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RESEARCH ARTICLE

Connecting higher-order interactions with ecological stability in experimental aquatic food webs

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Abstract

Community ecology is built on theories that represent the strength of interactions between species as pairwise links. Higher-order interactions (HOIs) occur when a species changes the pairwise interaction between a focal pair. Recent theoretical work has highlighted the stabilizing role of HOIs for large, simulated communities, yet it remains unclear how important higher-order effects are in real communities. Here, we used experimental communities of aquatic protists to examine the relationship between HOIs and stability (as measured by the persistence of a species in a community). We cultured a focal pair of consumers in the presence of additional competitors and a predator and collected time series data of their abundances. We then fitted competition models with and without HOIs to measure interaction strength between the focal pair across different community compositions. We used survival analysis to measure the persistence of individual species. We found evidence that additional species positively affected persistence of the focal species and that HOIs were present in most of our communities. However, persistence was only linked to HOIs for one of the focal species. Our results vindicate community ecology theory positing that species interactions may deviate from assumptions of pairwise interactions, opening avenues to consider possible consequences for coexistence and stability.

KEYWORDS

indirect effects, interaction modifications, persistence, species interactions, time series

TAXONOMY CLASSIFICATION

Community ecology

1 | INTRODUCTION

Community ecology seeks to unravel the mechanisms that maintain the tremendous organismal diversity on our planet. Species interactions, such as competition and predation, are important processes determining community structure and ecosystem stability and hence are important drivers of diversity (HilleRisLambers et al., 2012; Hooper et al., 2005; Landi et al., 2018). Most research on species

coexistence has focused on pairwise interactions (Levine et al., 2017). However, in nature, species rarely interact only in a direct pairwise fashion, but rather in large networks of interacting species where a suite of indirect effects are likely to be important (Levine et al., 2017). Recently, these indirect effects have gained increasing attention, since in some cases they may explain the dynamics and stability of communities better than classic pairwise models (Grilli et al., 2017; Letten & Stouffer, 2019; Mayfield & Stouffer, 2017).

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Lotka–Volterra competition or predator–prey models assume that the direct interaction between two species is an intrinsic property, thus the strength and sign of the interaction are independent of the community in which these species are embedded (Werner & Peacor, 2003). However, when more than two species co-occur (Worthen & Moore, 1991), indirect effects can arise through chains of pairwise interactions, or through higher-order interactions (HOIs), that is, changes of the per capita effect of one competitor on another in the presence of additional species (Levine et al., 2017; Wootton, 1994). Since interaction chains and HOIs emerge from fundamentally different mechanisms, it is important to understand their underlying mechanisms for appropriate modeling and inference (Levine et al., 2017).

Interaction chains emerge when pairwise interactions are embedded in a network of interactions. A series of such direct interactions between species pairs can lead to connections between species that do not directly interact with each other (Wootton, 1993). Despite introducing indirect pathways between species, interaction chains are the result of a series of fixed, strictly pairwise interactions. In contrast, “higher-order interactions” (HOIs (Case & Bender, 1981; Letten & Stouffer, 2019; Mayfield & Stouffer, 2017); also called “interaction modifications” (Abrams, 1983)), encompass the nonadditive effects of a species on the pairwise interaction between a focal pair. Conventionally, the species altering the interaction is a third species (i.e., an interspecific HOI); however, it can also be one of the focal species (i.e., an intraspecific HOI) sensu Letten and Stouffer (2019).

Wootton (1994) observed both types of indirect effects in the upper zone of a rocky intertidal community. As an example of an interaction chain, bird predators indirectly increase acorn barnacle (*Balanus glandula*) abundance by consuming limpets (*Lottia digitalis*), which dislodge or consume young acorn barnacles. An example of an interaction modification, or interspecific HOI, is barnacles altering the bird–limpet interaction by changing the ability of birds to find limpets due to the similar color of *L. digitalis* and barnacle shells. HOIs are most commonly described in terms of how a third species impacts the interaction between a focal species pair. However, intraspecific HOIs are also important to consider and represent the cumulative impacts of interactions among the individuals of each “competitor species” (that is, intraspecific crowding) on the focal species (Letten & Stouffer, 2019; Mayfield & Stouffer, 2017). In the intertidal community, intraspecific HOIs could occur when birds influence the foraging behavior of barnacles, causing them to aggregate in areas sheltered from bird predation, which leads to increased intraspecific competition. Interaction chains can be predicted with only a knowledge of pairwise species interactions; in contrast, descriptions of intra- and interspecific HOIs require a knowledge of all species combinations involved (Wootton, 1994).

Theoretical work has suggested that empirical studies of interactions between species should consider HOIs (Grilli et al., 2017), given that simple models of pairwise interactions fail to explain the stable persistence in simulation models of very large ecological communities (Barabás et al., 2016; Clark, 2010; Gibbs et al., 2022;

Kleinhesselink et al., 2022; Levine et al., 2017). Recently, many theoretical advances have been made to understand the influence of HOIs on community dynamics (Gibbs et al., 2022; Grilli et al., 2017; Letten & Stouffer, 2019; Mayfield & Stouffer, 2017; Singh & Baruah, 2021). A particular focus has been on the effect of HOIs on the stability of communities, specifically the persistence of species in a community. However, simulation-based studies often consider highly complex and diverse communities (Bailey et al., 2016; Singh & Baruah, 2021), making it impractical to verify their findings through experimental manipulations.

A valuable approach to simplify the complexity of communities is to analyze their component community modules (Holt, 1997). Studies of community modules have revealed how interactions between species affect species persistence (Kondoh, 2008; Mayfield & Stouffer, 2017; McCann et al., 1998). Theoretical investigations on food web modules have highlighted the crucial role of weak interactions, especially omnivorous links, in maintaining community stability (Emmerson & Yearsley, 2004; McCann et al., 1998). Experimental manipulations of these modules have confirmed that weak interactions, coupled with strong interactions mediated by generalist consumers, enhanced community stability by reducing interaction strength (Rip et al., 2010). Experimental work has indicated that HOIs weaken intense pairwise competition and predator–prey interactions, thereby stabilizing the community and promoting coexistence (Kratina et al., 2007; Sundarraman et al., 2020).

Despite the theoretical support for the importance of HOIs for coexistence and stability, empirical evidence connecting HOIs with ecological stability remains very scarce (but see Mayfield & Stouffer, 2017; Mickalide & Kuehn, 2019; Sundarraman et al., 2020). This is partly due to the challenges of empirically quantifying HOIs (Billick & Case, 1994), which requires the evaluation of the interaction strength between two species accounting for changes in density or the presence of additional species (Adler et al., 2018). Even in simple communities, this can quickly become logistically infeasible. In addition, empirical research also requires detailed time series data to evaluate species persistence over extended periods of time, presenting a second logistical hurdle.

Our goal was to experimentally test the causal links between HOIs and the persistence of species within communities. Microcosms are a convenient tool to investigate concepts in community ecology because they are easily manipulated, can be highly replicated, and have large population sizes (Altermatt et al., 2015). Moreover, a range of analytical approaches is available to estimate intra- and interspecific interaction strengths within these systems (Carrara et al., 2015). We, therefore, used microcosms to experimentally examine HOIs and community persistence in aquatic microbial communities. We used six different community compositions, which all included a focal pair of consumers competing for the same resource. We tested the effect of two additional competitors as well as the presence of a predator on the interaction strength between the focal pair. Time series of population dynamics were collected to quantify the strength of species interactions and examine the effect of HOIs on persistence and address the following questions:

1. Are HOIs detectable in our communities?
2. Do HOIs differ depending on the identity and trophic role of the third species?
3. Is there a relationship between the presence of HOIs and population persistence?

We hypothesized that additional competitors would influence the effect of the two focal species on each other since all species engage in resource competition. For instance, in the presence of a third species, focal species may shift their foraging behavior and thereby their pairwise effect on one another. We also expected that higher-order effects would arise in communities with a predator whose behavior is affected by different consumer body sizes which could render one prey susceptible to predation while the other may be attacked but is too large to be consumed. All else being equal, we expected additional competitors to strengthen the competition for shared resources shortening population persistence, but additional predators to weaken competition between the focal pair of species, potentially extending species persistence.

2 | METHODS

2.1 | Data collection: Community experiment

2.1.1 | Food web construction and culture conditions

To experimentally test HOIs, we constructed an experimental food web. The ciliates of our microbial food web were primarily bacterivorous (Pennekamp et al., 2018); thus, the experimental communities were sustained on a mix of three species of bacteria (*Bacillus subtilis*, *Serratia fonticola*, and *Brevibacillus brevis*) decomposing the protozoan pellet medium (PPM; provided by Carolina Biological Supplies; concentration of 0.55 g/L, see Altermatt et al., 2015).

