Within-species trait diversity reorganizes consumer competitive hierarchy and prey community structure in an experimental microbial ecosystem

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Abstract

Phenotypic trait differences between species are expected to play an essential role in community assembly. However, the magnitude of trait variability between individuals of the same species is increasingly recognized to have significant ecological effects, including maintaining species diversity. Here we ask how within-species trait diversity at the consumer trophic level affects consumer competition and prey community dynamics at lower trophic levels in an experimental microbial ecosystem consisting of ciliated protozoa, nematode worms, and bacterial prey. Although the nematode had an inherent competitive advantage and rapidly excluded ciliates with low trait variance, we found that high trait variance in the ciliate inverted the consumer hierarchy, ultimately excluding the nematode. Competition between the high trait variance 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 performed pairwise co-cultures with each consumer and prey species and found that high trait variance in the ciliate increased the mean prey consumption rate over the low trait variance ciliate, which led to an increase in selective feeding in the presence of all prey species. Overall, our results are consistent with predictions from the coexistence theory framework and are compelling evidence that intraspecific trait diversity in consumer species 1) modulates competitive differences between species at higher trophic levels and 2) generates cascading effects on the community composition at lower trophic levels.

Introduction

Ecologists increasingly recognize that within-species trait variability can have significant consequences for species interactions, community assembly, and ultimately ecosystem function. For example, trait diversity within consumer species can have direct impacts on prey community composition as well as indirect effects on community primary productivity via trophic cascades[1–3]. Likewise, phenotypic trait diversity in host plant species can affect the composition of the host-associated microbiome and associated nutrient remineralization processes[4–6]. Intraspecific trait variability accounts for one-fourth

of trait variability in natural plant communities[7] and can have ecological consequences that are comparable in magnitude to those from different species[8].

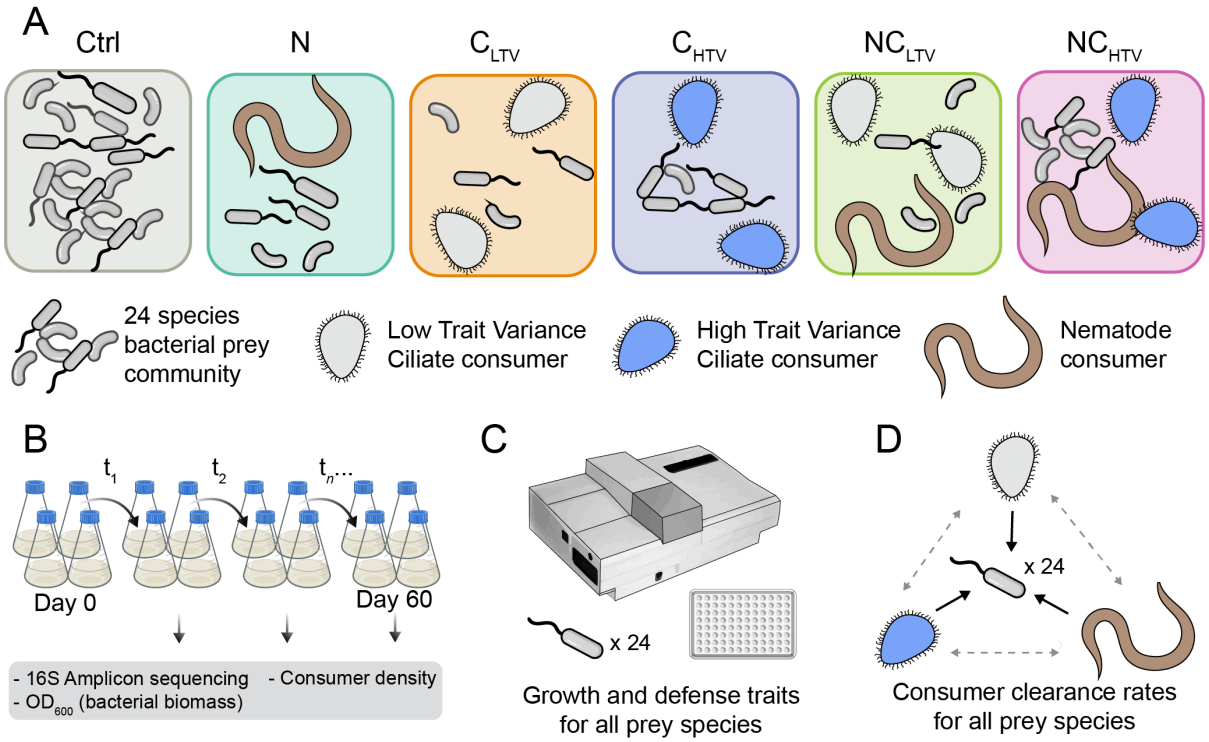

Figure 1. Study setup

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).

