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Predator trait evolution alters prey community composition

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Abstract. Predators have potentially strong effects on prey abundance, growth, life histories, and behavior, but the net direction and strength of these effects depend on traits in the predator population. Trait evolution that occurs on ecological timescales is common and may affect species interactions. Using laboratory microcosms of a bacterivorous ciliate (*Colpidium* sp.) and a diverse community of bacteria, we tested the hypothesis that predator trait evolution in response to temperature variability and nutrient concentration alters the composition of the prey community using a two-phase experiment. The first phase consisted of a selection experiment, in which microcosm communities were randomly assigned to one of eight fully factorial treatments of high or low nutrients, variable or stable temperatures, and with or without *Colpidium*. The second phase was a reciprocal transplant experiment, in which a subsample of each microcosm community from the selection experiment was transferred to one of four common garden environments, consisting of all combinations of high or low nutrients and variable or stable temperature. Nutrient enrichment directly decreased bacterial species richness and altered the community composition, but also indirectly increased bacterial species richness through decreases in ciliate peak density and, subsequently, ciliate abundance. Moreover, the evolutionary effects of nutrient enrichment on predator traits were more pronounced in some contemporary common garden environments, and undetectable in others. Our findings suggest that historical and contemporary environments are equally important to consider in understanding how trait evolution affects community structure.

Key words: community composition; environmental change; evolution; nutrients; predator–prey; temperature; traits.

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INTRODUCTION

A century of theory suggests that predators should have strong ecological effects on the community of organisms on which they prey (Lotka 1920, Volterra 1926, Holling 1959, Rosenzweig and MacArthur 1963, Arditi and Ginzburg 1989, Berryman 1992, Ives et al. 2005). Predators can reduce prey population size and decrease prey growth rates (Sih et al. 1985) or alter prey age distributions and life histories (Michod 1979). Predators may also have non-lethal effects on prey behavior, habitat use, and competitive interactions as well as other physiological and morphological traits (reviewed in Lima 1998, Peckarsky et al. 2008).

The effects of predators on their prey community depend on the predator's traits, particularly those that relate to foraging, such as prey detection, capture rates, and the efficiency with which predators convert prey biomass into offspring. Such traits have been shown to determine the stability of the predator–prey system and the population densities of both predator and prey (Abrams 1989, 2000). In cases where the predator feeds on a diverse community of prey, foraging traits also determine the prey community structure. For example, alewives in different lakes

vary in morphological (gill raker spacing and gape size) and behavioral traits (time of residence in freshwater) that drive differences in zooplankton community structure (Post et al. 2008). Predator foraging traits may vary spatially (Post et al. 2008) or temporally when predator traits change over time through development, seasonal changes, and evolution.

A large body of theoretical work (Schaffer and Rosenzweig 1978, Abrams 1986, 1989, Dieckmann et al. 1995) and several empirical studies (Brodie and Brodie 1999, Yoshida et al. 2003, Hiltunen et al. 2014) have described how the ecological dynamics of a system can be altered when predators and/or prey evolve in response to one another. Although this can lead to an evolutionary arms race between predator and prey (Cott 1940, Dawkins and Krebs 1979, Frickel et al. 2016), predator traits may evolve in response to many selective agents simultaneously. Trait evolution in response to environmental change has been reported in a variety of species (reviewed in Bradshaw and Holzapfel 2006) and can affect community ecology (Thompson 1998, terHorst et al. 2010, Palkovacs et al. 2012). Few studies have focused on how predator evolution in response to abiotic factors might influence the effect of predators on prey populations (Declerck et al. 2015).

Evolution of predator traits in response to changes in the abiotic environment could alter the interaction between predator and prey, which could ultimately affect the abundance or diversity of the prey community (Bolker et al. 2003). Short-term plastic changes in predator traits have been shown to affect prey populations (Rall et al. 2010). Spiders plastically increase feeding rates in response to warmer temperatures, leading to stronger short-term interaction strengths between spiders and their prey, as well as greater effects of spiders on prey abundance (Rall et al. 2010). Whether these dynamics play out in response to trait changes over longer timescales remains to be seen.

The effects of trait evolution on ecological interactions may depend on the contemporary context in which those traits are expressed. For instance, plants that evolved in response to drought affected soil microbial communities differently than those without this selective pressure. These differences were more pronounced when plants and soils were grown in contemporary drought conditions (terHorst et al. 2014). One reason for this may be genotype by environment (G × E) interactions, in which the phenotypic differences among genotypes are more pronounced in certain environments. This results in stronger potential for selection in environments where phenotypes differ, but weaker selection in environments where different genotypes produce similar phenotypes (Falconer and Mackay 1996). For example, plant genotypes that differ in traits that confer drought tolerance might produce similar phenotypes in wet environments. Genetic correlations or tradeoffs among traits may also be apparent in some environments, but obscured in others (Stearns and Kaiser 1996, Sgrò and Hoffmann 2004).

Here, we used controlled laboratory experiments to quantify how historical and contemporary environmental factors associated with global change (temperature variability and nutrient input) affected the evolution of bacterivorous ciliate traits in pitcher plant inquiline communities. We then used 16S rRNA gene sequencing to determine how historical and contemporary environments affect bacterial community diversity. Finally, we used structural equation modeling (SEM) to determine whether the nutrient input and temperature variability affected bacterial communities directly or indirectly through effects on protist abundance and traits.