Four bacterivorous ciliates (*Colpidium striatum*, *Dexiostoma campylum*, *Paramecium caudatum*, and *Spirostomum teres*) constitute the intermediate consumers in our experimental microcosms. Both *C. striatum* (length: 50–100 μm) and *D. campylum* (35–90 μm) are consumed by the top predator *Spathidium* sp. (40–300 μm), which cannot survive only on bacteria as prey (Woodruff & Spencer, 1921). Due to the large body size of *P. caudatum* (170–300 μm) (Foissner & Berger, 1996) and *S. teres* (150–400 μm) (Bick & World Health Organization, 1972), we expected these species to interfere with the predator because they are more difficult or impossible to consume; however, depending on the mode of attack of the predator, even failed consumption could result in death of prey.

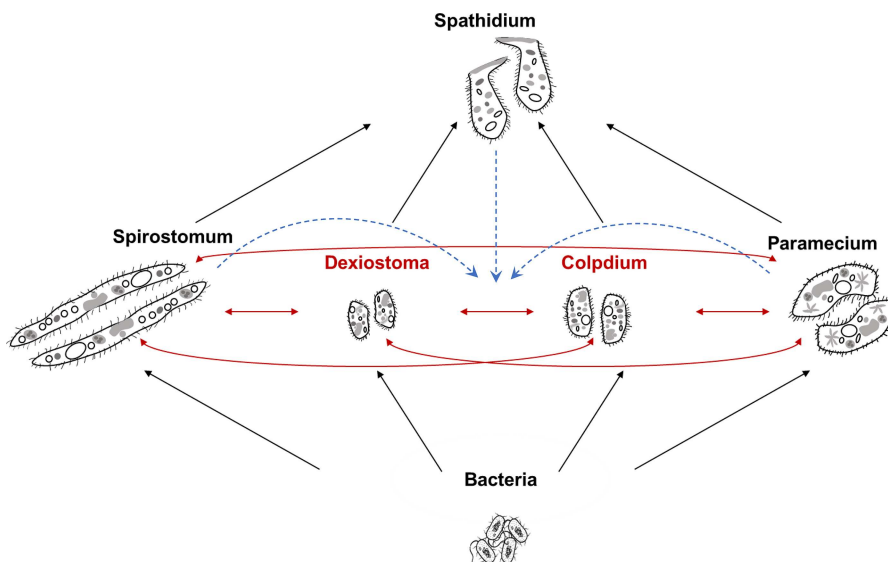
We chose these five eukaryotic ciliates (hereafter referred to by the genus name only) because their pairwise trophic interactions are well documented by previous studies (Daugaard et al., 2019; Pennekamp et al., 2018; Tabi et al., 2019). The consumptive and competitive interactions present in our experimental food web are shown in Figure 1.

2.1.2 | Experimental design

To assess how the interaction strength between two focal species is altered by adding a third or more species, we established the following treatments: (1) two prey (*Colpidium* and *Dexiostoma*) alone to investigate the direct effects between the two focal species (CD) (2) two prey and *Paramecium* (CDP). (3) two prey and *Spirostomum* (CDS). These communities were also grown in the presence of the top predator *Spathidium* (CDPd, CDPPd, CDSPd; predator = Pd) (Figure 2), yielding six treatments in total. For each treatment, we cultured four replicates resulting in 24 microcosms in total.

To start the experiment, we took out 20 or 30 mL of the bacterized PPM medium (depending on experimental community composition) and replaced it with 10 mL of the stock culture of each consumer species at their carrying capacity. Therefore, the starting

FIGURE 1 Microbial food web in our experiment. Consumptive interactions (in black) among the top predator (*Spathidium* sp.), the consumers (*Colpidium striatum*, *Dexiostoma campylum*, *Paramecium caudatum*, and *Spirostomum teres*) and a mix of three bacteria species (*Bacillus subtilis*, *Serratia fonticola*, and *Brevibacillus brevis*). All the consumers compete with each other (red arrows), with the focal pair marked in red. Hypothesized HOIs are indicated by the blue dashed lines with arrows.



Species — Colpidium — Dexiostoma — Paramecium — Spirostomum — Spathidium

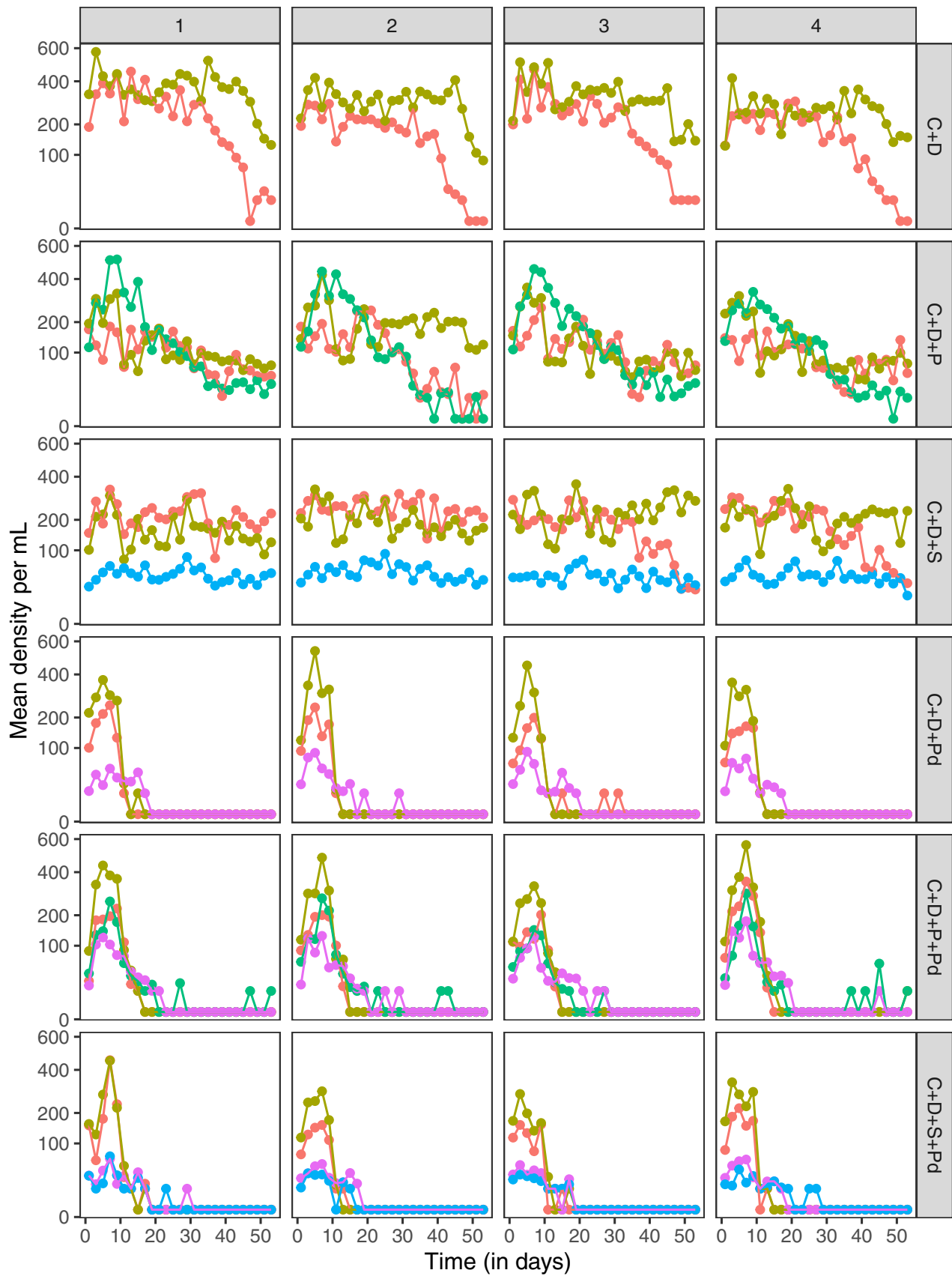


FIGURE 2 Population dynamics for each experimental community. Rows are six different treatments in our experiment. Each treatment is represented by the species it contains (C for *Colpidium*, D for *Dexiostoma*, P for *Paramecium*, S for *Spirostomum* and Pd for predator). The y-axes are plotted on a logarithmic scale. Results for each replicate are shown in columns. Most species in competition treatments (first three rows) survived to the last day of measurement but rapidly dropped below detection limits when housed with predators (last three rows).

densities of all consumers were 10% of their carrying capacities. In the predation treatments, 4 days after the introduction of the consumer species, ten predator individuals were pipetted from the maintenance plates to the microcosms. To assure establishment in each microcosm, another ten individuals of predators were added after 8 days. The experiments were conducted at 15°C with no light, and suitable growing conditions for ciliates (Altermatt et al., 2015).