The theoretical foundation for the effects of within-species trait diversity on species coexistence[9–11] is derived from models for between-species trait differences[12] and the modern ecological coexistence theory framework[13]. Here species coexistence reflects a balance between density-dependent ecological processes that promote positive growth of low-abundance species (stabilizing differences) and differences in competitive ability inherent to each species. From the perspective of modern coexistence theory, intraspecific trait diversity may blur competitive differences between species, thus operating as an equalizing mechanism [*sensu* Chesson[13]] favoring coexistence[14–16]. However, classic niche theory predicts that intraspecific trait diversity may increase the niche overlap of competing species, which would favor competitive exclusion[9, 10]. Whether or not intraspecific trait diversity favors coexistence is not currently understood, but it is likely to depend upon ecological context and the presence and strength of stabilizing mechanisms (e.g., resource partitioning) [9, 10, 17]. Furthermore, it is not yet established how trait variability at one trophic level affects species diversity at different trophic levels. This is important because the rapid evolution of trait diversity at the level of both prey[18] and consumer[19] can have dramatic effects on predator-prey dynamics and wider community structure and function[1, 20].

Within-species trait variability can emerge from phenotypic plasticity (e.g., maternal effects or learned behavior) and heritable evolutionary change (e.g., intergenerational changes in allele frequencies). Rapid, contemporary evolution is one mechanism that can allow genetic changes to accumulate on ecological

timescales. For example, prey can evolve traits over a few generations that facilitate consumer evasion[18], and consumers can also quickly adapt to nascent prey/host defense traits[19]. Rapid evolutionary change is particularly relevant for microbial ecosystems because microbes generally have large standing population sizes, quick generation times, and high mutation rates. Indeed, rapid evolution and its downstream ecological effects have been demonstrated in many experimental[21–23] and wild microbial systems[24–26]. Even though contemporary evolution can rapidly produce new or altered traits in microbes, the ecological consequences of this intraspecific trait diversity are unclear. One major question in microbial ecology is how the apparent coexistence of hundreds to thousands of microbial species in sympatry is related to intraspecific (i.e., strain-level) phenotype diversity.

Motivated by this knowledge gap, we conducted an experiment to determine 1) how consumer trait variability influences the coexistence of two consumer species and 2) how intraspecific trait variability and species composition at the consumer trophic level 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 Variance (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 variability - hereafter High Trait Variance (HTV) ciliate[27]. 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 and 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. Our results ultimately reveal an interaction between consumer competition and consumer trait diversity where interspecific consumer competition depends upon trait diversity in one key consumer with downstream impacts on the prey community composition largely mediated through key prey traits.

Results

Consumer trait variability flips the consumer competitive hierarchy The magnitude of trait variability 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 variability did not significantly alter the carrying capacity for the ciliate in the absence of competition (Tables S4, S5). Prey biomass differed by less than twofold across all consumer treatments (mean fold difference of 1.08, Table S3), 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 S4, S5, S6, S7). 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 (30 days).