Materials and Methods

Study system

Purple pitcher plants (*Sarracenia purpurea*) are carnivorous plants native to bogs and wetlands of North America. The plant's cup-shaped leaves retain rainwater to form a contained body of water. This contained water provides habitat for an inquiline community of aquatic invertebrates, unicellular protists, and bacteria. Nutrients enter the system in the form of insect prey. The pitcher plant inquiline food web is limited primarily by organic carbon and, to a lesser degree, phosphorus (Gray et al. 2006). Protists in these communities reproduce asexually every 4–8 h and traits, such as cell size, evolve on the timescale of days in response to predators and competitors (terHorst et al. 2010, terHorst 2011). Here, we assessed whether temperature variability and nutrient input affected two ecologically relevant traits, cell size and peak density, in the ciliate protozoan *Colpidium* sp. Interspecific variation in

protist cell size affects interactions with predators and competitors in these communities (Kneitel 2012), as does intraspecific variation in peak density (terHorst 2010, 2011, terHorst et al. 2010). Body size, as one of the main parameters in the metabolic theory of ecology (Brown et al. 2004), may be affected by temperature and resource availability. Finally, each trait represents an alternative strategy for resource allocation—increasing peak abundance prioritizes cell division and population growth, while increasing cell size prioritizes individual cell growth.

In contrast to the insect larvae and protists, the bacterial communities at the base of the inquiline food web in pitcher plants have been explored relatively little. Initial work suggests that the abundance and composition of the bacterial community can vary greatly among and within sampling locations (Gray et al. 2012) and is driven largely by leaf age (Miller and terHorst 2012). The bacterial community composition is not correlated with that of bacterial communities from the surrounding soil (Koopman et al. 2010) or adjacent pitchers (Peterson et al. 2008). Similarly, environmental predictors, such as pH and pitcher size, provide little explanation for variation in bacterial diversity among pitcher plant inquiline communities (Peterson et al. 2008, Miller and terHorst 2012). However, early evidence suggests that at least one species of protist altered the relative abundance of bacterial taxa (Paisie et al. 2014).

We isolated monocultures of the ciliate protozoan *Colpidium* sp. from naturally occurring pitcher plants at four sites within the Apalachicola National Forest in North Florida (Appendix S1: Fig. S1, Table S1) in May 2014. We mixed our isolated cultures together in order to maximize genetic diversity and used the resulting stock to inoculate our experimental microcosms. In addition to isolating *Colpidium*, we passed our field samples through a series of filters decreasing in size from 25 to 2.5 μm to exclude larger organisms and produced one mixed stock of bacteria that was used to inoculate all microcosms. This bacteria stock was monitored for several days prior to initiating experimental microcosms, in order to ensure that filtering was successful. We measured traits in this stock of *Colpidium* as well as community structure and diversity of the bacterial community stock (see *Protozoa trait measurements* and *Bacterial community measurements*).

Selection experiment

We experimentally manipulated temperature, nutrient levels, and predators in a fully factorial design in experimental laboratory microcosms. We established 40 microcosms in 50-mL macrocentrifuge tubes with 25 mL of sterile water, 4 mL of bacteria stock culture, and 6 mg of autoclaved freeze-dried bloodworms, which mimics the insect-based detritus found in pitcher plant leaves and serves as a long-term resource for bacteria (terHorst et al. 2010).

Half of the microcosms were maintained in a growth chamber with a stable temperature of 25°C. The remaining microcosms were maintained in an identical growth chamber with a higher and more variable temperature regime. The variable temperature treatment experienced a daily temperature cycle that gradually increased from 24° to 37°C and back to 24°C over four hours. On average, the variable treatment was 1°C warmer than the stable treatment, which reflects how temperatures are expected to change in North America over the next century (IPCC 2014).

We manipulated nutrient levels by establishing a standard sterile nutrient mixture composed of a 1:60 ratio (Gray et al. 2006) of sodium phosphate as a source of phosphorus, a mixture of eight different carbon sources ranging in complexity (glucose, xylose, cellobiose, glycine, *N*-acetylglucosamine, vanillin, lignin, and cellulose; Whitaker et al. 2014), and yeast extract as a source of micronutrients. The nutrient mixture contained 1.07 g of each carbon source (8.56 g in total), 0.14 g of sodium phosphate, and 0.08 g of yeast extract. We added 1 mg of this mixture to the low nutrient treatment and 10 mg to the high nutrient treatment every three days for the duration of the experiment. The level of nutrient input in the high nutrient treatment was designed to maintain eutrophic levels of enrichment (Nixon 1995).

Finally, we manipulated the presence of *Colpidium* by adding 1 mL of *Colpidium* stock (~582 cells/mL) to appropriate microcosms. We manipulated temperature, nutrients, and *Colpidium* in a fully factorial design, producing eight unique treatment combinations. We randomly assigned five replicate microcosms to each treatment for a total of 40 microcosms. The microcosms were maintained under these conditions for 28 d (~150 protozoan generations), at which time we measured protozoan

traits and bacterial communities in a reciprocal transplant design.

