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Species richness increases fitness differences, but does not affect niche differences

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Abstract

A key question in ecology is what limits species richness. Modern coexistence theory presents the persistence of species as a balance between niche differences and fitness differences that favor and hamper coexistence, respectively. With most applications focusing on species pairs, however, we know little about if and how this balance changes with species richness. Here, we apply recently developed definitions of niche and fitness differences, based on invasion analysis, to multi-species communities. We present the first mathematical proof that, for invariant average interaction strengths, the average fitness difference among species increases with richness, while the average niche difference stays constant. Extensive simulations with more complex models and analyses of empirical data confirmed these mathematical results. Combined, our work suggests that, as species accumulate in ecosystems, ever-increasing fitness differences will at some point exceed constant niche differences, limiting species richness. Our results contribute to a better understanding of coexistence multi-species communities.

Introduction

Explaining nature's biodiversity is a key challenge for science (Hutchinson, 1957). One type of approach
15 consists of focusing on the capacity of individual species to persist through time despite occasional pruning
to low density or species interactions (Turelli, 1978). Modern coexistence theory is such an approach, and
predicts species persistence in a community when niche differences overcome fitness differences. Niche
18 differences measure the strength of negative frequency dependence, i.e. whether a focal species i can recover
when reduced to low abundance (Adler *et al.*, 2007; Chesson, 2000; Spaak *et al.*, 2020). Fitness differences
measure the intrinsic strength of that species (Adler *et al.*, 2007; Hart *et al.*, 2018; Spaak *et al.*, 2020).

21 However, few applications of coexistence theory focused explicitly on explaining the persistence of
species in species-rich communities. Instead, most applications considered two-species communities (Ches-
son, 2000; Letten *et al.*, 2017), using a variety of approaches and case studies, including annual and peren-
24 nial plants (Adler *et al.*, 2018; Godoy & Levine, 2014), phytoplankton (Gallego *et al.*, 2019; Narwani *et al.*,
2013) and bacteria (Zhao *et al.*, 2016), evaluated under different environmental conditions (Bimler *et al.*,
2018; Cardinaux *et al.*, 2018; Grainger *et al.*, 2019; Lanuza *et al.*, 2018; Matías *et al.*, 2018; Napier *et al.*,
27 2016; Wainwright *et al.*, 2019). To our knowledge only three empirical studies report niche and fitness
differences in communities composed of more than two species (hereafter multi-species communities) (Chu
& Adler, 2015; Petry *et al.*, 2018; Veresoglou *et al.*, 2018). However, none of these three studies explained
30 how niche and fitness differences change with species richness. In order to understand how niche and fitness
differences co-determine species persistence in multi-species communities, we need to understand how both
variables change when adding species to a community.

33 Multi-species communities possess at least four complexities that are absent from two-species commu-
nities, which may affect niche and fitness differences, and therefore how we interpret coexistence in mul-
tispecies communities. (1) First, a multi-species community can host more interaction types than species
36 pairs, e.g. competitive or mutualistic interactions. Species richness increases the number of possible in-
teractions and the number of combinations of these interaction types. Several metrics exist to summarize
this diversity of interaction types and study their implications for community dynamics (Fontaine *et al.*,

39 2011). (2) Second, two-species communities are always fully connected (Carpentier *et al.*, 2021) and cor-
relations between interspecific interactions (Barabás *et al.*, 2016) become irrelevant since there is only a
single pair of interspecific interactions. In contrast, in an n -species community there may be anywhere from
42 $n - 1$ to $\frac{n}{2}(n - 1)$ connections, and the interspecific effects of species j on species i can be positively or
negatively correlated with the effects i has on j (Barabás *et al.*, 2016). May (1972) and Allesina & Tang
(2012, 2015) have shown that connectance and correlation can have large effects on the local stability of
45 multi-species communities. We therefore expect these factors to influence coexistence as well. (3) Third,
higher-order interactions, through which a third species changes the interaction between two species, are
usually considered absent from two-species communities (but see Letten & Stouffer (2019); Levine *et al.*
48 (2017) who include higher-order interactions into two-species communities). Such higher-order interactions
have been found empirically, for example, in communities composed of phytoplankton, bacteria, and ciliates
(Mickalide & Kuehn, 2019). In that study, bacteria coexisted with phytoplankton and ciliates, but all three
51 functional groups did not coexist. (4) Fourth, indirect effects, whereby a third species changes the dynamics
of a species pair by directly interacting with one or both partners, are by definition absent from two-species
communities (Walsh, 2013). We will refer to these four complexities throughout the text with (1) interaction
54 types, (2) interaction matrix structure, (3) higher-order interactions and (4) indirect interactions.

Studying multi-species coexistence is challenging both theoretically and experimentally. Theoretically
speaking, the methods to analyse coexistence via niche and fitness differences in a multi-species community
57 were not available until recently (Carmel *et al.*, 2017; Carroll *et al.*, 2011; Chesson, 2003; Saavedra *et al.*,
2017; Spaak *et al.*, 2020). Experimentally speaking, studying coexistence of multiple species is resource-
demanding. For instance, in the simple case of linear direct interactions among species (i.e. as in Lotka-
60 Volterra models) the number of experiments needed to parametrize the community is quadratic in species
richness (but see Maynard *et al.* (2020)). Considering higher-order interactions will consequently result
in an even higher experimental load. For example, measuring higher-order interactions, sensu Letten &
63 Stouffer (2019), would require 39 experiments in a three species community.

Here, we investigate the balance between niche and fitness differences along a gradient of species rich-
ness. More specifically, we ask how niche and fitness differences change as the number of species increases

66 in randomly assembled communities, and how the additional complexities (1)-(4) influence these changes. We do so using four independent methods that rely on a novel, species-specific definition for niche and fitness differences (Spaak *et al.*, 2020). First, we derive equations that quantify how niche and fitness
69 differences respond to species richness in a community with linear interactions and simple cases of higher-order interactions. Second, we give an intuitive explanation of these responses based on the Mac-Arthur consumer-resource model. Third, we perform simulations with more complex models. We run these sim-
72 ulations as a full-factorial virtual experiment, varying direct interactions (type, correlation, connectance), higher-order interactions and indirect interactions. Fourth, we compile data from the literature on empirically measured species interaction matrices and compute niche and fitness differences. We then compare
75 our results obtained via random species assembly to a community with non-random assembly. All methods support the same general conclusion: species richness does not affect niche differences, but increases fitness differences. Importantly, these conclusions are independent of the four complexities (1)-(4).

78 Methods

Model assumptions

To include the additional complexities of multi-species communities we use a generalized Lotka-Volterra
81 model with n species containing second-order interactions to model the per-capita growth rates $f_i(N_i, \mathbf{N}^{-i})$:

$$\frac{1}{N_i} \frac{dN_i}{dt} = r_i \underbrace{\left(1 + \sum_j \alpha_{ij} N_j \left(1 + \sum_k \beta_{ijk} N_k \right) \right)}_{f_i(N_i, \mathbf{N}^{-i})}, \quad (1)$$

where N_i is the density of the focal species i , \mathbf{N}^{-i} is the density of all species except the focal one (vector of length $n - 1$), r_i is its intrinsic growth rate, and α_{ij} and β_{ijk} are first (or linear) and second-order species
84 interactions, respectively. We focus on basal species, e.g. primary producers, and therefore assume $r_i > 0$. A positive α_{ij} indicates a positive interaction between species i and j (facilitation). Negative α_{ij} indicate negative interactions (competition). Additionally, two species i and j do not necessarily have the same effect
87 on each other as α_{ij} can differ from α_{ji} in both sign and magnitude. When β_{ijk} is positive or negative, species

k will intensify or weaken the relationship between species i and j , respectively (second-order interaction). The inclusion of third-order interactions did not affect any of our results. Throughout the manuscript we assume $\alpha_{ii} = -1$, which can be achieved by re-scaling of empirical data. Furthermore, we initially assume a random community assembly (but see section Non-random community assembly). That is, α_{ij} and α_{ik} ($j \neq k$) are sampled from any independent distributions (Appendix S1, similarly, the β_{ijk} are all sampled from any independent distributions for $k \neq j \neq i$ (we chose $\beta_{ijk} \in [-0.05, 0.05]$)).