2.1.3 | Video sampling and species classification

Experimental units were sampled every second day for 53 days to capture time series of the dynamical changes in the abundance of ciliates. For each sampling event, the microcosm was gently agitated, a subsample of 250 µL was mounted onto a glass slide and covered with a glass lid. Three five-second videos (at 25 frames per second) were taken using 25× magnification on a stereomicroscope (Leica M205 C) mounted with a digital CMOS camera (Hamamatsu Orca Flash 4.0 C11440, Hamamatsu Photonics, Japan) with dark field illumination. We took three subsamples for each microcosm to get a precise estimate of abundance and processed the videos with the R package BEMOVI (version 1.0.2) (Pennekamp et al., 2015). We took the mean of the three videos as our measure of density. If no individuals were detected, we assigned a zero. The volume lost from the microcosm by sampling during the course of the experiment was replaced with a fresh bacterized PPM medium.

For species classification, we trained a support vector machine (SVM) classifier on 350 to 400 randomly chosen and manually labeled trajectories of each species across the community compositions and time with the R package e1071 (Meyer et al., 2022). We also included a “noise” class in our classifier representing spurious trajectories due to background movement. Twenty morphological and movement features extracted from an established classification pipeline (Pennekamp et al., 2017) were selected to train the SVM to distinguish among classes based on information about body size and movement patterns (Table S1). As ciliate phenotypes may change over time (Pennekamp et al., 2017), we included “week number” to enhance the accuracy of the classifier. Further details about the classification can be found in the Appendix S1.

2.2 | Data analyses: Interaction strengths and persistence

2.2.1 | Abundance

To understand the effect of community composition on the abundance of the species, we calculated the mean abundance over the

entire 53-day experiment for each species in each of the six communities. Mean abundance of each of the five species was analyzed with two-way ANOVA (predictors: identity of the third species [levels: *Paramecium* or *Spirostomum*] and the presence of the predator [levels: present or absent]).

2.2.2 | Estimating species interaction strengths

We estimated the effect of species j on the population growth rate of species i within a regression framework, where the population growth rate of species i is regressed against the densities of species i and j to determine the intra- and interspecific effects on the population growth rate of species i (Pfister, 1995). The per capita population growth rate of each species i was calculated as $\frac{N_{i,t+1}/N_{i,t}}{(t+1)-t}$ where $N_{i,t+1}$ is the abundance at time $t+1$ and $N_{i,t}$ is abundance at time t . We used the `gauseR` package to calculate the per capita population growth rate (Mühlbauer et al., 2020). To explore how interaction strength and HOIs may differ between communities, we compared the fit of six models to the per capita population growth rate of the focal species (either *Dexiostoma* or *Colpidium*). First, we fitted the Lotka–Volterra model with no HOIs:

$$\frac{N_{i,t+1}}{N_{i,t}} = \lambda_i - \sum_{j=1}^n \alpha_{ij} N_j$$

For the simple additive model, λ_i is the intrinsic population growth rate and α_{ij} represents both the intra- and interspecific interaction coefficients. To test for intra- and interspecific HOIs, we then added interaction terms to the additive model LV, resulting in the interactive LV model (Letten & Stouffer, 2019). For the interactive LV model only including intraspecific HOIs, we included the interaction term β_{ijj} for the effect of species j on i , where β_{ijj} captures the cumulative impacts of intraspecific interactions on the focal species:

$$\frac{N_{i,t+1}}{N_{i,t}} = \lambda_i - \sum_{j=1}^n \alpha_{ij} N_j - \sum_{j=1}^n \beta_{ijj} N_j N_j$$

The identity of species j may or may not be the same as species i . Next, we fitted an interactive LV model that only includes interspecific HOIs, where β_{ijk} captures the cumulative impacts of interspecific interactions on the focal species:

$$\frac{N_{i,t+1}}{N_{i,t}} = \lambda_i - \sum_{j=1}^n \alpha_{ij} N_j - \sum_{j=1}^n \sum_{k=j+1}^n \beta_{ijk} N_j N_k$$

Here, the identity of species k strictly excludes the focal species i . Finally, we tested the fit of the interactive LV model including both intra- and interspecific HOIs (full HOI model):

$$\frac{N_{i,t+1}}{N_{i,t}} = \lambda_i - \sum_{j=1}^n \alpha_{ij} N_j - \sum_{j=1}^n \sum_{k=j+1}^n \beta_{ijk} N_j N_k - \sum_{j=1}^n \beta_{ijj} N_j N_j$$

To test whether the shape of the density dependence deviates from the linear form of the additive Lotka–Volterra model, we fitted the Ricker model with nonlinear density dependence. First, we fitted the additive model without HOIs:

$$\frac{N_{i,t+1}}{N_{i,t}} = \lambda_i e^{-\sum_{j=1}^n \alpha_{ij} N_j}$$

and interactive form only including interspecific HOIs (terms as previously defined):

$$\frac{N_{i,t+1}}{N_{i,t}} = \lambda_i e^{-\sum_{j=1}^n \alpha_{ij} N_j - \sum_{j=1}^n \beta_{ijk} N_j N_k}$$

We used generalized linear models with the “identity” link function assuming Gaussian errors to estimate the coefficients of the additive and interactive LV model, considering all errors as measurement errors. For the Ricker model, we used a GLM with the “log” link function, assuming also Gaussian errors. For the fitted models, the intercept can be interpreted as the maximum intrinsic population growth rate, while the regression coefficients represent the per capita effect of species i on itself and the per capita effects of species j on species i . Interaction terms describe the mediating effects that the density of species j can have on the effect of species i (and vice versa) on the population growth rate of the focal species (Letten & Stouffer, 2019). We performed an AICc model selection adjusting for the small sample size when identifying the model that best explained the population growth rate of each focal species (Burnham & Anderson, 2002). AICc was preferred over the Bayesian information criterion since the models which we fit are approximate and we do not necessarily expect to include the true model in our set (Aho et al., 2014). We then calculated the difference (Δ AICc) between the model with the lowest AICc and all other models. Models with a Δ AICc larger than 2 are considered to be different; thus, the model with the fewest parameters and a Δ AICc less than 2 is considered the most parsimonious model. Since the presence of predators drove some prey to early extinction, we only estimated interaction strengths in communities without predators.

2.2.3 | Estimating species survival (persistence)

We measured the persistence of the focal species *Colpidium* and *Dexiostoma*, as well as the predator *Spathidium* as a proxy of the persistence of these species using nonparametric survival analysis. We estimated the time to event (i.e., extinction), accounting for right-censoring, that is, the possibility of missing future extinctions since we only recorded the abundance for 53 days. We tested the effect of community composition on the Kaplan–Meier estimate of the focal species with log-rank tests, followed by pairwise tests of community composition in case of a significant community wide effect with the survminer package (Kassambara et al., 2021).

All of the above analyses including the species classification were performed with the statistical computing environment R (R Core Team, 2022).

3 | RESULTS

3.1 | Population dynamics over time

The population dynamics of the protists differed depending on the community context (Figure 2). When *Colpidium* and *Dexiostoma* competed only with each other, we consistently observed the extinction of *Colpidium* toward the end of the experiment, while *Dexiostoma* persisted. The addition of *Paramecium* or *Spirostomum* led to a longer period of coexistence of *Dexiostoma* and *Colpidium*. The addition of the top predator *Spathidium* considerably shortened the persistence of all species. Thus, the addition of a third competitor increased the period of coexistence of the focal species pair, whereas the addition of the predator led to the rapid collapse of the whole community.

The addition of the competitor *Paramecium* had a negative effect on the mean abundance of both *Colpidium* ($b = -0.60$, 95% CI = -0.89 to -0.32 , $n = 4$) and *Dexiostoma* ($b = -0.81$, 95% CI = -1.0 to -0.59 , $n = 4$, Figure 3). In contrast, adding the competitor *Spirostomum* only had a negative effect on the mean abundance of *Dexiostoma* ($b = -0.43$, 95% CI = -0.67 to -0.19 , $n = 4$, Figure 3).

