pattern. Algal abundance declined over time, whereas bacterial abundances increased. In the *Fronto* single species treatment, the algal proportion of total resource biovolume decreased from over 90% at the beginning of the experiment to 25% at the end. *Fronto* is primarily an algivore (Foissner et al. 1994), which explains its poor competitive ability in the end when resources were dominated by bacteria.

Disturbances and predation are further important factors regulating species coexistence in natural communities, but were not factors in our experiments. However, our data give some hints at the importance of these alternative mechanisms of coexistence for our model community. Our results stress the importance of temporal effects which, in combination with disturbances, probably enable coexistence in natural communities. Competitive effects changed over the time course of the experiment and were far from being instantaneous, an assumption of most modeling approaches. The strong competitive effects of the superior competitor (*Onycho*) became apparent only in the final phase of the experiment. Weak disturbances might be enough to eliminate this slow-growing species in natural communities. Conversely, *Tachy*, the smallest and fastest-growing species, negatively affected growth of other species in the initial phase of the experiment. *Tachy* may compensate for its low competitive ability with a high maximal growth rate. Fast-growing, early successional species are able to coexist with superior competitors and may even temporarily dominate by exploiting resource-rich conditions following a disturbance (successional niche; Pacala and Rees 1998; Rees et al. 2001). Reductions in density due to a generalist predator may have similar implications as disturbances, while effects of a specialized predator on species coexistence will strongly depend on its prey preferences.

Despite the limitation of a comparatively small species pool, we believe that this study provides some new insights into CC trade-offs by focusing on the traits and mechanisms underlying colonization and competitive ability. In our ciliate community, a CC trade-off is at most a mechanism of coexistence for a subset of our species. This trade-off does not extend over the whole community, indicating that some of the inferior competitors, common in nature, persist by other mechanisms. These results can be expected from a community of species where colonization and competitive ability are not constrained to trade off, since the underlying organismal traits are only weakly or complexly related.

# References

Amarasekare P (2000) Spatial dynamics in a host-multiparasitoid community. J Anim Ecol 69:201–213

Amarasekare P (2003) Competitive coexistence in spatially structured environments: a synthesis. Ecol Lett 6:1109–1122. doi:10.1046/j.1461-0248.2003.00530.x

Balčiūnas D, Lawler SP (1995) Effects of basal resources, predation, and alternative prey in microcosm food chains. Ecology 76:1327–1336

Berger H (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Kluwer, Dordrecht

Cadotte MW, Mai DV, Jantz S, Collins MD, Keele M, Drake JA (2006) On testing the competition–colonization trade-off in a multispecies assemblage. Am Nat 168:704–709

Calcagno V, Mouquet N, Jarne P, David P (2006) Coexistence in a metacommunity: the competition–colonization trade-off is not dead. Ecol Lett 9:897–907. doi:10.1111/j.1461-0248.2006.00930.x

Clark JS, Macklin E, Wood L (1998) Stages and spatial scales of recruitment limitation in southern Appalachian forests. Ecol Monogr 68:213–235

Cochran-Stafira DL, von Ende CN (1998) Integrating bacteria into food webs: studies with *Sarracenia purpurea* inquilines. Ecology 79:880–898

Coomes DA, Grubb PJ (2003) Colonization, tolerance, competition and seed-size variation within functional groups. Trends Ecol Evol 18:283–291. doi:10.1016/S0169-5347(03)00072-7

Foissner W, Blatterer H, Berger H, Kohmann F (1991) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems— Band I: Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea.

Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, München

Foissner W, Berger H, Kohmann F (1994) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems—Band III: Hymenostomata, Prostomatida, Nassulida. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, München

Fox JW (2002) Testing a simple rule of dominance in resource competition. Am Nat 159:305–319

Fox JW, Barreto C (2006) Surprising competitive coexistence in a classic model system. Commun Ecol 7:143–154. doi:10.1556/ComEc.7.20062.2

Grover JP (1997) Resource competition. Chapman & Hall, London

Guélat J, Jaquiéry J, Berset-Brändli L, Pellegrini E, Moresi R, Broquet T, Hirzel AH, Perrin N (2008) Mass effects mediate coexistence in competing shrews. Ecology 89:2033–2042

Haddad NM, Holyoak M, Mata TM, Davies KF, Melbourne BA, Preston K (2008) Species' traits predict the effects of disturbance and productivity on diversity. Ecol Lett 11:348–356. doi:10.1111/j.1461-0248.2007.01149.x

