Effects of phenotypic variation on consumer coexistence and prey community structure

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Abstract

A common expectation in ecology is that trait variation among individuals from the same species promotes the coexistence of competing species. However, theoretical and empirical studies testing this expectation have been equivocal. One complicating factor is that species exist within multitrophic networks characterized by direct and indirect linkages across trophic levels. It is currently unknown how intraspecific trait diversity at the consumer trophic level affects interspecific consumer competition and population dynamics at lower trophic levels. Here we use an experimental microbial ecosystem consisting of ciliated protozoa, nematode worms, and 24 species of bacterial prey to mechanistically show how intraspecific consumer variation can 1) modulate competitive and niche differences between competing consumers and 2) produce cascading effects on ecological dynamics at lower trophic levels. Although the nematode could competitively exclude ciliates with low trait variation, we found that ciliate trait variation inverted the consumer hierarchy, ultimately excluding the nematode. Competition between the high trait variation ciliate and the nematode altered the temporal trajectories of individual prey species in non-additive ways, mediated by prey traits related to growth rate and defense. We connected the competitive outcome to consumer prey preferences by performing pairwise co-cultures with each consumer and prey species and measuring the clearance of prey biomass. High trait variation in the ciliate increased the mean and variance of the species-specific prey clearance rate over the low trait variation ciliate, which resulted in increased selective foraging behavior. These findings provide a new multitrophic perspective on trait diversity and species coexistence.

Significance

Ecological theory posits that a balance of trait differences influencing competition (competitive differences) and the use of the environment (niche differences) allow different species to coexist in time and space. However, a substantial portion of trait variation exists within species. We manipulated intraspecific trait diversity in a microscopic ciliate competing with a nematode for bacterial prey and found that consumer trait variation inverted the competitive hierarchy that existed under low trait variation. These altered consumer competitive dynamics produced non-additive ecological effects on prey abundance, resulting in prey communities distinct from those forming under either predator individually. Our results suggest that predicting consumer competitive population dynamics and the assembly of prey communities will require understanding the extent of trait variation within consumer species.

Introduction

Comparative studies of species traits have historically focused on trait differences between species. However, ecologists increasingly recognize that a large amount of the trait variation in ecological communities exists between individuals from the same species. For example, trait variation within species accounts for up to one-fourth of total trait variation in plant communities[1] and can have ecological consequences comparable in magnitude to those from separate species[2]. Intraspecific trait variation may emerge from phenotypic plasticity (e.g., maternal effects or learned behavior) and heritable evolutionary change (e.g., intergenerational changes in allele frequencies).

While intraspecific trait variation is ubiquitous in natural ecological communities, its relevance for competitive interactions and species coexistence is unclear. Theory has considered intraspecific variation and species coexistence through the evolutionary lens of character displacement[3], but the purely ecological effects of intraspecific trait variation have historically been ignored. Work built from coexistence theory[4] suggests that the ecological effects of intraspecific variation should hinder species

coexistence and mostly reinforce existing competitive hierarchies[5, 6]. However, other studies have argued that intraspecific variation should promote coexistence - for example, by blending average competitive differences between species, reducing the fraction of individuals that strongly compete, or processes akin to bet-hedging [7–9]. From the perspective of coexistence theory, intraspecific trait variation can have both positive and negative effects on average trait values that drive both niche and competitive differences between species[10]. Therefore, intraspecific trait variation may have complex effects that can both promote and hinder species coexistence[11].

Studies on the effects of intraspecific trait variation have primarily focused on ecological effects at a single trophic level, predominantly with plants[10]. The handful of studies investigating the consequences of intraspecific variation for trophic interactions have concentrated on variable prey defense traits that reduce consumption risk[12]. Very few studies have examined trophic interaction from the perspective of consumers and offensive trait variation (but see [13, 14]). Furthermore, the simultaneous effects of consumer trait variation on trophic interactions and consumer competition are entirely unknown. This is important because most natural ecosystems contain multiple consumer species competing for prey[15]. Furthermore, a fundamental understanding of natural community assembly processes requires adopting multitrophic perspectives that account for indirect and direct linkages spanning trophic levels[16].

Motivated by these knowledge gaps, we conducted an experiment to determine 1) how intraspecific consumer trait variation influences the competitive dynamics of two consumer species and 2) how intraspecific trait variation and consumer species composition impacts bacterial species diversity at the prey trophic level (Fig. 1). We combined a 24-species isogenic bacterial prey community (Table S1) with an isogenic nematode worm, *Caenorhabditis elegans*, and an isogenic ciliated protozoan *Tetrahymena thermophila* - hereafter Low Trait Variation (LTV) ciliate. In some treatments, we replaced the LTV ciliate consumer with an even mixture of 20 different ciliate populations, each displaying high intraspecific phenotype variation - hereafter High Trait Variation (HTV) ciliate[17]. In this way, we could manipulate the standing stock of trait diversity in the ciliate consumer in the presence or absence of competition from the nematode. We then combined the LTV ciliate, the HTV ciliate, the nematode, and the prey bacterial community in experimental microcosms with a full-factorial design (six conditions) and followed consumer densities, prey density, and bacterial community composition over 60 days. We also measured key functional traits from both prey and consumer species to determine how community assembly and consumer composition were related to species phenotypes.

Results

Consumer trait variation flips the consumer competitive hierarchy The magnitude of trait variation in the ciliate consumer was sufficient to alter ecological dynamics of the competing consumer. Each consumer by itself (nematode, LTV ciliate, HTV ciliate) could feed and grow successfully on the bacterial prey community (Fig. 2A), and ciliate trait variation did not significantly alter the carrying capacity for the ciliate in the absence of competition (Table S3). Prey biomass differed by less than twofold across all consumer treatments (mean fold difference of 1.08, Table S2), indicating that each consumer or consumer pair drew down total prey resources to similar levels. The ciliate and nematode grew from starting densities of 10^4 and 10 individuals ml^{-1}, respectively. In the absence of competition, the LTV ciliate, the HTV ciliate, and the nematode stabilized at $4.2 \pm 2.3 \times 10^4$, $5.0 \pm 2.3 \times 10^4$, and $6.0 \pm 2.5 \times 10^3$ individuals ml^{-1}, respectively, after an exponential growth period of about 17 days (Fig. 2A). However, the density of each consumer was strongly dependent upon the competitive arrangement between species (Tables S3-S4). Competition between the LTV ciliate and the nematode excluded the ciliate from the microcosms by day 11, and by day 24, the nematode density had reached competition-free densities. However, competition between the HTV ciliate and the nematode flipped the consumer hierarchy, ultimately excluding the nematode but promoting longer species coexistence.