The addition of the predator had a negative effect on the mean abundance of all competing protist species (Figure 3). In communities composed of just the focal pair, the presence of the predator resulted in a similar reduction in mean abundance for both *Dexiostoma*: ($b = -1.8$, 95% CI = -2.0 to -1.5 , $n = 4$) and *Colpidium* ($b = -1.9$, 95% CI = -2.1 to -1.6 , $n = 4$, Figure 3). In communities with a third competitor, the predator had a greater negative effect on the mean abundance of *Spirostomum* ($b = -1.7$, 95% CI = -1.9 to -1.5 , $n = 4$) than *Paramecium* ($b = -1.5$, 95% CI = -1.7 to -1.2 , $n = 4$, Figure 3). The addition of *Spirostomum* had no effect on the mean abundances of *Dexiostoma*, *Colpidium* and the predator compared to the focal pair cultured with just the predator. Intriguingly, the presence of both *Paramecium* and the predator had a positive effect on the density of both *Colpidium* ($b = 0.83$, 95% CI = 0.43 to 1.2 , $n = 4$) and *Dexiostoma* ($b = 0.96$, 95% CI = 0.62 to 1.3 , $n = 4$; Figure 3) in comparison to when the focal species were exposed to the predator in isolation. This translated into higher density for the predator *Spathidium* when *Paramecium*, *Colpidium* and *Dexiostoma* were present ($b = 0.66$, 95% CI = 0.48 to 0.84 , $n = 4$; Figure 3).

3.2 | Higher-order interactions

Model selection revealed that HOIs influenced the per capita population growth rates of *Dexiostoma* and *Colpidium* in some communities (Table 1; Figures S1–S7). For *Colpidium*, the interactive LV model only including intraspecific HOIs was best supported in the

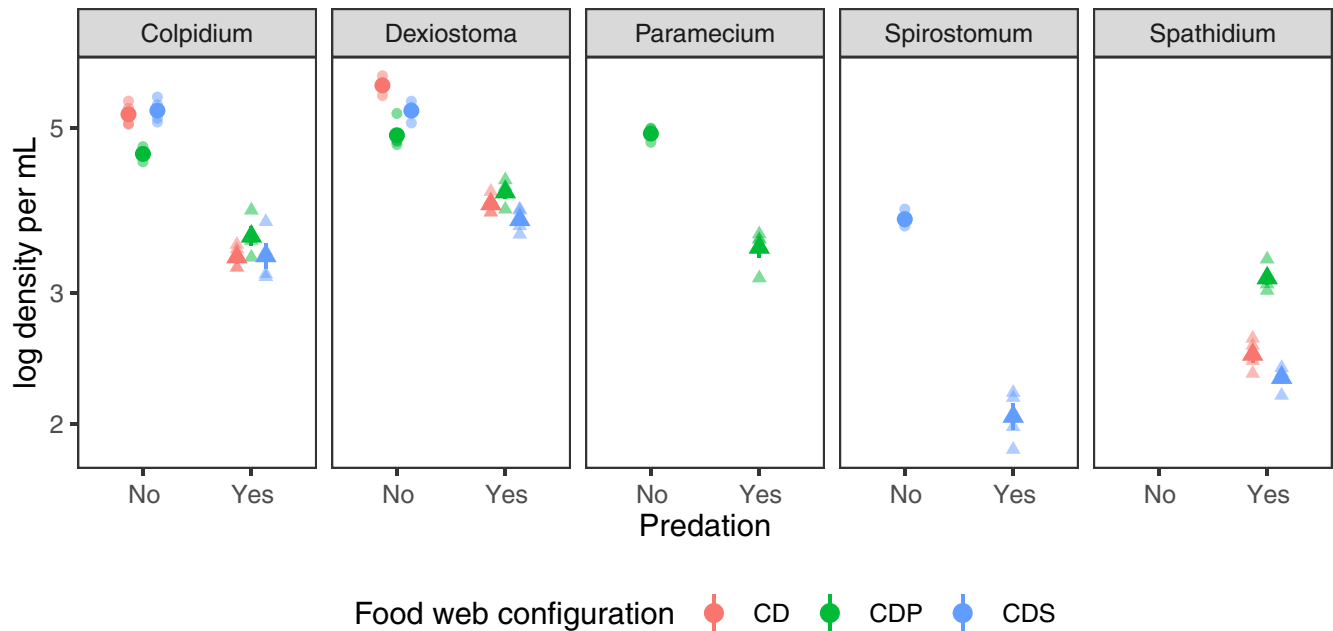


FIGURE 3 Density of focal species as a function of community composition. Each panel shows one of the protist species. Whether the predator was present is shown on the x-axis. The treatments with predators are shown with triangles, and the treatments without predators are shown with circles. The three competitive communities are shown in color (CD=*Colpidium* & *Dexiostoma*, CDP=*Colpidium*, *Dexiostoma* & *Paramecium*, CDS=*Colpidium*, *Dexiostoma* & *Spirostomum*). The solid shapes show the mean, and the error bars show the standard error of the mean (calculated across the duration of the experiment), and the transparent shapes show the actual data points.

two-species community of *Colpidium* and *Dexiostoma*, indicating that *Dexiostoma* modified the effect of *Colpidium* on itself. In culture with a third species, the best supported model differed: when *Colpidium* was present with *Dexiostoma* and *Paramecium*, the additive Ricker model was the most parsimonious ($\Delta\text{AIC}=0.06$ but fewer parameters). In contrast, when *Colpidium* was present with *Dexiostoma* and *Spirostomum*, the interactive Ricker model was best supported, suggesting interspecific HOIs. For the per capita population growth rate of *Dexiostoma*, the interactive LV model only including intraspecific HOIs ($\Delta\text{AIC}=0.59$ but fewer parameters) was most supported when only *Colpidium* was present, suggesting that *Colpidium* modified the effect of *Dexiostoma* on itself. When a third species was present, in both cases the additive Ricker model was best supported, suggesting that neither intra- nor interspecific HOIs were operating. Since all the best supported models were either the interactive LV or Ricker models, all intra- and interspecific interactions were nonlinear.

3.3 | Population persistence

In the absence of predators, *Colpidium* survival was contingent on the community composition (log-rank test $p=.0066$, $n=4$; Figure 4a). Without predators, adding *Spirostomum* increased *Colpidium* survival (pairwise log-rank test $p=.02$, $n=4$; Figure 4a), whereas adding *Paramecium* did not. In contrast, *Dexiostoma* persisted in the absence of predators regardless of the community composition (log-rank test $p=.37$, $n=4$; Figure 4c). In the presence of predators, *Colpidium* survival did not depend on the identity of the competitor species

(Figure 4b). In contrast for *Dexiostoma*, the community composition did impact survival (log-rank test $p=.024$, $n=4$), since adding *Paramecium* to the community increased *Dexiostoma* survival (Figure 4d). *Spathidium* survival depended on the prey community composition (log-rank test $p=.011$, $n=4$), with *Paramecium* increasing the survival of *Spathidium* (pairwise log-rank test $p=.018$, $n=4$; Figure 4e).

4 | DISCUSSION

4.1 | Patterns of population abundance

Overall, species' abundances and coexistence were influenced by community composition. *Colpidium* and *Dexiostoma* grew to lower densities when cultured with *Paramecium*, a sign of competition for shared resources. *Colpidium* abundance was not affected by the presence of *Spirostomum*. However, the mean abundance of *Dexiostoma* was lower in the presence of *Spirostomum*, suggesting competition between the two species, but to a lesser degree than with *Paramecium*. These patterns are in line with known phylogenetic relationships between these species, where *Colpidium* is most closely related to *Dexiostoma*, then to *Paramecium* and finally to *Spirostomum* (Violle et al., 2011). These relationships have been found to predict competitive exclusion due to the similarity in mouth size between species, which in turn defines the feeding niche of species (Violle et al., 2011).

