Supplementary Material for

Multitrophic assembly: a perspective from modern coexistence theory

Chuliang Song[1*] and Jurg W. Spaak[2*]

[1] Department of Ecology and Evolutionary Biology,
Princeton University, Princeton, NJ, USA

[2] Department of Ecology and Evolutionary Biology,
Cornell University, Ithaca, NY, USA

Contents

A Analytical derivations **S2**

B Simulations **S12**

C Testing in another perspective of assembly in multitrophic communities **S18**

D Analysis of tri-trophic communities **S20**

E Analysis of empirical food webs **S25**

A Analytical derivations

A.1 Model assumption

We here present the analytical computations of how changes in species richness in the different trophic levels affect niche and fitness differences according to the different focuses. For the analytic computation of niche and fitness differences, we assume that all inter-specific interactions are identical, i.e. $A_{ij}^{(11)} = A_{ik}^{(12)} = A_{lj}^{(21)} = A_{lk}^{(22)} = a$ for $i \neq j$ and $l \neq k$, as opposed to a random variable with mean a. The intraspecific interaction is set to 1, again independent of the trophic level, i.e. $A_{ii}^{(11)} = A_{ll}^{(22)} = 1$. Note, we use i and j for indices of a lower-trophic species and l and k for indices of a higher-trophic species. Furthermore, let n_1 be the lower-trophic species richness and n_2 be the higher-trophic species richness in the second trophic level. To compute niche and fitness differences according to the community focus, we first compute the equilibrium densities of the species. Because of symmetry, all lower-trophic species must have the same density at equilibrium, i.e. $N_i^{(1)} = N_j^{(1)}$ and similar for the second trophic level.

$$\frac{1}{N_i^{(1)}}\frac{dN_i^{(1)}}{dt} = 0 = \mu_i^{(1)} - \sum_j A_{ij}^{(11)} N_j^{(1)} - \sum_k A_{ik}^{(12)} N_k^{(2)} \tag{S1}$$

$$= \mu_i^{(1)} - (1 + a(n_1 - 1))N_i^{(1)} - an_2 N_k^{(2)} \tag{S2}$$

$$\frac{1}{N_l^{(2)}}\frac{dN_l^{(2)}}{dt} = 0 = \mu_l^{(2)} + \sum_j A_{lj}^{(21)} N_j^{(1)} - \sum_k A_{lk}^{(22)} N_k^{(2)} \tag{S3}$$

$$= \mu_l^{(2)} + an_1 N_j^{(1)} - (1 + a(n_2 - 1))N_k^{(2)} \tag{S4}$$

Solving the equation system, we get

$$\begin{pmatrix} N_i^{(1)} \\ N_l^{(2)} \end{pmatrix} = \begin{pmatrix} 1 + a(n_1 - 1) & an_2 \\ -an_1 & 1 + a(n_2 - 1) \end{pmatrix}^{-1} \begin{pmatrix} \mu^{(1)} \\ \mu^{(2)} \end{pmatrix} \tag{S5}$$

$$= \frac{1}{(1 + a(n_1 - 1))(1 + a(n_2 - 1)) + a^2 n_1 n_2} \begin{pmatrix} 1 + a(n_2 - 1) & -an_2 \\ an_1 & 1 + a(n_1 - 1) \end{pmatrix} \begin{pmatrix} \mu^{(1)} \\ \mu^{(2)} \end{pmatrix} \tag{S6}$$

$$= \frac{1}{1 + a(n_1 - 1) + a(n_2 - 1) + a^2(n_1 - 1)(n_2 - 1) + a^2 n_1 n_2} \begin{pmatrix} an_2(\mu^{(1)} - \mu^{(2)}) + (1 - a)\mu^{(1)} \\ an_1(\mu^{(1)} + \mu^{(2)}) + (1 - a)\mu^{(2)} \end{pmatrix} \tag{S7}$$

$$= \frac{1}{(1 - a)^2 + a(1 - a)(n_1 + n_2) + 2a^2 n_1 n_2} \begin{pmatrix} an_2(\mu^{(1)} - \mu^{(2)}) + (1 - a)\mu^{(1)} \\ an_1(\mu^{(1)} + \mu^{(2)}) + (1 - a)\mu^{(2)} \end{pmatrix} \tag{S8}$$

We note that $N_l^{(2)} > 0$ if and only if $n_1 > \frac{(1-a)|\mu_l^{(2)}|}{a(\mu_i^{(1)} + \mu_l^{(2)})}$.

For the traditional and the alternative approach we first convert the community model with two trophic levels into a community model with only one trophic level by setting the growth rates of the other community level to constant. Often this is done with a timescale separation, which in this case would not be meaningful, as we cannot assume at the same time that the dynamics of the higher trophic are faster than the lower trophic and vice versa. Rather, the justification for this stems from how we compute niche and fitness differences, which depends on the intrinsic growth rate μ_i, the invasion growth rate r_i, and the no-niche growth rate η_i, which are all evaluated at an equilibrium state. Therefore, we do not assume timescale separation, but rather we only evaluate the growth dynamics at equilibrium, such that we can assume the non-focal trophic level to be at equilibrium.

A.2 Traditional focus

We first investigate how a change in richness of the lower trophic level affects niche and fitness differences of the lower trophic level. The density of the higher trophic level is given by

$$\frac{1}{N^2}\frac{dN^2}{dt} = \mu^{(2)} + A^{(21)}N^{(1)} - A^{(22)}N^{(2)} = 0 \tag{S9}$$

$$\Leftrightarrow N^{(2)} = (A^{(22)})^{-1}(\mu^{(2)} + A^{(21)}N^{(1)}) \tag{S10}$$

Inserting this into the equation for the of the lower trophic level we get

$$\begin{aligned}\frac{1}{N^{(1)}}\frac{dN^{(1)}}{dt} &= \mu^{(1)} - A^{(12)}N^{(2)} - A^{(11)}N^{(1)} && \text{(S11)}\\ &= \mu^{(1)} - A^{(11)}N^{(1)} - A^{(12)}(A^{(22)})^{-1}\mu^{(2)} - A^{(12)}(A^{(22)})^{-1}A^{(21)}N^{(1)} && \text{(S12)}\\ &= (\mu^{(1)} - A^{(12)}(A^{(22)})^{-1}\mu^{(2)}) - (A^{(11)} + A^{(12)}(A^{(22)})^{-1}A^{(21)})N^{(1)} && \text{(S13)}\end{aligned}$$

We therefore obtain another Lotka-Volterra community model with different parameters of the form $\mu^{1'} = \mu^{(1)} - A^{(12)}(A^{(22)})^{-1}\mu^{(2)}$ and $A^{(11)'} = A^{(11)} + A^{(12)}(A^{(22)})^{-1}A^{(21)}$. To understand how species richness n_1 affects niche and fitness differences according to the traditional focus we must first understand how species richness n_1 affects $A^{(11)'}$.

We now compute $A^{(11)'} = A^{(11)} + A^{(12)}(A^{(22)})^{-1}A^{(21)}$. To do so, we note that $A^{(22)} = (1-a)I + aJ$, where I is the unit matrix and J is the matrix with all ones. We have $(A^{(22)})^{-1} = \frac{1}{1-a}\left(I - \frac{a}{1+(n_2-1)a}J\right)$, which can be verified as

$$\begin{aligned}A^{(22)}(A^{(22)})^{-1} &= ((1-a)I + aJ)\frac{1}{1-a}\left(I - \frac{a}{1+(n_2-1)a}J\right) && \text{(S14)}\\ &= I + \frac{1}{1-a}\left(-\frac{(1-a)a}{1+(n_2-1)a} + a - \frac{n_2a^2}{1+(n_2-1)a}\right) && \text{(S15)}\\ &= I + \frac{1}{1-a}\left(\frac{-a+a^2+a+(n_2-1)a^2-n_2a^2}{1+(n_2-1)a}\right) && \text{(S16)}\\ &= I && \text{(S17)}\end{aligned}$$

Given this, we can compute $A^{(12)}(A^{(22)})^{-1}A^{(21)}$ as

$$\begin{aligned}A^{(12)}(A^{(22)})^{-1}A^{(21)} &= A^{(12)}\frac{1}{1-a}\left(I - \frac{a}{1+(n_2-1)a}J\right)A^{(21)} && \text{(S18)}\\ &= aJ\frac{1}{1-a}\left(I - \frac{a}{1+(n_2-1)a}J\right)(aJ) && \text{(S19)}\end{aligned}$$

$$
\begin{aligned}
&= \frac{a^2}{1-a}\left(JIJ - \frac{a}{1+(n_2-1)a}J\cdot J\cdot J\right) && \text{(S20)}\\
&= \frac{a^2}{1-a}\left(n_2 J - \frac{n_2 a}{1+(n_2-1)a}n_2^2 J\right) && \text{(S21)}\\
&= \frac{a^2}{1-a}\left(n_2 - \frac{n_2 a}{1+(n_2-1)a}\right)J && \text{(S22)}\\
&= \frac{a^2}{1-a}\frac{n_2+n_2^2a-n_2a+n_2^2a}{1+(n2-1)a}J && \text{(S23)}\\
&= \frac{n_2a^2}{1+(n_2-1)a}J && \text{(S24)}
\end{aligned}
$$

Given this we can compute $A_{ij}^{(11)'} = a + \frac{n_2a^2}{1+(n_2-1)a}$ and $A_{ii}^{(11)'} = 1 + \frac{n_2a^2}{1+(n_2-1)a}$.

We can then compute niche differences of the lower-trophic species as

$$
\begin{aligned}
\mathcal{N}_i^{(1)} &= \frac{r_i-\eta_i}{\mu_i-\eta_i} && \text{(S25)}\\
&= \frac{(\mu_i' - n_1A_{ij}^{(11)'}N^{(1)}) - (\mu_i' - n_1A_{ii}^{(11)'}N^{(1)})}{(\mu_i') - (\mu_i' - n_1A_{ii}^{(11)'}N^{(1)})} && \text{(S26)}\\
&= 1 - \frac{A_{ij}^{(11)'}}{A_{ii}^{(11)'}} && \text{(S27)}
\end{aligned}
$$

We see that this does not depend on n_1, the lower-trophic species richness, as neither $A^{(11)'}$ is independent of n_1.

