Species interactions amplify the temperature dependence of microbial community respiration

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Running title: Species interactions amplify community thermal sensitivity

Keywords: warming, temperature dependence, species interactions, respiration, microbes

Word count: 2551 (Text), xx (Methods)

Figures: 3; **Text references:** 26; **Methods references:** 3

Summary

The sensitivity of microbial community respiration to temperature is crucial to understanding the effects climate change on the carbon cycle. However, large uncertainties about the magnitude of this response exist, owing to limited mechanistic understanding of how the thermal sensitivity of respiratory flux emerges in complex microbial communities. Here, we develop a new mathematical model that predicts how the nature and strength of species interactions affects the temperature sensitivity microbial community respiration. Our theory predicts that this temperature sensitivity is amplified as species interactions become more positive (e.g., move from competition to facilitation). This amplification is driven by positive feedbacks between the temperature-dependencies of species-level metabolic and biomass accumulation rates, which are enhanced by stronger, facilitatory interactions among species. We test this theory using laboratory experiments on communities of heterotrophic bacteria. Consistent with our predictions, we found that the temperature sensitivity of community-level respiration increases by 60% after a coevolution experiment which decreased inter-species competition and increased facilitation through cross-feeding on metabolic by-products. These findings demonstrate that changes in the nature and strength of species interactions over space and time can play a profound role in determining how of complex microbial communities and their functioning respond to changes in temperature.

Empirical data show that ecosystem-level respiration generally follows an exponential-like relationship with temperature[1], raising concerns that future warming will increase carbon emissions from the biosphere and accelerate rates of global warming[2-4]. A major component of this ecosystem thermal response is driven by the microbial community within ecosystems[3,5]. Indeed, microbes are a dominant component of the earth's total biomass[6], and by degrading the organic carbon from primary and secondary production contribute significantly to carbon cycling[7]. For example, bacterial contributions to ecosystem respiration have been estimated to be >50% in some ocean regions[5]. Consequently, even small differences in the thermal sensitivity of microbial community respiration could have a major impacts on projections of future global warming[7]. Yet, the response of microbial community respiration to temperature represents a critical uncertainty in climate-carbon cycle projections for the next century and an important question in microbial ecology [3,8,9].

Current models of the temperature responses of multispecies ecosystems typically assume that thermal responses can be scaled up from the individual to community- or ecosystem-level by the simple addition of the temperature responses of component taxa[10-13]. These models focus only on the direct effect of temperature on individual metabolism, ignoring the effects of species interactions which could alter the amount and distribution of biomass within ecosystems, affecting total ecosystem respiration and its thermal sensitivity[14,15]. Thus, current models are unlikely to be an accurate representation of the processes that contribute to the temperature sensitivity of respiration in natural ecosystems, where species interactions are critical for shaping the structure and dynamics of complex communities[16-18], particularly in response to changes in the abiotic environment[19].

In microbial communities in particular, where demographic processes and turnover rates occur over short timescales, temperature dependence of community respiration more likely reflects the impacts of temperature both directly on individual metabolism, and indirectly through species' interaction-driven population biomass dynamics. Microbial taxa interact in numerous ways, ranging from competition for limiting abiotic resources, to facilitation, typically through cross-feeding on metabolic by-produces[20–22]. Because key microbial metabolic traits are temperature-sensitive, as temperatures change these interactions inevitably result in shifts in biomass dynamics, and thus community respiration. For example, widespread facilitation may amplify the effects of temperature by creating a positive feedback loop due to enhanced metabolic and growth rates in warmer conditions. Conversely, if weak or neutral interactions dominate, such as when species partition resource use, the species' population biomasses should become relatively decoupled, and the response of community respiration to temperature should emerge through the simple addition of the thermal responses of individual taxa. In general, when interactions are strong, feedbacks between populations should be greater, amplifying the (positive or negative) effects of temperature across the whole system.

Here we investigate the role of biotic interactions in the temperature dependence of community metabolism by combining theory with laboratory experiments. We show that nonlinear species interactions drive changes in the thermal sensitivity of community respiration through their effects on species' biomass dynamics. This change occurs in a predictable way and allows us to characterise the relationship between the average interaction strength in the community and the thermal sensitivity of respiration.

