Fitness differences, not niche differences, limit species richness

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A key question in ecology is what limits species richness. Coexistence theory presents the persistence of species amidst heterospecifics as a balance between niche differences and fitness differences that favour and hamper coexistence, respectively. With most applications focusing on species pairs, we know little about how niche and fitness differences respond to species richness, i.e. what constraints richness most. We present analytical proof that, in absence of higher-order interactions, the average fitness difference increases with richness, while the average niche difference stays constant. Analysis of a simple model with higher-order interactions, extensive simulations that relaxed all assumptions, and analyses of empirical data, confirmed these results. Our work thus shows that fitness differences, not niche difference, limit species richness. Our results contribute to the expansion of coexistence theory towards multi-species communities.

niche differences | fitness differences | competition | mutualism | coexistence | multi-species

Explaining nature's biodiversity is a key challenge for science (1). Coexistence theory predicts species persistence when niche differences overcome fitness differences ($\mathcal{N}$ and $\mathcal{F}$). $\mathcal{N}$ measures the strength of negative frequency dependency, i.e. whether a species can recover when reduced to small abundance. $\mathcal{F}$ measures the intrinsic strength of a species in the absence of niche differences ($\mathcal{N} = 0$), when the species with the highest $\mathcal{F}$ will exclude all other species.

Given these two ingredients of coexistence theory, it can be asked what limits species richness: $\mathcal{N}$ becoming too small, or $\mathcal{F}$ becoming to strong, as we pack more species into a community. Available applications of coexistence theory do not address this question. This is because these have typically focused on two-species communities (2, 3), using a variety of experimental and theoretical approaches. $\mathcal{N}$ and $\mathcal{F}$ have been measured in various systems, including annual and perennial plants (4, 5), phytoplankton (6, 7) and bacteria (8), and under different environmental conditions including drought (9–11), biotic soil conditions (12, 13), and water availability (14). In these communities, environmental gradients affect $\mathcal{N}$ and $\mathcal{F}$ (15), phylogenetic distance increases $\mathcal{N}$ and $\mathcal{F}$ (4, 6), and $\mathcal{N}$ is a better predictor for coexistence than $\mathcal{F}$ (7). However, all these studies have been performed on two-species communities and only three studies report $\mathcal{N}$ and $\mathcal{F}$ in communities composed of more than two species (hereafter multi-species communities) (16–18). However, none of these last studies have addressed the question what limits species richness: too low $\mathcal{N}$, or too high $\mathcal{F}$.

Studying multi-species coexistence is challenging both theoretically and experimentally. Theoretically speaking, the methods to analyse coexistence via $\mathcal{N}$ and $\mathcal{F}$ in a multi-species community were not available until recently (19–22). 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-Volterra models) the number of experiments needed to parametrize the community is quadratic in species richness. Considering higher order interactions will consequently result in a higher experimental load. For example, measuring higher order interactions (sensu. (23)) would imply 39 experiments in a three species community.

It is far from sure if the main results obtained in two-species communities apply to multi-species communities (17, 23, 24). Multi-species communities possess at least four complexities that are absent from two-species communities, and therefore may affect $\mathcal{N}$ and $\mathcal{F}$ in ways that do not occur in two-species communities. (1) In a multi-species community multiple interaction types can co-occur. Species richness increases the number of possible interactions, the number of possible interaction types and the number of combinations of these interaction types. Several summary metrics exist to understand this vast quantity of different possible communities (25). (2) Two-species communities are always fully connected and there's no correlation between interspecific interactions, as there is only one link between species. In an n-species community there may be anywhere from $n-1$ (e.g. food chains) to $\frac{n}{2}(n-1)$ (e.g. fully connected competitive network) links and these interspecific interactions may be positively or negatively correlated (26). (27–29) have shown that connectance and correlation play a mayor role in multi-species stability, we therefore expect them to influence coexistence as well. (3) Higher-order interactions can make a third species change the interaction between a species pair. Such higher-order interactions have been found, for example, in communities composed of phytoplankton, bacteria, and ciliates (30). The bacteria coexisted with the phytoplankton species and with the ciliate, but all three functional groups did not coexist. The reason was that the phytoplankton inhibited bacterial aggregation, leaving the latter more vulnerable to predation. (4) A third species may change the dynamics of two-species communities via indirect effects, even without changing the interaction between two species. In the classic text-book example of Rock-Paper-Scissors communities, these indirect effects can allow three species to coexist via intransitive effects, while no two species can coexist without the third (31). We will refer to these complexities throughout the text with (1) Interaction types, (2) Interaction matrix structure, (3) Higher-order interactions and (4) indirect interactions

While it is known that species in species rich communities

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are less probable to coexist (27, 28, 32, 33), we do not know what limits species richness ($\mathcal{N}$, $\mathcal{F}$, or a combination of both), nor if this answer depend on the community investigated.

In this paper we therefore investigate what limits multi-species coexistence. More specifically, we ask how $\mathcal{N}$ and $\mathcal{F}$ changes as the number of species in a community increases, and how the additional complexities (1)-(4) influence these changes. We do so using three independent methods. First, we derive equations that quantify how $\mathcal{N}$ and $\mathcal{F}$ respond to species richness in a community with linear interactions and a model containing simple cases of higher order interactions. Second, we performed simulations in which we measured how $\mathcal{N}$ and $\mathcal{F}$ respond to the species richness in communities with more complex models. These simulations were run as a full-factorial virtual experiment, varying direct species interactions (type, correlation, connectance), indirect interactions, and higher order interactions. Third, we searched the literature for empirically measured Lotka-Volterra interaction matrices and computed $\mathcal{N}$ and $\mathcal{F}$ as a response to species richness. All three methods support the same general conclusion: $\mathcal{N}$ are unaffected by species richness while $\mathcal{F}$ increase with higher species richness. Furthermore, these conclusions are independent of the four complexities (1)-(4).