To compute $\mathcal{F}_l^{(2)}$ we first compute the equilibrium density of the higher-trophic species as

$$
\begin{aligned}
0 &= \mu^{1'} - A^{(11)'}N^{1'} && \text{(S28)}\\
&= \mu^{2'} - A_{ij}^{(11)'}(n_1-1)N^{1'} - A_{ii}^{(11)'}N^{1'} && \text{(S29)}\\
N^{1'} &= \frac{\mu_i^{1'}}{A_{ii}^{(11)'} + (n_1-1)A_{ij}^{(11)'}} && \text{(S30)}
\end{aligned}
$$

we therefore get $\eta_i = \mu_i^{1'} - \frac{A_{ii}^{(11)'}n_1\mu_i^{1'}}{A_{ii}^{(11)'}+(n_1-1)A_{ij}^{(11)'}}$

$$
\begin{aligned}
\mathcal{F}_i^{(1)} &= 1 - \frac{\mu_i}{\mu_i-\eta_i} && \text{(S31)}\\
&= 1 - \frac{\mu_i'}{\frac{A_{ii}^{(11)'}n_1\mu_i^{1'}}{A_{ii}^{(11)'}+(n_1-1)A_{ij}^{(11)'}}} && \text{(S32)}
\end{aligned}
$$

$$= 1 - \frac{1}{n_1} - \frac{(n_1-1)A^{(11)'}_{ij}}{n_1 A^{(11)'}_{ii}} \tag{S33}$$

$$= \left(1 - \frac{A^{(11)'}_{ij}}{A^{(11)'}_{ii}}\right)\left(1 - \frac{1}{n_1}\right) \tag{S34}$$

We therefore see that increasing n_1 increases $\mathcal{F}^{(1)}_i$. The same analysis applies if we change the species richness of the higher trophic level and analyse the niche and fitness differences of the higher trophic level. For the traditional focus we therefore see that the results from Spaak *et al.* (2021a) apply, that is species richness in the same trophic level have no effect on niche differences, but does increase fitness differences.

A.3 Alternative focus

Similar to the traditional focus we can compute $A^{(22)'} = A^{(22)} + A^{(21)}(A^{(11)})^{-1}A^{(12)}$ which gives

$$A^{(22)'}_{lk} = a + \frac{n_1 a^2}{1+(n_1-1)a} \tag{S35}$$

$$= \frac{a + n_1 a^2 - a^2 + n_1 a^2}{1+(n_1-1)a} \tag{S36}$$

$$= a\frac{1 - a + 2n_1 a}{1+(n_1-1)a} \tag{S37}$$

$$A^{(22)'}_{ll} = 1 + \frac{n_1 a^2}{1+(n_1-1)a} \tag{S38}$$

$$= \frac{1 + (n_1-1)a + n_1 a^2}{1+(n_1-1)a} \tag{S39}$$

$$= \frac{1 - a + a n_1(1+a)}{1+(n_1-1)a} \tag{S40}$$

Next we can compute the relative interspecific competition, i.e.

$$\frac{A^{(22)'}_{lk}}{A^{(22)'}_{ll}} = \frac{a\,(1 - a + 2n_1 a)}{1 - a + a(1+a)n_1} \tag{S41}$$

$$= a\frac{1 - a + 2n_1 a + a(1+a)n_1 - a(1+a)n_1}{1 - a + a(1+a)n_1} \tag{S42}$$

$$= a\left(1 + \frac{2n_1 a - a(1+a)n_1}{1 - a + a(1+a)n_1}\right) \tag{S43}$$

$$= a\left(1 + \frac{a(1-a)n_1}{1 - a + a(1+a)n_1}\right) \tag{S44}$$

Therefore, the relative interspecific competition strength is increasing in the species richness of the lower trophic level. As a consequence, we expect niche differences to decrease with increasing n_1.

To compute $\mathcal{F}^{(2)}_l$ we note that $c_{ij} = 1$ because of symmetry. To compute the no-niche growth rate we first compute the equilibrium density of the species as

$$
\begin{aligned}
0 &= \mu^{2'} - A^{(22)'} N^{2'} && \text{(S45)}\\
&= \mu^{2'} - A^{(22)'}_{lk}(n_2 - 1)N^{2'} - A^{(22)'}_{ll} N^{2'} && \text{(S46)}\\
N^{2'} &= \frac{\mu^{2'}}{A^{(22)'}_{ll} + (n_2 - 1)A^{(22)'}_{lk}} && \text{(S47)}
\end{aligned}
$$

We therefore get $\eta_i = \mu^{2'} - \frac{A^{(22)'}_{ll} n_2 \mu^{2'}}{A^{(22)'}_{ll} + (n_2-1)A^{(22)'}_{lk}}$ and therefore

$$
\begin{aligned}
\mathcal{F}^{(2)}_l &= 1 - \frac{\mu_i}{\mu_i - \eta_i} && \text{(S48)}\\
&= 1 - \frac{\mu^{2'}}{\frac{n_2 \mu^{2'} A^{(22)'}_{ll}}{A^{(22)'}_{ll} + (n_2-1)A^{(22)'}_{lk}}} && \text{(S49)}\\
&= 1 - \frac{1 + (n_2 - 1)\frac{A^{(22)'}_{lk}}{A^{(22)'}_{ll}}}{n_2} && \text{(S50)}\\
&= 1 - \frac{A^{(22)'}_{lk}}{A^{(22)'}_{ll}} - \frac{1 - \frac{A^{(22)'}_{lk}}{A^{(22)'}_{ll}}}{n_2} && \text{(S51)}\\
&= \left(1 - \frac{A^{(22)'}_{lk}}{A^{(22)'}_{ll}}\right)\left(1 - \frac{1}{n_2}\right) && \text{(S52)}
\end{aligned}
$$

As shown above, $1 - \frac{A^{(22)'}_{lk}}{A^{(22)'}_{ll}}$ decreases in n_1, therefore fitness differences decrease with increasing species richness of the adjacent trophic level.

Conversely, focusing on the effect of changing the species richness in the higher trophic level and assessing it's effect on the lower trophic level. We know that $\frac{A^{(11)'}_{lk}}{A^{(11)'}_{ll}}$ increases in n_2, the species richness in the higher trophic level. Therefore, we expect niche differences of the lower trophic level to decrease with increasing species richness of the higher trophic level. For the same arguments as mentioned above we therefore expect fitness differences to decrease as well.

A.4 Community focus

We note that the $c_{ij} = 1$ for all species, as all inter-specific species interactions have the same strength. With this we can compute the relevant niche and fitness differences of the two trophic levels

$$
\mathcal{N}^{(1)}_i = 1 - \frac{\mu^{(1)}_i - r^{(1)}_i}{\mu^{(1)}_i - \eta^{(1)}_i} \tag{S53}
$$

$$= 1 - \frac{\mu_i^{(1)} - (\mu_i^{(1)} - an_1N^{(1)} - an_2N^{(2)})}{\mu_i^{(1)} - (\mu_i^{(1)} - n_1N^{(1)} - n_2N^{(2)})} \tag{S54}$$

$$= 1 - a, \tag{S55}$$

which is independent of species richness n_1 and n_2.

For the other niche and fitness differences we first observe that the derivative of the function $\frac{d}{dx}\left(-\frac{\alpha x+\beta}{\gamma x+\delta}\right) = -\frac{\alpha(\gamma x+\delta)-(\alpha x+\beta)\gamma}{(\gamma x+\delta)^2} = -\frac{\alpha\delta-\beta\gamma}{(\gamma x+\delta)^2}$ is negative if $\alpha\delta - \beta\gamma > 0$. Therefore, $-\frac{\alpha x+\beta}{\gamma x+\delta}$ is a decreasing function if $\alpha\delta - \beta\gamma > 0$.

$$\begin{aligned}
\mathcal{F}_i^{(1)} &= 1 - \frac{\mu_i^{(1)}}{\mu_i^{(1)} - \eta_i^{(1)}} && \text{(S56)}\\
&= 1 - \frac{\mu_i^{(1)}}{\mu_i^{(1)} - (\mu_i^{(1)} - n_1N^{(1)} - n_2N^{(2)})} && \text{(S57)}\\
&= 1 - \frac{\mu_i^{(1)}}{n_1N_i^{(1)} + n_2N_l^{(2)}} && \text{(S58)}\\
&= 1 - \mu_i^{(1)}\frac{(1-a)^2 + a(1-a)(n_1+n_2) + 2a^2n_1n_2}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} && \text{(S59)}\\
&= 1 - \mu_i^{(1)}\frac{(1-a)^2 + \frac{a}{\mu_i^{(1)}}\left((1-a)(n_1+n_2)\mu_i^{(1)} + 2an_1n_2\mu_i^{(1)}\right)}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} && \text{(S60)}\\
&= 1 - \mu_i^{(1)}\left(\frac{a}{\mu_i^{(1)}} + \frac{(1-a)^2 + \frac{a}{\mu_i^{(1)}}\left((1-a)n_2\mu_i^{(1)} - (1-a)n_2\mu_l^{(2)}\right)}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})}\right) && \text{(S61)}\\
&= 1 - a - \frac{\mu_i^{(1)}(1-a)^2 + a(1-a)n_2(\mu_i^{(1)} - \mu_l^{(2)})}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} && \text{(S62)}\\
&= (1-a)\left(1 - \frac{\mu_i^{(1)}(1-a) + an_2(\mu_i^{(1)} - \mu_l^{(2)})}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})}\right) && \text{(S63)}
\end{aligned}$$

We therefore see that increasing n_1 increases $\mathcal{F}_i^{(1)}$, as the last term in the brackets is of form $-\frac{0n_1+\beta}{\gamma n_1+\delta}$ with $\beta, \gamma, \delta > 0$. Additionally, for increasing n_1 $\mathcal{F}_i^{(1)}$ approaches $1-a$. To assess the effect of n_2 on $\mathcal{F}_i^{(1)}$ we note that the last term has the form $-\frac{\alpha n_2+\beta}{\gamma n_2+\delta}$ with
$\alpha = a(\mu_i^{(1)} - \mu_l^{(2)}), \beta = \mu_i^{(1)}(1-a)$, $\gamma = 2an_1\mu_i^{(1)} + (1-a)\mu_l^{(2)}, \delta = (1-a)n_1\mu_i^{(1)}$, which leads to

$$\begin{aligned}
\alpha\delta - \beta\gamma &= a(\mu_i^{(1)} - \mu_l^{(2)})(1-a)n_1\mu_i^{(1)} - \mu_i^{(1)}(1-a)(2an_1\mu_i^{(1)} + (1-a)\mu_l^{(2)}) && \text{(S64)}\\
&= (1-a)\mu_i^{(1)}\left(an_1(\mu_i^{(1)} - \mu_l^{(2)} - 2\mu_i^{(1)}) - (1-a)\mu_l^{(2)}\right) && \text{(S65)}\\
&= (1-a)\mu_i^{(1)}\left(-an_1(\mu_i^{(1)} + \mu_l^{(2)}) - (1-a)\mu_l^{(2)}\right) && \text{(S66)}
\end{aligned}$$

This is negative if $n_1 > \frac{(1-a)|\mu_l^{(2)}|}{a(\mu_i^{(1)}+\mu_l^{(2)})}$. This condition is equivalent to the condition that the higher trophic level have a positive equilibrium density, i.e. $N_l^{(2)} > 0$, see S8 We therefore conclude that $F_i^{(1)}$ increases with increasing n_2.