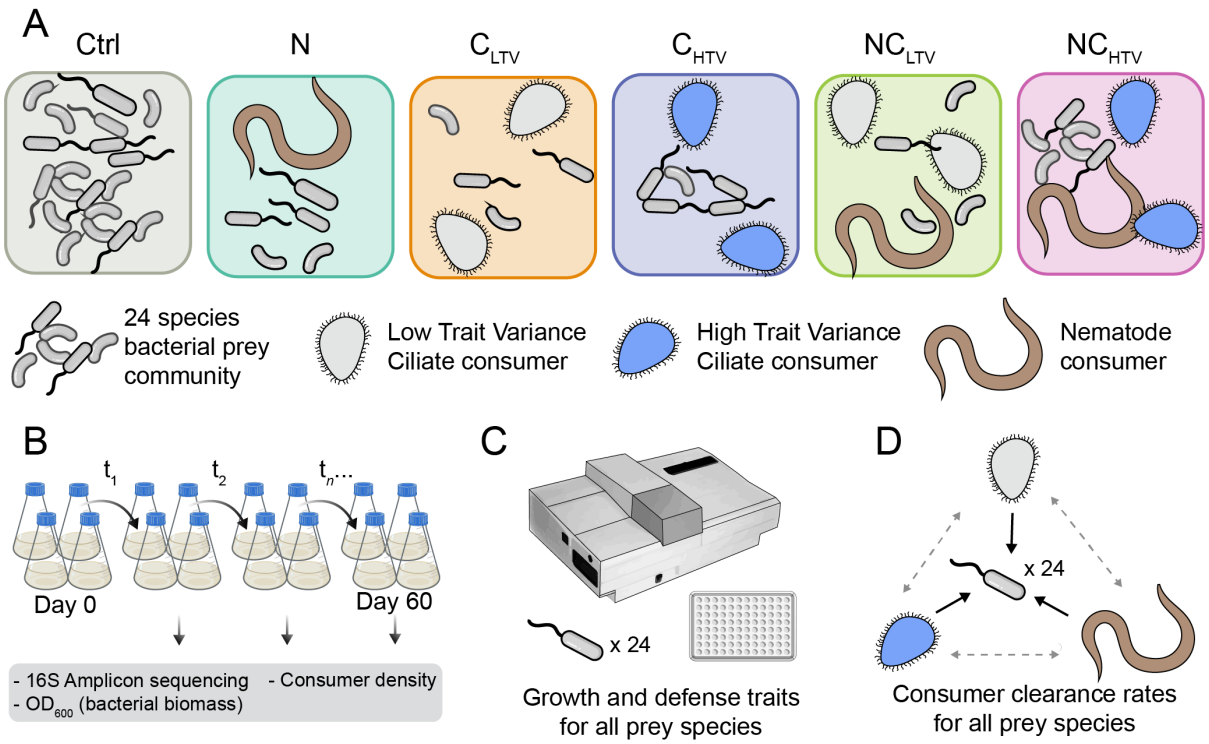

Figure 1. textbfStudy setup

A) The experimental bacterial community with different consumer combinations. Ctrl - bacteria only, N - bacteria with nematode, C_{LTV} - bacteria with low trait diversity isogenic ciliate, C_{HTV} - bacteria high trait diversity mixed ciliate populations, NC_{LTV} - bacteria + nematode + low trait diversity ciliate, NC_{HTV} - bacteria + nematode + high trait diversity ciliate. **B)** Schematic representation of the serial transfer experiment. Each experimental condition from A) was performed in four biological replicates and transferred/sampled every four days. **C)** Traits of individual bacterial species were measured in monoculture and inferred from genome sequences (see methods). **D)** Consumers were grown on each bacterial species to assess consumer grazing efficiency (see methods).

We next attributed consumer dynamics to changes in niche differences and competitive differences following coexistence theory. Coexistence theory describes the coexistence of two species as a balance between stabilizing and equalizing mechanisms[4]. Equalizing mechanisms relate to inherent competitive differences between species that describe dominance hierarchies in a community. Stabilizing mechanisms (i.e., niche differences) relate to the ability of each species to recover from low density after perturbation - e.g., when species can use the environment in different ways to avoid competition. Niche and competitive differences can be quantitatively estimated by fitting population density data to a model of competitive population dynamics, the parameters of which represent within-species and between-species interactions[18]. Using consumer density data and coefficients derived from the model we estimated niche differences ($1 - \rho$) and the competitive differences ($k_{ciliate}/k_{nematode}$) between the HTV or LTV ciliate and the nematode (see methods). Results indicate that relatively strong niche differences separated the LTV ciliate and nematode, but these differences were more than compensated by a strong competitive advantage for the nematode (Fig. 2B). Surprisingly, the model predicted the LTV ciliate and nematode were at or near the boundary of coexistence. In both HTV and LTV competitive arrangements we observed very low abundances of the inferior competitor (< 100 ciliates ml^{-1}, < 5 nematodes ml^{-1}) in some of the replicates near day 40. Accurate manual counting is challenging at these low densities, and it may be that the inferior competitor never truly became extinct. Finally, high trait variation in the ciliate strongly increased competitive differences between the ciliate and nematode while simultaneously *decreasing* niche differences. The magnitude of the competitive change was much larger than the niche change, which suggests that high trait variation in the ciliate predominantly drove equalizing competitive