Predation lowered the abundances of the two focal prey species, but this effect could be partially mitigated in the presence

of additional species. For instance, *Colpidium* benefitted from the presence of *Paramecium* when predators were present, having a higher average abundance than would be expected if the effects of both predation and competition were additive. This might be

explained by the interference of *Paramecium* with the predator, indirectly leading to a decrease in interaction strength between the prey and the predator. Ciliate predators show a preferred predator-prey size ratio of 8:1 (Hansen et al., 1994), explaining

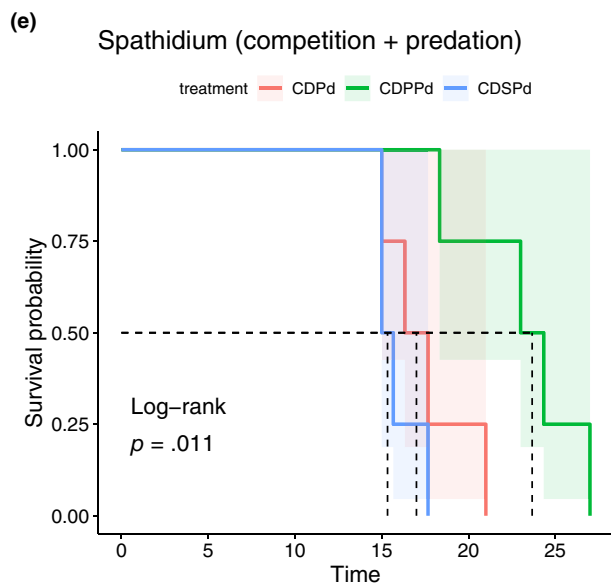
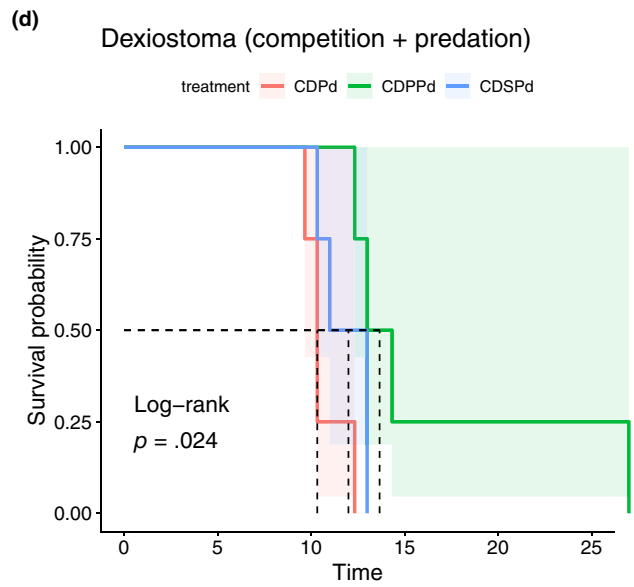
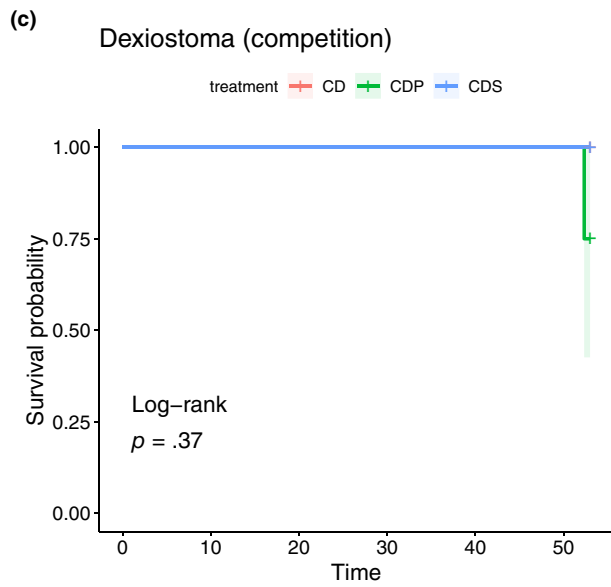
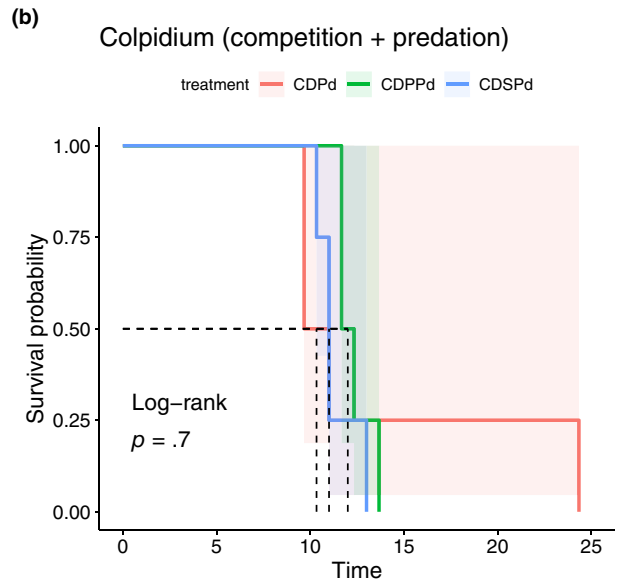
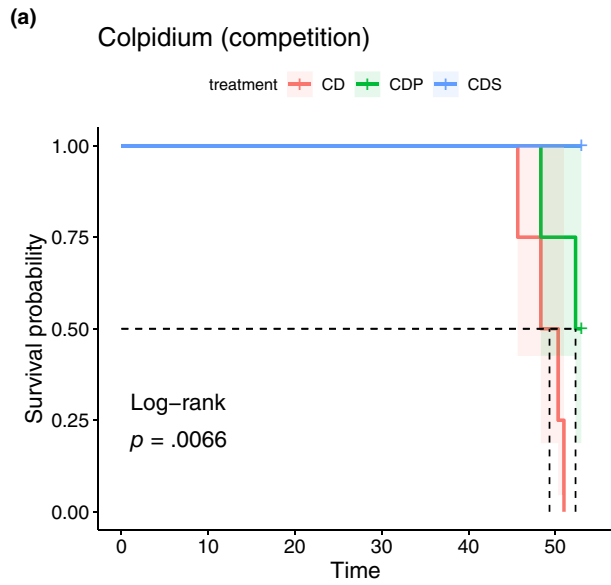
Species	Community	Model	K	AICc	Δ AICc		
Colp	CD	Interactive LV (intra HOI)	6	-48.74	0.00		
		Interactive LV (full HOI)	7	-48.42	0.32		
		Additive Ricker	4	-41.81	6.93		
		Additive LV	4	-41.61	7.13		
		Interactive Ricker	5	-39.59	9.15		
	CDP	CDP	Interactive LV (inter HOI)	5	-39.39	9.35	
			Interactive LV (intra HOI)	8	63.30	0.00	
			Additive Ricker	5	63.37	0.06	
			Interactive Ricker	8	68.21	4.90	
			Interactive LV (full HOI)	11	68.81	5.51	
		CDS	CDS	Additive LV	5	75.50	12.20
				Interactive LV (inter HOI)	8	77.96	14.65
				Interactive Ricker	8	-75.94	0.00
				Interactive LV (inter HOI)	8	-74.09	1.85
				Interactive LV (full HOI)	11	-68.82	7.12
Dexio	CD	Additive Ricker	5	-66.40	9.54		
		Additive LV	5	-65.64	10.30		
		Interactive LV (intra HOI)	8	-61.48	14.46		
		Interactive LV (full HOI)	7	-87.01	0.00		
		Interactive LV (intra HOI)	6	-86.42	0.59		
	CDP	CDP	Additive Ricker	4	-80.56	6.46	
			Interactive Ricker	5	-79.61	7.40	
			Additive LV	4	-75.10	11.92	
			Interactive LV (inter HOI)	5	-73.87	13.14	
			Additive Ricker	5	3.88	0.00	
			Additive LV	5	5.96	2.08	
			Interactive LV (intra HOI)	8	7.80	3.92	
			Interactive Ricker	8	8.80	4.92	
	CDS	CDS	Interactive LV (inter HOI)	8	10.12	6.25	
			Interactive LV (full HOI)	11	12.03	8.16	
Additive Ricker			5	-60.05	0.00		
Additive LV			5	-56.38	3.67		
Interactive LV (intra HOI)			8	-56.21	3.84		
Interactive Ricker			8	-54.32	5.73		
Interactive LV (full HOI)			11	-51.22	8.83		
		Interactive LV (inter HOI)	8	-50.82	9.23		

TABLE 1 Model selection table showing the AICc values for the per capita population growth rate of the two focal species across the three community compositions.

Note: Additive and interactive versions of the Lotka-Volterra (LV) and Ricker models were fitted to the data. K is the number of estimated parameters, Δ AICc is the difference between the model with the lowest AICc and the AICc of the specific model.

Bold indicates the most parsimonious model (Δ AICc < 2 and lowest number of parameters).

FIGURE 4 Survival curves (lines) of *Colpidium*, *Dexiostoma* and *Spathidium* as a function of community composition (CD = *Colpidium* & *Dexiostoma*, CDP = *Colpidium*, *Dexiostoma* & *Paramecium*, CDS = *Colpidium*, *Dexiostoma* & *Spirostomum*, CDPd = CD + predator, CDPPd = CDP + predator, CDSPd = CDS + predator). 95% confidence intervals are shown by shaded boxes. While for *Colpidium*, survival depends on additional species in the competitive communities (a) but not the communities with predation (b), the opposite is observed for *Dexiostoma* (c, d). *Spathidium* survival depended on the prey community composition (e).



why some species may interfere with predator foraging. *Dexiostoma* similarly benefited from *Paramecium* when the predator was present, reaching on average higher abundance than when *Colpidium* and *Spirostomum* were present.