$$\begin{aligned}
\mathcal{N}_l^{(2)} &= 1 - \frac{\mu_l^{(2)} - r_l^{(2)}}{\mu_l^{(2)} - \eta_l^{(2)}} \tag{S67}\\
&= 1 - \frac{-an_1N_i^{(1)} + an_2N_l^{(2)}}{n_1N_i^{(1)} + n_2N_l^{(2)}} \tag{S68}\\
&= 1 - a\frac{-n_1N_i^{(1)} + n_2N_l^{(2)}}{n_1N_i^{(1)} + n_2N_l^{(2)}} \tag{S69}\\
&= 1 - a\frac{-n_1(an_2(\mu_i^{(1)} - \mu_l^{(2)}) + (1-a)\mu_i^{(1)}) + n_2(an_1(\mu_i^{(1)} + \mu_l^{(2)}) + (1-a)\mu_l^{(2)})}{n_1(an_2(\mu_i^{(1)} - \mu_l^{(2)}) + (1-a)\mu_i^{(1)}) + n_2(an_1(\mu_i^{(1)} + \mu_l^{(2)}) + (1-a)\mu_l^{(2)})} \tag{S70}\\
&= 1 - a\frac{2\mu_l^{(2)}an_1n_2 - (1-a)(n_1\mu_i^{(1)} - n_2\mu_l^{(2)})}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} \tag{S71}\\
&= 1 - a\left(\frac{\mu_l^{(2)}}{\mu_i^{(1)}} + \frac{-(1-a)(n_1\mu_i^{(1)} - n_2\mu_l^{(2)}) - (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})\frac{\mu_l^{(2)}}{\mu_i^{(1)}}}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})}\right) \tag{S72}\\
&= 1 - a\left(\frac{\mu_l^{(2)}}{\mu_i^{(1)}} - (1-a)\frac{n_1(\mu_i^{(1)} + \mu_l^{(2)}) - n_2\mu_l^{(2)}\frac{\mu_i^{(1)} - \mu_l^{(2)}}{\mu_i^{(1)}}}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})}\right) \tag{S73}\\
&= 1 - a\frac{\mu_l^{(2)}}{\mu_i^{(1)}} - \frac{a(1-a)n_2\mu_l^{(2)}\frac{\mu_i^{(1)} - \mu_l^{(2)}}{\mu_i^{(1)}}}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} + \frac{a(1-a)n_1(\mu_i^{(1)} + \mu_l^{(2)})}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} \tag{S74}\\
&= 1 - a\frac{\mu_l^{(2)}}{\mu_i^{(1)}} - a(1-a)\frac{n_2\mu_l^{(2)}\frac{\mu_i^{(1)} - \mu_l^{(2)}}{\mu_i^{(1)}} - n_1(\mu_i^{(1)} + \mu_l^{(2)})}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} \tag{S75}
\end{aligned}$$

The last expression is of the form $-\frac{\alpha n_1 + \beta}{\gamma n_1 + \delta}$ with $\alpha = -(\mu_i^{(1)} + \mu_l^{(2)}), \beta = n_2\mu_l^{(2)}\frac{\mu_i^{(1)} - \mu_l^{(2)}}{\mu_i^{(1)}}$, $\gamma = 2a\mu_i^{(1)}n_2 + (1-a)\mu_i^{(1)}, \delta = (1-a)n_2\mu_l^{(2)}$ where α, β and δ are negative, hence $\alpha\delta - \beta\gamma$ is positive and hence $\mathcal{N}_l^{(2)}$ decreases with increasing n_1.

To assess the dependence on n_2 we not that the last expression is of the form $-\frac{\alpha n_2 + \beta}{\gamma n_2 + \delta}$ with $\alpha = \mu_l^{(2)}\frac{\mu_i^{(1)} - \mu_l^{(2)}}{\mu_i^{(1)}}, \beta = -n_1(\mu_i^{(1)} + \mu_l^{(2)})$, $\gamma = 2\mu_i^{(1)}an_1 + (1-a)\mu_l^{(2)}, \delta = (1-a)n_1\mu_i^{(1)}$ which leads to

$$\begin{aligned}
\alpha\delta - \beta\gamma &= \mu_l^{(2)}\frac{\mu_i^{(1)} - \mu_l^{(2)}}{\mu_i^{(1)}}(1-a)n_1\mu_i^{(1)} - (-n_1(\mu_i^{(1)} + \mu_l^{(2)}))(2\mu_i^{(1)}an_1 + (1-a)\mu_l^{(2)}) \tag{S76}\\
&= n_1\left((1-a)\mu_l^{(2)}((\mu_i^{(1)} - \mu_l^{(2)}) + (\mu_i^{(1)} + \mu_l^{(2)})) + (\mu_i^{(1)} + \mu_l^{(2)})2\mu_i^{(1)}an_1\right) \tag{S77}
\end{aligned}$$

$$= 2n_1\mu_i^{(1)}\left((1-a)\mu_l^{(2)} + an_1(\mu_i^{(1)} + \mu_l^{(2)})\right) \tag{S78}$$

This last expression is positive if $n_1 > \frac{|\mu_l^{(2)}|(1-a)}{a(\mu_i^{(1)}+\mu_l^{(2)})}$, which is the same expression as for $\mathcal{F}_i^{(1)}$. We therefore note that $\mathcal{N}_l^{(2)}$ decreases in n_2.

$$\begin{aligned}
\mathcal{F}_l^{(2)} &= 1 - \frac{\mu_l^{(2)}}{\mu_l^{(2)} - \eta_l^{(2)}} && \text{(S79)}\\
&= 1 - \frac{\mu_l^{(2)}}{n_1N_i^{(1)} + n_2N_l^{(2)}} && \text{(S80)}\\
&= 1 - \mu_l^{(2)}\frac{(1-a)^2 + a(1-a)(n_1+n_2) + 2a^2n_1n_2}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} && \text{(S81)}\\
&= 1 - \mu_l^{(2)}\frac{(1-a)^2 + \frac{a}{\mu_i^{(1)}}\left((1-a)(n_1+n_2)\mu_i^{(1)} + 2an_1n_2\mu_i^{(1)}\right)}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} && \text{(S82)}\\
&= 1 - \mu_l^{(2)}\left(\frac{a}{\mu_i^{(1)}} + \frac{(1-a)^2 + \frac{a}{\mu_i^{(1)}}\left((1-a)n_2\mu_i^{(1)} - (1-a)n_2\mu_l^{(2)}\right)}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})}\right) && \text{(S83)}\\
&= 1 - a\frac{\mu_l^{(2)}}{\mu_i^{(1)}} - (1-a)\frac{\mu_l^{(2)}(1-a) + \mu_l^{(2)}an_2\frac{\mu_i^{(1)}-\mu_l^{(2)}}{\mu_i^{(1)}}}{2an_1n_2\mu_i^{(1)} + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} && \text{(S84)}\\
&= 1 - a\frac{\mu_l^{(2)}}{\mu_i^{(1)}} - \frac{a(1-a)n_2\mu_l^{(2)}\frac{\mu_i^{(1)}-\mu_l^{(2)}}{\mu_i^{(1)}}}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} - \frac{\mu_l^{(2)}(1-a)^2}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} && \text{(S85)}\\
& && \text{(S86)}
\end{aligned}$$

The expression in S84is of the form $-\frac{0n_1+\beta}{\gamma n_1+\delta}$ with negative β (note that $\mu_l^{(2)} < 0$) and positive γ, therefore $\mathcal{F}_l^{(2)}$ decreases in n_1.

The expression in S84is of the form $\frac{\alpha n_2+\beta}{\gamma n_1+\delta}$ with $\alpha = \mu_l^{(2)}a\frac{\mu_i^{(1)}-\mu_l^{(2)}}{\mu_i^{(1)}}, \beta = \mu_l^{(2)}(1-a)$, $\gamma = 2an_1\mu_i^{(1)} + (1-a)\mu_l^{(2)}$ and $\delta = (1-a)n_1\mu_i^{(1)}$ which leads to

$$\begin{aligned}
\alpha\delta - \beta\gamma &= \mu_l^{(2)}a\frac{\mu_i^{(1)} - \mu_l^{(2)}}{\mu_i^{(1)}}(1-a)n_1\mu_i^{(1)} - \mu_l^{(2)}(1-a)(2an_1\mu_i^{(1)} + (1-a)\mu_l^{(2)}) && \text{(S87)}\\
&= (1-a)\mu_l^{(2)}\left(an_1(\mu_i^{(1)} - \mu_l^{(2)}) - 2an_1\mu_i^{(1)} - (1-a)\mu_l^{(2)}\right) && \text{(S88)}\\
&= -(1-a)\mu_l^{(2)}\left(an_1(\mu_i + \mu_l^{(2)}) + (1-a)\mu_l^{(2)}\right) && \text{(S89)}
\end{aligned}$$

This last expression is positive if $n_1 > \frac{|\mu_l^{(2)}|(1-a)}{a(\mu_i^{(1)}+\mu_l^{(2)})}$, which is the same expression as for $\mathcal{F}_i^{(1)}$ and for

$\mathcal{N}_l^{(2)}$. We therefore note that $\mathcal{N}_l^{(2)}$ decreases in n_2.

A.5 Relative change of niche and fitness differences

For the higher trophic level we note that the expression for niche and fitness differences are almost equivalent (see equations S74 and S85). Specifically, they differ only in the last term of each expression.

$$\mathcal{F}_l^{(2)} - \mathcal{N}_l^{(2)} = -\frac{a(1-a)n_1(\mu_i^{(1)} + \mu_l^{(2)})}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} - \frac{\mu_l^{(2)}(1-a)^2}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} \tag{S90}$$

$$= -\frac{(1-a)\left(an_1(\mu_i^{(1)} + \mu_l^{(2)}) + \mu_l^{(2)}(1-a)\right)}{2\mu_i^{(1)}an_1n_2 + (1-a)(n_1\mu_i^{(1)} + n_2\mu_l^{(2)})} \tag{S91}$$

Which is negative if $n_1 > \frac{|\mu_l^{(2)}|(1-a)}{a(\mu_i^{(1)} + \mu_l^{(2)})}$, i.e. if the higher trophic level has positive equilibrium density. This expression is of the form $-\frac{\alpha n_1 + \beta}{\gamma n_1 + \delta}$ with negative α, γ and δ, but positive β, hence $\alpha\delta - \gamma\beta > 0$. Consequentially, $\frac{d}{dn_1}\mathcal{F}_l^{(2)} - \frac{d}{dn_1}\mathcal{N}_l^{(2)}$ is negative, or said differently, changes in species richness of the lower trophic level have a stronger effect on niche than on fitness differences of the higher trophic level.