Niche and fitness differences

There exist a total of eleven definitions for niche and fitness differences with various assumptions about the community model (Spaak *et al.*, 2020). Five of these definitions apply to multi-species communities (Carmel *et al.*, 2017; Carroll *et al.*, 2011; Chesson, 2003; Saavedra *et al.*, 2017; Spaak *et al.*, 2020). While these definitions highlight different aspects of a community model, they all capture the idea that higher niche differences facilitate coexistence, while higher fitness differences hamper coexistence. Optimally, we would compute niche and fitness differences according to all five methods to understand how species richness affects coexistence.

However, the available definitions all are based on different understanding of what niche and fitness differences should intuitively mean. We have shown that these different intuitions make it hard to compare across definitions. Here, we focus on one specific definition that we feel is best suited to address our specific research question (Spaak *et al.* (2020), rationale explained in Appendix S2). Yet, we acknowledge that other interpretations of the concepts of \mathcal{N}_i and \mathcal{F}_i (i.e. different definitions of \mathcal{N}_i and \mathcal{F}_i) will lead to different answers on how species richness affects \mathcal{N}_i and \mathcal{F}_i . To illustrate this variation, we have applied four different methods to competitive Lotka-Volterra community models (Appendix S2).

Importantly, the definition of Spaak *et al.* (2020) for niche and fitness differences capture the intuitive concepts of niche and fitness differences known from the Lotka-Volterra community model (Chesson, 1990; Chesson & Kuang, 2008). Specifically, negative niche differences imply positive frequency dependence (Ke & Letten, 2018), niche differences exceeding 1 imply facilitation (Koffel *et al.*, 2021) and intermediate niche differences imply negative frequency dependence. Stronger intrinsic strength is associated with lower

114 fitness differences (more positive \mathcal{F}_i) and species persist, i.e. have a positive invasion growth rate, if niche
differences overcome fitness differences ($-\mathcal{F}_i > \frac{\mathcal{N}_i}{1-\mathcal{N}_i}$). Additionally, these definitions align with the niche
and fitness differences for a two-species Lotka-Volterra community model Spaak *et al.* (2020).

117 Spaak *et al.* (2020) base their definition of niche and fitness differences for a focal species i (\mathcal{N}_i and
 \mathcal{F}_i) on the comparison of growth rates in various conditions. If the two species (i and j) of a two-species
community have completely separated niches ($\mathcal{N}_i = \mathcal{N}_j = 1$), the species i will grow in absence of j as it
120 would in its presence. Which implies that intrinsic growth rate of species i ($f_i(0,0)$) is equal to the invasion
growth rate, the per-capita growth rate of species i when it invades a community with j at equilibrium
density N_j^* ($f_i(0, N_j^*)$, Carroll *et al.* (2011); Chesson (1994); Ellner *et al.* (2018)), i.e. $f_i(0,0) = f_i(0, N_j^*)$.
123 Conversely, if the two species have exactly the same niche ($\mathcal{N}_i = \mathcal{N}_j = 0$) they have equivalent effects on
each other. It then holds that $f_i(0, N_j^*) = f_i(c_{ij}N_j^*, 0)$ where c_{ij} is the conversion factor allowing to express
individuals of species j as individuals of species i .

126 Unfortunately, c_{ij} are defined implicitly as the solution of an equation, which often does not have a
closed form solution, but can be computed numerically. However, intuitively the c_{ij} are simple. The con-
version factors c_{ij} change the frequency of species i and j , but do not affect the total density. That is, two
129 communities with densities $(0, N_j^*)$ and $(c_{ij}N_j^*, 0)$ have the same total density, but different frequencies of
species i . In the first community species i has 0 frequency, while in the second it has 100% frequency, as
 $c_{ij}N_j^*$ is a density of species i . We refer to Spaak *et al.* (2020) and the appendix S3 for a mathematical
132 definition of c_{ij} .

Further intuitive insight can be gained from a Lotka-Volterra model in which the $c_{ij} = \sqrt{\left| \frac{\alpha_{ij}\alpha_{jj}}{\alpha_{ji}\alpha_{ii}} \right|}$ (Ap-
pendix S4). Note that the arrangement of the interaction coefficients α_{ij} differs from both, the niche and
135 the fitness differences. The numerator ($\alpha_{ij}\alpha_{jj}$) is the competitive effect of species j , while the denominator
($\alpha_{ji}\alpha_{ii}$) is the competitive effect of species i . Therefore, c_{ij} keeps total density constant by scaling the effects
of the two species.

138 Niche differences are then defined via interpolation between these two extreme cases of $\mathcal{N}_i = 0$ and

$\mathcal{N}_i = 1$:

$$\mathcal{N}_i = \frac{f_i(0, N_j^*) - f_i(c_{ij}N_j^*, 0)}{f_i(0, 0) - f_i(c_{ij}N_j^*, 0)} \quad (2)$$

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This equation of niche differences represents the relative frequency dependence of species i . The numerator assesses frequency dependence, as it compares the growth rate of species i when it has 0% frequency ($f_i(0, N_j^*)$) to when it has 100% frequency ($f_i(c_{ij}N_j^*, 0)$), notably at the same converted density. The denominator assesses density dependence, as it compares the growth rate of species i at 0 density ($f_i(0, 0)$) to when it has non-zero density ($f_i(c_{ij}N_j^*, 0)$). Importantly, in both growth rates species i has 100% frequency.

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Similarly, Spaak *et al.* (2020) define fitness differences as the scaled growth rate in the absence of niche differences

$$\mathcal{F}_i = \frac{f_i(c_{ij}N_j^*, 0)}{f_i(0, 0)} \quad (3)$$

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Zero fitness differences imply that the species have equal intrinsic strength, as $N_i^* = c_{ij}N_j^*$. That is, species will have the same equilibrium density after conversion with c_{ij} . Competitive subordinate species then have negative fitness differences, as $N_i^* < c_{ij}N_j^*$, conversely competitive dominant species have positive fitness differences. Note that $\mathcal{F}_i = 0$ means no fitness differences and more negative \mathcal{F}_i means stronger fitness differences. Importantly, these definitions of niche and fitness differences agree with the original definitions on the two species Lotka-Volterra models (Chesson, 2013; Chesson & Kuang, 2008), they are therefore not newly defined niche and fitness differences, but rather a generalization thereof (Appendix S4, Spaak *et al.* (2020)).

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Additionally, this definition, illustrated so far for a two-species community, naturally generalizes to multi-species communities. The three growth rates remain conceptually the same. The intrinsic growth rate $f_i(0, \mathbf{0})$, where $\mathbf{0}$ denotes the absence of all other species. The invasion growth rate $f_i(0, \mathbf{N}^{(-i,*)})$, where $\mathbf{N}^{(-i,*)}$ denotes the equilibrium densities of the community in absence of species i . And the no-niche growth rate $f_i(\sum_j c_{ij}N_j^{(-i,*)}, 0)$, where all densities of the resident species have been converted to densities of the focal species, while keeping total density constant due to the c_{ij} . Doing so makes clear that \mathcal{N}_i and \mathcal{F}_i are species-specific properties, i.e. in general we have $\mathcal{N}_i \neq \mathcal{N}_j$ and $\mathcal{F}_i \neq \mathcal{F}_j$ in multi-species communities (Adler *et al.*, 2007), Appendix S2. However, \mathcal{N}_i and \mathcal{F}_i compare the effect of the focal species on itself to

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the effect of the rest of the community on the focal species. They therefore do depend on the traits of the
168 other species in the community.