Reciprocal transplant experiment

We performed a reciprocal transplant experiment from each of the eight environments in the selection experiment to each of four different contemporary environments. These four contemporary environments were identical to the four different abiotic environments in the selection experiment (stable vs. variable temperature and high vs. low nutrients). To do this, we used four 1-mL random subsamples from each of the 40 microcosms from the selection experiment ("historical microcosms") to inoculate each of four new microcosms ("contemporary microcosms"), which were randomly assigned to one of the four contemporary environments. We established each contemporary microcosm in a sterile 50-mL macrocentrifuge tube with 29 mL of sterile water and 6 mg of autoclaved freeze-dried bloodworms. This design allowed us to partition the effects of historical environment (temperature, nutrients, or predation) from the effects of contemporary environments (temperature and nutrients). We sampled protozoa and bacteria after three days in their contemporary environment. This period (~15 protozoa generations) minimized any protozoan maternal effects from the historical environment and allowed enough time for ecological interactions to occur, but minimized the time for further evolution.

We sampled *Colpidium* by removing 1 mL from each well-mixed contemporary microcosm. We fixed each sample by adding 20 μL of Lugol's iodine solution. We sampled bacterial abundance in each microcosm by fixing a 100-μL sample in 900 μL of a mixture of 10% formalin in phosphate-buffered saline (PBS) solution to create a 10-fold dilution; 100 μL of this was added to another 900 μL of 10% formalin–PBS solution to make a 100-fold dilution. All of the remaining volume from each microcosm was passed through a 0.22-μm vacuum filter. The filter was frozen to be used for sequencing to determine bacterial community structure.

Protozoa trait measurements

We used light microscopy to measure protozoan abundance and two traits (cell size and peak density) in the *Colpidium* stock culture and in each microcosm at the end of the reciprocal transplant experiment. We gently centrifuged fixed protozoan samples (8 rcf for 1 min) to concentrate cells at the bottom of the tube, after which we counted cells from the bottom 100 μL of each sample using a Palmer counting cell (Sandgren and Robinson 1984). We used protist abundance at the end of the reciprocal transplant experiment to calculate per-capita growth rates, which we used as a proxy for fitness. To measure peak density, we transferred a 100-μL sample from each microcosm to a sterile macrocentrifuge tube with 20 mL of sterile water and 6 mg of freeze-dried bloodworms and allowed microcosms to grow at room temperature (approximately 24°C) for six days. We fixed 1 mL from each microcosm using 20 μL of Lugol's iodine every 24 h. Although Lugol's iodine reduces cell volume by up to 40% over one month of storage (Zinabu and Bott 2000), it reduces the volume of all cells equally and, therefore, did not affect comparisons between treatments. We counted fixed samples as above. Over this six-day period, the density of *Colpidium* increased exponentially and then decreased; we used the maximum density over this time as our estimate of peak population density. To determine cell area, we analyzed digital images of approximately 80–100 cells for each microcosm using Image J (National Institutes of Health, Maryland, Bethesda, USA).

We examined the effects of historical and contemporary temperature and nutrients on protozoa traits (cell size and peak density) and fitness (per-capita growth rates) using a series of nested generalized linear models by employing the glm function in the base-R package (R Core Team 2016). Historical and contemporary temperature and nutrients were included as fixed effects in these models. Each historical microcosm identity was nested within the interaction between historical temperature and historical nutrients in order to account for variance at the start of the reciprocal transplant based on shared historical identity (e.g., population size at the start of the reciprocal transplant). We also included each trait in the model as a covariate of the other trait (i.e., cell size as a covariate of peak density and vice versa). Akaike's information criterion (AIC) values were used for model comparison (via the "AICtab" function in R package "bbmle"; R Core Team 2016, Bolker and R Core Development

Team 2016) to determine the most suitable distribution and whether or not to include higher-order terms in the final model. For brevity, we only report the highest order significant interactions for each analysis; a complete summary of all statistical results can be found in the supporting information (Appendix S2: Tables S1, S2).

Bacterial community measurements

We estimated total bacterial abundance at the end of the reciprocal transplant experiment from direct cell counts using 4′,6-diamidino-2-phenylindole (DAPI) stain, a fluorescent stain that binds to DNA, and fluorescent microscopy. We mixed 10 μL of DAPI stain (5 mg/mL), 100 μL of the diluted samples, and 900 μL of PBS solution to stain cells. We filtered this solution onto 0.22-μm black polycarbonate filters for visualization using fluorescent light microscopy.

We analyzed bacterial community composition in the original bacterial and *Colpidium* stocks and in each experimental microcosm using an Illumina MiSeq platform (Illumina, San Diego, California, USA) for paired-end 16S rRNA gene sequencing. We extracted DNA from the vacuum filters using a MoBio RapidWater DNA Isolation Kit (Mobio Laboratories, Carlsbad, California, USA) following the manufacturer's instructions. With this genomic DNA, we amplified approximately 300 base pairs of the V3–V4 region of the 16S rRNA gene using the archaeal and bacterial primers 515F and 806R and standard PCR protocols established by the Earth Microbiome Project (Bates et al. 2011, Caporaso et al. 2011). We used a PicoGreen fluorescence assay to determine the molar concentration of dsDNA in each PCR product and used these data to pool samples at the same molar concentration. Pooled samples were cleaned up using a Wizard DNA Clean-Up System (Promega, Madison, Wisconsin, USA). Samples were sequenced on an Illumina MiSeq platform using an Illumina MiSeq v2 Reagent Kit (Illumina).