Similarly, this expression is of the form $-\frac{0n_2 + \beta}{\gamma n_2 + \delta}$ with positive β and γ, hence $\alpha\delta - \gamma\beta < 0$. Consequentially, $\frac{d}{dn_1}\mathcal{F}_l^{(2)} - \frac{d}{dn_1}\mathcal{N}_l^{(2)}$ is positive, or said differently, changes in species richness of the higher trophic level have a stronger effect on fitness than on niche differences of the higher trophic level.

A.6 Summary of key results

Method	type	Equation	Effect low	Effect high
Trad.	$\mathcal{N}_i^{(1)}$	$1-\frac{A_{ij}^{(11)'}}{A_{ii}^{(11)'}}$	Constant	NA
Trad.	$\mathcal{F}_i^{(1)}$	$\left(1-\frac{A_{ij}^{(11)'}}{A_{ii}^{(11)'}}\right)\left(1-\frac{1}{n_1}\right)$	Increase	NA
Trad.	$\mathcal{N}_l^{(2)}$	$1-\frac{A_{lk}^{(22)'}}{A_{ll}^{(22)'}}$	NA	Constant
Trad.	$\mathcal{F}_l^{(2)}$	$\left(1-\frac{A_{lk}^{(22)'}}{A_{ll}^{(22)'}}\right)\left(1-\frac{1}{n_2}\right)$	NA	increase
Alt.	$\mathcal{N}_i^{(1)}$	$1-a\left(\frac{a(1-a)n_2}{1-a+a(1+a)n_2}\right)$	NA	decrease
Alt.	$\mathcal{F}_i^{(1)}$	$\left(1-a\left(\frac{a(1-a)n_2}{1-a+a(1+a)n_2}\right)\right)\left(1-\frac{1}{n_1}\right)$	NA	decrease
Alt.	$\mathcal{N}_l^{(2)}$	$1-a\left(\frac{a(1-a)n_1}{1-a+a(1+a)n_1}\right)$	decrease	NA
Alt.	$\mathcal{F}_l^{(2)}$	$\left(1-a\left(\frac{a(1-a)n_1}{1-a+a(1+a)n_1}\right)\right)\left(1-\frac{1}{n_2}\right)$	decrease	NA
Com.	$\mathcal{N}_i^{(1)}$	$1-a$	Constant	Constant
Com.	$\mathcal{F}_i^{(1)}$	$(1-a)\left(1-\frac{\mu_i^{(1)}(1-a)+an_2(\mu_i^{(1)}-\mu_l^{(2)})}{2an_1n_2\mu_i^{(1)}+(1-a)(n_1\mu_i^{(1)}+n_2\mu_l^{(2)})}\right)$	increase	increase
Com.	$\mathcal{N}_l^{(2)}$	$1-a\frac{\mu_l^{(2)}}{\mu_i^{(1)}}-a(1-a)\frac{n_2\mu_l^{(2)}\frac{\mu_i^{(1)}-\mu_l^{(2)}}{\mu_i^{(1)}}-n_1(\mu_i^{(1)}+\mu_l^{(2)})}{2\mu_i^{(1)}an_1n_2+(1-a)(n_1\mu_i^{(1)}+n_2\mu_l^{(2)})}$	decrease	decrease
Com.	$\mathcal{F}_l^{(2)}$	$1-a\frac{\mu_l^{(2)}}{\mu_i^{(1)}}-(1-a)\frac{\mu_l^{(2)}(1-a)+\mu_l^{(2)}an_2\frac{\mu_i^{(1)}-\mu_l^{(2)}}{\mu_i^{(1)}}}{2an_1n_2\mu_i^{(1)}+(1-a)(n_1\mu_i^{(1)}+n_2\mu_l^{(2)})}$	decrease	decrease

Table S1: We summarize the expressions for niche and fitness differences (second and third column) according to the three different focus (first column). We report the effect of increasing the lower ("Effect low") or increasing the higher ("Effect high") trophic level. NA implies that this focus can not be applied to the given change in species richness.

B Simulations

To validate that our analytic results apply beyond the strict assumptions, we used simulations to relax the assumptions. We performed a large set of additional simulations.

B.1 Alternative presentation of Figure 3

Figure S1 reproduces Figure 3 except that the x-axis now shows quantifies the rate of change in niche or fitness differences with an increased proportion of lower-trophic species (i.e., slope in Figure 2). A higher value signifies stronger effects.

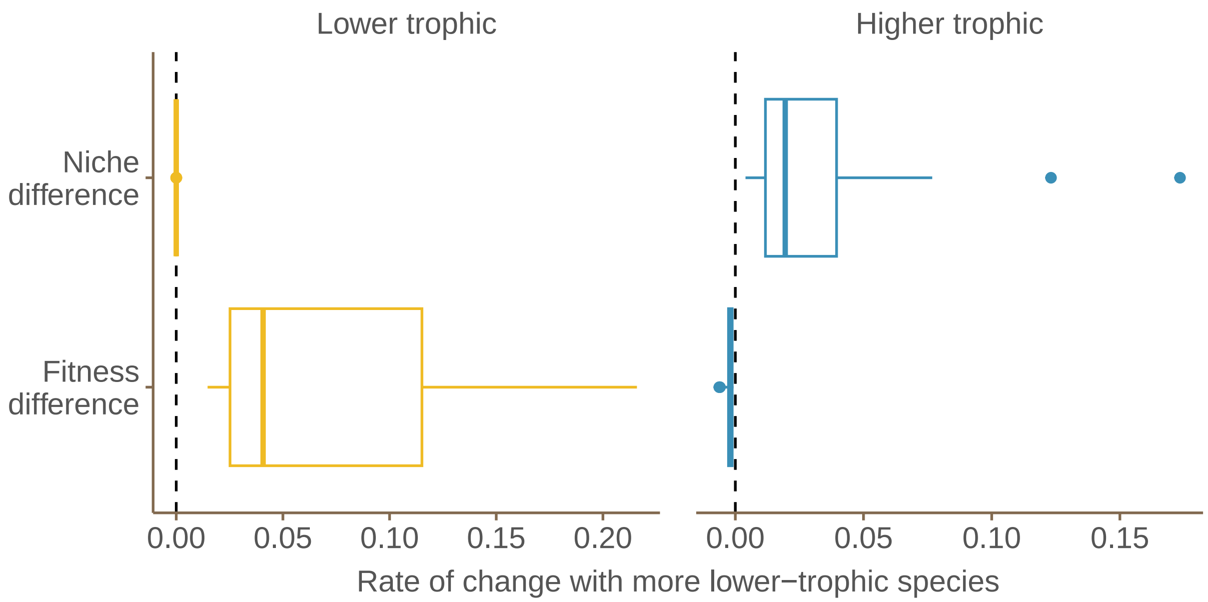


Figure S1: **Diversity at lower (respectively higher) trophic is primarily constrained by fitness (respectively niche) differences**. To validate the generality of our analytic results, we adopted a full-factorial simulation approach by relaxing various assumptions (see Methods for details). The horizontal axis quantifies the rate of change in niche or fitness differences with an increased proportion of lower-trophic species, where higher values signify stronger effects. The vertical dashed line represents the baseline of no effect. Despite adopting more relaxed and realistic conditions, the observed trends closely mirror our analytical predictions. For lower-trophic species (denoted in yellow), a higher proportion of lower-trophic species shows an almost null impact on their niche differentiation (Upper Left) but a strong increase in fitness disparity (Lower Left). Conversely, for higher-trophic species (denoted in blue), the trend is reversed, with their niche differences becoming higher and fitness differences remaining almost constant. These findings further confirm that species diversity in trophic levels is constrained by contrasting coexistence mechanisms.

B.2 Low niche dimension

In the main text, we assumed that interspecific interaction strengths are drawn from a probability distribution. This could correspond to the assumption that the species compete for many different resources and have many traits that affect their interaction strength (Barbier *et al.*, 2021). We therefore simulate a community model where the interaction strength between species is derived mechanistically from an underlying resource competition model known as the MacArthur resource model (Macarthur & Levins, 1967). To expand this model to a two-trophic community, we follow the notation of (Spaak *et al.*, 2023).

Specifically, we assume that the species in the lower trophic level compete for a resource continuum, the carrying capacity of the resources is given by $K(r) = C\exp\left(-\frac{r^2}{2\omega^2}\right)$, where C is the maximum carrying capacity, r is the resource and ω is the niche breadth of the environmental niche (Fig. S2A, black line). Each species from the lower trophic level is defined by two traits, their resource consumption preference $x_i^{(1)}$ and the niche width $\sigma_i^{(1)}$. Given these two traits, the resource consumption of a species is given by $u_i(r) = \frac{1}{\sigma_i^{(1)}\sqrt{2\pi}}\exp\left(-\frac{(x_i^{(1)}-r)^2}{2(\sigma_i^{(1)})^2}\right)$ (Fig. S2A, blue line). The inter-specific competition $a_{ij}^{(11)}$ between two species is then given as the integral of the two resource uptake functions, i.e. $a_{ij} = \int u_i(r)u_j(r)dr$. Similarly, the intrinsic growth rate $\mu_i^{(1)}$ is given by the integral of the resource uptake function multiplied by the carrying capacity of the resources, i.e. $\mu_i^{(1)} = \int K(r)u_i(r)dr$.

Similarly, each predator has a preferred prey size $x_j^{(2)}$ as well as a niche width $\sigma_j^{(2)}$. For simplicity, we assume that the resource preference $x_i^{(1)}$ is equal to the body size of the prey and hence determines how strongly a predator will consume this prey (Fig. S2A, red line). The interaction strength between a predator and a prey is then given by a Gaussian kernel, i.e. $a_{ij}^{(12)} = \frac{1}{\sigma_j^{(2)}\sqrt{2\pi}}\exp\left(-\frac{\left(x_j^{(2)}-x_i^{(1)}\right)^2}{2(\sigma_j^{(2)})^2}\right)$. The predators have to digest the consumed prey and therefore the effect of the prey on the predator is multiplied with a conversion factor $e = 0.2$, i.e. $a_{ji}^{(21)} = ea_{ij}^{(12)}$. We assume that the predators have no direct interspecific interactions, i.e. $a_{lk}^{(22)} = 0$ for $l \neq k$, however, each predator has a self-limitation, which we assumed to be equal to 0.5. This was necessary to ensure stable coexistence. Finally, we assume that the predators have a mortality rate of $\mu_j^{(2)} = 0.1$.

We created 1000 communities. We first randomly defined the available resources by choosing $C \in [3, 8]$ and $\omega \sim \mathcal{N}(20, 3)$. We then randomly set species richness from 10 to 26 species, with each species having a 70% chance of being a prey species and 30% chance of being a predator species. The resource preferences $x_i^{(1)}$ of the prey were then evenly spaced on the resource axis, and similarly the preferred prey size $x_j^{(2)}$ of the predators (Fig. S2). Finally, we chose a random niche width $\sigma_i^{(1)} \in [0.3, 0.6]$. The niche width of the predators was chosen to be 3 times larger than that of the prey to avoid continuous assembly cycles (Spaak *et al.*, 2023).