Analyses and Simulations

We first examined analytically how \mathcal{N}_i and \mathcal{F}_i change with species richness. We found a generic solution
171 for first-order interactions and for a simplified case of higher-order interactions.

Second, we designed a full-factorial virtual experiment in which we numerically computed \mathcal{N}_i and \mathcal{F}_i
for a wider range of different simulated communities (Table 1). For these we solve numerically for equi-
174 librium densities and invasion growth rates using the 'fsolve' function from the scipy package in Python.
Communities with higher-order interactions can have multiple equilibria (Aladwani & Saavedra, 2019). To
compare communities with and without higher-order interactions we computed \mathcal{N}_i and \mathcal{F}_i only for the equi-
177 librium which was closest to the equilibrium without higher-order interactions (Appendix S1). We did so
because the chosen higher-order interactions are small, i.e. $\beta_{ijk} \in [-0.05, 0.05]$, such that the higher-order
interactions should be seen as a perturbation of the linear interaction. The factors used in our simulations
180 were (i) first-order interaction type (competitive, facilitative or both, i.e. $\alpha_{ij} < 0, > 0$ or unrestricted); (ii)
first-order interaction strength (strong or weak); (iii) connectance of the interspecific interaction matrix ($c \in$
 $\{1, \frac{4}{5}, \frac{2}{3}\}$); (iv) correlation between the interspecific interaction ($\rho(\alpha_{ij}, \alpha_{ji}) = \rho_{ij}(\beta_{ijk}, \beta_{jik}) \in \{-1, 0, 1\}$);
183 (v) inclusion of indirect effects (present or absent); (vi) second-order interaction type ($\beta_{ijk} < 0, > 0$, un-
restricted, or absent). To exclude indirect effects we set equilibrium densities of resident species to their
monoculture equilibrium density. In this way, we cancel out interactions among residents that will change
186 the residents' densities. The intrinsic growth rate r_i does not affect \mathcal{N}_i and \mathcal{F}_i , therefore we set it to 1 in all
simulations. However, the r_i may affect the community dynamics from stable coexistence to chaotic fluc-
tuations and competitive exclusion (Song *et al.*, 2020). In this case, invasion growth rates do not correctly
189 predict coexistence and should not be analyzed with \mathcal{N}_i and \mathcal{F}_i (see limitations).

This design leads to a total of $3 \cdot 2 \cdot 3 \cdot 3 \cdot 2 \cdot 4 = 432$ parameter settings. We ran 1000 repetitions for
each of the five species richness levels ($2 \leq n \leq 6$), leading to a total of $432 \cdot 5 \cdot 1000 = 2'160'000$ simu-
192 lations. We parametrized the first-order interactions using distributions of empirically obtained first-order

interactions (Appendix S1). We sampled “strong” first-order interactions between the $Q_1 - 1.5(Q_3 - Q_1)$ and $Q_3 + 1.5(Q_3 - Q_1)$ of those distributions, where Q_1 and Q_3 are the first and third quartile, to remove
195 outliers (Appendix S1). Similarly, we sampled “weak” first-order interactions between the Q_1 and Q_3 of the empirical distributions of interaction strength. We fitted linear regressions to measure the effect of species richness on \mathcal{N}_i and \mathcal{F}_i . With this approach we were able to investigate the effects of all complexities (1)-(4).

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Literature data

We found three review papers that collected multi-species Lotka-Volterra interaction matrices (Adler *et al.*, 2018; Fort & Segura, 2018; Keddy & Shipley, 1989), representing a total of 33 interaction matrices, ranging
201 from 3 to 9 species, and containing 29 plant, 2 phytoplankton, 1 zooplankton and 1 ciliate communities. We normalized all these data such that $\alpha_{ii} = -1$. The interaction matrices were obtained through pairwise experiments, measuring the interspecific effect of one species on the other. For each multi-species community
204 we constructed all possible sub-communities with at least two species, leading to a total of 2544 communities that varied in species richness from 2 to 9. We excluded all communities in which not all interaction strengths were available, e.g. because of a “NA” entry in the sampled sub-community, leading to 2296
207 communities. For 1376 communities we could not compute \mathcal{N}_i and \mathcal{F}_i . That is because, like any method seeking to quantify frequency dependence, our approach is based on invasion analysis: the capacity of an invader to grow with the other species at their non-zero equilibrium. \mathcal{N}_i and \mathcal{F}_i are thus only computable for
210 communities where all species in each subcommunity (the community without the invading species) coexist stably. We therefore computed \mathcal{N}_i and \mathcal{F}_i for the remaining 920 communities (Appendix S5).

For each interaction matrix obtained from the literature we computed \mathcal{N}_i and \mathcal{F}_i using equation 2 and 3.
213 For each of the 33 interaction matrices, we regressed \mathcal{N}_i against species richness of the sub-communities. We used a Theil-Sen estimator for the slope, which is more robust to outliers than linear regression based on least squares (Sen, 1968). We fitted (using least squares) a saturating function $\mathcal{F}_i = \frac{n-2}{(n-2)+H}$ for the fitness
216 differences, where n is the species richness and H is the free parameter. This saturating response was chosen for \mathcal{F}_i , because our analytical results suggested such a response.

Results

Analytical solutions

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For the linear Lotka-Volterra model without higher-order interactions (i.e. $\beta_{ijk} = 0$), we can explicitly compute the fitness \mathcal{F}_i and niche differences \mathcal{N}_i of species i in the multispecies community (Appendix S4):

$$1 - \mathcal{F}_i = \sum_{j, \alpha_{ij} \neq 0} \frac{N_j^{-i,*}}{N_j^*} \left(1 - \mathcal{F}_{ij}^{(2)} \right) \quad (4)$$

$$\mathcal{N}_i = \frac{\sum_{j, \alpha_{ij} \neq 0} (c_{ij} N_j^{-i,*}) \mathcal{N}_{ij}^{(2)}}{\sum_{j, \alpha_{ij} \neq 0} c_{ij} N_j^{-i,*}} \quad (5)$$

222 with $\mathcal{F}_{ij}^{(2)}$ and $\mathcal{N}_{ij}^{(2)}$ the fitness and niche difference of species i in a two-species community consisting of species i and j . $N_j^{-i,*}$ is the equilibrium density of species j in the absence of species i , N_j^* is the equilibrium density of species j in monoculture and c_{ij} is the conversion factor from species j to species i (Methods).