Raw FASTQ sequence files of 16S rRNA genes were processed with the UPARSE pipeline (Edgar 2013). We used a custom Python script to demultiplex and prepare sequence files for paired-end assembly and clustering (Ramirez et al. 2014). Paired sequences were assembled using UPARSE according to the following parameters: fastq_truncqual 3, fastq_maxdiffs 1, fastq_minovlen 20, fastq_minmergegelen 200. Assembled sequences were then filtered at a maxee value of 0.5 (signifying that, on average, only one nucleotide in every two sequences is potentially incorrect) to remove low-quality sequences. The remaining sequences were dereplicated and singletons were removed. Operational taxonomic units (OTUs) were assigned with UPARSE at a 97% sequence identity threshold. Taxonomy was assigned to sequences for each OTU using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) with a confidence threshold of 0.5 against the Greengenes 13_5 database (DeSantis et al. 2006, McDonald et al. 2012) as implemented by QIIME version 1.6.0 (Caporaso et al. 2010). We also used QIIME to calculate alpha (species richness; i.e., the total number of OTUs present in a sample) and beta diversity (UniFrac; Lozupone and Knight 2005) metrics. To eliminate any bias due to sequencing depth, all samples were rarefied to 25,000 sequences before calculating diversity metrics. We choose to use the UPARSE pipeline because it has been shown to reduce the number of spurious OTUs in comparison with other pipeline protocols (Edgar 2013).

We compared the effects of historical and contemporary environments and *Colpidium* on bacterial community composition using permutational analysis of variance, or PERMANOVA, which is a non-parametric extension of multivariate analysis of variance based on pairwise UniFrac distances (Kelly et al. 2015). We visualized the effects of historical and contemporary temperature and nutrients and the presence of *Colpidium* on bacterial beta diversity using the first three axes of a redundancy analysis (Van Den Wollenberg 1977), which is a constrained ordination technique. We also plotted species scores, which describe how taxa are correlated with each axis, from the redundancy analysis over the site scores to produce a biplot. This allowed us to visualize which taxa are strongly associated with which treatments.

Partitioning ecological and evolutionary effects

Although the treatment manipulations may have affected bacterial communities directly, the ecological (abundance) and evolutionary (trait change) responses of protozoan populations to temperature and nutrient regimes may have also affected the bacterial communities. To partition these multiple pathways by which treatments could affect bacterial diversity, we utilized the

graph-theoretic approach to SEM (Mitchell 1992) as described by Grace et al. (2012). To do so, we employed the "sem" function in the R package "lavaan" (Rosseel 2012, R Core Team 2016). We treated the calculated bacterial alpha and beta diversity metrics as our response variables, experimental treatments as main factors, and protist traits and abundance as mediating factors. To compare the strength of multiple predictors on response variables, we calculated standardized regression coefficients for each path in the model using the documentation provided in the "lavaan" package in R (Rosseel 2012, R Core Team 2016), which standardizes regression coefficients by rescaling the variance of each observed variable to 1 (Grace and Bollen 2005).

Results

Evolution of protist traits

We found a significant ecological interaction between contemporary nutrients and contemporary temperature on protist cell size ($F_{1,63}$ = 6.951, P = 0.011). *Colpidium* grown in high contemporary nutrients had larger cells, but this effect was more pronounced in the variable contemporary temperature treatment (Fig. 1A). We also found a significant eco-evolutionary interaction between contemporary nutrients and historical nutrients ($F_{1,63}$ = 5.589, P = 0.021) on cell size. *Colpidium* from high historical nutrient environments had larger cell sizes than *Colpidium* from low nutrient environments, but we only observed this difference in low contemporary nutrient environments (Fig. 1B).

We found a significant eco-evolutionary interaction of contemporary and historical nutrient environments on *Colpidium* peak density ($F_{1,63}$ = 5.225, P = 0.026). *Colpidium* populations from low nutrient environments evolved significantly higher peak densities relative to populations that evolved in high nutrient environments, but we only observed this evolutionary effect in contemporary high nutrient environments (Fig. 2). One protist population did not fix properly in Lugol's iodine and was excluded from analyses of cell size (n = 79; peak density n = 80). Model fit to both traits was improved without historical microcosm identity ($dAIC_{34}$ = 8.5), so it was excluded from the final model (full statistical results can be found in Appendix S2: Table S1).

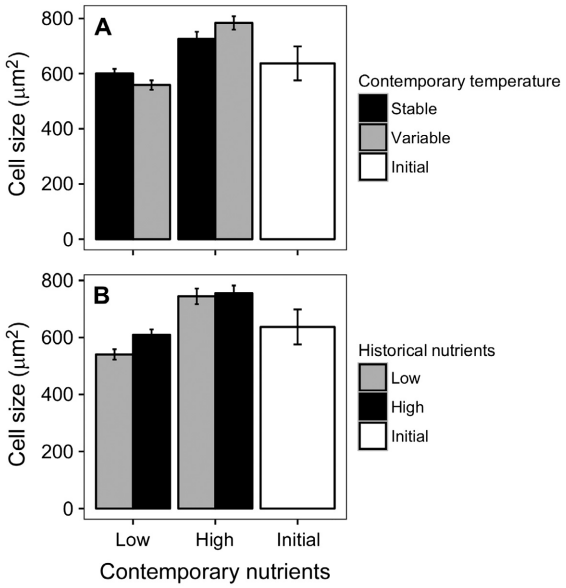

Fig. 1. Effects of (A) contemporary nutrients and temperature and (B) contemporary nutrients and historical nutrients on *Colpidium* cell size (means ± SE). All other fixed effects and interactions were not significant, so the means here were pooled across (A) historical treatments and (B) temperature treatments. Ancestral trait values are shown in white.