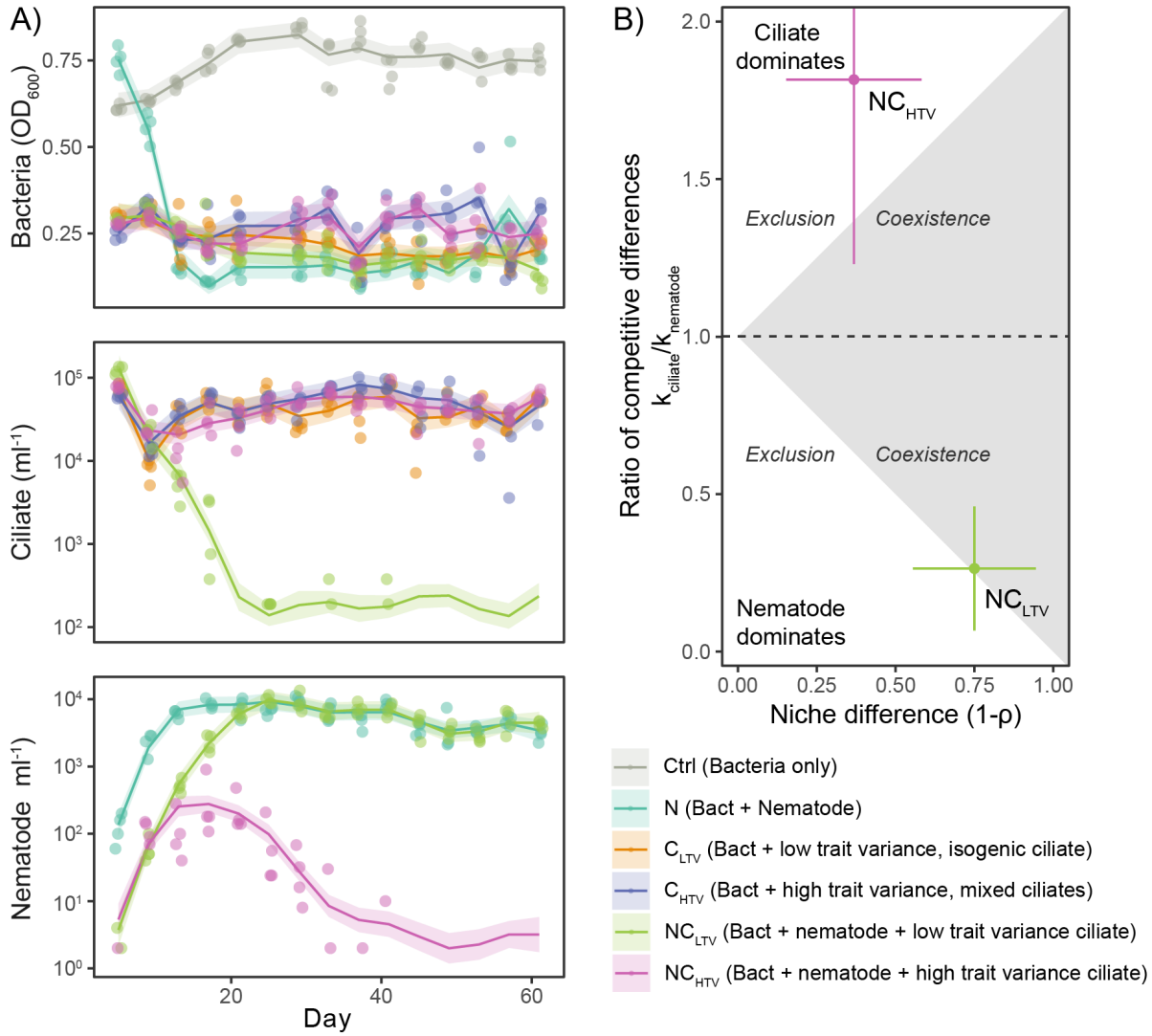

Figure 2. Consumer competitive hierarchy depends upon ciliate trait variability

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, S3, S4, S5, S6, S7). **B)** Niche and competitive differences between the ciliate and the nematode depending on whether the ciliate had low trait variability (NC_{LTV}) or high trait variability (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.

We next attempted to attribute 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[13]. 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[28]. 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, which implies that the outcome we observed between the LTV ciliate and nematode might not have been stable. 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 variability 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 variability in the ciliate predominantly drove equalizing competitive differences between competitors rather than stabilizing niche differences (Fig. 2B).

Prey Shannon diversity follows repeatable longitudinal trajectories: We next asked how consumer trait variability influenced prey diversity. We reasoned that neutral dynamics and stochasticity of individual lineages in the HTV mixed ciliate treatment might increase the dissimilarity of replicate prey populations[29]. However, replicate prey communities were remarkably consistent in composition across all consumer combinations and time (Fig. S1, 3A). We quantified the degree of reproducibility of individual species trajectories by calculating the scaled Shannon diversity dissimilarity (D') across treatments at each distinct sampling point. When $D' = 0$, replicate populations have identical species frequencies, and when $D' = 1$, replicate populations share no species in common. The observed D' values (Fig. 3A) were close to 0 and significantly lower than expected if cross-replicate variance equaled cross-treatment variance (Table S8). This result indicates that 1) technical variation in laboratory conditions (e.g., temperature or transfer volumes) were minimal for these experiments and 2) the process of prey community assembly alongside each consumer or consumer pair was strongly deterministic[29].

The prey species abundance distribution across all populations/treatments followed the canonical form[30, 31] with a handful of abundant species and many rare species (Fig. 3B). Generally, we observed a rapid peak and then monotonic decline of the rarest species during the first quarter of the experiment prior to the stabilization of the consumer (Fig. 3B). In the latter half of the experiment, these rare species would occasionally and 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 in the second quarter, these common species displayed dynamic peaks and declines for the remainder of the experiment.

Although prey species abundance trajectories were dynamic and species turnover rapid, we were able to identify two distinct phases of prey species diversity in the experiment (Fig. 3C). Species diversity linearly declined in the first phase - hereafter sorting phase - which was followed by oscillation around a longitudinally zero-trend mean in the second phase - hereafter equilibrium phase (Supplementary methods). We found that the presence of a a consumer or co-consumer pair slowed the rate of diversity decline in the sorting phase relative to the control, with the nematode slowing diversity decline by the largest magnitude on average (Fig. 2, Tables S9, S10, S11)). In the equilibrium phase, predation generally supported higher mean diversity, with the exception of the LTV ciliate, which decreased prey diversity to the lowest mean value of all treatments (Tables S12, S13)). Consumer competition did not change either the rate of diversity decline in the sorting phase (Table S11) or the mean diversity in the equilibrium phase relative to consumer monocultures (Tables S12, S13)). Thus, the effect of two competing consumers on the Shannon diversity of the prey community was generally equivalent to that of a single predator.