225 The sums are taken across all species j with which species i interacts directly, i.e. $\alpha_{ij} \neq 0$.

Equation 4 and 5 illustrate two main results. First, $1 - \mathcal{F}_i$ is the weighted *sum* of the two-species fitness differences $1 - \mathcal{F}_{ij}^{(2)}$, across all species pairs with which i interacts. The weights $\frac{N_j^{-i,*}}{N_j^*}$ are the relative yields, 228 as known from biodiversity ecosystem functioning research (Fox, 2005; Hector & Loreau, 2001). The effect of species richness on fitness differences will therefore be similar to the effect of species richness on the sum of the relative yield, known as the relative yield total ($\sum_{j \neq i} \frac{N_j^{-i,*}}{N_j^*}$). The relative yield total is known 231 to increase with species richness in many communities (Carroll *et al.*, 2011; Grace *et al.*, 2016; Loreau, 2004). Hence, $1 - \mathcal{F}_i$ will, on average, increase with species richness. Second, \mathcal{N}_i is the weighted *average* of the two-species niche differences $\mathcal{N}_{ij}^{(2)}$. Hence, species richness will, on average, not affect \mathcal{N}_i . Since 234 we did not make assumptions about the distributions of α_{ij} , these results are independent of the details of interspecific interactions, i.e. the results apply regardless of complexities (1) and (2).

Equation 4 and 5 can be approximated by assuming constant interspecific interaction strength $\bar{\alpha}$ (Ap- 237 pendix 4). This yields $\mathcal{N}_i \approx 1 - \bar{\alpha}$ and $\mathcal{F}_i \approx 1 - \frac{n-1}{1-(n-2)\bar{\alpha}}$, from which it is clear that \mathcal{N}_i is independent of species richness n and \mathcal{F}_i is an increasing but saturating function of species richness. The saturation occurs

because the sum of the weights $\frac{N_j^{-i,*}}{N_j^*}$, the relative yield total, will saturate as well in the Lotka-Volterra model
 240 (Loreau, 2004; Spaak *et al.*, 2017).

Link to resource competition

The fact that $1 - \mathcal{F}_i$ is a weighted sum, while \mathcal{N}_i is a weighted average makes intuitive sense when realising
 243 that the interaction coefficients α_{ij} can, under certain conditions, be related to resource utilisation (Chesson,
 1990; MacArthur, 1970). Consider a focal species (yellow, Fig. 1) utilizing a range of resources. We ask
 how adding competitors changes this species \mathcal{N}_i and \mathcal{F}_i (Fig. 1 A-E). We assume that the species only differ
 246 in resource utilisation rates, not in other parameters such as mortality.

In a two species community, the species with the higher total utilisation rate will have a competitive
 advantage and consequently the higher fitness difference. The total resource utilisation rate, denoted $\|A_i\|$,
 249 is the area under the curve A_i . One could therefore intuit that the fitness difference is linked to the ratio
 of total resource utilisation rates, i.e. $\mathcal{F}_1 \approx 1 - \frac{\|A_2\|}{\|A_1\|}$. Fitness differences therefore increase with species
 richness, as each competitor increases the total resource utilisation rates of all competitors combined (Fig.
 252 1 F), i.e. $\mathcal{F}_1 \approx 1 - \frac{\sum \|A_j\|}{\|A_1\|}$. It turns out that this intuition is almost correct; we only have to add weights to
 the sum according to the densities of the species at equilibrium (compare this equation to eq. 4). \mathcal{F}_1 thus
 increases (becomes more negative), as species richness increases.

255 Intuitively, $1 - \mathcal{N}_i$ is the proportion of the shared resources between the focal species and its competitors,
 that is the amount of the shared resources scaled by the total consumption of the species. In a two species
 community, we therefore intuit $\mathcal{N}_1 \approx 1 - \frac{\|A_1 \cap A_2\|}{\|A_1\| \cdot \|A_2\|}$. Increasing species richness will increase the sum of
 258 the shared resources, as the focal species will share resources with each competitor (Fig. 1 G), but also
 the sum of the total consumption of the species (Fig. 1 F). We therefore expect $\mathcal{N}_1 \approx 1 - \frac{\|A_1 \cap \sum_j A_j\|}{\|A_1\| \cdot \sum_j \|A_j\|}$ to be
 independent of species richness (Fig 1 H). Again, this intuition is correct up to the inclusion of the weights
 261 according to the species equilibrium densities.

Full-factorial simulations

The simulations with strong first-order interactions only partially seem to confirm the predictions made by theory (Fig. 2). That is, \mathcal{N}_i is not invariant but approaches 1 as species richness increases (Fig. 2 A), which seems to contradict the theoretical results. Yet, species richness does not directly affect \mathcal{N}_i , but rather affects the average interaction strength $\bar{\alpha}$, which in turn affects \mathcal{N}_i , which can be approximated by $\mathcal{N}_i \approx 1 - \bar{\alpha}$ (Appendix S4). That is because, by design, any method based on invasion growth rates (such as those to compute \mathcal{N}_i and \mathcal{F}_i) can only be applied to communities in which invasion analysis is possible. Hence, too strong negative interactions prevent the invasion into highly-diverse communities, and will often impede feasible $n - 1$ subcommunities to begin with (Kokkoris *et al.*, 2002). Hence, species richness selects against communities with overly strong negative interactions, which leads to, on average, weaker interactions at higher species richness (Appendix Fig.S3). Similarly, species densities in communities with strong positive interactions will tend to grow to infinity, and more so in species-rich communities, because interspecific facilitation is stronger than intraspecific limitation (self-regulation). Again, species richness selects against strong positive interactions, weakening the average interaction strength (Appendix Fig.S3). This selection of weak (negative and positive) interspecific interactions causes \mathcal{N}_i to approach 1. \mathcal{F}_i increases with species richness for all parameter settings, as predicted by the theory (Fig 2 B).

The simulation results based on weak interaction strengths allow assessing the direct effect of species richness on \mathcal{N}_i and \mathcal{F}_i without the confounding effect of species richness on interspecific interaction strength α_{ij} . In these simulations, the effect of species richness on interspecific interactions was much weaker (Appendix Fig.S3). These simulations confirmed our theoretical results; \mathcal{N}_i was on average unaffected by species richness (Fig. 3 A) and \mathcal{F}_i increased with species richness (Fig. 3B). We illustrate how \mathcal{N}_i and \mathcal{F}_i values jointly varied with species richness, using weak interaction strength: no higher-order interactions ($\beta_{ijk} = 0$), no correlation between the α_{ij} , and maximum connectance (Fig. 3 C). Again, these results hold independently of the complexities (Appendix S1).

As expected, using other definitions of \mathcal{N}_i and \mathcal{F}_i to the simplest case (competitive Lotka-Volterra communities) leads to a variety of responses of \mathcal{N}_i and \mathcal{F}_i to species richness. Some of these definitions

288 respond in the same way to richness (e.g. Chesson (2003) for \mathcal{F}_i), while others respond in an differently
(e.g. (Carroll *et al.*, 2011) for \mathcal{N}_i). This comparison shows that it is crucial to use the appropriate definition
of \mathcal{N}_i and \mathcal{F}_i for the question at hand (Appendix S2).

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Literature Data

The results for the empirical communities reflect those obtained for the simulated communities. The absolute
values of the slopes of the linear regressions of \mathcal{N}_i were small (< 0.05) for all but 6 datasets. The slope for
294 the overall regression of \mathcal{N}_i against species richness (Fig. 4A, black line) was small (-0.028). \mathcal{F}_i increased
with richness in all but one dataset. Overall, we conclude that the response of \mathcal{N}_i and \mathcal{F}_i to richness for
empirical communities did not qualitatively differ from that of randomly generated communities.

297 The empirical data also revealed cases in which coexistence is possible even though some of the species
have negative \mathcal{N}_i . This is possible as long as \mathcal{F}_i is sufficiently positive such that $\mathcal{F}_i \geq \frac{-\mathcal{N}_i}{1-\mathcal{N}_i}$. A total of 95
(4.1%) communities were found with species persisting despite having negative \mathcal{N}_i .