Surprisingly, both *Paramecium* and *Spirostomum* densities decreased in the presence of the predator, indicating that the predator had a negative effect on the two largest protist species. Furthermore, the abundance of the predator was greater when *Paramecium* was present compared to when it was cultured with only the focal species pair, or the focal pair and *Spirostomum*. It thus appears that *Paramecium* was consumed in addition to the two focal prey and that while *Spirostomum* was attacked, it was potentially too large to be consumed. This observation could be explained by the mode of prey capture employed by the predator *Spathidium*. The cell mouth (cytostome) of *Spathidium* is furnished with a rod-like tip (toxicyst) to paralyze or kill other microorganisms for easier consumption (Fyda et al., 2005). But while the toxicyst make it possible to paralyze large prey, phagocytosis still requires that prey organisms can be engulfed.

4.2 | Evidence for higher-order interactions

Comparing additive and interactive LV models revealed the significant effect of intraspecific HOIs on the population growth rate for both *Colpidium* and *Dexiostoma* in the two species communities (Figure 5a). While our experiment was not designed to test the underlying mechanisms that give rise to intraspecific HOIs, one plausible explanation could be the presence of an “information cascade,” wherein individual behaviors within the same species are regulated by the actions of others (Bikhchandani et al., 1992; Potts, 1984). Studies have shown that the behavior of neighboring fish influences the direction and speed of the school (Ioannou et al., 2011). If a fish senses a signal of danger and turns, it creates a pressure wave in the water, and other fish respond to this pressure wave by turning as well (Ioannou et al., 2011). Although

ciliates do not have as elaborate sensory systems as fish, they are capable of sensing changes in local population density and adjusting their movement strategies accordingly (Fronhofer et al., 2015; Pennekamp et al., 2014). Since movement and foraging are often closely linked (Van Dyck & Baguette, 2005), it is possible that movement-related crowding effects could lead to the emergence of intraspecific HOIs.

Interspecific HOIs were only found on the population growth rate of *Colpidium* when *Dexiostoma* and *Spirostomum* were present (Figure 5b). *Spirostomum* most likely changed the effect of *Dexiostoma* on *Colpidium*, because both *Dexiostoma* and *Colpidium* strongly competed for the same resources, whereas the direct effect of *Spirostomum* on *Colpidium* was more limited. It is possible that there is an overlap in the size of the bacteria consumed by *Dexiostoma* and *Spirostomum*, while *Colpidium* uses a different size class. In such a case, *Dexiostoma* would compete with both species but *Colpidium* would only compete with *Dexiostoma*. This would also explain why we did not observe an interspecific HOI on the population growth rate of *Dexiostoma* when *Spirostomum* and *Colpidium* were present. Interestingly, there was no HOI of *Paramecium* on either *Dexiostoma* or *Colpidium*, although *Paramecium* was a stronger competitor than *Spirostomum*. Previous work has found strong evidence that interspecific HOIs can affect the coexistence and dynamics of aquatic microbial communities (Mickalide & Kuehn, 2019). Mickalide and Kuehn (2019) observed that *Escherichia coli* can invade cultures of the alga *Chlamydomonas reinhardtii* or the ciliate *Tetrahymena thermophila* but fails to invade a community where both species are present. The invasion resistance of the algae-ciliate community arises from an HOI caused by the algal inhibition of bacterial aggregation, which leaves bacteria vulnerable to predation.

The prevalence of intra- versus interspecific HOIs is noteworthy, since most studies have so far focused on interspecific HOIs. Work on plant communities has shown that the prevalence of intra- or interspecific HOIs can vary across focal species and that neither type is generally more or less important (Mayfield & Stouffer, 2017). Since coexistence will be determined by the relative strengths of

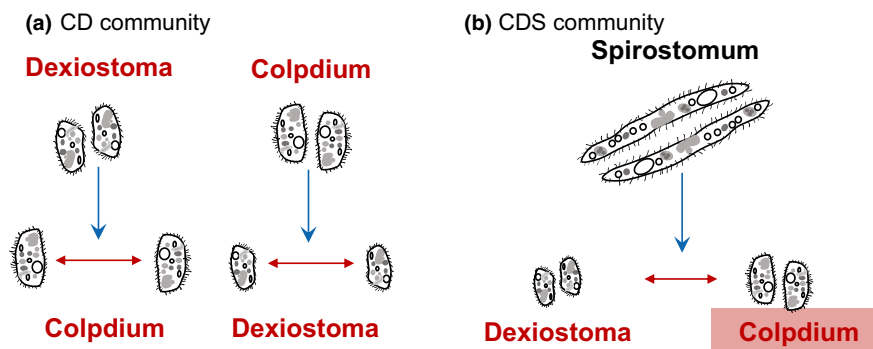


FIGURE 5 Higher-order interactions detected in our experiment. Direct pairwise interactions are shown as red arrows, HOIs are shown in blue. (a) In the two-species community of *Colpidium* and *Dexiostoma*, intraspecific HOIs were detected, indicating *Dexiostoma* modified the effect of *Colpidium* on itself while *Colpidium* modified the effect of *Dexiostoma* on itself. (b) In the community with *Colpidium*, *Dexiostoma*, and *Spirostomum*, an interspecific HOI was detected (the name of the affected species is marked with a light-colored box), suggesting *Spirostomum* modified the interaction between the two focal species.

intra- and interspecific competition (Gibbs et al., 2022; Singh & Baruah, 2021), changes in intraspecific interaction strength alone can influence the persistence of communities. We therefore recommend that empirical studies investigating HOIs embrace a definition of HOIs that includes both intra- and interspecific effects (e.g., Mayfield & Stouffer, 2017).

4.3 | The nature of the detected HOIs

Some authors have argued that HOIs are not ecological processes in their own right but are instead emergent properties of phenomenological models (Letten & Stouffer, 2019). For example, a Lotka–Volterra competition model imposes a linear relationship between the density of one species and the growth of the other. However, if this relationship is not linear in nature, the addition of HOI terms may improve the fit, but the model would not accurately describe the species' interactions. Properly accounting for nonlinear density dependence is an important prerequisite to estimating interaction strength (Hart et al., 2018) and avoiding erroneous conclusions about the presence of HOIs when nonlinearity is present (Kleinhesselink et al., 2022; Letten & Stouffer, 2019). To investigate the potential for, and source of, nonlinear density dependence, we fit models that included either linear (i.e., additive LV) or nonlinear (i.e., additive Ricker) competition coefficients, as well as nonlinear intra- and interspecific HOI terms (i.e., the interactive models). We observed nonlinear density dependence in all communities, as either the Ricker or the interactive LV model had the lowest AICc values. The observation that models which included HOIs had the lowest AICc suggests that there are processes resulting in nonlinearity which the simple additive LV and the nonlinear Ricker model are missing. Therefore, we believe the HOIs detected are not an artifact of nonlinear density dependence, but true behavioral responses. A mechanistic model that allows for such nonlinearity may provide further insights into the ecological processes driving community dynamics.

4.4 | Do HOIs affect the abundance and persistence in competitive communities?

Persistence was different for the two focal prey species. *Dexiostoma* showed high persistence regardless of the additional species present, while *Colpidium* had higher persistence in the presence of a third species. The increased persistence of *Colpidium* when cultured with *Paramecium* was not driven by an HOI, since the additive Ricker model was best supported by the data in the CDP community. But the highest persistence of *Colpidium* was detected in the presence of *Dexiostoma* and *Spirostomum*, where the interactive model was best supported. This pattern is consistent with a stabilizing effect due to interspecific HOIs.

Dexiostoma showed a similar persistence across all combinations of competitors, despite a nonsignificant trend to lower persistence

when *Paramecium* was present. *Dexiostoma* also showed variation in the presence of HOIs, but the pattern is not consistent with a stabilizing role of HOIs: when present with only *Colpidium*, intraspecific HOIs were observed and *Dexiostoma* persisted till the end of the experiment. When *Paramecium* or *Spirostomum* was added, HOIs were no longer detected but neither did the persistence change. This suggests that changes in HOIs do not always result in changes in the persistence of focal species.