We first developed a mathematical model to explore the effects of species interactions on the thermal response of the community-level respiration. Consider a community comprising of N interacting species. Within this system, the total temperature (T)-dependent respiration rate ($R^{eco}(T)$) depends on the sum of contributions of each population's respiration ($R_i^{pop}(T)$), which in turn can be expressed as the product of mass-specific respiration ($R_i(T)$) and biomass ($C_i(T)$) of each population:

$$R^{eco}(T) = \sum_{i=0}^{N} R_i^{pop}(T) = \sum_{i=0}^{N} R_i(T)C_i(T) \ .$$

Thus, temperature can affect community respiration by changing mass-specific respiration of individual populations, by changing their biomasses, or both. Next, we derive the thermal sensitivity of $R^{eco}(T)$ (the magnitude of change of community respiration to a unit change in temperature), which we denote by an apparent activation energy, E_{eco} (Supplementary xxx):

$$E_{eco} = \frac{\sum_{i=0}^{N} R_i C_i (E_{R,i} + E_{C,i})}{\sum_{i=0}^{N} R_i C_i} \ , \qquad (1)$$

where $E_{R,i}$ and $E_{C,i}$ are the thermal sensitivities (apparent activation energies) of mass-specific respiration and biomass of the ith species' population, respectively. Eq 2 shows that E_{eco} is given by the average thermal sensitivities of biomass and respiration across all species in the system, weighted by each species total respiratory output, R_iC_i. Next, we consider how E_{eco} (Eq 2) is affected by interspecific interactions. Assuming interactions do not significantly affect species' mass-specific respiration (R_i), we focus on how they affect the biomass terms (C_i's and $E_{C,i}$'s) in Eq 2. Using the generalised Lotka-Volterra (LV) model for interaction-driven biomass dynamics in the early stages of community growth (Supplementary xxx), we show that the thermal sensitivity of community-level respiration can be partitioned as

$$E_{eco} \approx \overline{a}\gamma + N \operatorname{Cov}\left(w_i(\overline{a}), \left(E_{R,i} + \omega_i\right)\right) + N\left(\overline{E_R} + \overline{\omega}\right), \qquad (3)$$

where $\overline{a}$ is the average of the interaction coefficients between all species pairs (a_{ij}'s), $\gamma = \frac{(N-1)\overline{C_0}t^2}{2}\frac{d\overline{r}}{dT}$ represents the time (t)-dependent change in average (across all species) biomass of the community with temperature (driven by average temperature-dependent changes in average population growth rate $\overline{r}$), $\omega_i = \left(t + \frac{t^2 a_{ii} C_{0,i}}{2}\right)\frac{dr_i}{dT}$ represents the time dependent change in biomass of the ith species with temperature (taking into account only its growth rate r_i and intra-specific density-dependence, a_{ii}), and $w_i(\overline{a}) = \frac{R_i C_i(\overline{a})}{\sum_{i=0}^{N} R_i C_i(\overline{a})}$ is the weighting of the ith species, given by it's the normalised respiratory output. The dependence of these weights on $\overline{a}$ arises through the effects of species interactions on population biomasses ($C_i(\overline{a})$).

Eq 3 shows how the thermal sensitivity of community respiration is determined by three main components: (i) the average effect of interspecies interactions across the community ($\overline{a}\gamma$), (ii) the covariation in species responses to interactions (via the weighting terms) and their thermal sensitivities $\left(N\operatorname{Cov}(w_i(\overline{a}), E_{R,i} + \omega_i)\right)$ and (iii) the average thermal sensitivities of respiration and biomass growth (not accounting for interactions) across the community ($\overline{E_R} + \overline{\omega}$). When interactions are neutral ($\overline{a} = 0$) the first of these terms is zero and community sensitivity is dependent only on internal factors affecting species growth (growth rates and intra-specific interactions in the ω and covariance term). When interactions are non-neutral ($\overline{a} \neq 0$) they will either amplify or dampen the sensitivity of community respiration relative to this neutral case (Fig 1B). More facilitatory (positive) interactions will result in

higher community-level thermal sensitivity whilst competitive interactions will result in a reduction in sensitivity. Generally, stronger interactions (i.e. greater absolute values of $\overline{a}$) will result in greater changes in community-level sensitivity. These changes occur as interactions amplify the change in biomass with temperature across the community (due to altered rates of biomass accumulation) represented in the γ term. This makes mathematically-explicit the amplification effect of species interactions on the thermal sensitivity of whole community (or ecosystem) respiration. Interactions can also alter community sensitivity through the covariance term in Eq 3 which occurs when species affected strongly by interspecific interactions (i.e. those with more extreme $w_i(\overline{a})$ values) also tend to have higher temperature sensitivities of respiration and internal biomass growth $(E_{R,i} + \omega_i)$. Assuming that the net effect of interactions on each species is roughly equivalent, the effects of interactions on this covariance term will be negligible relative to the main effect in the first term.