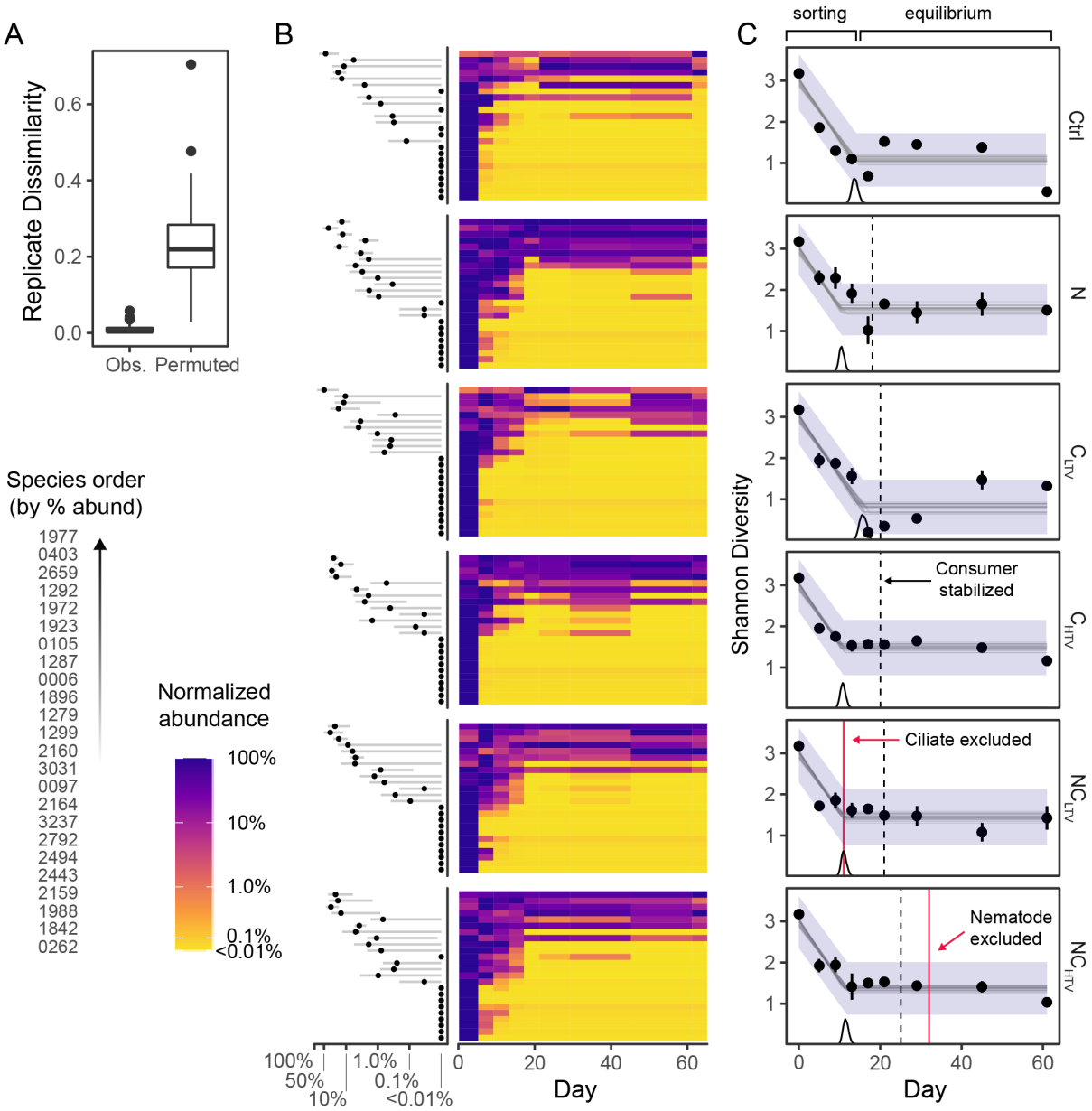

Figure 3. Prey species responses are unique but total diversity invariant to the consumer arrangement **A)** Shannon dissimilarity of lineages over replicate microcosms for each sampling time and consumer treatment. **B)** Abundance trajectories for each of the 24 bacterial prey species. Left plot shows the average relative abundance of each species while the heatmap shows the mean abundance trajectory over time (normalized to each species maximum). **C)** Shannon diversity of prey species over time. Points are observations with 95% confidence interval. The gray lines are 25 samples from the posterior of the model fit, the purple shading is the 95% credible interval of predictions from in-sample data, and the black lines on the horizontal show the equilibrium change point posterior distribution. Red lines in treatments NC_{LTV} and NC_{HTV} show when a competing consumer falls below starting density and is effectively excluded. Black dashed lines represent the time when consumer density stabilized.

Consumer trait variability modulates non-additive multiple predator effects on prey: Although there was no significant difference in total prey diversity between two-consumer or single-consumer treatments, we hypothesized that individual prey species might display distinct responses to multiple consumers. In animal communities[32–34], 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[35] 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[36] 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.

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. S3). 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. S4), 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. S5). Thus, the dynamics of *Niabella yanshanensis* 3031 were an emergent property of the HTV ciliate/nematode system [37] and provide a concrete example of how trait variability at one trophic level can have nonlinear ecological effects on a lower trophic level.