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Non-random community assembly

So far we have focused on random community assembly, where the location and width of the resource
utilization A_i of species i were chosen randomly (Fig. 1 A-E). We here expand our results towards two
303 different possibilities of non-random community assembly. First, given a species pool, we rearranged the
order of species arrival. By choosing a non-random community assembly, species richness can increase
or decrease niche differences, but will always increase fitness differences (Appendix S6). Additionally,
306 averaged over all possible community assemblages, species richness again does not affect niche differences.

Alternatively, one might ask how species richness affects niche and fitness differences, if species opti-
mize their resource utilisation according to the prevailing species richness (Barabás *et al.* (2016), Fig. 5 A-E,
309 Appendix S7). Importantly, in this case, the two-species community is *not* a subcommunity of the three-
species community. This links to the traditional question of species packing, which is how close species can
be packed in a given environment and still coexist (Barabás *et al.*, 2014; MacArthur, 1970). In this scenario,

312 species richness still increases fitness differences (Fig. 5 F). However, because of the limited niche space,
species in species rich communities are located closer to each other and therefore have a stronger interaction
strength on average (Fig. 5 G). Niche differences therefore decrease with species richness (Fig. 5 H).

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Discussion

It is well-established that the likelihood for stable coexistence drops with species richness (Godoy *et al.*,
2017; Goh & Jennings, 1977; May, 1972; Serván *et al.*, 2018). Here, we explain this well known result
318 in the context of modern coexistence theory by examining how niche and fitness differences (\mathcal{N}_i and \mathcal{F}_i)
change with species richness. We found that species richness has no direct effect on \mathcal{N}_i but directly increases
 \mathcal{F}_i . However, species richness may have an indirect effect on \mathcal{N}_i (Figure 2 and 5). This conclusion is based
321 on four independent approaches: mathematical computation, biological intuition, numerical simulations,
and analysis of experimental data. Overall, the influence of species richness on \mathcal{N}_i and \mathcal{F}_i is robust to inclu-
sion or omission of the complexities (1)-(4), and all their combinations. The fitness differences of a species
324 increases with species richness, as fitness differences measure the fitness of a species compared to the com-
bined fitness of all other species. In multi-species communities, most species will therefore have negative
fitness differences, as one species will rarely have higher fitness than all other species combined. Moreover,
327 \mathcal{N}_i in multi-species communities is approximately $1 - \bar{\alpha}$, where $\bar{\alpha}$ is the average inter-specific interac-
tion strength. This new result facilitates considerably the computation of \mathcal{N}_i in multi-species communities.
Taken together, our findings shed new light on the causes of coexistence in multi-species communities.

330 The niche differences of a species measure the proportion of limiting factors, e.g. resources, that are
limiting to other species as well. Increasing species richness increases the amount of limiting factors shared
with other species, but also the amount of limiting factors that are not shared with other species. The
333 proportion of shared limiting factors is therefore unaffected, on average. Species-rich communities are
therefore less likely to coexist (all else being equal), as fitness differences become too strong to be overcome
by niche differences.

Limitations

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The available experimental data only represent fully connected communities, with no correlation among interactions (complexity (2)) and, most notably, did not contain cases of higher-order interactions (complexity (3)). We do therefore not know whether the parameter values used to describe these higher-order interactions in our simulations (and therefore the simulation results) are realistic. The available experimental data were biased towards competitive communities of terrestrial plants with relatively low species richness. Our simulations suggest that our conclusions hold for other networks as well. Computing \mathcal{N}_i and \mathcal{F}_i on a larger collection of natural communities would help refine our understanding of this process. However, obtaining the full interaction matrix for species-rich communities is challenging. To obtain interaction matrices, various approaches exist. For example, one can use the frequency of interaction between species (e.g. number of visits of a pollinator on a plant) as a proxy for interaction strength. The robustness of this approach, however, still needs to be tested (García-Callejas *et al.*, 2018). Other methods rely, for example, biomass (Moore *et al.*, 1996; Zhao *et al.*, 2019), mass ratio (Emmerson & Raffaelli, 2004) or production and consumption rates of species (Christensen V. & D., 1992; Jacquet *et al.*, 2016). These different methods rely on different assumptions and may therefore influence the resulting matrix estimate (Carrara *et al.*, 2015).

As mentioned before, the computation of niche and fitness differences is based on invasion growth rates, which may not always be defined (Barabás *et al.*, 2018; Spaak *et al.*, 2020) or may not predict coexistence (Pande *et al.*, 2019). This makes it especially challenging to assess the importance of indirect species interactions, and more specifically, intransitivity. For example, a community coexisting solely through intransitive coexistence could not be analyzed with our method as invasion analysis will not be possible.

Given these limitations, one can ask to what extent addressing them would change our conclusions. In communities where species richness increases total abundance, which is the case for various communities (Grace *et al.*, 2016; Loreau, 2004; Turnbull *et al.*, 2013), we expect the no-niche growth rate $f_i(\sum_j c_{ij} N_j^{-i,*}, 0)$ to become more negative, as $\sum_j c_{ij} N_j^{-i,*}$ increases (eq. 2, 3). Consequently, we expect species richness to increase fitness differences, i.e. make \mathcal{F}_i more negative, as it is linear in the no-niche growth rate. Conversely, in communities where species richness decreases total abundance we expect the

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opposite, that is: fitness differences might decrease with species richness. It is less clear how species richness will affect niche differences in models not explored in the current paper, e.g. with different per-capita growth rates functions f_i , or with a community with age structure (Chu & Adler, 2015). Niche differences depend on the invasion growth rate and the no-niche growth rates, which both depend on the species richness and total abundance. When species richness has a stronger negative effect on the no-niche growth rate than on the invasion growth rate, then niche differences will increase with species richness. Conversely, if the invasion growth rate decreases more than the no-niche growth rate, niche differences will decrease.

Additionally, we have mostly assumed that species richness is independent of other factors such as the average interaction strength, connectance or correlation (Cohen & Briand, 1984; Kokkoris *et al.*, 2002). It is well-established that the number of links scales nonlinearly with species richness, causing connectance to decrease with species richness (Carpentier *et al.*, 2021; Cohen & Briand, 1984; Martinez, 1992). However, this will not affect our findings, as the sums in the equations 4 and 5 only run over species with which the focal species i interacts. We ran additional simulations to illustrate this point (Appendix S8). As predicted by theory, niche differences are unaffected and fitness differences increase with species richness. However, fitness differences increase more slowly than in the other investigated cases, as the number of links (and therefore the number of summands in equation 3) increases more slowly.

The main determinant of niche differences is the average interaction strength, which might decrease with species richness due to coexistence requirements (Fig. 2A) or might increase due to increasing overlap of resource requirements (Fig. 5 G). How species richness covaries with average interaction strength will affect how species richness covaries with niche differences, it is therefore very likely that species richness and niche differences will covary in natural communities.

New insights

Our results yield two new insights, other than the main result on how \mathcal{N}_i and \mathcal{F}_i varies with species richness.

A first insight is that negative niche differences do not necessarily preclude coexistence. Negative niche differences have been attributed to priority effects and therefore viewed as precluding coexistence (Fukami *et al.*, 2016; Ke & Letten, 2018). Our framework confirms this finding for the case of competitive two-

species communities (Spaak *et al.*, 2020). However, species in multi-species communities will not all have the same niche differences (example community in Fig 4). This implies that species a , with negative niche differences and low fitness differences, can coexist with species b and c that have positive niche differences and negative fitness differences. Consequently, multiple species can have negative niche differences in a multi-species communities and still persist. In our empirical data set, we found six three-species communities in which all but one species had negative niche differences.