We tested these theoretical predictions using experiments with heterotrophic microbes. To investigate how changes in interaction structure (e.g., from competitive to facilitatory) affect the temperature sensitivity of community respiration, we assembled replicated communities of 8 bacterial taxa isolated from geothermal streams in Iceland, and allowed them to coevolve in a minimal, single carbon source media (M9 + glucose) at ambient temperature (20°C) for over 100 generations. This allowed us to (a) use a diverse array of natural isolates that vary considerably in their thermal physiology; and (b) exploit the tendency of bacterial taxa to evolve cross feeding (i.e. facilitation between taxa by the exchange of metabolic by-products) when subject to resource limitation, enabling us to generate replicated communities with the same taxa but different interaction structures [23-25].

In all communities biomass dynamics stabilised after ~50 generations (7 transfers), with 5 of the original 8 taxa from the ancestral species pool persisting. At the end of the experiment, after approximately 100 generations (14 transfers), we re-isolated the taxa from each of these replicated "coevolved" communities. We first assessed whether long-term coevolution in a community affected the thermal response of respiration at the taxon-level by quantifying rates of respiration along a broad temperature gradient (15-35°C) for both ancestral and coevolved populations of each of the 5 taxa. We found no significant difference in any of the parameters characterising the temperature response of respiration between the ancestral and coevolved populations (Fig. 2a, Fig. S2), showing that coevolution had no discernible effect on the temperature response at the population level.

Based on our theory, we predicted that the thermal sensitivity of total community respiration should be higher in the 'coevolved' communities where we expected facilitatory interactions to be more common compared to communities assembled from the same ancestral taxa that had not coevolved together (hereafter termed 'de-novo'). As we expected metabolic facilitation (i.e. the exchange of metabolic by-products between populations) to be the main mechanism for any change in interaction structure, we preformed the community respiration assays in both M9+glucose and "spent" media (obtained by allowing communities to grow until all the initial in glucose was depleted and only metabolic by-products remained, see Methods). If an increase in metabolic facilitation was the main mechanism for changes in species interactions, then we expected to see an amplification of the thermal sensitivity of respiration in the coevolved communities in the spent media, because the taxa that comprise these communities will have adapted to persisting on the metabolic by-products of their conspecifics whereas the de-novo assembled communities will not. By contrast, in the M9+glucose media, populations will be able to independently access glucose, free from exploitative or interference competition (as the assays were carried out at low densities in the exponential phase of growth) leading to neutral or weakly competitive interactions. When interactions are weak or neutral, our theory predicts an additive scaling of the temperature sensitivity from population to community levels, therefore we anticipate comparable temperature sensitivities between the population- and community level in both de-novo and coevolved treatments.

Consistent with our expectations, we found that the average temperature sensitivity of respiration was statistically indistinguishable between the de-novo and coevolved communities measured in M9+glucose (Fig. 2b, Table S3). Furthermore, the apparent activation energy of respiration at the population- and community-levels in the M9+glucose media (where interactions are expected to be neutral) were

162 indistinguishable (species = 0.96 eV ± 0.28, community = 1.0 eV ± 0.2) as predicted by Eqn (3) for
163 neutral interactions. However, when we quantified the temperature response of community respiration
164 in spent media, as expected from the theory, we found a marked increase in the thermal sensitivity of
165 respiration compared to the de-novo communities (Fig. 2c, Fig. S4, Table S5) and the activation energy
166 of the coevolved community in the spent media was 60% higher (1.6 eV ± 0.35) than the average
167 population-level activation energy. These results are consistent with our hypothesis that positive biotic
168 interactions resulted in population dynamical feedbacks that amplify the scaling of the temperature
169 sensitivity between levels of organisation.