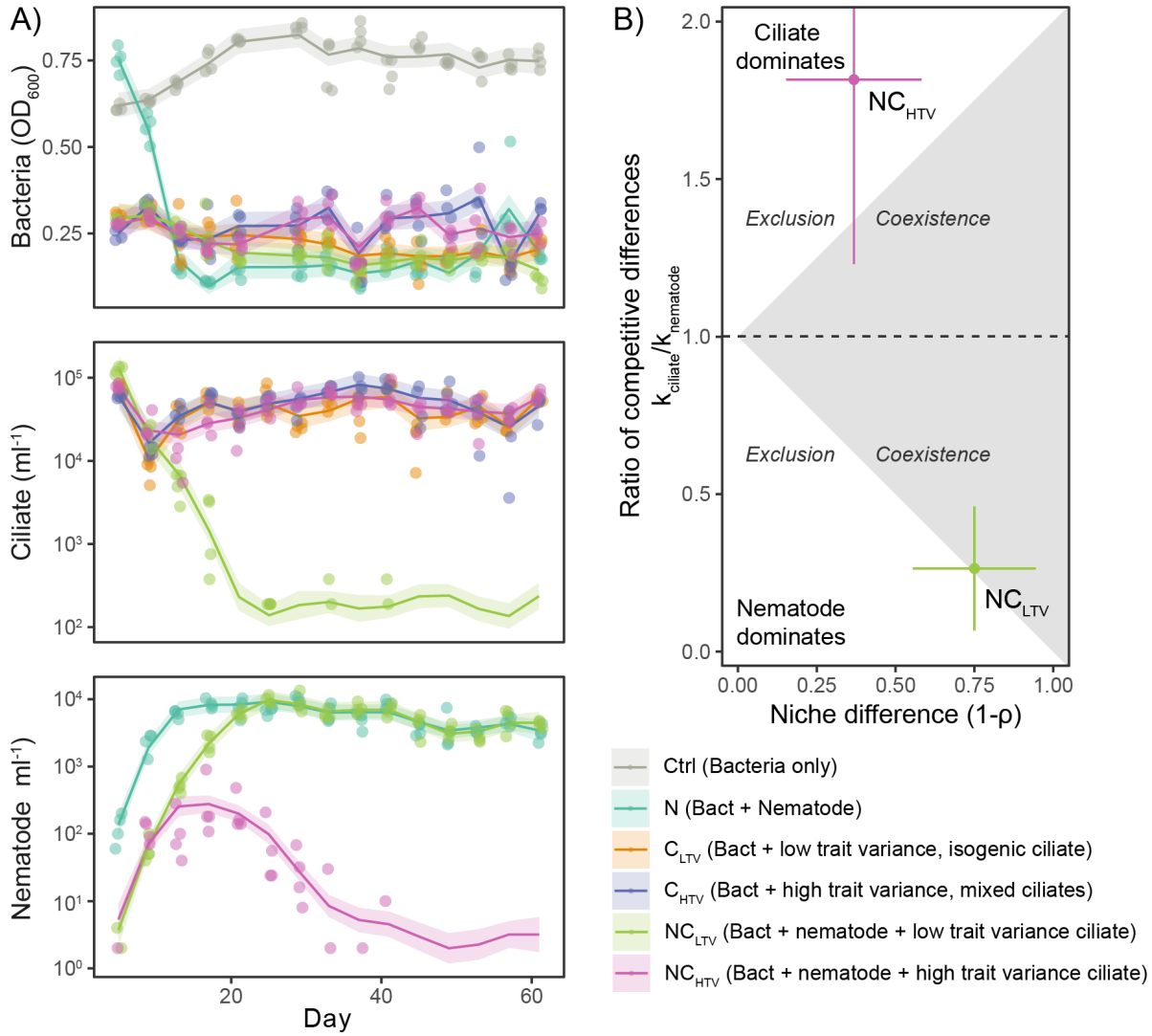

Figure 2. Consumer competitive hierarchy depends upon ciliate trait variation
A) Consumer and bacterial biomass during the course of the experiment. Points are observations, lines are estimated marginal means (mean response and 95% confidence levels adjusted for all other variables) from generalized additive models. Model summaries and contrasts of marginal means are available in Tables S2-S4). **B)** Niche and competitive differences between the ciliate and the nematode depending on whether the ciliate had low trait variation (NC_{LTV}) or high trait variation (NC_{HTV}). Coexistence (grey region) occurs when when stabilizing niche differences ($1-\rho$) are greater than competitive differences ($k_{ciliate}/k_{nematode}$). Coexistence is defined by the inequality $\rho < k_{ciliate}/k_{nematode} < 1/\rho$. Points and line ranges shows the mean and standard deviation of niche and competitive differences from four biological replicates.

differences between competitors rather than stabilizing niche differences (Fig. 2B).

Prey community assembly follows consumer dynamics: Our experimental setup allowed us to investigate the prey community response to consumer trait variation. We first asked how consumer trait variation influenced the repeatability of prey responses across biological replicates. Overall, the prey species abundance distribution across all populations/treatments followed the canonical form[19] with a handful of abundant species and many rare species (Fig. 3A). We quantified the repeatability of prey

communities by calculating the scaled Shannon diversity dissimilarity (D') across treatments at each sampling point. Replicate prey communities were highly consistent in composition across all consumer combinations and time (Fig. S1, Table S5). These results show that 1) prey communities generally assembled under each treatment condition so that the majority of prey species were represented by the minority of sequencing reads, 2) technical variation in laboratory conditions (e.g., temperature or transfer volumes) were minimal for these experiments, and 3) the process of prey community assembly alongside each consumer or consumer pair was strongly deterministic.

Next, we asked how consumers affected both the local abundance and evenness of prey species present in each treatment (i.e., alpha diversity). Generally, we observed a rapid peak and then monotonic decline of the rarest species during the first 15 days prior to the stabilization of the consumer (Fig. 3B). In the last 30 days, these rare species would briefly increase in abundance above 0.01%, then rapidly decline. The more common species generally established themselves at high abundances ($> 10\%$) after 14 days when both total consumer and prey densities had stabilized. Afterwards, these common species displayed dynamic peaks and declines for the remainder of the experiment. We next noticed that prey Shannon diversity varied in two distinct phases across treatments, which we then operationally defined using multiple change point regression (Fig. S2). Prey Shannon diversity declined linearly with time in the first phase - hereafter sorting phase - which was followed by oscillation around a temporally zero-trend mean in the second phase - hereafter equilibrium phase (Fig. 3B, Supplementary methods). We found that the presence of a a consumer or co-consumer pair slowed the rate of Shannon diversity decline in the sorting phase relative to the control, with the nematode slowing decline by the largest magnitude on average (Fig. S3, Table S6). In the equilibrium phase, predation generally supported higher mean Shannon diversity, with the exception of the LTV ciliate, which decreased prey diversity to the lowest mean value of all treatments (Tables S7). Consumer competition did not change either the rate of diversity decline in the sorting phase or the mean diversity in the equilibrium phase relative to consumer monocultures. Thus, the effect of two competing consumers on the Shannon diversity of the prey community was generally equivalent to that of a single predator.

We then examined how the differentiation of prey community composition (i.e., beta diversity) changed between consumer treatments and through time. We used ordination plots to visualize the temporal trajectory of each community through system phase space (i.e., community space) and to quantify relative state changes under each consumer treatment[20]. The LTV ciliate prey community traversed the longest distance in community space followed by the no-consumer control and then the combination of the LTV ciliate and nematode (Fig. 3C). The prey communities assembling with only the HTV ciliate or the nematode both traversed the smallest distance and had significantly smaller jumps through community space than the no-consumer control and the LTV ciliate (Table S8). Thus, the HTV ciliate or the nematode rapidly stabilized the prey community in a relatively small region of community state space. Alternatively, the LTV ciliate appeared to drive the prey community across a wider range of community states similar to the no-consumer treatment. The effect of two consumers was generally to reduce the distance traversed in prey community from the no-consumer control, but not to the extent of the HTV ciliate or nematode alone.

Consumer trait variation results in non-additive effects on prey abundances: We next asked how ciliate trait variation affected the response of individual prey species to the presence of multiple predators. In animal communities[21, 22], the effects of multiple predators on prey often do not combine additively but result in higher (risk reduction) or lower (risk enhancement) prey abundances than would be predicted by a linear combination of individual predator effects. These emergent multiple predator effects[15] cannot be predicted based on the knowledge of the effects of a single predator on prey but are dependent upon the context of the consumer arrangement. We used additive longitudinal Gaussian process regression[23] to decompose prey abundance trajectories into components corresponding to the effect of each consumer and a statistical interaction (i.e., an emergent multiple predator effect) between consumer types.