For these communities, we then computed niche and fitness differences according to the community focus. However, some of the communities were not able to coexist or some of the invasion growth rates did not exist. These were removed from the analysis. As in the main text, we see a wider spread of fitness differences than of niche differences for the lower trophic level (Fig. S2C), however, we see a wider spread of niche differences than of fitness differences for the higher trophic level (Fig. S2D).

Finally, we asked how sensitive these niche and fitness differences are to perturbations. To do so, we randomly selected a species from each community and removed this species. We then computed the niche and fitness differences of all other species, with this one species absent. We then computed how the perturbation of removing one species affects the niche and fitness differences of each species. We see the same pattern as before, but even clearer. Specifically, the niche difference of the lower trophic level is almost not affected by the perturbation, but fitness differences are affected (Fig. S2E). Conversely, the niche differences of the higher trophic level are affected but not the fitness differences of the higher trophic level (Fig. S2D).

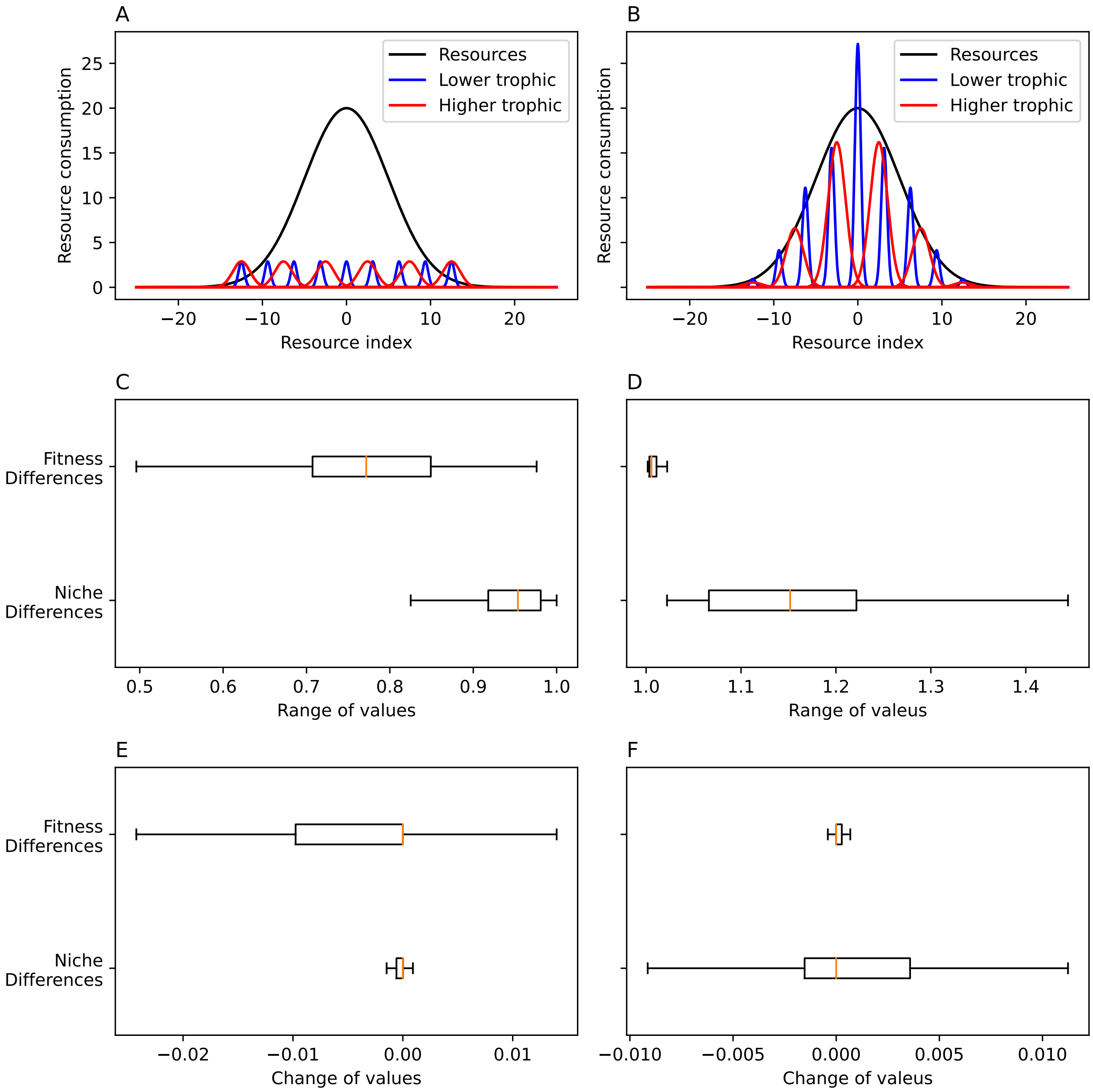


Figure S2: We computed niche and fitness differences for a community model with only one niche axis. A: The species of the lower trophic levels compete for the available resources (black line). Each blue line represents the resource consumption of one species. The predation preferences of the predators are shown in red, which consume the lower trophic levels. B: We simulated the community model to equilibrium and multiplied the resource consumption vectors by the equilibrium density, the scaled resource consumption rates losely track the available resources. C,D: We computed niche and fitness differences for 1000 communities and show the distribution of niche and fitness differences for the lower (C) and higher (D) trophic level. E,F: Finally, we removed one species and computed how much this affects the niche and fitness differences of the remaining species for the lower (E) and higher trophic levels (F).

B.3 Decreasing mean interaction strength with increasing species richness

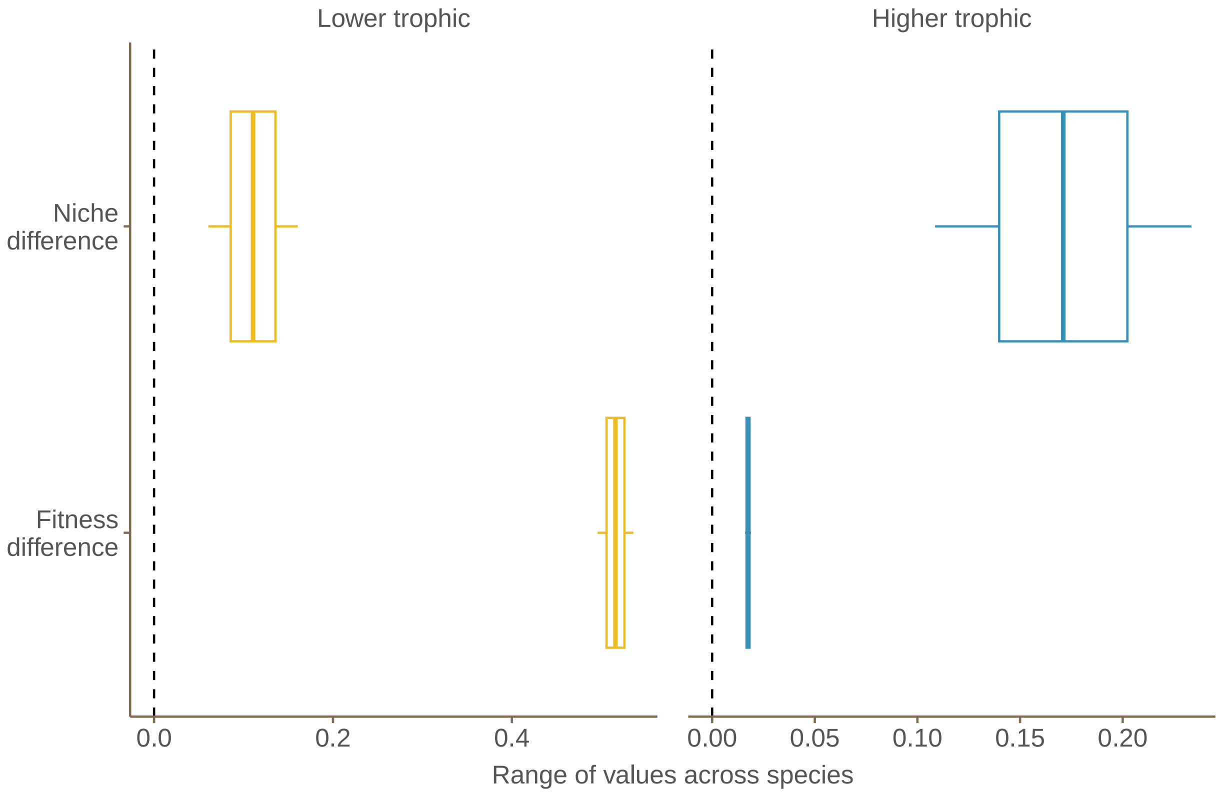


Figure S3: The same as Figure S5 except that the mean interaction strength scales with the square root of total species. This scaling is a typical assumption in random matrix theory. While we observe a higher range of niche differences for lower-trophic species (as well as fitness differences for higher-trophic species), the qualitative patterns still hold.

B.4 Different interaction strength depending on the trophic levels

Previously, we assumed that all interspecific interactions are drawn from the same underlying distribution (e.g., $A_{ij}^{(11)}, A_{jl}^{(12)}, A_{lj}^{(21)}, A_{kl}^{(22)} \sim N(\alpha, \sigma)$). This is likely not the case in natural communities, as these interaction strengths might depend on the trophic community. To address this, we simulated communities where the underlying distributions for interspecific interactions depend on the trophic levels, i.e.
$A_{ij}^{(11)} \sim N(\alpha^{(11)}, \sigma^{(11)}), A_{jl}^{(12)} \sim N(\alpha^{(12)}, \sigma^{(12)}), A_{lj}^{(21)} \sim N(\alpha^{(21)}, \sigma^{(21)})$ and $A_{kl}^{(22)} \sim N(\alpha^{(22)}, \sigma^{(22)})$.