170 Having established that the temperature sensitivity of respiration was markedly higher in the coevolved
171 communities, we next quantified the strength and nature of interactions among the 5 taxa in the co-
172 evolved and de-novo communities to confirm that interactions had become more facilitatory (or less
173 competitive). First, we looked at how the asymptotic biomass of the communities and the individual
174 taxa (i.e. carrying capacity, *K*) changed following coevolution. If interactions had become more facili-
175 tatory, we expected to see higher biomass attained in these communities due to more efficient use of
176 limiting resources. When each taxa was grown in monoculture on the M9+glucose media for 72 h at
177 20°C, we saw no statistically significant difference in *K* before and after coevolution (ANOVA: $F_{1,58}$ =
178 0.33, P = 0.57, Table S2) (Fig S2). However, when the same test was performed with multi-species
179 communities, *K* was higher in the coevolved community (Fig. 3a, M9 media, ANOVA: $F_{1,10}$ = 6.73, P
180 = 0.03, Table S2). To further explore these effects we repeated the experiment, but this time, incubated
181 the communities in the spent media. We found that that the coevolved community reached higher *K*
182 compared to the de-novo community on the spent media (Fig 2, Spent media, ANOVA: $F_{1,10}$ = 5.49, P
183 = 0.04, Table S2). Furthermore, the *K* attained by the coevolved community in the spent media was
184 even higher than it attained in the M9+glucose media. Together these results provide clear evidence
185 that that the coevolved communities were better able to exploit the available resources either in the form
186 of M9+glucose or the by-products of one another's metabolism.

187 We further explored the change in interaction structure after coevolution by estimating the direction
188 and strength of interactions between each taxon pairs. We first grew isolates of each taxon before and
189 after co-evolution for 72 h at 20°C in M9+glucose and the spent media, as well as all possible pairwise
190 combinations of taxa. We then estimated pairwise interaction strengths using a metric based on the
191 comparison of growth trajectories in monoculture vs biculture (see Methods). When grown on M9+glu-
192 cose media, interactions were weak between taxa ($\bar{a}$ = 0.005 ± 0.01) and showed no significant change
193 between the coevolved and de-novo populations (Fig. 3b, est = 0.002, t =0.013, P = 0.99). This result
194 aligns with our expectation that in M9+glucose media, interactions should be weak due to the abun-
195 dance of resources in the environment. When the same experiment was attempted in the spent media,
196 most of the pairwise trials and monoculture experiments exhibited no growth and resulted in population
197 extinctions. This indicates that the enhanced performance of the coevolved communities (Fig. 3a) stems
198 from a network of cross-feeding that higher-order interactions beyond the level of pairs, i.e., it depends
199 on the resource environment created by the metabolic by-products of the community as a whole.

200 Overall, these results show that changes in the nature of species interactions can profoundly alter the
201 temperature sensitivity of community-level respiration. Using experiments with a model system of het-
202 erotrophic microbes, we found that after long-term coevolution in resource poor environments, com-
203 munities displayed enhanced performance compared to de-novo assembled communities composed of
204 the same ancestral taxa, implying a shift in the interaction network towards a prevalence of facilitation.
205 The temperature sensitivity of respiration was also amplified in the coevolved communities, consistent
206 with our theory which predicted that stronger, positive interaction networks lead to higher thermal sen-
207 sitivity due to the amplification of the effects of temperature on population growth through feedback
208 between populations.

209 The nature of interactions within ecosystems are likely to be critically important in determining re-
210 sponses of ecosystem functioning to temperature change, having the capacity to both amplify or dampen
211 temperature sensitivities based on the nature and strength of interactions within the community. These

findings will be particularly relevant for systems where the nature of interactions are biased in one direction or the other, as is often seen in microbial communities where whole communities tend to be biased towards either competitive or facilitatory interactions across space[22], or over time[16,24]. In particular, microbially-mediated decomposition of organic matter, which is a key component of CO_2 and CH_4 fluxes in the carbon cycle, depends on facilitation among diverse consortia of bacteria and archaea. Indeed, our finding that coevolved microbial facilitation amplifies the temperature sensitivity of community metabolism may go some way to explaining the relatively high thermal sensitivity seen in aquatic microbe dominated methane decomposing communities[26] and other aquatic ecosystems[4,14].

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Acknowledgements: This work was supported by a European Research Council Starting Grant awarded to G.Y-D (ERC StG 677278 TEMPDEP).