Bacterial diversity

Bacterial community composition was explained by three significant three-way interactions between four factors: historical nutrients, historical temperature, contemporary nutrients, and the presence or absence of *Colpidium* (Appendix S2: Table S2). To determine which of these significant effects was most important, we referred to the eigenvalues and eigenvectors for the first five constrained axes of the redundancy analysis (Table 1). The first and second axes were largely explained by contemporary nutrients and *Colpidium*, and the third axis was explained by historical nutrients (Table 1). Collectively, these three axes explained over 92% of the total variance in bacterial community composition. For this reason, we reserve our interpretations here to the first three axes, although historical temperature and contemporary temperature explained most of variation captured by the fourth and fifth axes, respectively (Table 1).

The contemporary nutrient treatment had a significant effect on bacterial community composition (indicated by shape of points; Fig. 3A).

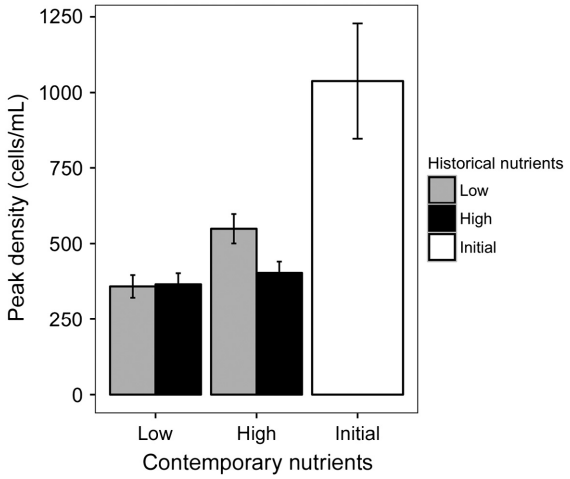

Fig. 2. Effects of historical nutrients and contemporary nutrients on *Colpidium* peak density, measured as peak density (means ± SE). Historical and contemporary temperature and interactions were not significant, so the means represented were pooled across temperature treatments. Ancestral trait values are shown in white.

Samples from the high contemporary nutrient treatment (triangles) showed tight clustering with one another but showed little overlap with samples from the low contemporary nutrient treatments (circles). The presence of *Colpidium* significantly affected bacterial communities, but this effect was more pronounced in communities grown in low contemporary nutrients (indicated by size of circles; Fig. 3A). Historical nutrients explained less, but significant, variation in community composition, associated primarily with the third constrained axis of the ordination (indicated by open or solid points; Fig. 3B).

Through visualization of species scores on a biplot, we found that samples with low first axis values were dominated by *Enterobacter* and those with high first axis values were dominated by *Azospirillum* (Fig. 4). *Enterobacter* was most abundant in eutrophic environments, while *Azospirillum* thrived in oligotrophic environments. More specifically, *Azospirillum* was the dominant taxon found in samples grown in low historical and contemporary nutrient environments with *Colpidium*. Samples with high values along the second ordination axis tended to include bacteria of the family Chitinophagaceae (Fig. 4). Samples in the region of the ordination plot associated with chitinolytic taxa were those grown in low contemporary nutrients without *Colpidium* (Fig. 4). Taxa associated with low values along the second ordination axis, such as *Burkholderia*, were abundant in samples when *Colpidium* was present.

Partitioning ecological and evolutionary effects

In the structural equation model that included *Colpidium* cell size, contemporary nutrients decreased bacterial species richness indirectly (Fig. 5A). An increase in contemporary nutrients resulted in a significant increase in protist abundance ($P = 0.010$), which in turn significantly decreased bacterial species richness ($P = 0.005$). Increased historical nutrients also had a direct negative effect on bacterial species richness that was larger in magnitude than that of protist abundance (Fig. 5A).

In the structural equation model that included *Colpidium* peak density, historical nutrients had both direct and indirect effects on bacterial species richness. Increased historical nutrients decreased *Colpidium* peak density ($P = 0.016$), which in turn increased *Colpidium* abundance ($P = 0.001$) and subsequently decreased bacterial species richness ($P = 0.022$; Fig. 5B). This evolutionary effect was countered by negative direct effects of historical

Table 1. Eigenvectors for the first five axes of the redundancy analysis (RDA) of bacterial community composition using a UniFrac distance matrix.