Hanski I, Ranta E (1983) Coexistence in a patchy environment: three species of Daphnia in rock pools. J Anim Ecol 52:263–279

Harrison S, Thomas CD, Lewinsohn TM (1995) Testing a metapopulation model of coexistence in the insect community on ragwort (Senecio jacobaea). Am Nat 145:546–562

Higgins SI, Cain ML (2002) Spatially realistic plant metapopulation models and the colonization–competition trade-off. J Ecol 90:616–626

Holmes EE, Wilson HB (1998) Running from trouble: long-distance dispersal and the competitive coexistence of inferior species. Am Nat 151:578–586

Hunt JJFG, Bonsall MB (2009) The effects of colonization, extinction and competition on co-existence in metacommunities. J Anim Ecol 78:866–879. doi:10.1111/j.1365-2656.2009.01532.x

Jakobsson A, Eriksson O (2003) Trade-offs between dispersal and competitive ability: a comparative study of wind-dispersed Asteraceae forbs. Evol Ecol 17:233–246

Kneitel JM, Chase JM (2004) Trade-offs in community ecology: linking spatial scales and species coexistence. Ecol Lett 7:69–80. doi:10.1046/j.1461-0248.2003.00551.x

Laska MS, Wootton JT (1998) Theoretical concepts and empirical approaches to measuring interaction strength. Ecology 79:461–476

Lei G, Hanski I (1998) Spatial dynamics of two competing specialist parasitoids in a host metapopulation. J Anim Ecol 67:422–433

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett 7:601–613. doi:10.1111/j.1461-0248.2004.00608.x

Levine JM, Rees M (2002) Coexistence and relative abundance in annual plant assemblages: the roles of competition and colonization. Am Nat 160:452–467

Levins R, Culver D (1971) Regional coexistence of species and competition between rare species. Proc Nat Acad Sci USA 68:1246–1248

Liess A, Diehl S (2006) Effects of enrichment on protist abundances and bacterial composition in simple microbial communities. Oikos 114:15–26

Marie D, Simon N, Vaulot D (2005) Phytoplankton cell counting by flow cytometry. In: Andersen RA (ed) Algal culturing techniques. Elsevier, Burlington, pp 253–267

McEuen AB, Curran LM (2004) Seed dispersal and recruitment limitation across spatial scales in temperate forest fragments. Ecology 85:507–518

Orrock JL, Watling JI (2010) Local community size mediates ecological drift and competition in metacommunities. Proc R Soc Lond B 277:2185–2191. doi:10.1098/rspb.2009.2344

Pacala SW, Rees M (1998) Models suggesting field experiments to test two hypotheses explaining successional diversity. Am Nat 152:729–737. doi:10.1086/286203

Rees M, Condit R, Crawley MJ, Pacala SW, Tilman D (2001) Long-term studies of vegetation dynamics. Science 293:650–655. doi:10.1126/science.1062586

Rodríguez A, Jansson G, Andrén H (2007) Composition of an avian guild in spatially structured habitats supports a competition–colonization trade-off. Proc R Soc Lond B 274:1403–1411. doi:10.1098/rspb.2007.0104

Steiner CF (2005) Impacts of density-independent mortality and productivity on the strength and outcome of competition. Ecology 86:727–739

Suding KN, Goldberg DE, Hartman KM (2003) Relationships among species traits: separating levels of response and identifying linkages to abundance. Ecology 84:1–16

Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton

Tilman D (1994) Competition and biodiversity in spatially structured habitats. Ecology 75:2–16. doi:10.2307/1939377

Tilman D, Wedin D (1991) Plant traits and resource reduction for five grasses growing on a nitrogen gradient. Ecology 72:685–700

Turnbull LA, Rees M, Crawley MJ (1999) Seed mass and the competition/colonization trade-off: a sowing experiment. J Ecol 87:899–912

Westoby M, Leishman M, Lord J (1996) Comparative ecology of seed size and dispersal. Philos Trans R Soc Lond B 351:1309–1318

Yu DW, Wilson HB, Frederickson ME, Palomino W, De la Colina R, Edwards DP, Balareso AA (2004) Experimental demonstration of species coexistence enabled by dispersal limitation. J Anim Ecol 73:1102–1114