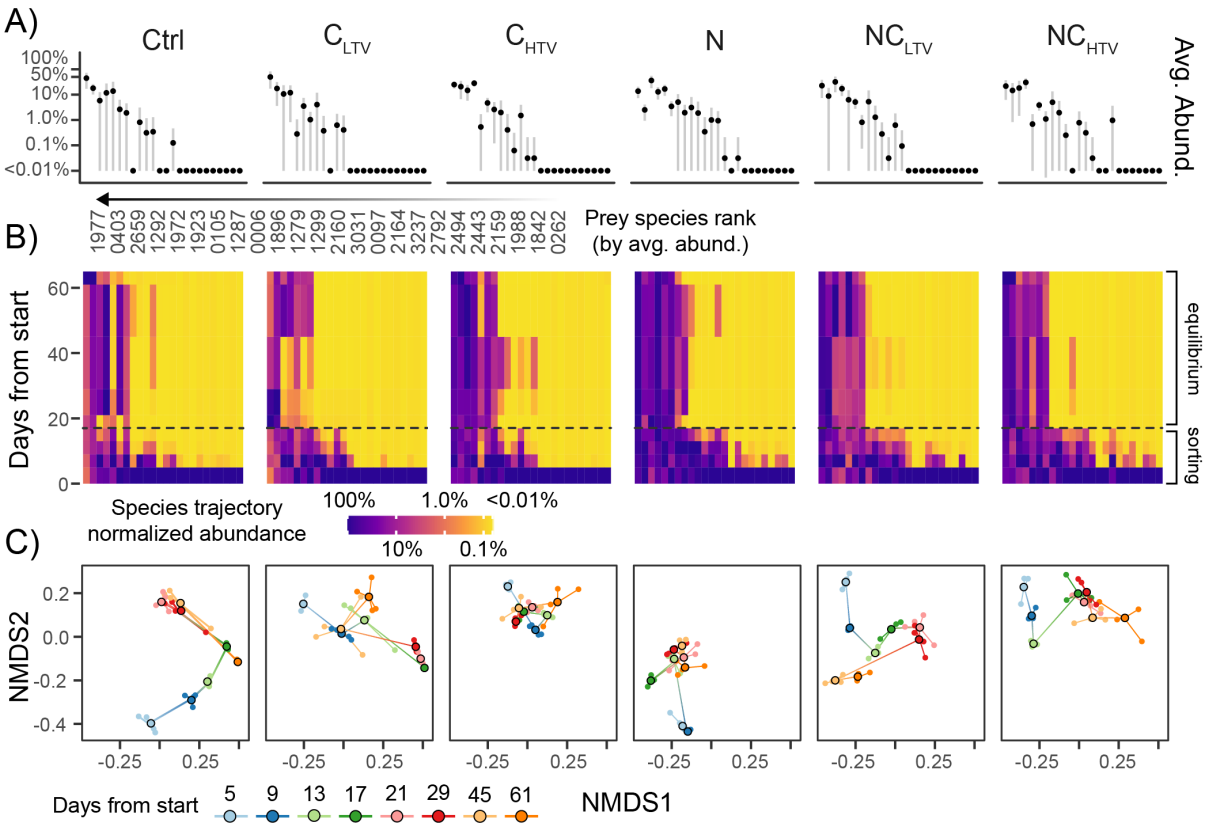

Figure 3. Prey community response to consumer trait variation

A) Prey species abundance distributions from each experimental treatment. X-axis ranks prey species (1977 most abundant) by average across all conditions. Y-axis shows relative abundance. **B)** Replicate mean abundance trajectories over time (normalized to each species maximum) for each of the 24 bacterial prey species. **C)** Non-metric multidimensional scaling ordination of trajectories through community space. Larger, outlined circles show means across replicates, while small circles are replicates. Colors represent days from the experiment start. Means are connected in chronological order.

We found that different consumer combinations resulted in significant non-additive effects - i.e., emergent multiple predator effects - for both abundant and rare prey species (Fig. S4). The effects were generally strongest when the HTV ciliate was paired with the nematode. For example, the ciliate had the largest predicted effect on *Aeromonas caviae* 1972 during exponential growth of the consumer (first 20 days) and before the ciliate density had stabilized (Fig. 4). The nematode had the largest predicted effect on *A. caviae* 1972 after its exponential growth phase (30 days). The model did not support the existence of a significant statistical interaction between the LTV ciliate and nematode - i.e., the effect of the combined consumer arrangement was effectively the sum of the two individual consumers. However, competition between the HTV ciliate and nematode resulted in a substantial risk enhancement for *A. caviae* 1972 (Fig. 4), which explained the greatest proportion of variance in the model. In the case of *C. testosterone* 0403, there was a significant but small non-additive effect between the LTV ciliate and nematode, but the greatest amount of variance was explained by the statistical interaction between the HTV ciliate and the nematode model terms. Surprisingly, this effect was persistent after the nematode was effectively excluded from the microcosm (Fig. S5), implying that prior dynamics from the co-consumer arrangement influenced the state of the microcosms long after one consumer had been competitively excluded. Finally, we also observed instances where a prey species could only exist in the presence of two consumers but not with either consumer alone. For example, the rare species *Niabella yanshanensis* 3031 only reached appreciable abundance in the presence of both the HTV ciliate and nematode, making it

impossible to predict the longitudinal trajectory of this strain from individual consumer effects or the effect of trait variance alone (Fig. S6). Thus, the dynamics of *Niabella yanshanensis* 3031 were an emergent property of the HTV ciliate/nematode system and provide a concrete example of how trait variation at one trophic level can have nonlinear ecological effects on a lower trophic level.

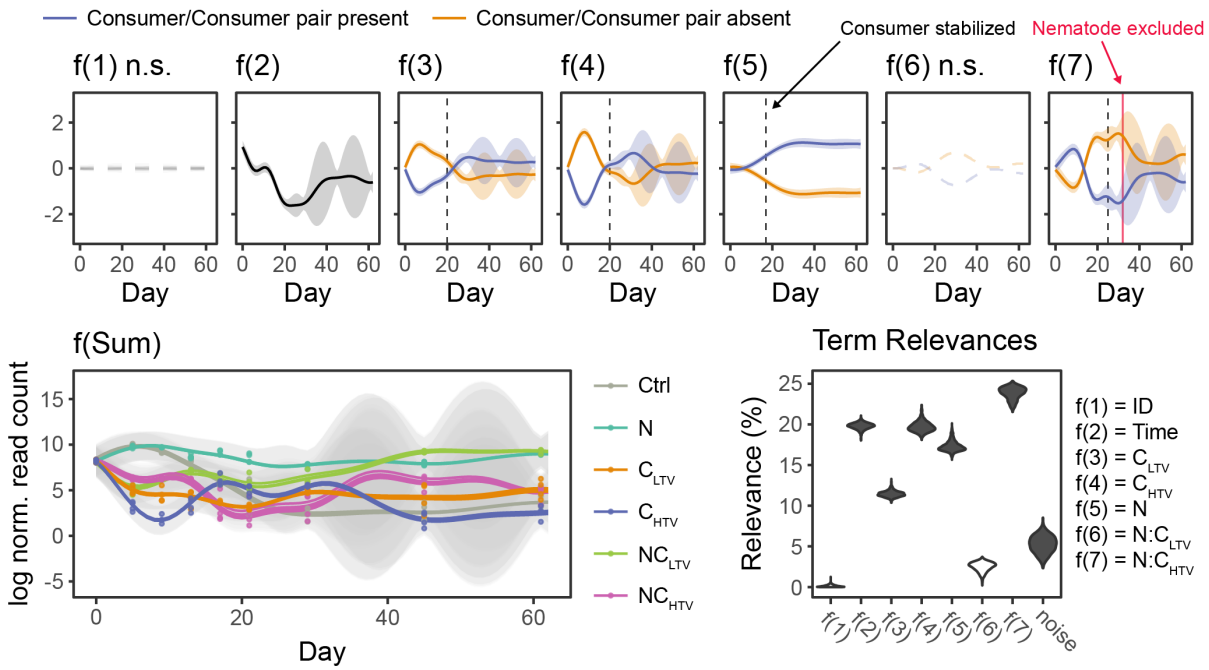

Figure 4. Nonadditive effects of consumer trait variation and consumer competition on prey trajectories

A) f(1) - f(7) are decomposed function components inferred from longitudinal Gaussian process regression for the abundance of *A. caviae* 1972. Components include a microcosm-specific deviation from the shared time effect f(1) and the shared time effect f(2). The remaining components are deviations from the shared time effect and include the low trait diversity ciliate specific effect f(3), the high trait diversity ciliate effect f(4), the nematode effect f(5), an interaction effect for low trait diversity ciliate and nematode f(6), and an interaction effect for high trait diversity ciliate and nematode. Red and dashed lines show when a consumer was excluded or stabilized as in Fig. 3. N.S. indicates that the term explained insufficient variance to be selected in the final model. The sum f(Sum) of the decomposed components is shown in the bottom row. Shading is 2x std. deviations from the posterior mean. Solid lines are posterior means, and points are experimental observations. Term relevances are shaded if combined with noise they explain over 95% of variance of the data.

Overall, the consumer arrangement had an important effect on the abundance trajectories for individual prey species and frequently manifested as non-additive emergent multiple predator effects. Different consumer combinations produced a distinct compositional succession of both rare and abundant prey species relative to the control (Fig. 3), even though bacteria and consumer densities were similar across treatments (Fig. 2). Most prey species displayed multiple predator effects due to the presence of both the nematode and ciliate, but usually, the relevance of one of these interaction terms to the model was significantly larger than the other (Fig. S4). Thus, risk modifying emergent multiple predator effects were universal to this ecological system, but the magnitude and direction of these effects could be modulated at the prey level via consumer trait diversity and competition.