Parameters	RDA1	RDA2	RDA3	RDA4	RDA5
Proportion variance	0.691	0.133	0.100	0.066	0.009
Contemporary nutrients	−0.703	−0.692	0.133	−0.092	−0.017
Contemporary temperature	−0.031	0.002	−0.01	0.099	0.995
Colpidium	0.705	−0.641	0.291	−0.079	0.028
Historical nutrients	0.107	−0.331	−0.868	0.353	−0.034
Historical temperature	0.048	0.007	0.381	0.919	−0.084

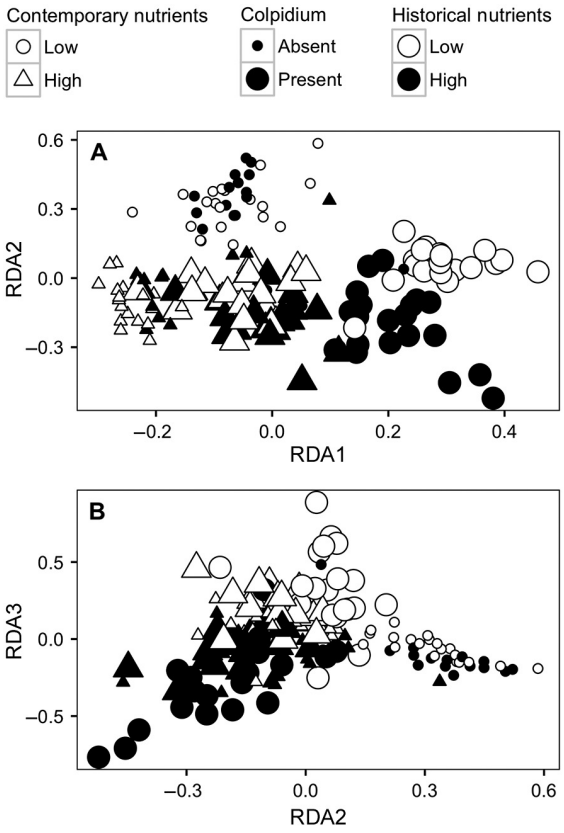

Fig. 3. Ordination plot of bacterial community beta diversity based on weighted UniFrac distances. Each point represents one replicate, and those closer together have a more similar bacterial community. Shown are (A) the first two and (B) first three axes of site scores from the redundancy analysis. Contemporary nutrients are represented by shape, historical nutrients are represented by fill, and *Colpidium* presence/absence is represented by size. RDA, redundancy analysis.

nutrients on bacterial species richness (Fig. 5B). Contemporary nutrients indirectly affected bacterial species richness through two pathways (Fig. 5B). Similar to the model with cell size, increased contemporary nutrients increased *Colpidium* abundance ($P = 0.016$), which decreased bacterial species richness ($P = 0.022$). Additionally, increased nutrients increased *Colpidium* peak density ($P = 0.001$), which increased *Colpidium* abundance ($P = 0.001$) and subsequently decreased bacterial species richness ($P = 0.022$). We found no significant effects of abiotic treatments or *Colpidium* traits or abundance on bacterial abundance and very little of the variation in bacterial abundance was explained by these factors (Appendix S3: Fig. S1).

Discussion

Here, we have provided evidence that protist traits evolve in response to the abiotic environment on ecological timescales (four weeks, or ~150 generations), but the effects of trait evolution were only observed in certain environments. When grown in low nutrient conditions, protists that evolved in high nutrient environments became larger than those that evolved in low nutrient conditions. Similarly, when grown in high nutrient conditions, protists that evolved in high nutrient environments had lower peak density than those that evolved in low nutrient environments. The evolution of peak density affected the abundance of *Colpidium*, which affected bacterial species richness. This demonstrates that evolution of traits in a predator over in response to changes in the abiotic environment can alter the diversity and community composition of the prey community.

Although we also observed plastic changes in protist traits in response to the contemporary

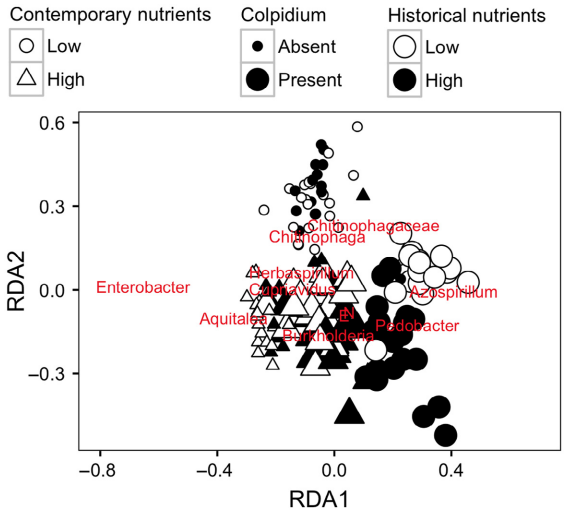

Fig. 4. Biplot of bacterial community beta diversity based on UniFrac distances. Each point represents one replicate, and those closer together have a more similar bacterial community. The positions of red text represent taxa scores for those taxa ("E" = Enterobacteriaceae, "N" = Novosphingobium) taken from the redundancy analysis (RDA).