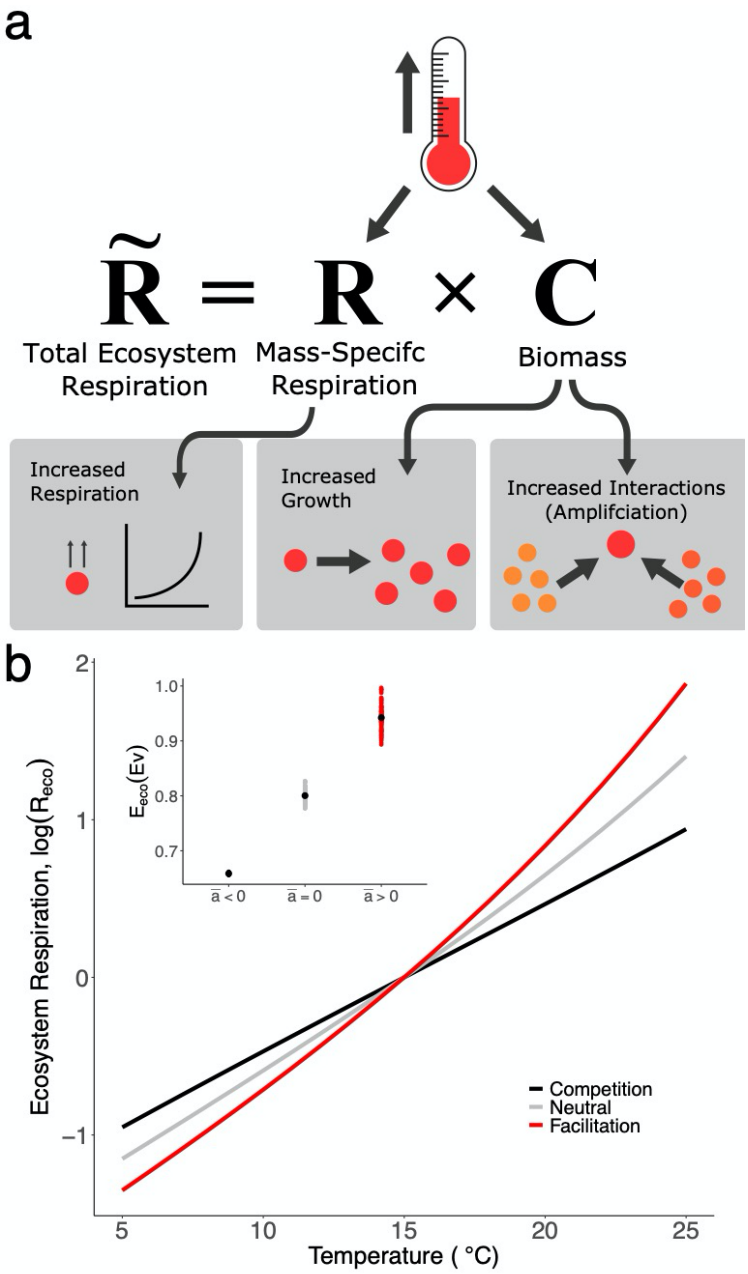

Fig 1. Changes in biotic interactions alter the temperature sensitivity of community respiration. a) Illustration of the mechanisms through which temperature can act on ecosystem respiration, either by affecting individual metabolism directly (increasing respiration) or the amount of biomass, which is determined by the effects of temperature on growth rates and interactions between species. b) Analytical prediction of the relationship between temperature and community respiration under different interaction structures. Respiration becomes more sensitive to temperature change as interactions become more positive. Main plot shows ecosystem respiration, log(R_{eco}), at three levels of interaction strength across the community. Inset plot shows the resultant thermal sensitivity E_{eco}. Results are obtained by randomly generating communities which parameterise the model detailed in Eq 3 (see Methods).