Prey community response to consumer trait variation is mediated through defense traits: We next asked how consumer trait variation and competition influenced community-scale prey responses in the microcosms. We used a joint species distribution model (JSDM) framework[24] to simultaneously

evaluate prey species and community responses to different consumer competitive arrangements. We modeled the sorting and equilibrium phases separately to account for the strong temporal dependence in the sorting phase (supplementary text). The average explanatory power was excellent for the sorting phase ($R^2 = 0.90 \pm 0.09$) due to the strong species changes with time (Fig. 5A). In the equilibrium phase, the average model power was lower ($R^2 = 0.58 \pm 0.19$) but still appreciable. Models of both phases showed the most predictive power through their fixed effects, with random effects contributing largely in the equilibrium phase due to temporal oscillations around the mean (Table S9). Variance partitioning over the explanatory variables included in the models showed that the fixed effects explained a substantial amount of variation in the abundance model. Time and treatment-specific differences accounted for most variation in the sorting phase, which reflected 1) the rapid decline in abundance of the rare species under all treatments and 2) the condition-specific responses of the more abundant species (Fig. 5A). The direction of these effects generally reflected the same patterns we observed from the individual species models. Specifically, ciliate evolution generally strengthened the non-additivity of multiple consumers on prey abundances (supplementary text).

Our primary goal with the JSDM was to obtain a mechanistic understanding of the prey community assembly process by identifying specific prey traits associated with the different consumer arrangements. We used the JSDM framework to ask whether phylogenetic relationships and prey traits were associated with specific community responses to the different consumer arrangements. This is important as bacterial taxonomy is an important determinant for colonization success of the *C. elegans* intestine[25], certain bacterial taxonomic classes are associated with different stages of *C. elegans* growth in the wild[26], and prey trait variation affects prey coexistence under *T. thermophila* predation[27]. In the final JSDMs, we included traits for defense, growth rate, diversity of carbon compounds used, and biofilm formation. These particular traits represented approximately 80% of the variance from the total collection of traits acquired for the synthetic community (Fig. S7). The estimated posterior of phylogenetic strength was 0.05 ± 0.11 in sorting and 0.32 ± 0.34 in equilibrium with the 95% credibility interval including zero for both phases. Thus, closely related bacteria were no more likely to share a common response to the experimental consumer arrangements than distant relatives. In contrast to phylogeny, prey traits explained a substantial amount of variation in both experimental phases (Table S10, Fig. 5C,F), indicating a shared phenotypic response of the prey community under each treatment. In the sorting phase, the diversity of carbon compounds used was positively associated with the average community response to the LTV ciliate, which reflected the dominance of *Morganella morganii* 1292 and *Pseudomonas chlororaphis* 1977 - the two strains that could use the most carbon compounds. However, carbon use versatility was negatively associated with changes in prey abundance with the nematode, which mirrored the higher species diversity supported by the nematode Fig. 5C). Prey defense and biofilm traits were consistently associated with community responses in both the sorting and equilibrium phases. These traits were mostly associated with the community response to the HTV ciliate either alone or through an interaction with the nematode (Fig. 5C,F). Overall traits explained 22% and 44% of the variation in species abundances in the sorting and equilibrium phases, respectively. Traits explained the greatest proportion of the community response to the HTV ciliate and the combination of HTV ciliate and nematode (Table S10).

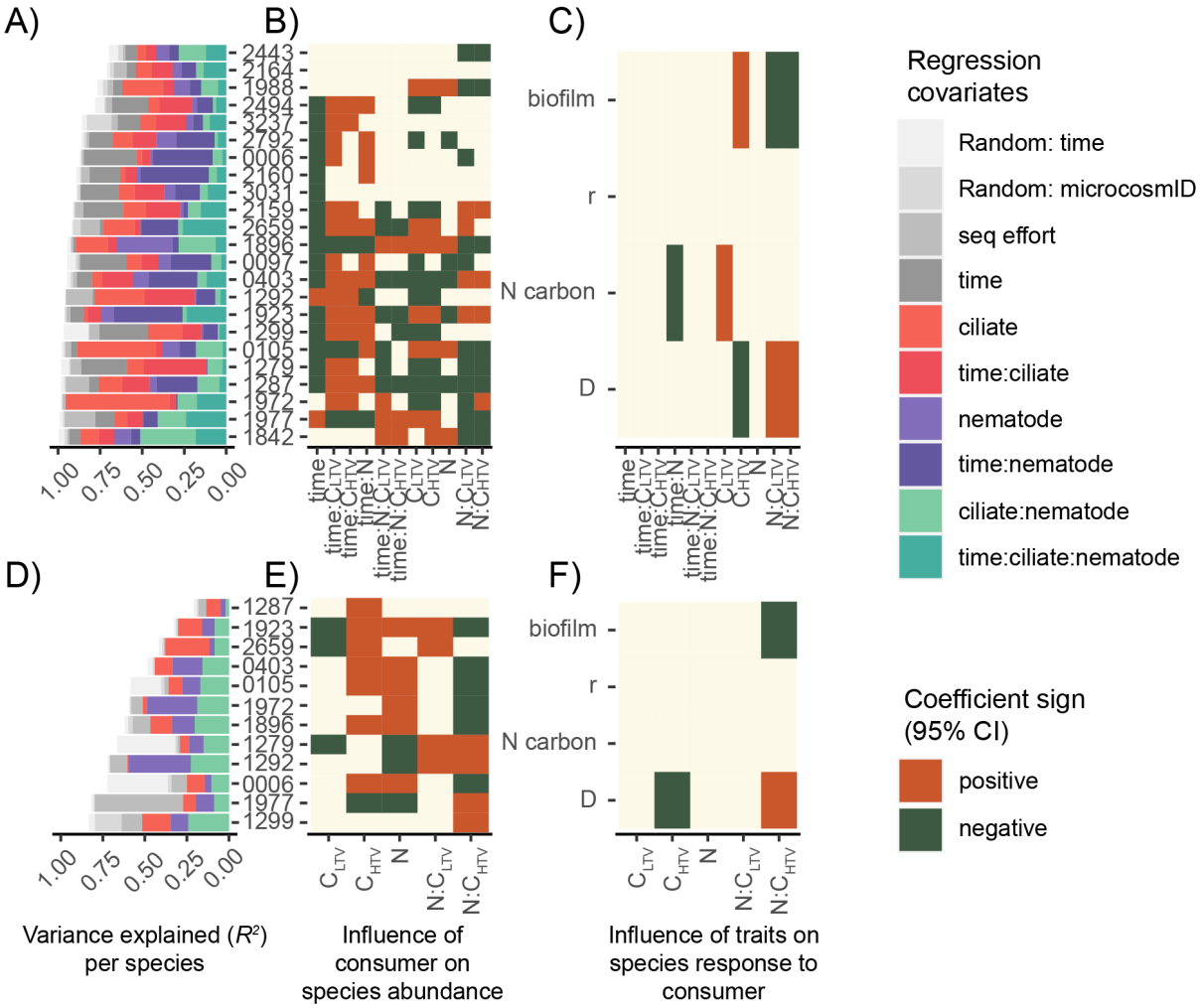

Figure 5. Determinants of prey community assembly

JSDM results from **A-C)** the sorting phase (days 0-13) and **D-F)** the equilibrium phase (days 17-61). **A** and **D** show the explanatory power for each bacterial prey species in the JSDM. For each species, the bars are colored by the proportion of variance explained by fixed (i.e. consumer evolution and/or co-consumer competition, etc) and random effects (time, sequencing effort, etc) and their interactions. **B)** and **E)** show the influence of fixed effect covariates on bacterial prey species. **C)** and **D)** show the influence of prey traits on bacterial prey species association with fixed effects from the JSDM. The sign of the coefficient (i.e the direction of the effect) is brown if positive and green if negative. Coefficients are included only if the 95% credibility interval excludes zero (i.e the probability the effect is not zero is 95%). **D)**, defense against C_{LTV}; N carbon, number of carbon sources the strain can use; r, maximum specific growth rate; biofilm, biofilm production.