We randomly created 100 communities types where the mean and variance of the inter-specific distributions were all drawn randomly from a uniform distribution, i.e. $\alpha^{(xy)} \sim U[0.1, 0.4]$ and $\sigma^{(xy)} \sim U[0.005, 0.02]$. For each community type we created two communities, one community with high species richness in the lower trophic level and one community with high species richness in the higher trophic level. Species richness was always set to 10, with a composition of 8 species in one trophic level and 2 species in the other trophic level. We then computed niche and fitness differences according to the community focus for these communities and compared the mean niche differences for each trophic level. Finally, we computed how much the change in community composition affects the mean niche and fitness differences per trophic level. The results confirm the

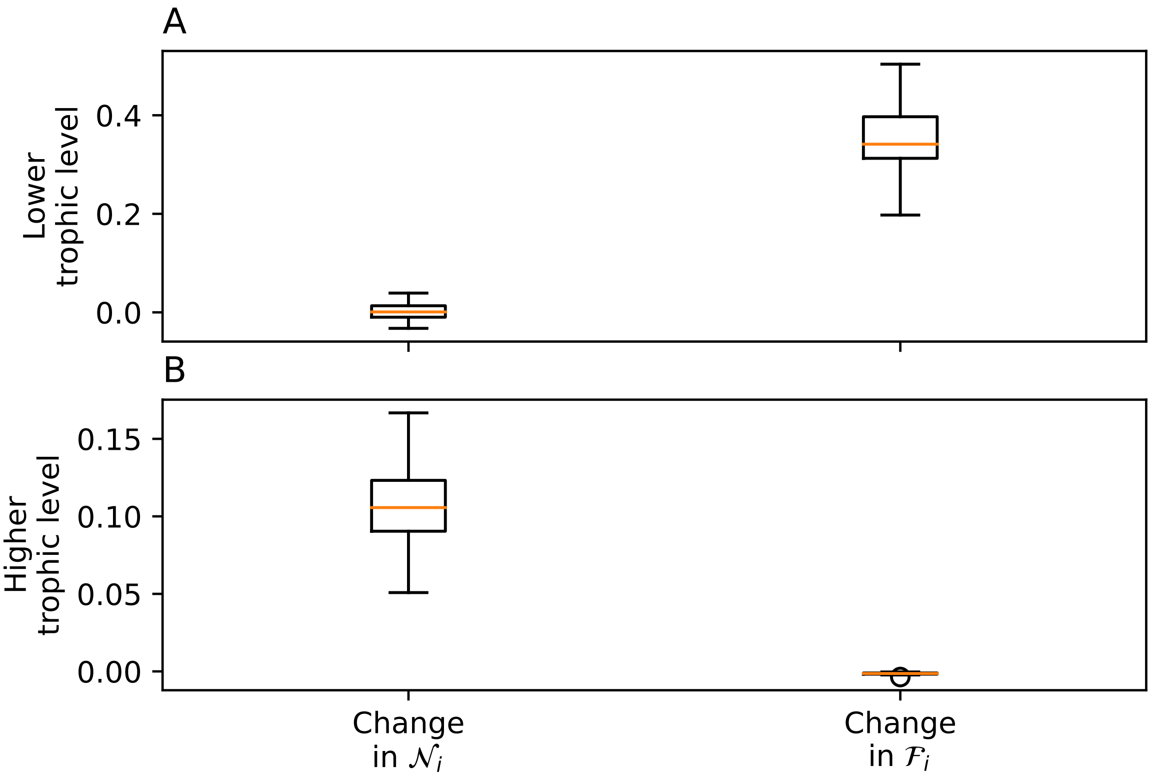


Figure S4: For different interspecific interaction distributions we computed how much a change in community composition affects niche and fitness differences of the lower (panel A) and higher (panel B) trophic level. As found in the main text, community composition affects fitness differences of the lower trophic level but affects the niche differences of the higher trophic level, even under these more general assumptions.

results found in the main text with more generality, change in community composition affect the fitness differences of the lower trophic level but the niche differences of the higher trophic level.

C Testing in another perspective of assembly in multitrophic communities

We now study how community composition affects niche and fitness differences by changing the proportion of lower-trophic species while keeping total species richness constant. We did not use the traditional or the alternative focus here because altering the proportion of lower-trophic species affects both higher and lower trophic levels, resulting in no changes to overall species richness.

Focusing on niche differences, increasing the proportion of lower-trophic species does not affect the niche differences of lower trophic levels, but increases the niche differences of higher trophic levels (Figure 3 left panel). Heuristically, niche difference is calculated as the mean difference between a species and a randomly chosen individual from the community. For a higher-trophic species, a higher proportion of lower-trophic species implies that the higher-trophic more often interacts with a lower-trophic species, leading to an increase in its niche differences. However, in our simulations, a lower-trophic species has the same niche difference with other lower-trophic species as with the higher-trophic species (see Methods), so the proportion of lower-trophic species does not affect its niche difference. More generally, the effect on lower-trophic species' niche differences depends on whether their differences with higher-trophic species are smaller or larger than those with another lower-trophic species; yet this effect is generally small.

Focusing on fitness differences, increasing the proportion of lower-trophic species increases the fitness differences of the lower trophic level and slightly decreases the fitness differences of the higher trophic level (Figure 3 right panel). Heuristically, increasing the proportion of lower-trophic species increases the total species density, which decreases the no-niche growth rate η_i, a main driver of fitness differences (Eqn. 3). For a lower-trophic species, higher total density implies more fierce competition, which increases fitness differences. In contrast, for a higher-trophic species, higher total density implies more potential lower-trophic species, which decreases their fitness differences.

The results obtained from the community focus are consistent with the traditional and alternative focuses, which suggest that a high or low proportion of lower-trophic species can hinder the persistence of either lower- or higher-trophic species. However, while the predicted patterns are the same, only by considering the community focus can we recognize that different trophic levels are influenced by distinct coexistence mechanisms.

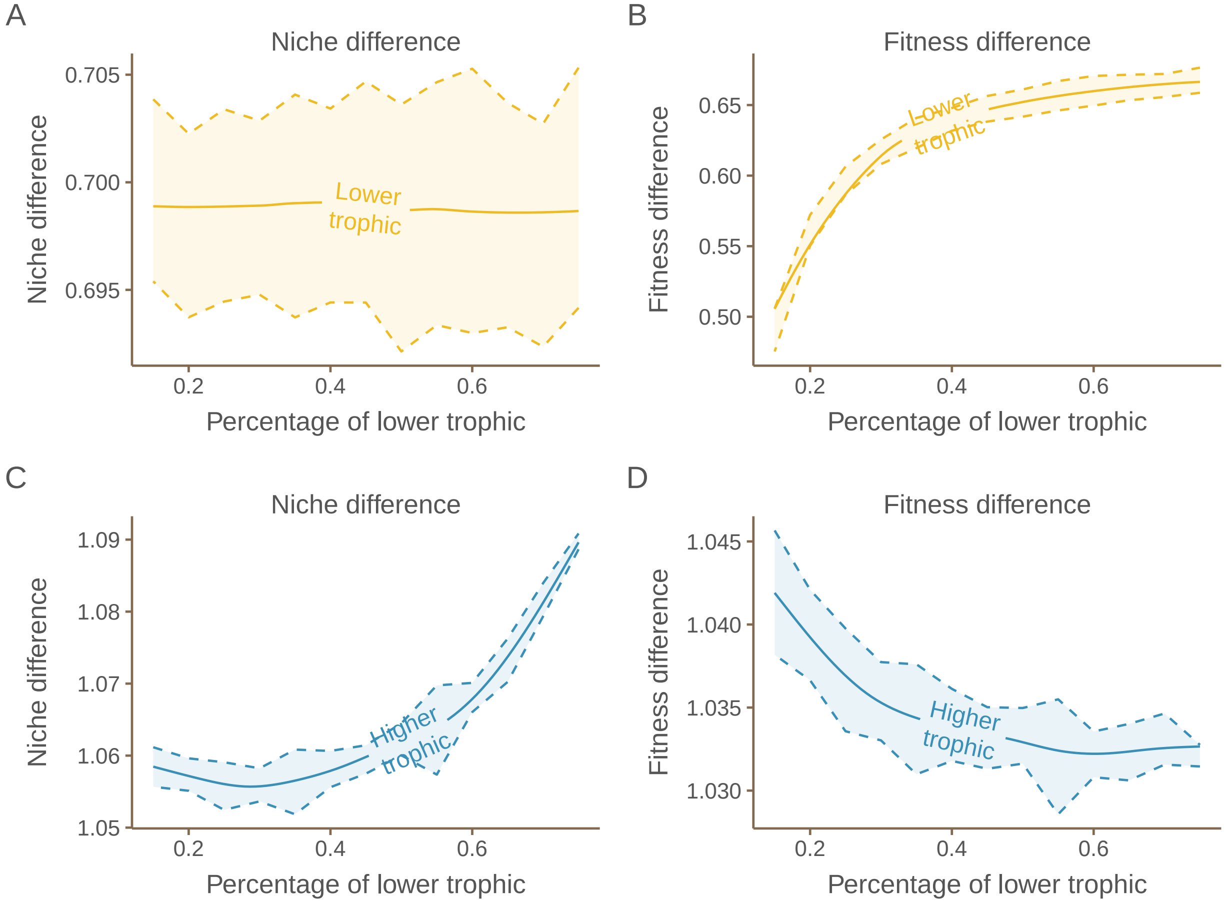


Figure S5: Diversity at lower (respectively higher) trophic is primarily constrained by fitness (respectively niche) differences. We alter the trophic composition of the community while keeping total species richness constant. As with changes in species richness, the community composition does not affect niche differences of the lower trophic level (Panel A), but increases its fitness differences (Panel B). In contrast, niche differences of the higher trophic level increase with increasing proportion of lower-trophic species (Panel C), while fitness differences of the higher trophic level decrease with increasing proportion of lower-trophic species (Panel D). The pattern in Panel C is driven by the decrease of higher-trophic species richness (see Figure 2 I), while the pattern in Panel D is driven by the increase of lower-trophic species richness (see Figure 2 F). As with changes of species richness, the changes of niche differences are stronger than changes of fitness differences for the higher trophic level.

D Analysis of tri-trophic communities

We then test whether our findings in two-trophic communities generalize to tri-trophic community. Specifically, for a community with 21 species in total (the median species richness of the empirical data), we altered the proportion of each trophic level and computed niche and fitness differences of the community as a whole (community focus). To assess the robustness of coexistence we computed the distance of a species niche and fitness differences to the persistence line, which is given by $\mathcal{N}_i - \mathcal{F}_i = \frac{r_i}{\mu_i - \eta_i}$ and can be interpreted how strongly the niche differences compensate for the fitness differences. Intuitively, a species with a high invasion growth rate r_i is less prone to extinction (Ellner *et al.*, 2019; Barabás *et al.*, 2018; Spaak *et al.*, 2021b), but see Pande *et al.* (2020). However, species with fast life-cycles will generally have higher invasion growth rates, independent of their actual robustness against extinction, we therefore scale r_i with $\mu_i - \eta_i$, a measure of how strongly a species limits itself. This approach is conceptually similar to the scaling of the invasion growth rate proposed by Pande & Shnerb (2022).

In general, the results from three-trophic communities align with those of two-trophic communities. In both cases, coexistence is most likely when trophic levels have low species richness (Figure S6G-I). Niche differences in the first trophic level remain unaffected by the trophic composition (Figure S6 A), while the niche differences of the second and third levels are affected (Figure S6B and C). The fitness differences of the second and third trophic levels are largely unaffected by the trophic composition, except for a slight increase fitness when there is a high percentage of lower-trophic species present (Figure S6E,F), conversely the trophic composition affects the fitness differences of the lower-trophic trophic level. These observations can be explained heuristically using similar reasoning as in two-trophic communities.