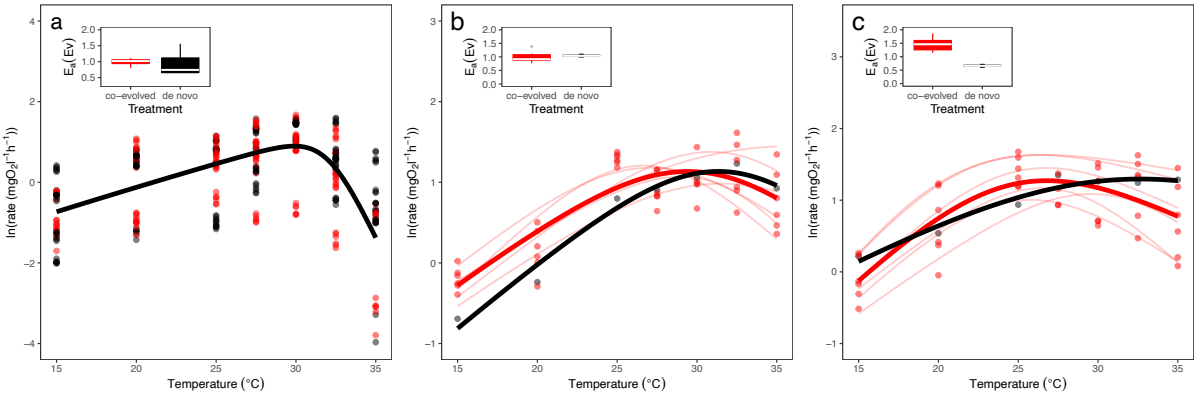

Fig 2. Coevolution of microbial facilitation amplifies the temperature sensitivity of community respiration. Temperature dependence of respiration at the population (a) and community level in (b) M9+glucose and spent media (c). Colours denote whether populations or communities have coevolved histories (red) or were assembled de-novo from the ancestral isolates (black). Inset plots show the distribution of thermal sensitivities (*E,* activation energy) for each treatment where box plots depict the median (centre line) and the first and third quartiles (lower and upper bounds). Whiskers extend to 1.5 times the interquartile range (the distance between the first and third quartiles). Note that for the community-level temperature responses (b, c) there is only a single combination of the 5 ancestral taxa in the de-novo community, hence there is only a single estimate of the activation energy which is presented along with the 95% confidence intervals of the model fit. The solid lines represent the average temperature sensitivity estimated by fitting the Sharpe-Schoofield equation using non-linear least squares (Materials and methods).

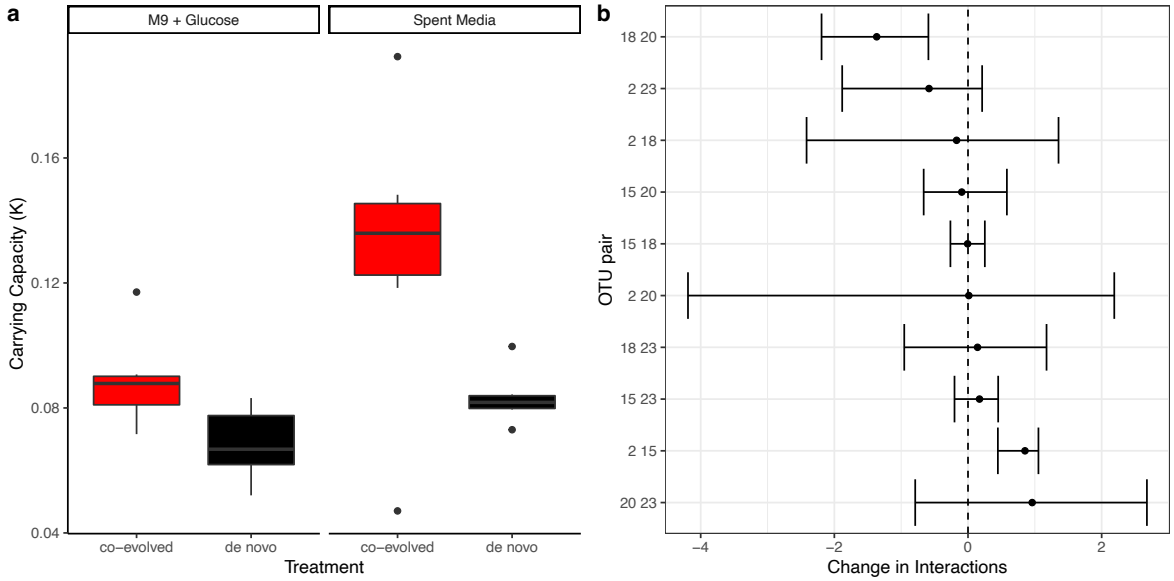

Fig 3. Coevolution of microbial metabolic facilitation. (a) Carrying capacity of the coevolved and den-nova assembled communities grown in either M9+glucose or spent media for 72 h at 20°C (table S2). (b) Change in interaction strength between pairwise combinations of taxa from the de-novo and co-evolved communities. Each row shows the average (points) and 95% confidence interval (ranges) of the estimated change in interactions for each taxa pair. Negative values indicate interactions have become more competitive whilst positive values indicate that interactions have become more facilitatory. Across most pairs, the interaction strength are indistinguishable from zero, which is consistent with the neutral case discussed in the main text.