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 total bacteria and total consumer biomass curves were highly similar across consumer 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. S3). 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 variability is mediated through defense traits: We next asked how consumer trait variability and competition influenced community-scale prey responses in the microcosms. We used a joint species distribution model (JSDM) framework[38] 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 effect in the sorting phase (supplementary text). The average explanatory power was excellent

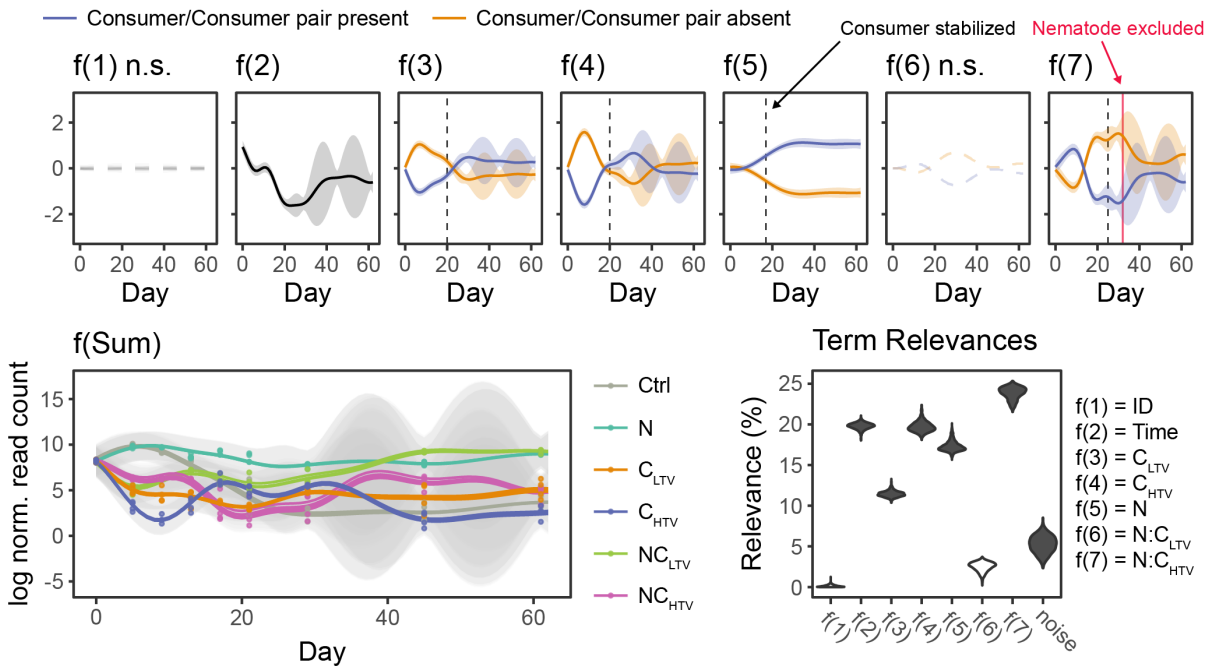

Figure 4. Nonadditive effects of consumer trait variance and competition on individual prey longitudinal trajectories

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.

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 S14). 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[39], certain bacterial taxonomic classes are associated with different stages of *C. elegans* growth in the wild[40], and prey trait variability affects prey coexistence under *T. thermophila* predation[41]. 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. S6). 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 S15, Fig. 5C,F), indicating a shared phenotypic response to different consumer combinations. 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 S15).