Finally, to explore the most stable multitrophic structures, we randomly chose a mean interaction strength α, standard-deviation of interaction strength σ, species richness n and mortality rate of higher trophic level m_i. For each of the 100 random parametrization, we kept the five most stable community compositions. This lead to a distribution of communities where approximately 60-80% of the species are in the first trophic level (Figure S7A)

We then compared our results on tri-trophic communities with empirical data. We analyzed 358 food webs from web of life (WWW.WEB-OF-LIFE.ES). We determined the trophic level of each species by considering species without prey as lower-trophic and assigned them a trophic level of 1. For all other species, we calculated their trophic level by taking the mean of their prey's trophic levels and adding 1, We were able to compute the trophic levels for 347 of the 358 food-webs. We grouped species with trophic levels between 2 and 2.5 into the second trophic level, species with trophic levels between 2.5 and 3.5 into the third trophic level and so on (see Figure S8B). We excluded 157 communities where the maximum trophic level exceeded 3.5 (Figure S8A). Finally, we excluded 60 communities which had no species in the third trophic level, resulting in a total of 130 tri-trophic communities.

We then quantified the divergence between the theoretical null expectation (Figure S8A) and the empirical pattern (Figure S8B). While the Kullback–Leibler divergence is a standard measure, the limited size of the empirical data set renders a kernel density estimate challenging. Thus, we utilized a nearest-neighbor estimator of distribution distance. The calculated divergence was found to be 0.04. To interpret this divergence, we compared it with a divergence from a random expectation, where the number of species at each trophic level is randomly drawn. In this case, the divergence averaged 0.73, with a standard deviation of 0.0023. The resulting z-score was -13.8, which indicates that our null expectation has significantly better explanatory power than the random expectation. This comparative analysis demonstrates that our theoretical null expectations are a more robust explanation of the patterns observed in empirical data than would be expected from a random distribution.

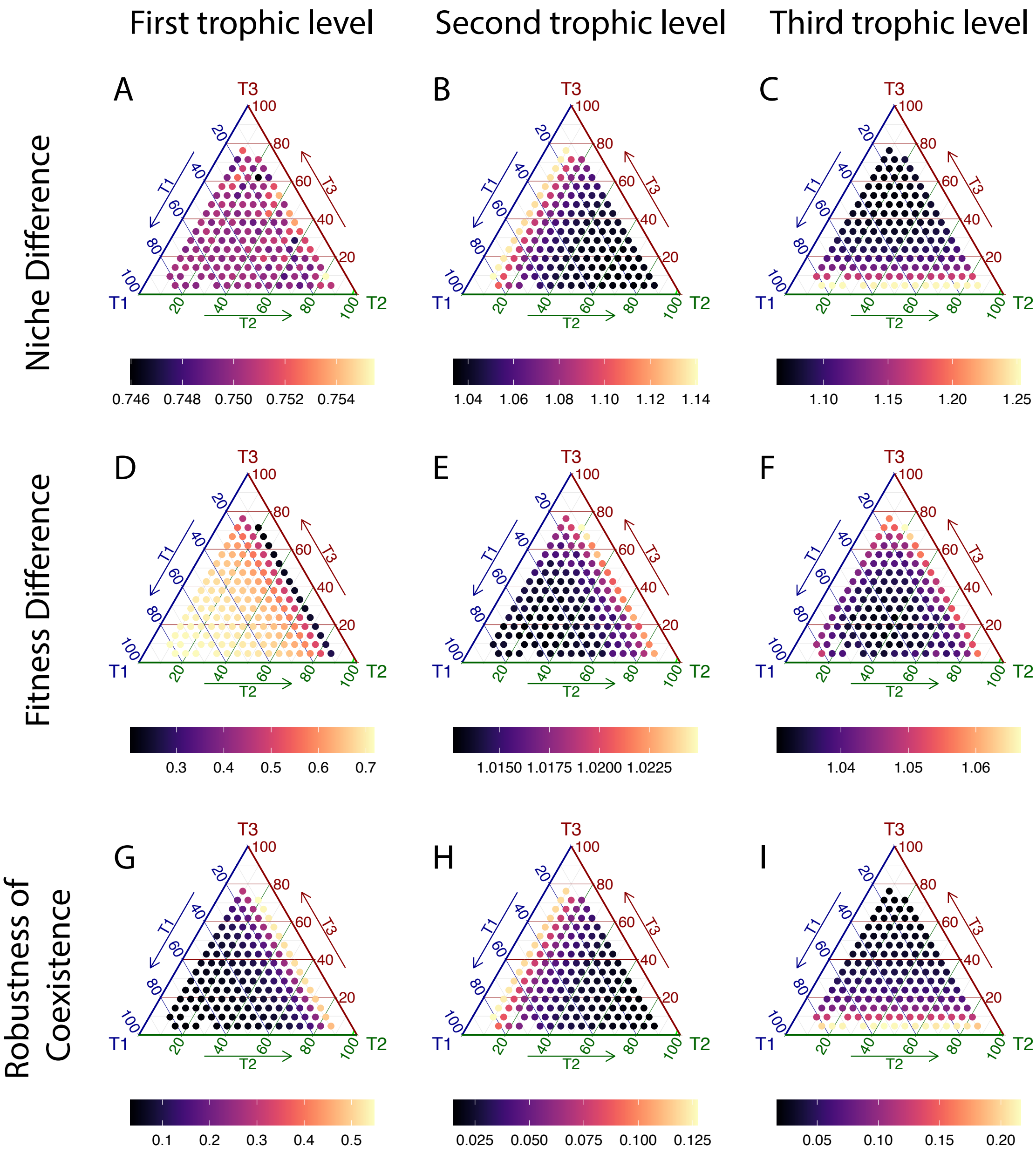
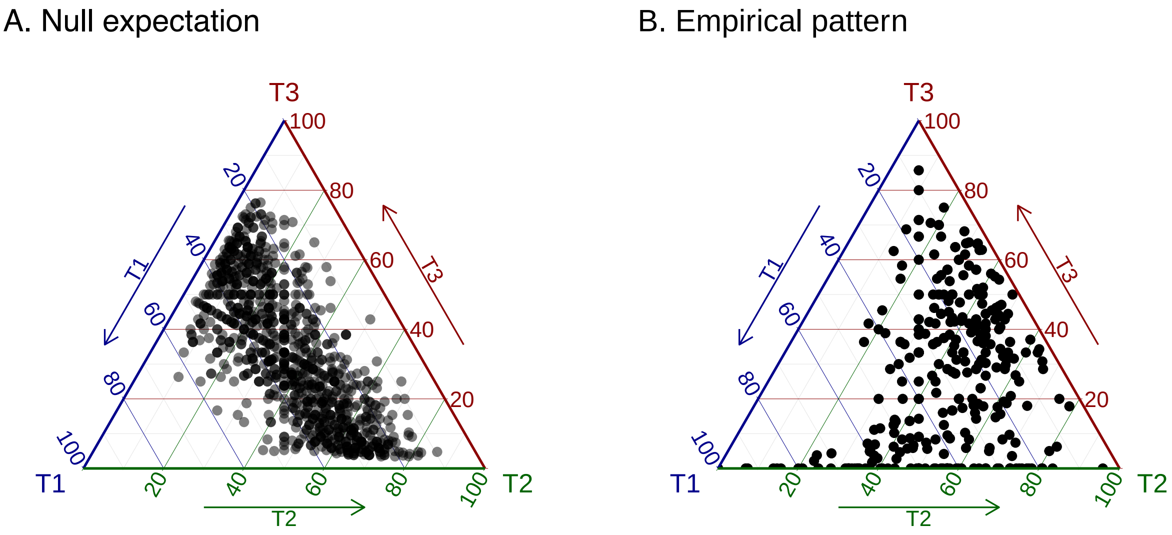


Figure S6: **How community composition affects niche and fitness differences in three-trophic communities.** We computed niche differences (A-C), fitness differences (D-F) and robustness of coexistence, i.e. $\mathcal{N}_i - \mathcal{F}_i$ (G-I) for tri-trophic communities with 21 species in total, but varying the percentage of species per trophic level (the axis of the ternary plots), and show the mean per trophic level (A,D,G for the first trophic level, B,E,H for the second and C,F,I for the thrid). As found with the two-trophic communities, community composition affects the fitness differences of the first trophic level (Panel D), but not the niche differences of the first trophic level (Panels A). However, community composition affects niche differences and to a lesser extent fitness differences of the higher trophic levels (Panels B, C, E & F). Finally, coexistence within each trophic level is most robust, i.e. $\mathcal{N}_i - \mathcal{F}_i$ is large (yellow dots), if the richness in this trophic level is low (G-I). For the first trophic level this pattern is driven by the low fitness differences, by the higher trophic levels this pattern is driven by the large niche differences.


A. Null expectation

B. Empirical pattern

Figure S7: **Comparing theoretical null expectation with the empirical pattern.** The location in the ternary plots shows the respective proportions of each trophic level. T1, T2, T3 represent the proportion of the first, second, and third trophic, respectively. Panel A: For 500 different parameter settings we computed the top 5 most stable trophic configurations similar to those in figure S6. Panel B: Trophical composition of empirical food webs from Web of Life. Species with intermediate trophic levels were rounded to the nearest integer. Species with trophic levels 4 and higher were lumped with species from level 3.