A second insight is that one can infer \mathcal{N}_i and \mathcal{F}_i in multi-species communities from \mathcal{N}_i and \mathcal{F}_i measured in pairwise interaction experiments. If one measures \mathcal{N}_i and \mathcal{F}_i for each two-species sub-community of an n species community, which is typically done (Gallego *et al.*, 2019; Godoy & Levine, 2014; Narwani *et al.*, 2013; Petry *et al.*, 2018), one can estimate $\mathcal{N}_i \approx \frac{\sum_j \mathcal{N}_{ij}^{(2)}}{(n-1)}$. With one additional multi-species experiment to estimate the relative yield RY_i we obtain an estimation of $\mathcal{F}_i \approx 1 - \sum_j (1 - \mathcal{F}_{ij}^{(2)}) \cdot \text{RY}_j$ as well. This indicates that two-species experiments are sufficient to predict \mathcal{N}_i and \mathcal{F}_i in multi-species communities. To validate this finding one would optimally conduct all the necessary n multi-species experiments to measure the invasion growth rates, and fit a community model to the multi-species communities. Given these invasion growth rates and the community model one can compute exact niche and fitness differences and compare them to their estimates.

Finally, one of the key questions in community ecology is whether niche differences are strong enough to overcome fitness differences and allow coexistence. Often, niche differences are found to be not only sufficiently strong, but much stronger than strictly needed (Chu & Adler, 2015; Levine & HilleRisLambers, 2009). The present results offer a potential explanation for this observation. That is, niche differences not only need to be sufficiently strong to overcome fitness differences of one or few competitors, as typically considered in empirical studies, but sufficiently strong to overcome fitness differences of the entire resident community, as niche differences are independent of species richness. Our results therefore allow asking the more general question of how many species one can pack in a community, given its niche difference.

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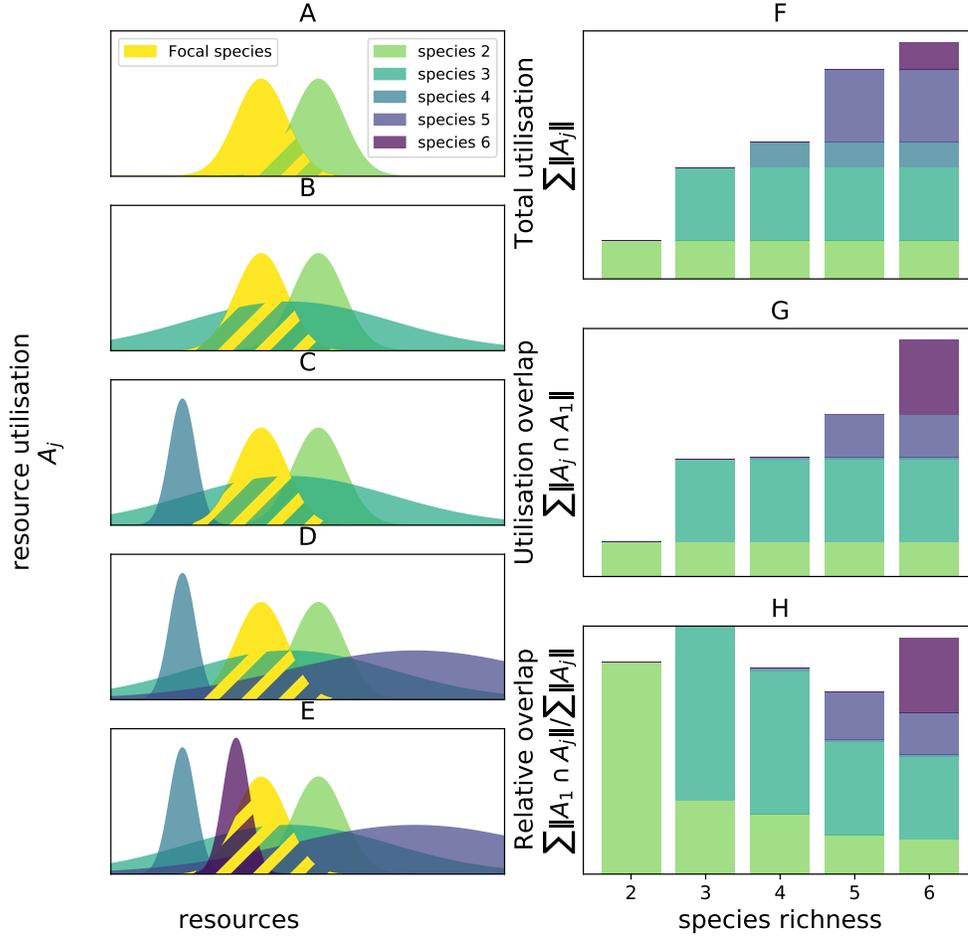


Figure 1: A-E: Resource utilisation of the yellow focal species and its competitors. F: Increasing the species richness will increase the total utilisation of the resident species $\sum_j \|A_j\|$. Similarly, we expect $\mathcal{F}_i \approx 1 - \frac{\sum_j \|A_j\|}{\|A_1\|}$ to increase with species richness, as it scales with the ratio of total resource consumption. G: The amount of shared resources (hatched region from panels A-E) increases with species richness. H: As both the amount of shared resources increase (panel G) and the total utilisation (panel F) increase, we expect the ratio to be independent of species richness. Similarly, we expect $\mathcal{N}_i \approx 1 - \frac{\|A_i \cap \sum_j A_j\|}{\|A_i\| \cdot \sum_j \|A_j\|}$ to be, on average, independent of species richness. F-H: The colours of the bar correspond to the contribution of each of the resident species to the total of the bar.