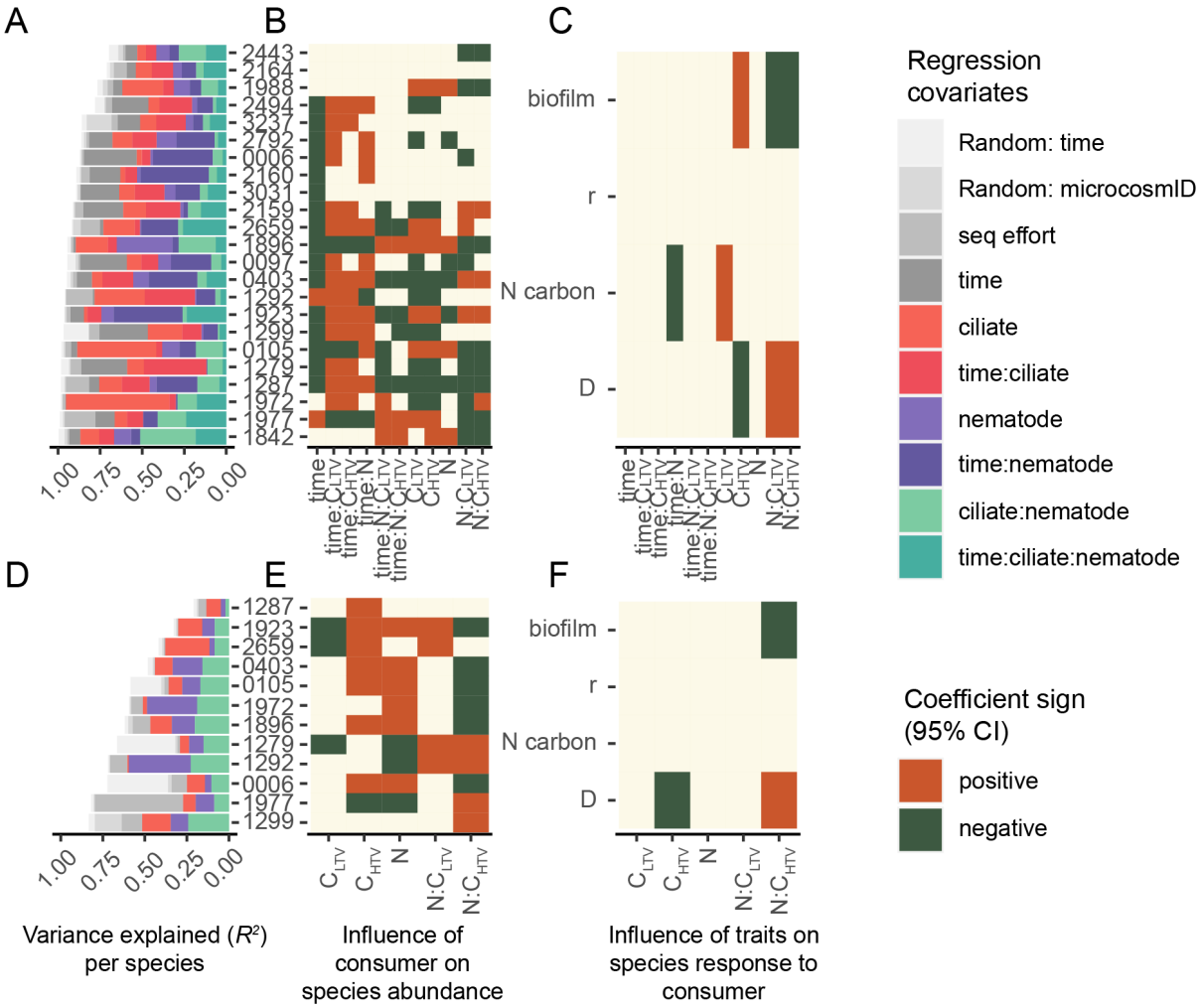

Figure 5. Determinants of prey community assembly

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 model 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[27, 41]. 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[42]. 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[35]. 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 results in non-selective foraging: We next asked whether the competitive outcome between consumer pairs were correlated with differences in specific consumer traits. Given the importance of prey defense traits in explaining prey community assembly, we also expected that consumer feeding traits to be a determinant of the competitive outcome. 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 variability significantly increased prey clearance by the ciliate, while the nematode was the most efficient consumer overall (Tables S16, S17). The coefficient of variation of prey clearance was smallest for the LTV ciliate, then the nematode, and then the HTV ciliate ($\mathrm{CV}_{\mathrm{LTV}} = 0.59$, $\mathrm{CV}_{\mathrm{N}} = 0.70$, $\mathrm{CV}_{\mathrm{HTV}} = 1.25$), 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 smallest clearance rate variability, 2) adaptive trait variability of the ciliate significantly increased feeding efficiency on some, but not all, species while also drastically increasing prey clearance variability, and 3) the nematode removed the most prey per capita on average but also displayed some clear prey species biases.

In a community context, we might expect greater relative variability in consumer prey clearance to manifest as more uneven foraging across prey species. We estimated foraging preference from sequencing data by regressing the relative abundance of bacterial species in the presence and absence of each consumer (Fig. 6B, Table S18). Uniform foraging should produce a prey community of similar composition to the no-consumer control because the consumer would remove all species equally, while uneven foraging should preferentially remove some taxa[43, 44]. Thus, a non-specific forager should have a slope approaching unity, while a selective feeder should have a slope approaching zero[43]. We found that the slope of the LTV ciliate did not significantly differ from unity, consistent with it having the most uniform per capita prey clearance rate and smallest relative variability in clearance across all prey species. The slopes of the HTV ciliate and nematode were significantly smaller than unity, suggesting these consumers foraged on some prey species preferentially (Fig. 6B, Table S19). In particular, the nematode and the HTV ciliate removed the dominant taxa (e.g., *Pseudomonas chlororaphis* 1977) to a much greater extent than the rare taxa.