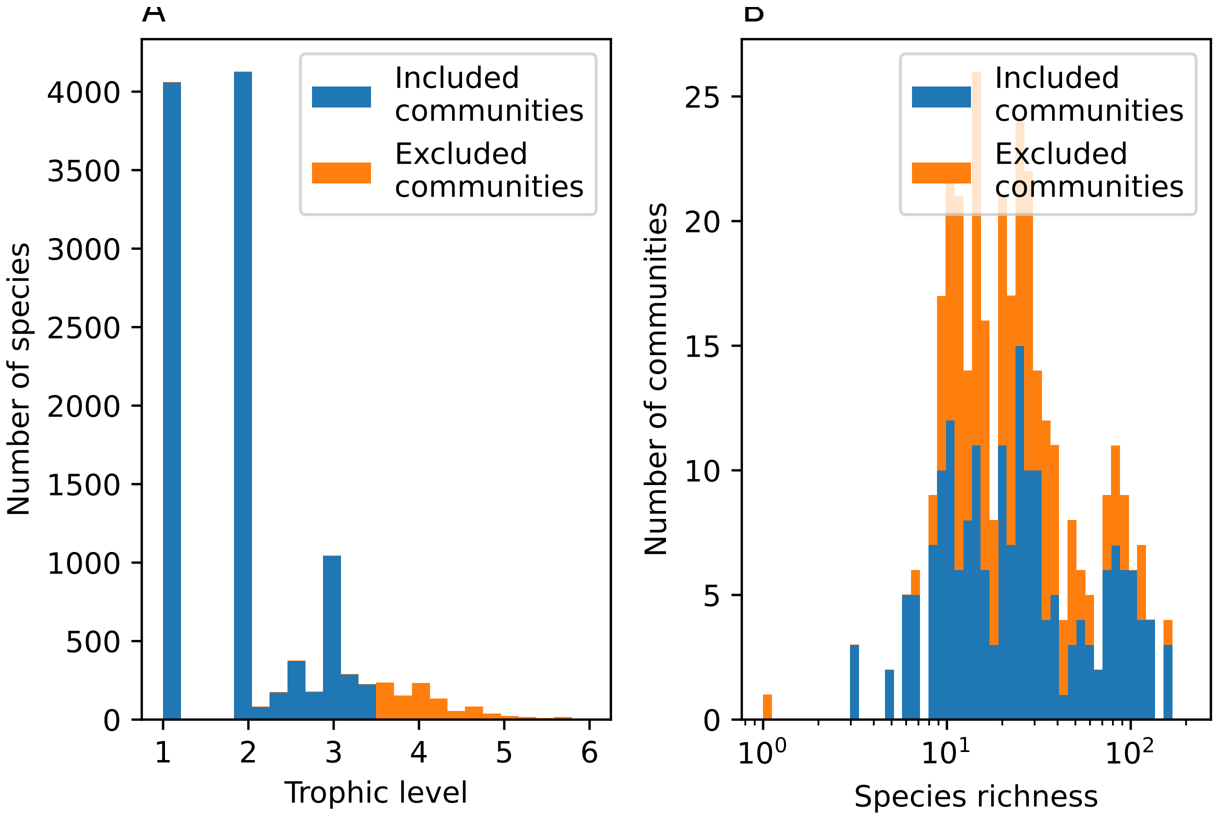


Figure S8: **Distribution of trophic levels and species richness in the empirical data.** A: Histogram of the trophic level of all species from all communities. The datasets contained considerable amount of omnivory leading to non-integer trophic levels. For figure S7, the trophic levels were rounded to the nearest integer. B: Species richness of the different communities, any community containing a species with a trophic level above 3.5 was excluded from the analysis in figure S7.

E Analysis of empirical food webs

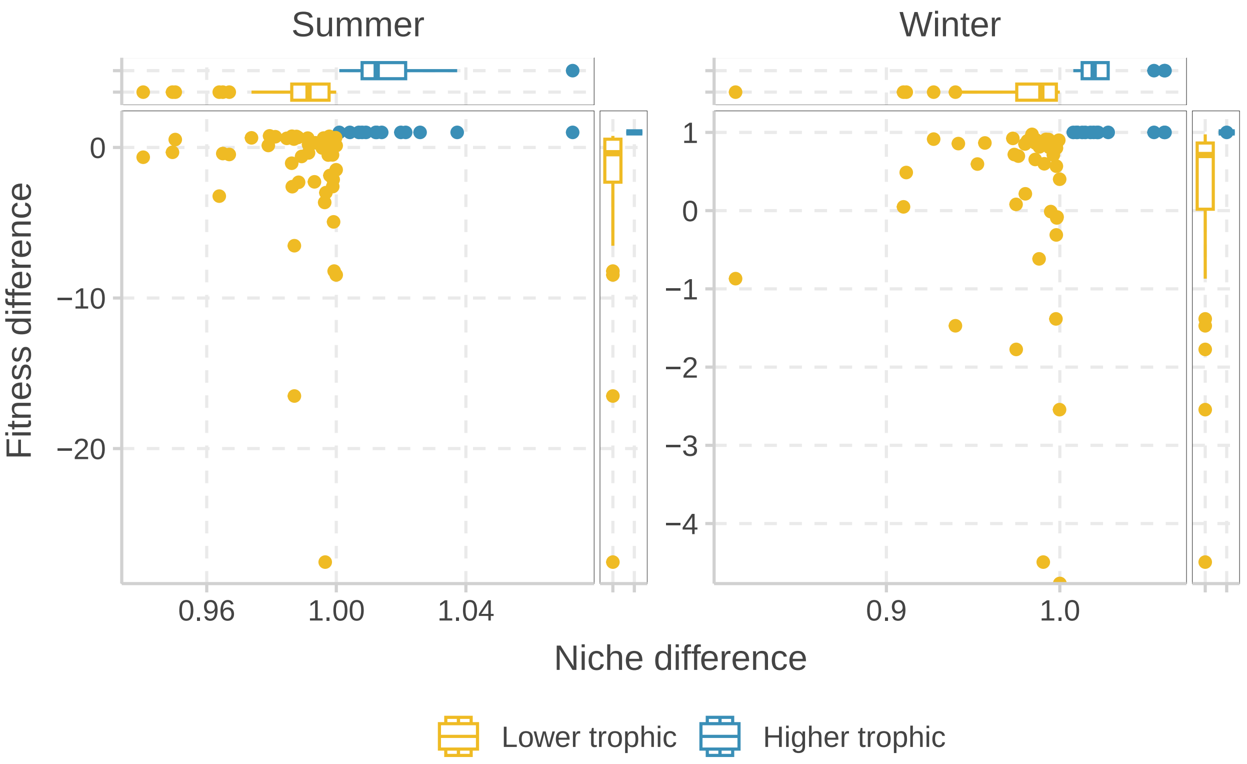


Figure S9: Niche and fitness differences map for the empirical data of temperate forest in the summer (left panel) and the winter (right panel). Subplots show the distribution of niche and fitness differences for the higher and lower trophic level. Both lower and higher trophic level have a comparable variance in niche differences. However, the lower trophic level has a much higher variance in fitness differences, while the higher trophic level has a much smaller variance of fitness differences. We hence conclude that the lower trophic level is driven by fitness differences, while the higher trophic level is driven by niche differences.

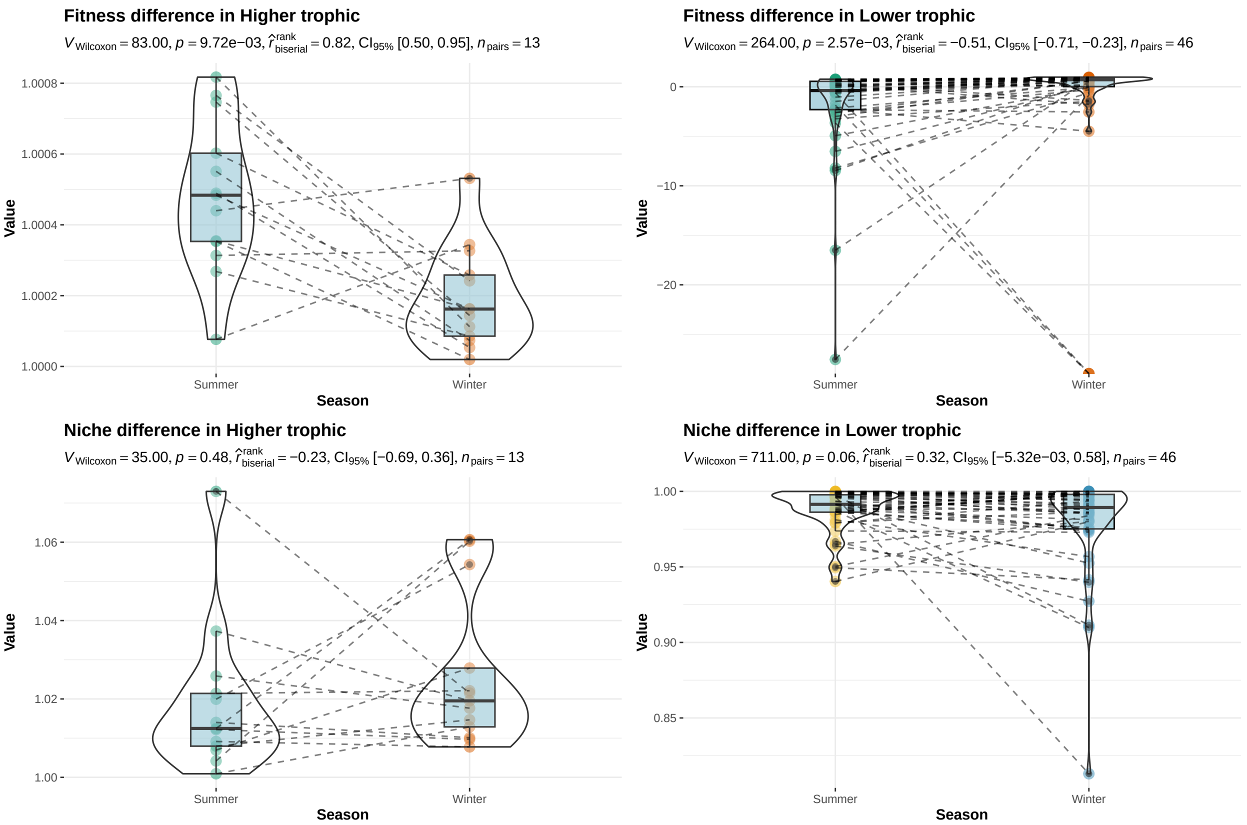


Figure S10: Changes of niche and fitness differences across seasons. Each panel shows fitness/niche difference for lower/higher-trophic species. Each point denotes a different species, and the line connects the same species in two seasons. Overall, we find that niche differences change the most in higher trophic species, while fitness differences change the most in lower trophic species.

References

Barabás, G., D'Andrea, R. & Stump, S. M. (2018). Chesson's coexistence theory. *Ecological Monographs*, 88, 277–303.

Barbier, M., de Mazancourt, C., Loreau, M. & Bunin, G. (2021). Fingerprints of high-dimensional coexistence in complex ecosystems. *Physical Review X*, 11, 011009.

Ellner, S. P., Snyder, R. E., Adler, P. B. & Hooker, G. (2019). An expanded modern coexistence theory for empirical applications. *Ecology Letters*, 22, 3–18.

Macarthur, R. & Levins, R. (1967). The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist*, 101, 377–385.

Pande, J., Fung, T., Chisholm, R. & Shnerb, N. M. (2020). Mean growth rate when rare is not a reliable metric for persistence of species. *Ecology Letters*, 23, 274–282.

Pande, J. & Shnerb, N. M. (2022). How temporal environmental stochasticity affects species richness: Destabilization, neutralization and the storage effect. *Journal of Theoretical Biology*, 539, 111053.

Spaak, J. W., Adler, P. B. & Ellner, S. P. (2023). Continuous assembly required: Perpetual species turnover in two-trophic-level ecosystems. *Ecosphere*, 14, e4614.

Spaak, J. W., Carpentier, C. & De Laender, F. (2021a). Species richness increases fitness differences, but does not affect niche differences. *Ecology Letters*, 24, 2611–2623.

Spaak, J. W., Godoy, O. & De Laender, F. (2021b). Mapping species niche and fitness differences for communities with multiple interaction types. *Oikos*, 130, 2065–2077.