The predator itself showed the highest persistence when feeding on communities that contained *Colpidium*, *Dexiostoma* and *Paramecium*. When feeding on the focal pair without competitors, it showed intermediate persistence times, while communities of the focal pair with *Spirostomum* persisted the least well. Hammill et al. (2015) found a similar effect for nontrophically interacting species in a food web: if species that do not interact trophically are present, prey persists for longer in diverse food webs. Since we could not quantify the interaction strength of the predator on *Colpidium* and *Dexiostoma* in the presence of *Paramecium* or *Spirostomum*, we could not determine whether the change in predator persistence was due to HOIs or simply the direct and indirect effects among the members of the food web. Quantifying the role of trophic interaction modifications with functional responses is an exciting area for future research (Kratina et al., 2007; Terry et al., 2017).

4.5 | Limitations of our work

While our study provides insights into the occurrence and implications of HOIs in aquatic microcosms, the small sample size used in our experiment limits the generalizability of our findings. Detecting interactions usually requires larger sample sizes, unless deviations from the additive model are very large (Burgess et al., 2022). Due to the small sample size of our experiment, we may have missed important but weaker interactive effects. In addition, we were only able to examine the interaction of a single focal pair across a few community compositions. To completely disentangle the drivers of community persistence would require to measure all interaction strengths between species, which was logistically impossible in our study. Since our study only investigated HOIs between a focal pair, it is possible that we missed additional interspecific HOIs between the focal pair and the predator. Future research with a larger sample size and a wider range of competitors and predators is warranted to further elucidate the complex nature of HOIs in ecological communities.

5 | CONCLUSIONS

The role of HOIs in community dynamics remains a key issue in community ecology (Gibbs et al., 2022; Levine et al., 2017). Currently, very few empirical studies measure HOIs in communities of real species and study how HOIs affect stability. Our study demonstrates that HOIs can play a role for species persistence and provides some support for the stabilizing effects of HOIs on competitive communities

shown in theoretical studies (Grilli et al., 2017). Our study further provides an example of how to identify HOIs by combining experiments with time series analyses and hence pave the way for more studies that study the occurrence and consequences of HOIs in natural communities. Our study adds to the growing body of research that shows that species interactions can deviate from the pairwise expectations when additional species are present, and a new theory needs to explore the consequences for community coexistence and stability.

AUTHOR CONTRIBUTIONS

Chenyu Shen: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (supporting); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Kimberley Lemmen:** Conceptualization (supporting); formal analysis (supporting); investigation (equal); methodology (equal); writing – original draft (supporting); writing – review and editing (equal). **Jake Alexander:** Conceptualization (supporting); investigation (supporting); methodology (supporting); supervision (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Frank Pennekamp:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); resources (lead); supervision (lead); validation (lead); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

None.

OPEN RESEARCH BADGES



This article has earned Open Data and Open Materials badges. Data and materials are available at [<https://doi.org/10.5281/zenodo.7896204>].

DATA AVAILABILITY STATEMENT

Data and code are available from the following github repository: <https://doi.org/10.5281/zenodo.7896204>.

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REFERENCES

- Abrams, P. A. (1983). Arguments in favor of higher order interactions. *The American Naturalist*, 121(6), 887–891. <https://doi.org/10.1086/284111>
- Adler, P. B., Smull, D., Beard, K. H., Choi, R. T., Furniss, T., Kulmatiski, A., Meiners, J. M., Tredennick, A. T., & Veblen, K. E. (2018). Competition and coexistence in plant communities: Intraspecific competition is stronger than interspecific competition. *Ecology Letters*, 21(9), 1319–1329. <https://doi.org/10.1111/ele.13098>
- Aho, K., Derryberry, D., & Peterson, T. (2014). Model selection for ecologists: The worldviews of AIC and BIC. *Ecology*, 95(3), 631–636. <https://doi.org/10.1890/13-1452.1>
- Altermatt, F., Fronhofer, E. A., Garnier, A., Giometto, A., Hammes, F., Klecka, J., Legrand, D., Mächler, E., Massie, T. M., Pennekamp, F., Plebani, M., Pontarp, M., Schtickzelle, N., Thuillier, V., & Petchey, O. L. (2015). Big answers from small worlds: A user's guide for protist microcosms as a model system in ecology and evolution. *Methods in Ecology and Evolution*, 6(2), 218–231. <https://doi.org/10.1111/2041-210X.12312>
- Bairey, E., Kelsic, E. D., & Kishony, R. (2016). High-order species interactions shape ecosystem diversity. *Nature Communications*, 7(1), 12285. <https://doi.org/10.1038/ncomms12285>
- Barabás, G., Michalska-Smith, M. J., & Allesina, S. (2016). The effect of intra- and interspecific competition on coexistence in multispecies communities. *The American Naturalist*, 188(1), E1–E12. <https://doi.org/10.1086/686901>
- Bick, H., & World Health Organization. (1972). *Ciliated protozoa: An illustrated guide to the species used as biological indicators in freshwater biology*. World Health Organization <https://apps.who.int/iris/handle/10665/40066>
- Bikhchandani, S., Hirshleifer, D., & Welch, I. (1992). A theory of fads, fashion, custom, and cultural change as informational cascades. *Journal of Political Economy*, 100(5), 992–1026. <https://doi.org/10.1086/261849>
- Billick, I., & Case, T. J. (1994). Higher order interactions in ecological communities: What are they and how can they be detected? *Ecology*, 75(6), 1530–1543. <https://doi.org/10.2307/1939614>
- Burgess, B. J., Jackson, M. C., & Murrell, D. J. (2022). Are experiment sample sizes adequate to detect biologically important interactions between multiple stressors? *Ecology and Evolution*, 12(9), e9289. <https://doi.org/10.1002/ece3.9289>
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multi-model inference: A practical information-theoretic approach*. Springer.
- Carrara, F., Giometto, A., Seymour, M., Rinaldo, A., & Altermatt, F. (2015). Inferring species interactions in ecological communities: A comparison of methods at different levels of complexity. *Methods in Ecology and Evolution*, 6(8), 895–906. <https://doi.org/10.1111/2041-210X.12363>
- Case, T. J., & Bender, E. A. (1981). Testing for higher order interactions. *The American Naturalist*, 118(6), 920–929.
- Clark, J. S. (2010). Individuals and the variation needed for high species diversity in Forest trees. *Science*, 327(5969), 1129–1132. <https://doi.org/10.1126/science.1183506>
- Daugaard, U., Petchey, O. L., & Pennekamp, F. (2019). Warming can destabilize predator–prey interactions by shifting the functional response from type III to type II. *Journal of Animal Ecology*, 88(10), 1575–1586. <https://doi.org/10.1111/1365-2656.13053>
- Emmerson, M., & Yearsley, J. M. (2004). Weak interactions, omnivory and emergent food-web properties. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 271(1537), 397–405. <https://doi.org/10.1098/rspb.2003.2592>
- Foissner, W., & Berger, H. (1996). A user-friendly guide to the ciliates (protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biology*, 35(2), 375–482. <https://doi.org/10.1111/j.1365-2427.1996.tb01775.x>