The JSDM results were generally consistent with a growth/defense trade-off scenario in the prey community. Rapidly growing, generalist prey species (1977 and 1292) quickly dominated the community in the absence of a predator and with the LTV ciliate even though these species were relatively undefended against the LTV ciliate (Fig. S6). The most defended prey species were less abundant with the HTV ciliate than with the LTV ciliate, presumably because the HTV ciliate was better adapted to consume these prey species[17, 27]. However, biofilm-forming prey species were more abundant with the HTV ciliate, potentially because it was poorly adapted to consuming prey biofilms. Although biofilm formation is one specific defense mechanism against protozoan grazing, bacterial defense against consumers ranges from motility to toxin production to cell surface masking[28]. Surprisingly, defended prey were more abundant than would be predicted from the summed effects of individual consumers in the presence of the HTV ciliate and nematode, while the opposite was true for biofilm-forming prey. In the sorting phase, this pattern also held for the interaction between the HTV ciliate and nematode. This may reflect prey risk enhancement and reduction from predation by multiple consumers[15]. For example, the nematode may have been able to mechanically disrupt prey aggregates while foraging, subsequently rendering these species more vulnerable to the HTV ciliate. Alternatively, nematode foraging behavior may have induced new traits or modulated existing traits in well-defended prey species that also increased resistance to the HTV ciliate. Overall, the model revealed that prey traits were key components mediating the prey community response to both consumer evolution and interspecific competition.

Ciliate trait diversity alters prey preference: We next asked whether the competitive outcome between consumer pairs was correlated with differences in specific consumer traits. We experimentally assayed the ability of each consumer to clear prey biomass in pairwise grazing trials with each of the 24 bacterial strains (Fig. 6A). We defined prey clearance as the ratio of removed bacteria to consumer individuals at the end of a 144-hour incubation for each consumer/prey species combination (see methods). The nematode had the highest prey clearance ($7.6 \pm 5.3 \times 10^4$, observed median and std. dev.), followed by the HTV ciliate ($7.1 \pm 9.0 \times 10^3$), and the LTV ciliate ($2.7 \pm 1.6 \times 10^3$). Indeed, trait variation significantly increased prey clearance by the ciliate, while the nematode was the most efficient consumer overall (Table S11). The coefficient of variation of prey clearance was smallest for the LTV ciliate, then the nematode, and then the HTV ciliate ($CV_{LTV} = 0.59$, $CV_N = 0.70$, $CV_{HTV} = 1.25$), reflecting a range of per species prey clearances for each consumer. Overall, the consumers displayed a hierarchy of prey clearance rates: 1) the LTV ciliate removed the fewest prey cells per capita with the most uniform clearance rates across prey species, 2) trait variation significantly increased ciliate feeding efficiency on some prey species, which increased prey clearance variation overall, and 3) averaged across all prey species the nematode removed the most prey per capita but also grazed on some species more efficiently than others.

We might expect greater relative variation in consumer prey clearance to manifest as more uneven foraging across prey species in a community context. In the short term, a consumer with the most uniform clearance rates should remove each prey species in proportion to their abundance in the community. The consequence would be lower total prey biomass but a prey composition with greater resemblance to the prey community without consumers. Alternatively, a consumer displaying nonuniform prey preferences should remove prey in a way that deviates from this proportionality[29, 30]. This approach assumes limited indirect consumer effects, but this assumption should hold during exponential growth (sorting phase) when resources should not limiting for prey or consumers[31]. We estimated consumer foraging preferences from sequencing data by regressing the relative abundance of bacterial species in the presence and absence of each consumer across all time points in the sorting phase of the experiment. We found that the slope of this relationship was largest for the LTV ciliate(Fig. 6B), consistent with it having the smallest relative variation in clearance across all prey species. Both the slopes for the HTV ciliate and nematode were significantly smaller than the LTV ciliate slope suggesting that these consumers foraged on some higher abundance prey species preferentially (Table S12).

Our results suggest that trait variation in the HTV ciliate increased both per capita prey removal and

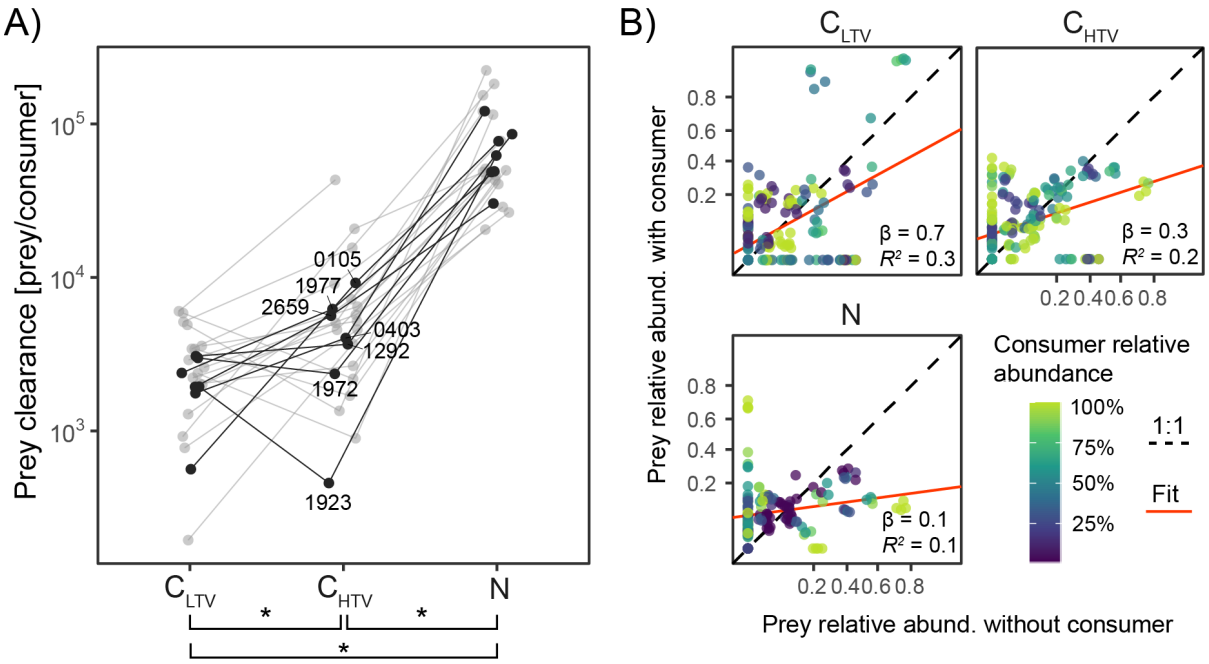

Figure 6. Consumer trait variation alters consumer prey preference
A) Distribution of prey clearances on the 24 different bacterial prey species. Prey clearance is the number of bacterial cells removed per consumer individual after 144 hours. Species (connected by lines) in black/labeled are those with > 1% mean relative abundance across consumer arrangements. Asterisks show significant differences in mean prey clearance between consumers (Table S11). B) Relative abundance of prey species in the presence of ciliate or nematode consumers (vertical axis) versus the corresponding relative abundance in the consumer-free controls. The regression includes prey species > 1% relative abundance and samples from first 21 days. The axes are arcsin square-root transformed. β, R^2 is the median for the posterior of the slope and Bayesian R^2, respectively from linear regression (Table S12).

selective feeding behavior over the LTV ciliate. What physiological differences might account for this outcome? Ciliate individuals from the HTV populations were, on average, 40 μm larger, 1.25× faster, and turned less often than isogenic, LTV ciliate individuals[17]. These traits primarily influence the "search phase" of prey capture[32] because they influence the search volume covered by each consumer. We speculate that altered body size and swimming speed account for the greater average prey clearance of the HTV ciliate relative to the LTV ciliate (Fig. 6A). The average *C. elegans* individual is over 100 times the volume of a *Tetrahymena* cell (BNIDs: 114967, 101424) [33], which likely explains why it removed about an order of magnitude more prey cells per capita than the ciliate (Fig. 6A). The physiological mechanisms driving the apparent shift in prey preference of the HTV ciliate are more difficult to explain. Prior work has demonstrated that both *C. elegans* and *Tetrahymena* ciliates can selectively forage for optimal prey[34–36]. Ciliate selective foraging occurs through multiple mechanistic steps ranging from searching to prey handling to prey digestion[32], any of which may have been affected by trait variation in the HTV ciliate.