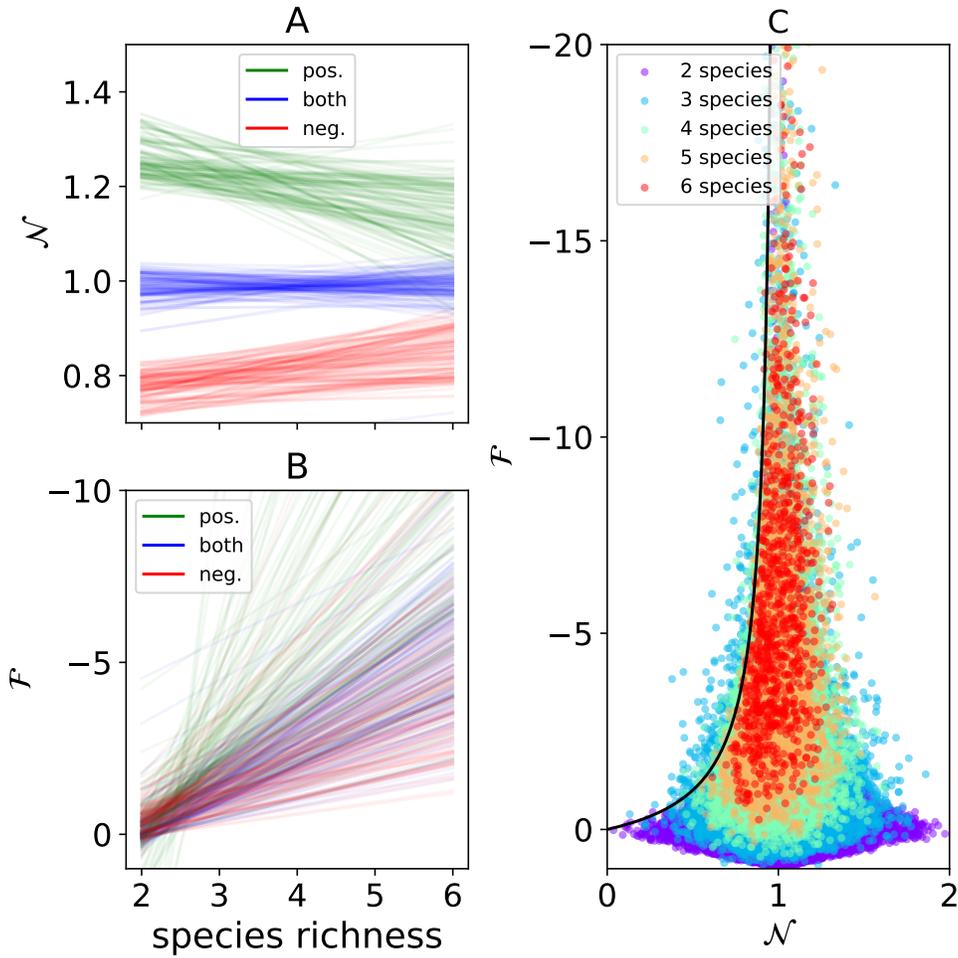


Figure 2: \mathcal{N}_i and \mathcal{F}_i as a function of species richness in simulated communities with strong first-order interspecific interactions. A: Contrary to predictions from theory, niche differences change with species richness when first-order interspecific interactions are either positive (green) or negative (red; results for unconstrained interspecific interactions are shown in blue). However, this is not a direct effect of species richness on niche differences, but rather an indirect effect through a decreasing average interaction strength with increasing species richness (Fig. S2). Each line represents a linear regression of niche differences as a function of species richness for one factorial setting of the full-factorial experiment (Table 1). B: Species richness, however, makes fitness differences more negative (i.e. larger). Note the differences in y-scale between panel A and B. C: Distribution of \mathcal{N}_i and \mathcal{F}_i for simulated theoretical communities that are fully connected, and exhibit first-order interactions without correlations, i.e. similar to the experimental communities (Fig. 4). Each dot represents \mathcal{N}_i and \mathcal{F}_i of one species in a community composed of 2-6 species (see colour legend). The black line indicates the persistence line, species below this line are assumed to persist in the community. Note the inverted y-axis.

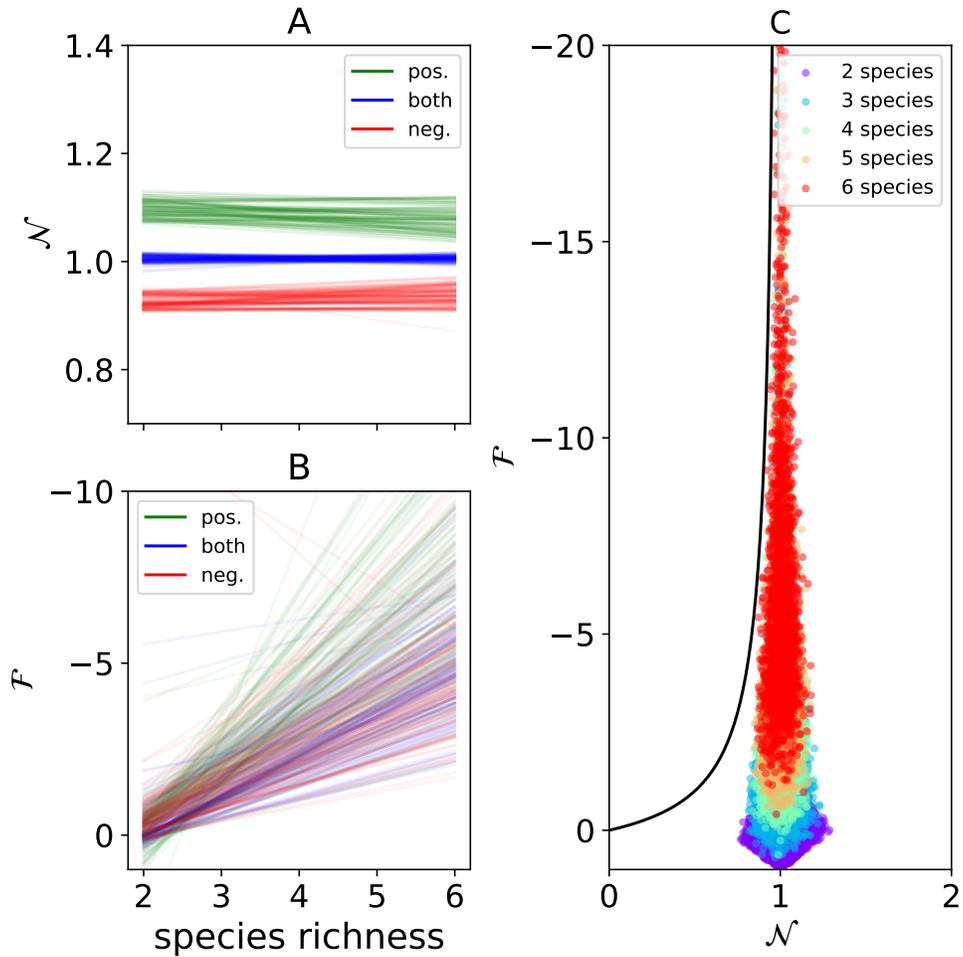


Figure 3: As Fig. 2, but with weak first-order interspecific interactions. A: As predicted by the mathematical results, species richness has no direct effect on niche differences, because communities with different species richness had comparable interaction strengths. B: Species richness, however, makes fitness differences more negative (i.e. larger). C: Distribution of \mathcal{N}_i and \mathcal{F}_i for simulated theoretical communities that are fully connected, and exhibit first-order interactions without correlations, i.e. similar to the experimental communities (Fig. 4).