- Fronhofer, E. A., Kropf, T., & Altermatt, F. (2015). Density-dependent movement and the consequences of the Allee effect in the model organism *Tetrahymena*. *Journal of Animal Ecology*, 84(3), 712–722. <https://doi.org/10.1111/1365-2656.12315>
- Fyda, J., Warren, A., & Wolinriska, J. (2005). An investigation of predator-induced defence responses in ciliated protozoa. *Journal of Natural History*, 39(18), 1431–1442. <https://doi.org/10.1080/0022293040004396>
- Gibbs, T., Levin, S. A., & Levine, J. M. (2022). Coexistence in diverse communities with higher-order interactions. *Proceedings of the National Academy of Sciences*, 119(43), e2205063119. <https://doi.org/10.1073/pnas.2205063119>
- Grilli, J., Barabás, G., Michalska-Smith, M. J., & Allesina, S. (2017). Higher-order interactions stabilize dynamics in competitive network models. *Nature*, 548(7666), 210–213. <https://doi.org/10.1038/nature23273>
- Hammill, E., Kratina, P., Vos, M., Petchey, O. L., & Anholt, B. R. (2015). Food web persistence is enhanced by non-trophic interactions. *Oecologia*, 178(2), 549–556. <https://doi.org/10.1007/s00442-015-3244-3>
- Hansen, B., Bjornsen, P. K., & Hansen, P. J. (1994). The size ratio between planktonic predators and their prey. *Limnology and Oceanography*, 39(2), 395–403. <https://doi.org/10.4319/lo.1994.39.2.0395>
- Hart, S. P., Freckleton, R. P., & Levine, J. M. (2018). How to quantify competitive ability. *Journal of Ecology*, 106(5), 1902–1909. <https://doi.org/10.1111/1365-2745.12954>
- HilleRisLambers, J., Adler, P. B., Harpole, W. S., Levine, J. M., & Mayfield, M. M. (2012). Rethinking community assembly through the lens of coexistence theory. *Annual Review of Ecology, Evolution, and Systematics*, 43(1), 227–248. <https://doi.org/10.1146/annurev-ecolsys-110411-160411>
- Holt, R. D. (1997). Community modules. In *Multitrophic interactions in terrestrial ecosystems, 36th symposium of the British Ecological Society* (pp. 333–339). Blackwell Science.
- Hooper, D. U., Chapin, F. S., III, Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A. J., Vandermeer, J., & Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75(1), 3–35. <https://doi.org/10.1890/04-0922>
- Ioannou, C. C., Couzin, I. D., James, R., Croft, D. P., & Krause, J. (2011). Social organisation and information transfer in schooling fish. In *Fish cognition and behavior* (pp. 217–239). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781444342536.ch10>
- Kassambara, A., Kosinski, M., Biecek, P., & Fabian, S. (2021). *survminer: Drawing survival curves using "ggplot2"* (0.4.9) [computer software]. <https://cran.r-project.org/web/packages/survminer/index.html>
- Kleinhesselink, A. R., Kraft, N. J. B., Pacala, S. W., & Levine, J. M. (2022). Detecting and interpreting higher-order interactions in ecological communities. *Ecology Letters*, 25(7), 1604–1617. <https://doi.org/10.1111/ele.14022>
- Kondoh, M. (2008). Building trophic modules into a persistent food web. *Proceedings of the National Academy of Sciences*, 105(43), 16631–16635. <https://doi.org/10.1073/pnas.0805870105>
- Kratina, P., Vos, M., & Anholt, B. R. (2007). Species diversity modulates predation. *Ecology*, 88(8), 1917–1923. <https://doi.org/10.1890/06-1507.1>
- Landi, P., Minoarivelo, H. O., Brännström, Å., Hui, C., & Dieckmann, U. (2018). Complexity and stability of ecological networks: A review of the theory. *Population Ecology*, 60(4), 319–345. <https://doi.org/10.1007/s10144-018-0628-3>
- Letten, A. D., & Stouffer, D. B. (2019). The mechanistic basis for higher-order interactions and non-additivity in competitive communities. *Ecology Letters*, 22(3), 423–436. <https://doi.org/10.1111/ele.13211>
- Levine, J. M., Bascompte, J., Adler, P. B., & Allesina, S. (2017). Beyond pairwise mechanisms of species coexistence in complex communities. *Nature*, 546(7656), 56–64. <https://doi.org/10.1038/nature22898>
- Mayfield, M. M., & Stouffer, D. B. (2017). Higher-order interactions capture unexplained complexity in diverse communities. *Nature Ecology & Evolution*, 1(3), 1–7. <https://doi.org/10.1038/s41559-016-0062>
- McCann, K., Hastings, A., & Huxel, G. R. (1998). Weak trophic interactions and the balance of nature. *Nature*, 395(6704), 794–798. <https://doi.org/10.1038/27427>
- Meyer, D., Dimitriadou, E., Hornik, K., Weingessel, A., Leisch, F., Chang, C. C., & Lin, C. C. (2022). *Misc Functions of the Department of Statistics, Probability Theory Group (Formerly: E1071), TU Wien* (1.7–11). e1071. [Computer software]. <https://CRAN.R-project.org/package=e1071>
- Mickalide, H., & Kuehn, S. (2019). Higher-order interaction between species inhibits bacterial invasion of a phototroph-predator microbial community. *Cell Systems*, 9(6), 521–533.e10. <https://doi.org/10.1016/j.cels.2019.11.004>
- Mühlbauer, L. K., Schulze, M., Harpole, W. S., & Clark, A. T. (2020). gauseR: Simple methods for fitting Lotka-Volterra models describing Gause's "struggle for existence". *Ecology and Evolution*, 10(23), 13275–13283. <https://doi.org/10.1002/ece3.6926>
- Pennekamp, F., Griffiths, J. I., Fronhofer, E. A., Garnier, A., Seymour, M., Altermatt, F., & Petchey, O. L. (2017). Dynamic species classification of microorganisms across time, abiotic and biotic environments—A sliding window approach. *PLoS One*, 12(5), e0176682. <https://doi.org/10.1371/journal.pone.0176682>
- Pennekamp, F., Mitchell, K. A., Chaîne, A., & Schtickzelle, N. (2014). Dispersal propensity in *Tetrahymena thermophila* ciliates—A reaction norm perspective. *Evolution*, 68(8), 2319–2330. <https://doi.org/10.1111/evo.12428>
- Pennekamp, F., Pontarp, M., Tabi, A., Altermatt, F., Alther, R., Choffat, Y., Fronhofer, E. A., Ganesanandamoorthy, P., Garnier, A., Griffiths, J. I., Greene, S., Horgan, K., Massie, T. M., Mächler, E., Palamara, G. M., Seymour, M., & Petchey, O. L. (2018). Biodiversity increases and decreases ecosystem stability. *Nature*, 563(7729), 109–112. <https://doi.org/10.1038/s41586-018-0627-8>
- Pennekamp, F., Schtickzelle, N., & Petchey, O. L. (2015). BEMOVI, software for extracting behavior and morphology from videos, illustrated with analyses of microbes. *Ecology and Evolution*, 5(13), 2584–2595. <https://doi.org/10.1002/ece3.1529>
- Pfister, C. A. (1995). Estimating competition coefficients from census data: A test with field manipulations of tidepool fishes. *The American Naturalist*, 146(2), 271–291. <https://doi.org/10.1086/285798>
- Potts, W. K. (1984). The chorus-line hypothesis of manoeuvre coordination in avian flocks. *Nature*, 309(5966), 344–345. <https://doi.org/10.1038/309344a0>
- R Core Team. (2022). *R: A language and environment for statistical computing*. [Computer software]. <https://www.r-project.org/>
- Rip, J. M. K., McCann, K. S., Lynn, D. H., & Fawcett, S. (2010). An experimental test of a fundamental food web motif. *Proceedings of the Royal Society B: Biological Sciences*, 277(1688), 1743–1749. <https://doi.org/10.1098/rspb.2009.2191>
- Singh, P., & Baruah, G. (2021). Higher order interactions and species coexistence. *Theoretical Ecology*, 14(1), 71–83. <https://doi.org/10.1007/s12080-020-00481-8>
- Sundarraman, D., Hay, E. A., Martins, D. M., Shields, D. S., Pettinari, N. L., & Parthasarathy, R. (2020). Higher-order interactions dampen pairwise competition in the zebrafish gut microbiome. *MBio*, 11(5). <https://doi.org/10.1128/mbio.01667-20>
- Tabi, A., Petchey, O. L., & Pennekamp, F. (2019). Warming reduces the effects of enrichment on stability and functioning across levels of organisation in an aquatic microbial ecosystem. *Ecology Letters*, 22(7), 1061–1071. <https://doi.org/10.1111/ele.13262>

- Terry, J. C. D., Morris, R. J., & Bonsall, M. B. (2017). Trophic interaction modifications: An empirical and theoretical framework. *Ecology Letters*, 20(10), 1219–1230. <https://doi.org/10.1111/ele.12824>
- Van Dyck, H., & Baguette, M. (2005). Dispersal behaviour in fragmented landscapes: Routine or special movements? *Basic and Applied Ecology*, 6(6), 535–545. <https://doi.org/10.1016/j.baae.2005.03.005>
- Violle, C., Nemergut, D. R., Pu, Z., & Jiang, L. (2011). Phylogenetic limiting similarity and competitive exclusion. *Ecology Letters*, 14(8), 782–787. <https://doi.org/10.1111/j.1461-0248.2011.01644.x>
- Werner, E. E., & Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology*, 84(5), 1083–1100.
- Woodruff, L. L., & Spencer, H. (1921). The food reactions of the infusorian *Spathidium spathula*. *Proceedings of the Society for Experimental Biology and Medicine*, 18(6), 183–184. <https://doi.org/10.3181/00379727-18-90>
- Wootton, J. T. (1993). Indirect effects and habitat use in an intertidal community: Interaction chains and interaction modifications. *The American Naturalist*, 141(1), 71–89.
- Wootton, J. T. (1994). Predicting direct and indirect effects: An integrated approach using experiments and path analysis. *Ecology*, 75(1), 151–165. <https://doi.org/10.2307/1939391>
- Worthen, W. B., & Moore, J. L. (1991). Higher-order interactions and indirect effects: A resolution using laboratory drosophila communities. *The American Naturalist*, 138(5), 1092–1104.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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