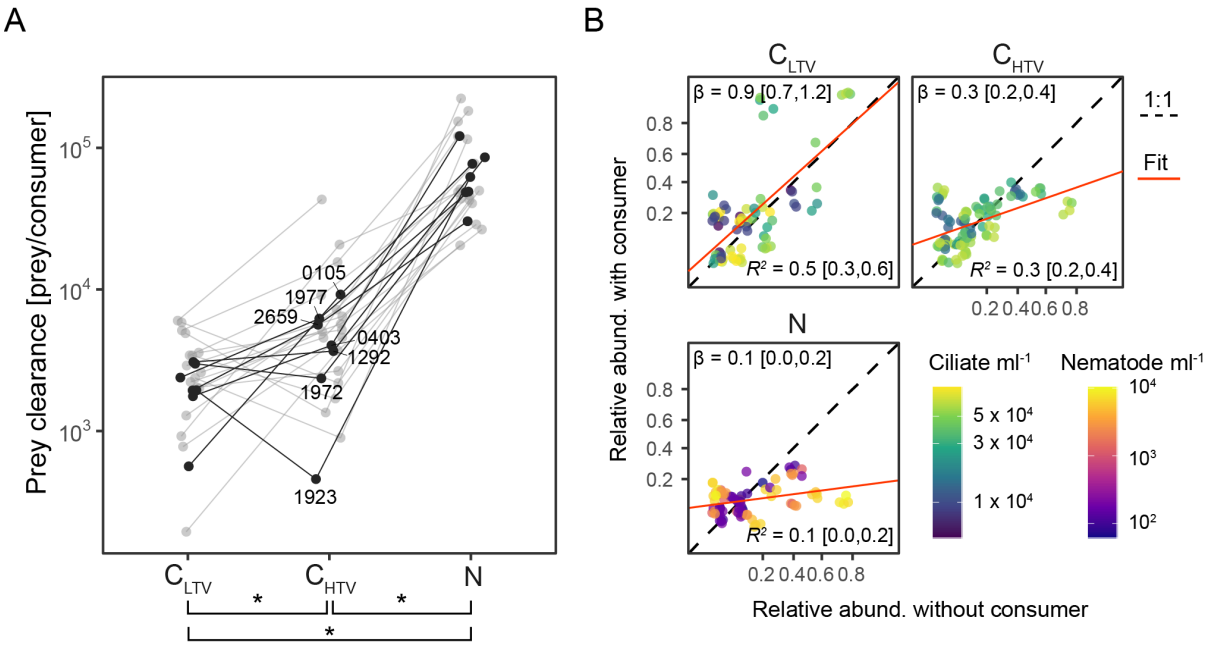

Figure 6. Consumer trait variability alters prey clearance and consumer specialization
A) Distribution of prey clearances on the 24 different bacterial prey species. Prey clearance is the number of bacterial cells removed per consumer individual after 144 hours. Species (connected by lines) in black/labeled are those with > 1% mean relative abundance across consumer arrangements. Asterisks show significant differences in mean prey clearance between consumers (Table S16, S17). 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 before day 21. The axes are arcsin square-root transformed. β, R^2 is the median and 95% CI for the posterior of the slope and Bayesian R^2, respectively from linear regression (Tables S18, S19). C_{LTV}, low trait variance isogenic ciliate; C_{HTV}, high trait variance mixed ciliate; N, nematode.

These findings, taken together, show that the LTV ciliate had the most generalist predation strategy, while the strategies of the HTV ciliate and nematode represented enhanced prey specialization. This is consistent with studies showing that *C. elegans* selectively forages for optimal prey on solid media[45] and that its growth rate is constrained by prey composition rather than total prey diversity[46]. It is unknown whether filter-feeding ciliates can selectively forage for reasons other than prey size[47], but there is some evidence for selective feeding based on prey quality[48, 49]. In our experiment, high trait variability increased the median prey clearance of the ciliate while also increasing the relative variability of prey clearance. For example, the HTV ciliate was a less efficient forager than the LTV ciliate for some key species (e.g., *Myroides odoratus* 1923), which likely reflects foraging trade-offs resulting from specific evolved responses in the HTV ciliate consumer[27]. However, high trait variability increased ciliate foraging efficiency for other abundant (> 1%) prey species that were also preferentially grazed by the nematode. Thus, the HTV ciliate population displayed distinct, prey-targeted traits, which significantly increased the mean and variance of per-species prey clearance over the LTV ciliate.

Discussion

Our results show that within-species trait variability can favor the competitive exclusion of one consumer over another when trait variance is asymmetric (i.e., unequal between species). We quantified consumer density during the experiment and found that the nematode could exclude the isogenic LTV ciliate, but

the HTV ciliate could exclude the nematode. Following coexistence theory, we estimated that the inversion of the competitive 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 next quantified one aspect of consumer trait variability by measuring the pairwise clearance rate for each prey species and connected that variability to competitive dynamics. We found that the HTV ciliate displayed both the broadest range of clearance rates across prey species and a higher mean prey clearance than the isogenic LTV ciliate. Furthermore, in the presence of the whole prey community, we found that both the HTV ciliate and nematode could remove high-abundance prey species preferentially, while the LTV ciliate generally removed all prey species uniformly. These findings suggest that high trait variability allowed the ciliate to preferentially forage across prey species in the same way as the nematode, which likely increased the niche overlap of the two consumers. Indeed, our estimates of niche and competitive differences derived from coexistence theory suggest that trait variability in the ciliate decreased niche differences between competing consumers (Fig. 2B). Still, trait variability had the largest impact on competitive differences between the ciliate and nematode, which likely drove the inversion of the competitive hierarchy. These competitive differences likely reflect consumer foraging specificity, but may also be related to other life history traits. For example, ciliate individuals from the HTV populations were, on average, 40 μm larger, 1.25$\times$ faster, and turned less often than isogenic, LTV ciliate individuals[27]. These offensive traits likely altered the sensitivity of both consumers to interspecific competition which altered mean competitive differences.