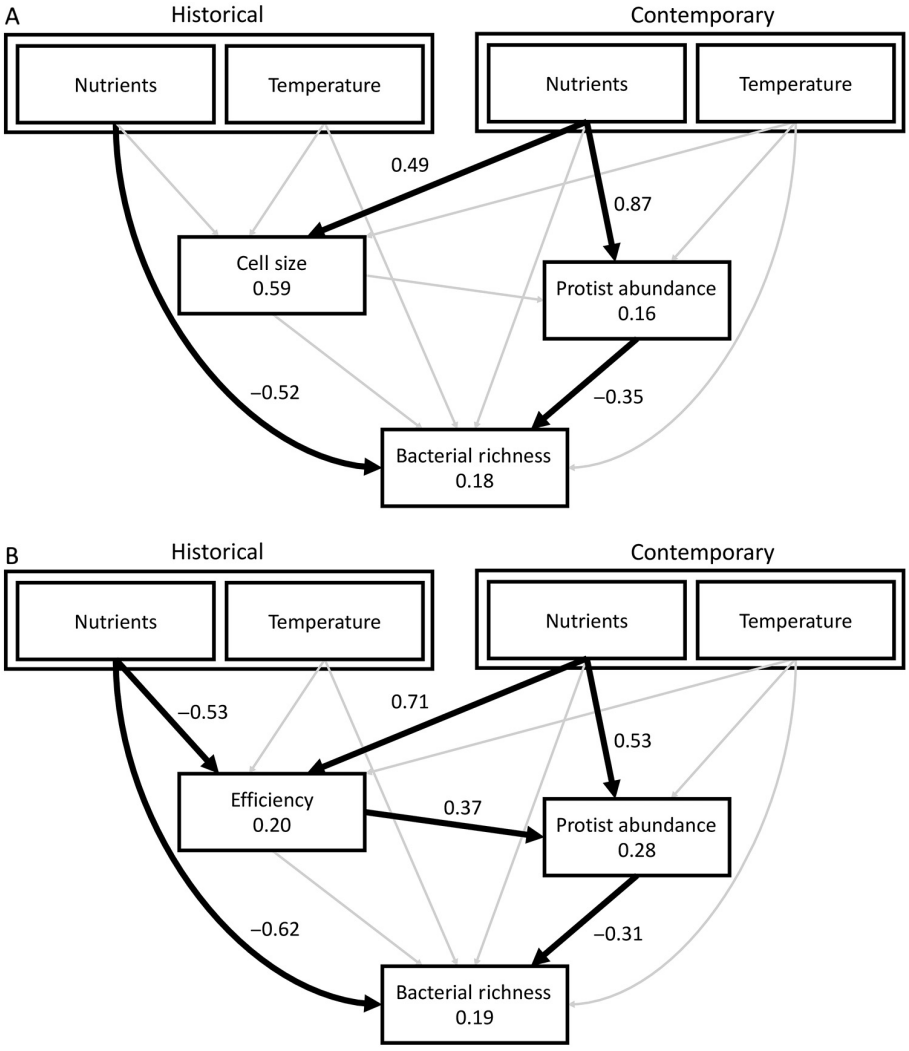

Fig. 5. Structural equation models showing the causal effects of treatments as well as (A) protist cell size and (B) protist peak density and abundance on bacterial species richness. Values inside of boxes are R^2 values, and those besides arrows are standardized coefficients from linear models. Bolded arrows show significant effects, while gray arrows are non-significant.

environment, we found significant evolutionary changes in cell size and peak density. High nutrient environments selected for cells that allocate more resources to cell growth (Fig. 1B) and less to cell division (Fig. 2). These effects of historical nutrients on both traits were measured after 9–18 protist generations in their contemporary nutrient environment, indicating that the evolutionary effects observed here may be conservative estimates if populations evolved back toward their ancestral state. This time period also minimized any plastic or maternal effects that persisted from previous generations, indicating that the trait changes we observed here are due to genetic changes in the population, as traits were measured in common garden environments.

In high nutrient environments, *Colpidium* populations were more dense (Appendix S4: Fig. S1) and likely experienced more intense intraspecific competition (Holdridge et al. 2016). This could be the selective agent driving the increase in cell size in the historical high nutrient environment.

Previous work found that larger protists in this system are better interspecific competitors (Kneitel 2012), so it is possible that this trait is also beneficial in intraspecific competitive interactions. In contrast, low nutrient environments were associated with a relative increase in *Colpidium* peak density, which was likely due to resource limitation in these treatments. These data suggest that cell size and peak density may evolve independently of one another or that different mechanisms of competitive ability (e.g., exploitation vs. interference; Holdridge et al. 2016) are advantageous in different resource environments.

Interestingly, we saw weak or no response to temperature variability in any of the response variables measured. One explanation for this is that the temperature fluctuations used in this experiment were more mild than those experienced by pitcher plant inquiline communities in nature (Holdridge 2015). Protist traits may not have evolved in response to temperature variation because the diurnal cycle of the fluctuations, intended to reflect natural fluctuations that pitcher plant inquiline communities experience, only allows the populations to experience the full range of variation roughly every four generations. The relative infrequency of this pressure would weaken any response to selection that may have occurred under more rapid fluctuations.

One critical assumption of eco-evolutionary feedbacks is that evolutionary effects depend on ecological context (Kokko and López-Sepulcre 2007). Our results support this assumption, as the observed evolutionary effects of nutrients on *Colpidium* traits were dependent on the contemporary nutrient environment. One possible explanation is that *Colpidium* experiences a tradeoff between large cell size and another unmeasured trait, but that this tradeoff only occurs in nutrient-limited environments. Many such tradeoffs among traits only manifest when nutrients are limited and the energetic requirements needed to optimize both traits cannot be reached (Drent and Daan 1980). In contemporary low nutrient environments, *Colpidium* that evolved in low nutrient environments may have produced smaller cells, relative to *Colpidium* that evolved in high nutrient environments, because their cells were already marginally smaller at the start of the reciprocal transplant (Appendix S4: Fig. S2). In this way, the response of cell size to the contemporary environment may be exacerbated by historical conditions. Similarly, we found that the effect of nutrients on the evolution of *Colpidium* peak density was only observed when measured in high contemporary nutrient treatments (Fig. 2). However, in high nutrient environments, it is apparent that *Colpidium* populations that evolved in low nutrients evolved the ability to exploit abundant resources to a larger degree. This indicates that *Colpidium* evolved the ability to better exploit resources in general, and this effect on peak population density is more pronounced when resources are abundant. This suggests that the critical assumption of eco-evolutionary feedbacks tested here—that evolutionary effects depend upon the ecological context in which they are expressed—is a reasonable one for this system.