Discussion

Many studies (e.g., discussed in [8, 10]) have focused on how the ecological effects of intraspecific trait variation should promote species coexistence. Our results suggest that trait variation in a single competitor (here, the LTV ciliate) can result in competitive exclusion. Following coexistence theory, we

estimated that the inversion of the competitive consumer hierarchy was due to the development of greater niche overlap between competitors and substantial competitive differences that favored the HTV ciliate over the nematode. We confirmed this prediction by measuring prey clearance rates for each consumer and morphological trait variation in the HTV and LTV ciliate. We found that 1) the nematode consumed the most prey per capita, 2) the HTV ciliate displayed both a broader range of clearance rates across prey species and a higher mean prey clearance than the isogenic LTV ciliate, 3) on average, the HTV ciliate was larger, faster, and turned less than the LTV ciliate[17] which likely allowed HTV individuals to forage across a larger habitat volume, and 4) the HTV ciliate and nematode suppressed the growth of the most abundant prey species from the no-consumer control while the LTV ciliate could not. We speculate that changes in ciliate morphological traits and prey preference increased the relative strength of competition with the nematode, while the convergence of HTV prey preferences towards the most abundant prey species also reduced the niche difference.

Consumer trait variation also strongly influenced the community composition of the prey trophic level. The HTV ciliate or the nematode rapidly stabilized the composition of the prey community, while the LTV ciliate and no-consumer treatment caused the prey community to make the largest oscillations through community state space (Fig. 3C). The combination of two consumers caused the prey community to traverse state space in a way that was distinct from either consumer alone. Indeed, we found that the combined effects of the ciliate and nematode on individual prey species were often non-additive - i.e., the combined effects of the consumers were not simple linear extrapolations of their individual effects. Emergent multiple predator effects are well-known for macroscopic predators and their prey[15] but have not been documented at the microbial scale. Importantly, we also found that trait variation in the ciliate significantly altered the magnitude of non-additive consumer effects across prey species. We also found that prey community assembly in the presence of consumers was associated with distinct prey traits related to consumer defense and resource use. Thus, prey communities may assemble partly due to how consumer trait heterogeneity and consumer competition operate on the distribution of defense traits present in a prey community. Consumer trait diversity generally strengthened the non-additivity of how prey defense traits influenced prey community assembly in the presence of the competing consumer. This suggests that the role of trait variation at one trophic level may have complex downstream effects on other trophic levels in the wild. The number and magnitude of these nonlinear relationships may be critical feedbacks stabilizing the coexistence of many bacterial prey species in natural microbial communities. Our findings also challenge the simple pairwise rules often invoked to explain microbial community assembly[37, 38] and suggest that nonlinear interactions across trophic levels - for example, driven by consumer trait variation - are important determinants of structure in natural, more complex microbial communities[39].

Our study leaves many avenues open for future research. One major question is what role prey trait variation plays in community dynamics across trophic levels. Future studies should investigate community assembly and species coexistence while systematically manipulating trait variation in both consumers and prey. Furthermore, we could not examine the role of rapid contemporary evolution during the time course of our experiments. This is important because recent theory suggests that the rapid evolution of a single prey species can facilitate the coexistence of multiple consumers both by broadening niche differences [40] and equalizing consumer competitive differences[41]. Longer experiments coupled with genome sequencing will better allow us to characterize the role of rapid evolution during the experiment time course for species coexistence. Recent studies have shown consumer co-evolution contributes significantly to eco-evolutionary dynamics in one and two species systems[27, 42]. Here we show that feedback between consumer competition and consumer trait variation can also add a significant layer of complexity to our understanding of species coexistence and the maintenance of diversity across trophic levels in more complex communities.

Materials and Methods

Study species: The prey bacterial community consisted of 24 species (Table S1) from the University of Helsinki Culture Collection. These species are derived from soil, aquatic, plant, animal, and human sources and have been described in detail elsewhere [43]. The consumer species consisted of the ciliate *Tetrahymena thermophila* strain 1630/1U (CCAP), obtained from the Culture Collection of Algae and Protozoa at the Scottish Marine Institute (Oban, Scotland, United Kingdom) and the clonal hermaphrodite nematode *Caenorhabditis elegans* strain N2, obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN, USA). The mixture of HTV genetically diverse ciliates is derived from 20 continuously transferred, clonally reproducing, long-term selection lines. Each of the ciliate lines is from a 600 generation co-evolution experiment initiated with the isogenic ciliate and one of seven different bacterial species (three replicates for each species, one ciliate line lost) with common anti-predatory defense mechanisms[17]. Only one of the bacterial species from the co-evolution experiment (*Comamonas testosterone* 0403) was included in the experimental prey community. These HTV ciliate populations each displayed altered phenotypic traits related to predation, including increased swimming speed, straighter swimming trajectories, and increased body size [17].

Culture conditions and experimental preparations: Bacteria were revived from cryopreserved glycerol stocks (-80°C) in protease peptone yeast (PPY) broth at 28°C with shaking for 24 hours. Individual colonies were picked from cultures plated on 50% PPY agar. Clones were then grown in 5% King's Broth (KB) until mid-log phase and cell densities (colony forming units) determined via plating on 50% PPY agar. Ciliates were revived from liquid nitrogen and cultured axenically at room temperature in PPY as described before[17, 44]. 50 ml of each revived ciliate culture was harvested by centrifugation (8 minutes, 3300 rpm, 4°C), then the cell pellet was resuspended in 25 ml M9 buffer. Ciliates were then counted by microscope using 40× magnification. Cryopreserved (-80°C) nematode stocks were revived following the standard protocol [45]. Briefly, frozen nematodes were spread on NGM-plates with *E. coli* OP50, then individuals transferred to fresh plates by "chunking" after eight days. Eggs were collected from 7-8 plates after six days by bleaching live worms. Developmentally synchronized nematodes were hatched in 125 ml M9 buffer with gentle rotation in tissue flasks overnight. Flasks were chilled on ice, 75 ml of buffer was manually decanted, and the remaining buffer and nematodes were centrifuged (3 mins, 1300 rpm, 8°C). Afterward, half the remaining buffer was decanted, and nematodes were counted directly by microscope.

Experimental setup and sampling: The general experimental design with different consumer treatment combinations is outlined in Fig. 1. Each microcosm was performed in four biological replicates in 150 ml plastic bottles using 20 ml of 5% King's Broth (KB) liquid medium containing M9 salts and supplemented with cholesterol in ethanol (5 μg ml^{-1}) (5% King's B: 1 g L^{-1} peptone number 3 and 0.5 ml L^{-1} 85% glycerol). For each treatment/replicate we inoculated 1×104 cfu ml^{-1} of each 24 bacterial species (total $\approx 2.4 \times 10^5$ cfu ml^{-1}). Consumer densities were inoculated at 1×10^4 ciliate cells ml^{-1} and 10 nematode cells ml^{-1}. Microcosms were maintained at 22°C with gentle shaking. Two ml of each microcosm was transferred to fresh growth medium every four days for a total of 21 transfers over 61 days. One ml was sampled at each transfer, combined with 0.5 ml 85% glycerol, and frozen at -80 °C for later DNA extraction. Another 0.5 ml was sampled at each transfer for quantifying consumer density (ciliate: 1:3 vol/vol with 10% Lugol, nematode: 1:1 vol/vol with 20% Lugol). Nematode cell densities were measured directly under a microscope. Ciliate densities were measured using Fiji/ImageJ with the cell counter plugin[46]. Total bacterial biomass was estimated using optical density (OD_{600}) as a proxy.