Many studies (e.g., discussed in [11, 15]) have focused on how intraspecific trait variability may promote species coexistence. However, the coexistence theory framework predicts that the ecological effects of intraspecific trait variability may actually impede the maintenance of species diversity[10]. This is because individual trait variation mostly 1) reinforces existing competitive hierarchies when traits are non-linearly linked to population growth and 2) niche variation between individuals weakens intraspecific competition, which decreases niche differences. Only when traits in an inferior competitor vary significantly more than in the superior competitor is trait variability predicted to promote coexistence [9, 10]. Interestingly, our results suggest that trait variability in a single inferior competitor (here, the LTV ciliate) can result in competitive exclusion if traits more strongly affect competitive competitive differences over niche differences. Recently, it was shown that rapid trait evolution in response to a competitor resulted in stronger between-species competition rather than greater niche differentiation as would be expected[50]. Additionally, our results suggest that adaptive trait variability in response to prey also emphasizes interspecific competition over stabilizing niche differences even when trait variability is asymmetric. Thus, trait variability that is not directly induced by competition may also have important impacts on the coexistence of competing species.

Consumer trait variability also strongly influenced community composition of the prey trophic level. We found that the combined effects of the ciliate and nematode on the prey community were 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[35] but have not been documented at the microbial scale. Importantly, we also found that trait variability in the ciliate significantly altered the magnitude of non-additive consumer effects across prey species (Fig. 4, 5, S3). We also found that prey community assembly in the presence of consumers was associated with distinct prey traits related to defense and resource use. Thus, prey communities may assemble partly as a consequence of 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 variability at one trophic level may have complex and nonlinear downstream effects on other trophic levels in the wild. The number and magnitude of these nonlinear relationships may be key 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[51, 52] and suggest that nonlinear interactions across trophic levels - for example, driven by consumer trait variability - are important

determinants of structure in natural, more complex microbial communities[53].

Our study leaves many avenues open for future research. One major question is what role prey trait variability plays in community dynamics across trophic levels. Future studies should investigate community assembly and species coexistence while systematically manipulating trait variability 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 [54] and equalizing consumer competitive differences[55]. 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[19, 41]. Here we show that feedback between consumer competition and consumer trait variability 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 [56]. 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[27]. 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 [27].

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[27, 57]. 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 [58]. 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[59]. 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[41]. 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[60]. We determined biofilm formation capacity for each using the crystal-violet method[61]. We determined bacterial growth on 31 different carbon substrates using BioLog EcoPlates (https://www.biolog.com). We predicted additional traits from bacterial genomes[56] using Traitar[62].

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[63] 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[56, 64].

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 [65] 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 [66] was used to filter out amplicons with more than two expected errors (`-fastq_maxee 2`)[67] 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. All statistical analyses were done in R version 3.6.1 (2019-07-05)[68]. Probabilistic modeling was performed using the Stan programming language v2.24[69] and the RStan v2.21.1 R interface to Stan. We assessed MCMC chain mixing and convergence using effective sample sizes [70] and potential scale reduction factors ($\hat{R} < 1.05$ for all covariates)[71]. For all Bayesian analyses, we used default priors.

Ciliate, nematode and bacterial densities were modeled with hierarchical generalized additive models [72] as implemented within the frequentist framework in mgcv v1.8-33 [73]. All other generalized linear models, beta regressions were performed in the Bayesian framework using rstanarm v2.21-1 [74]. Posterior distributions were summarized using BayestestR v0.8-2[75]. In particular, we used 95% Credible Intervals [CI] (the posterior range containing 95% of probable values), Regions of Practical Equivalence [ROPE] (the posterior percentage that overlaps with a null region of negligible effect magnitude), and The Probability of Direction [PD] (an index representing the certainty that an effect is positive or negative). When fitting models with alternative assumptions, we compared the predictive performance using Leave-One-Out Cross-Validation in loo v2.3.1[76].

We estimated differences in competitive ability and niche overlap by parameterizing phenomenological Lotka-Volterra competition models following Letten[28] and using gauseR v1.0[77]. The Lotka-Volterra models make no specific assumptions about the mechanisms underlying species interactions so they can flexibly parameterized such that they approximate any combination of potential mechanisms.

Because we knew the density at which each prey species was initially inoculated, we could estimate the compositional bias of amplicon-derived species abundances due to the combined steps of DNA extraction, PCR amplification, and sequencing[78]. We used the metacal v0.1.0 R package to calibrate species relative abundances. We estimated Shannon diversity using DivNet[79], which provides population-level estimates of species diversity that account for the inherent compositional structure of microbiome data and species co-occurrence patterns/dependencies. We calculated the Shannon diversity dissimilarity index following Tuomisto[80, 81] and Jasinska[82] and references therein. We performed segmented regressions of Shannon diversity using MCP v0.3-0[83] to identity sorting and equilibrium phases from the Shannon diversity data.

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 [84]. This transformation moderates variance across the mean abundance for each species and normalizes with respect to library size. The log transform of this normalized value is approximately Gaussian distributed. We modeled the transformed abundance of each species using a longitudinal Gaussian process regression model implemented in lpgr v0.33.3 [85]. 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[86], we used the Hierarchical Modelling of Species Communities (HMSC) package[87, 88]. 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[87–89].

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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