Unlike previous studies that have documented evolutionary change of protist traits in the pitcher plant inquiline community (terHorst 2010, 2011, terHorst et al. 2010, Miller et al. 2014), we have also demonstrated that evolutionary change can cascade down the food web to affect lower trophic levels. The effect of *Colpidium* on the bacterial prey community depended on the nutrient environment in which *Colpidium* evolved. Our structural equation models suggest that the historical nutrient treatment decreased bacterial species richness directly, which may have occurred through ecological changes in species composition or evolutionary changes within the species comprising the bacterial community (Kearns et al. 2016). However, the historical environment also affected the bacterial community indirectly, via evolutionary changes in *Colpidium* traits (Fig. 5A, B). Specifically, increased nutrients in the historical environment decreased *Colpidium* peak density. Decreases in peak density decreased the abundance of *Colpidium*. Decreases in *Colpidium* abundance increased bacterial species richness. We hypothesize that increased consumption by *Colpidium* reduced the number of bacterial species through prey species preference or variation in species vulnerability to predation (Fenchel 1980, Dopheide et al. 2011).

The effect of *Colpidium* on the bacterial prey community also depended on the ecological context in which that interaction occurred. In low contemporary nutrient environments, the presence of *Colpidium* significantly altered the composition of the bacterial community (Fig. 3A). However, in

the high contemporary nutrient environment, there was a less pronounced difference between bacterial communities grown with or without *Colpidium* (Fig. 3A). This result suggests that nutrient enrichment can mitigate the effects of predators on prey communities (Davis et al. 2010), perhaps because additional resource availability allows prey species that would otherwise be vulnerable to extinction via predation to maintain their abundance in spite of predation pressure. This implies that a tradeoff between competitive ability and predator tolerance may be less severe in enriched environments. This equalizing mechanism thereby minimizes fitness differences between species (Chesson 2000) and results in more similar prey community composition with and without predators in high nutrient environments.

Similar to a previous study in this system using a different protist species (Paisie et al. 2014), we found that protist consumers altered the composition of the bacterial prey community. Bacterial communities grown in low nutrient environments without *Colpidium* had a higher abundance of bacteria from the family Chitinophagaceae (Fig. 4), which is a group known for hydrolyzing chitin (Kämpfer et al. 2011). Chitin is the primary molecule in the exoskeletons of insects, which are the prey of *Sarracenia purpurea*. High abundance of chitinolytic taxa should be beneficial to *S. purpurea* because it would allow for more efficient decomposition of insect prey and faster release of nutrients to the plant itself. The fact that these beneficial bacteria were more abundant when *Colpidium* was absent suggests that *Colpidium* may have a negative effect on the mutualistic relationship between *S. purpurea* and its bacterial inquilines. This provides empirical support for a theoretical model that suggests that protozoa have a parasitic relationship with *S. purpurea* (Mouquet et al. 2008). Bacterial taxa, such as *Burkholderia*, *Pedobacter*, *Azospirillum*, and others that were found in high abundance when *Colpidium* was present (Fig. 4), may be more resistant to the effects of predation by bacterivores due to their larger cell sizes (0.7–6.0 μm). However, these taxa are unlikely to fill the same functional role or provide the same ecosystem service as the Chitinophagaceae. To achieve a more mechanistic understanding of these interactions, future research should focus on the functional metagenome and metatranscriptome of bacterial inquiline communities of *S. purpurea* with and without *Colpidium*, as well as other ciliates that feed on the bacterial community, to determine whether these ciliates reduce the ability of the bacterial community to decompose insect prey.

In this paper, we focused on the changes in protist traits. However, bacterial evolution is an important feature of bacterial communities that could have implications for ecological interactions and community response to environmental change (Bohannan and Lenski 2000). For example, bacterial communities may evolve to be more resistant to grazing by ciliates, which could reduce ciliate growth rates. Furthermore, increases in resistance to grazers or pathogens often come with physiological consequences that could affect bacterial growth rates (Bohannan and Lenski 2000). Bacterial generations are typically shorter than those of protists, so these changes may occur more quickly than changes in protists traits. In addition, bacteria are known to be highly plastic in their traits, especially those relating to metabolism (Justice et al. 2008), which could allow bacteria to respond rapidly to changes in resource availability. The ways in which bacterial traits factor into how communities respond to environmental change is an area of research that is ripe for exploration.

Environmental change is expected to have profound effects on communities, but most hypotheses that attempt to explain ecosystem response to environmental change fail to account for the effects of rapid evolution (Strauss et al. 2008). Our results suggest that evolutionary changes in predator traits in response to both historical and contemporary environments can have large effects on prey communities. Furthermore, these compositional changes to the prey community can affect their functional role in the environment. Without accounting for rapid evolutionary changes, we are likely to fail in predicting the ecological and ecosystem responses of communities to future environmental change.

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Supporting Information

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