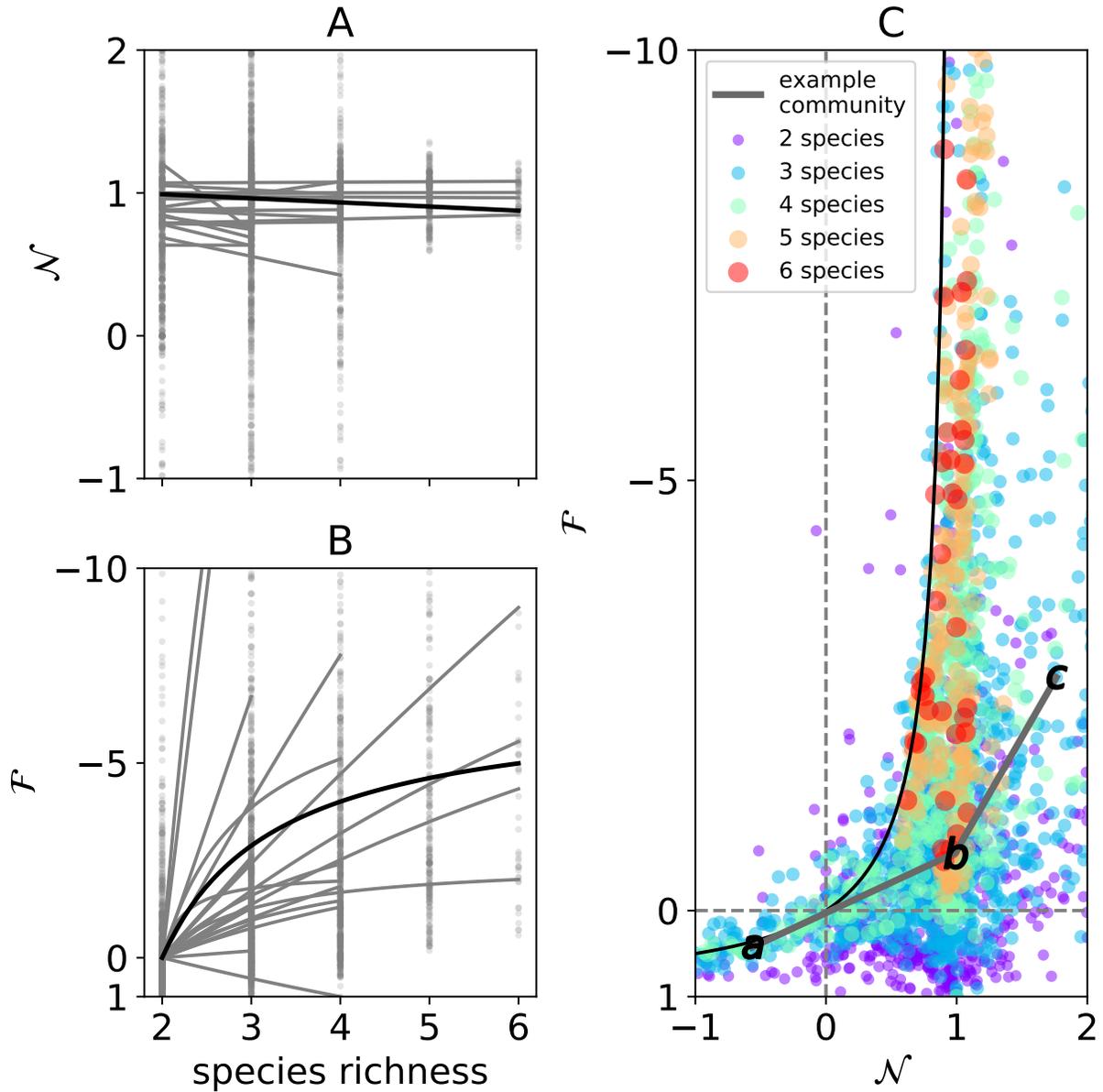


Figure 4: Similar to Fig 2 for empirically observed communities. Each grey line corresponds to a fit of a linear (\mathcal{N}_i , A) and saturating (\mathcal{F}_i , B) regression model to one dataset. The black line represents a fit through all \mathcal{N}_i (A) and \mathcal{F}_i (B) values. Grey dots in panel A and B represent the raw \mathcal{N}_i and \mathcal{F}_i values. Facilitation, i.e. species having a positive net effect on another, and therefore $\mathcal{N}_i > 1$ is common in the datasets we found. We highlight one specific three-species community (grey line) where all species coexist, even though species *a* has $\mathcal{N}_i < 0$, a property associated with priority effects and therefore exclusion. Axis from C are truncated to show $\sim 95\%$ of all data points.

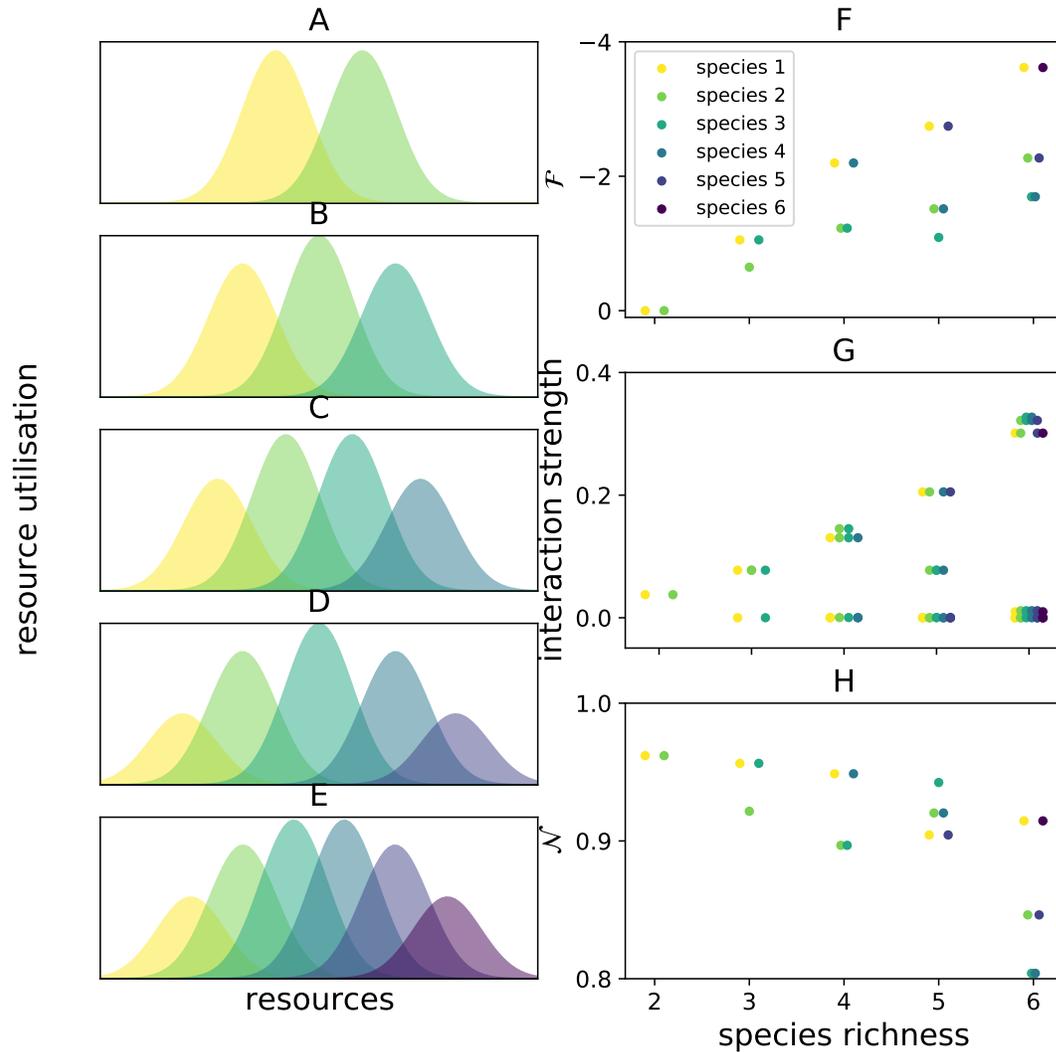


Figure 5: A-E: Resource utilisation of two to six competing species. Species evolve to optimal foraging strategies according to Barabás *et al.* (2016). F: Increasing species richness increases fitness differences, as predicted by theory G: As the niche width is limited, species are located closer to each other in species rich communities and therefore have a stronger interaction strength on average. Each dot represents one value of the interaction matrix α_{ij} . H: Consequently, niche differences decrease with species richness, contrary to theory. The example deviates from the theoretical predictions, as one of the key hypothesis of random assembly is not met.

Factor	Parameter	Levels	Interpretation	Complexity investigated
Interaction type first-order	α_{ij}	< 0 > 0 no restriction	competition facilitation mixed	(1)
Strength of interaction first-order	α_{ij}	strong weak		(1)
Connectance	$P(\alpha_{ij} \neq 0)$	$1, \frac{4}{5}, \frac{2}{3}$		(2)
Interaction correlation	$\text{cor}(\alpha_{ij}, \alpha_{ji})$ $\text{cor}_{ij}(\beta_{ijk}\beta_{jik})$	1 0 -1	equal unrelated opposite	(2)
Presence of indirect effects		Yes No	absent present	(4)
Interaction type second-order	β_{ijk}	> 0 < 0 no restriction = 0	intensify weaken mixed no second-order	(1) and (3)

Table 1: Design of full factorial virtual experiment.

Acknowledgements

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