Bacterial trait measurements and processing: We measured innate predator defense, carrying capacity and growth rate for each bacterial strain as described in detail earlier[27]. Briefly, we revived

each bacterial species in fresh medium (1% Kings Broth), acclimated them 24 hours, then pin replicated strains to fresh medium in 100 well plates for use with a Bioscreen C spectrophotometer (Growth Curves ABLtd, Helsinki, Finland). Plates were incubated at 28°C at constant shaking in the Bioscreen C and measurements were taken at 5 min intervals for 24 hours. After 48 hours, LTV ciliates were added to Bioscreen C wells to estimate biomass loss due to predation. For each bacterial species k we quantified growth potential using area under the logistic growth curve (AUC), and estimated innate defense of species k as $AUC_{k+consumer}/AUC_k$. We inferred maximum growth rate, the doubling time, the carrying capacity using growthcurveR[47]. We determined biofilm formation capacity for each using the crystal-violet method[48]. We determined bacterial growth on 31 different carbon substrates using BioLog EcoPlates (https://www.biolog.com). We predicted additional traits from bacterial genomes[43] using Traitar[49].

Consumer feeding efficiency experiments: The 24 bacteria species, LTV ciliate, HTV ciliate, and nematode stocks were revived from cryopreservation as described above. Each of the bacterial species was grown as monocultures in 100 ml of Reasoner's 2A liquid medium[50] at room temperature for 96 hours with shaking at 50 RPM. Afterward, bacteria were harvested by centrifugation (13000 RPM, 10 minutes, 21°C), the supernatant was discarded, and cells were resuspended in 100 ml of M9 saline solution. The optical density was then adjusted to $OD_{600} = 0.5$ with additional M9. We added 5 ml of suspension from each bacterial species to 6 well culture plates and then added either 2.5×10^3 nematode individuals or 2.5×10^4 of HTV or LTV ciliate individuals. One well for each bacterial species was reserved as a no-consumer control. All conditions were assayed in biological triplicates. Plates were incubated at room temperature with shaking at 50 RPM. After 144 hours of incubation, we measured bacterial optical density (OD_{600}) and counted consumers as described above. We defined prey clearance as the difference between the OD_{600} in each consumer treatment and the no consumer control. We converted optical density to cells ml^{-1} using a conversion of 8×10^6 bacterial cells per 0.01 OD_{600} unit. We defined prey clearance as the number of prey cells consumed per consumer individual after 144 hours.

DNA extraction and sequencing: Total DNA was extracted using the DNeasy Blood & Tissue 96-well extraction kit (Qiagen) according to the manufacturer's instructions. The V3-V4 region of the bacterial 16S rRNA was amplified using a primer set with Illumina sequencing adapters and multiplexing barcodes. Samples were sequenced on an Illumina MiSeq using v3 600 cycles reagent kit. The procedure is outlined in detail in earlier studies[43, 51].

DNA sequence analysis: Quality control and processing were performed using BBTools (version 38.61b (https://sourceforge.net/projects/bbmap/). 16S amplicon read pairs were processed using BBDuk to remove contaminants, trim reads that contained adapter sequence, and right quality trim reads where quality drops below Q10. BBMerge [52] was used to merge surviving read pairs into single amplicon sequences, and `msa.sh` and `cutprimers.sh` were used to remove any forward and reverse primer sequences. VSEARCH [53] was used to filter out amplicons with more than two expected errors (`-fastq_maxee 2`)[54] and excluded sequences outside of the 360-480 bp range. Quality controlled amplicon sequences were assigned to bacterial species using BBMap by mapping against a database of 30 full-length 16S rRNA sequences using the best possible mapping position. Only amplicons mapping unambiguously between position 341 to 805 in the aligned 16S rRNA region with at least a 95% mapping identity were retained and assigned to a species for subsequent analysis. Nearly all reads from each sample were perfectly mapped to a single species 16S rRNA sequence using these criteria, and the greatest number of unmapped reads was 0.1% of the total.

Statistics and data analysis: Detailed descriptions of the statistics and data analysis procedures are available in the supplementary text and `https://github.com/slhogle/consumer-competition`. All

statistical analyses were done in R version 3.6.1 (2019-07-05)[55]. Probabilistic modeling was performed using the Stan programming language v2.24[56] and the RStan v2.21.1 R interface to Stan. We assessed MCMC chain mixing and convergence using effective sample sizes [57] and potential scale reduction factors ($\hat{R} < 1.05$ for all covariates)[58]. For all Bayesian analyses, we used default priors.

Ciliate, nematode and bacterial densities were modeled with hierarchical generalized additive models using mgcv v1.8-33[59]. All other regression was performed in the Bayesian framework using rstanarm v2.21-1 [60] or MCPv0.3-0[61]. Posterior distributions were summarized using BayestestR v0.8-2[62]. We estimated differences in consumer competitive ability and niche overlap by parameterizing phenomenological Lotka-Volterra competition models using gauseR v1.0[63]. We then used the inferred per capita growth rates, intraspecific, interspecific coefficients to calculate competitive and niche differences[18]. We estimated the compositional bias of amplicon-derived species abundances using metacal v0.1.0[64] using abundance information from control samples (day 0) where all prey species were inoculated at equal cell densities based on colony counts. We calculated the Shannon diversity dissimilarity index following Jasinska[65]. We estimated population level alpha and beta diversities (Shannon diversity and Bray Curtis dissimilarity) over the four biological replicates per treatment using DivNet v0.3.6 [66]. To identity sorting and equilibrium phases of the experiment, we performed segmented regressions of Shannon diversity using MCP v0.3-0[61]. We also calculated Beta diversity for individual replicates using Bray and Curtis dissimilarity on species relative abundances. We used non-metric multidimensional scaling for ordination.

For modeling absolute species abundances with Gaussian Process Regression, we transformed calibrated species count matrices to the log scale using the regularized logarithm transform from DESeq2 v1.28.1 [67]. This transformation moderates variance across the mean abundance for each species and normalizes with respect to library size. We modeled the transformed abundance of each species using a longitudinal Gaussian process regression model implemented in lpgr v0.33.3 [68]. To infer whether covariates were informative for the models, we used a probabilistic covariate selection method which estimates the proportion of variance explained for each covariate and noise and selects those covariates that alongside noise explain 95% of the variance.

For joint species distribution modeling[69], we used the Hierarchical Modelling of Species Communities (HMSC) package[70, 71]. We used a two-step hurdle model approach to account for zero inflation in the log transformed count data where we first fit a probit model for presence/absence and then a Gaussian model for regularized log-transformed species abundances conditional upon presence. We accounted for differences in sequencing depth by including log-transformed total reads as a fixed effect in the model. We followed the approach from earlier studies using sequencing count data with HMSC[70–72].

Data availability:

Raw sequencing data is available from the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA725120. Preprocessed count tables are available from `https://github.com/slhogle/consumer-competition`.

Code availability:

Scripts reproducing all figures and steps in the data analysis are available from `https://github.com/slhogle/consumer-competition`.

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