Results

Analytical solutions. We first focus on the linear Lotka-Volterra model without higher order interactions (i.e. $\beta_{ijk} = \gamma_{ijkl} = 0$). For this case, we can compute explicitly (see appendix 1):

$$\mathcal{E}_i^m = \sum_{\alpha_{ij}\neq 0} \mathcal{E}_{ij} \frac{N_j^{-i,*}}{N_j^*} \quad [1]$$

$$\rho_i^m = \frac{\sum_{\alpha_{ij}\neq 0} \rho_{ij} c_j^i N_j^{-i,*}}{\sum_{\alpha_{ij}\neq 0} c_j^i N_j^{-i,*}} \quad [2]$$

where $\mathcal{E}_i^m = 1 - \mathcal{F}_i^m$ and $\rho_i^m = 1 - \mathcal{N}_i^m$ are the fitness equivalence and the niche overlap of species i in the multi-species community (superscript m), $\mathcal{E}_{ij}$ and ρ_{ij} are the fitness equivalence and the niche overlap of species i in the two-species community consisting of species i and j. c_j^i is the conversion factor from species j to species i , $N_j^{-i,*}$ is the equilibrium density of species j in the absence of species i and N_j^* is the equilibrium density of species j in monoculture (see methods). The sum is taken over all species j with which i interacts directly, i.e. $\alpha_{ij} \neq 0$

Eq. 1 and 2 show three main results. First, $\mathcal{E}_i^m$ is the weighted *sum*, across all species pairs, of the two-species fitness equivalences $\mathcal{E}_{ij}$. The sum of the weights $\sum_{j\neq i} \frac{N_j^{-i,*}}{N_j^*}$ is the relative yield total known from biodiversity ecosystem functioning research (34, 35). In case species coexist, which is the focus of the current manuscript, one expects the relative yield total to increase with species richness (19, 36). Hence, the mean and variance of $\mathcal{E}$ (and therefore of $\mathcal{F}$) in general increase with species richness. Second, ρ_i^m is the weighted *average* of the two-species niche overlaps ρ_{ij}. Hence, species richness has on average no effect on niche overlap ρ, and consequently neither on $\mathcal{N}$. Third, the variance of $\mathcal{N}$ decreases with species richness, because $var(\frac{1}{n}\sum_i X_i) = \frac{1}{n^2}\sum_i var(X_i)$, i.e. variance decreases with sample size. Since we did not make assumptions about the α_{ij}, these results are independent of them, i.e. the results apply regardless of complexities (1) and (2).

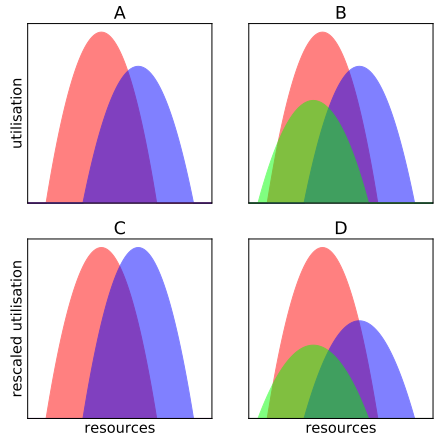

Fig. 1. Illustration of how to compute $\mathcal{F}$ (A,B) and $\mathcal{N}$ (C,D) for a two (A,C) and a three (B,D) species community. As an illustration, we chose the Mac Arthur resource model in which several species (red, blue and green) consume a resource continuum at different frequencies (e.g. birds with different beak size consuming seeds of different size). All else being equal, $\mathcal{F}$ measures how much resources the focal species consumes, compared to the consumption by its competitors (i.e. $\mathcal{F}_{red}^m = 1 - \frac{\sum\|A_{residents}\|}{\|A_{red}\|}$) (22). To compute $\mathcal{N}$, the resource consumption of the focal species and all its competitors combined must first be scaled to have the same area, this is done via c_{blue} (i.e. $c_{blue} = \frac{\|A_{blue}\|}{\|A_{red}\|}$) (22). $\mathcal{N}$ is the proportion of red area , not shared with the competitor species, when both areas have been scaled to equal size (i.e. $\mathcal{N}_{red}^m = 1 - \left\| \frac{A_{red}}{\|A_{red}\|} \cap \frac{\sum A_{residents}}{\sum\|A_{residents}\|} \right\|$).

That $\mathcal{E}_i^m$ is a weighted sum while ρ_i^m is a weighted average makes intuitive sense when realising that the interaction coefficients α_{ij} can under certain conditions be related to the Mac-Arthur resource competition model (37, 38). Consider three species (noted "red", "blue" and "green" hereafter) that consume a resource continuum at different frequencies (Fig. 1 A). We assume that the species only differ in their resource consumption, not in other parameters such as mortality. We want to compute the $\mathcal{N}$ and $\mathcal{F}$ of the red focal species in presence of the blue (only) or blue and green (combined) competitors. The species with the higher total consumption will have a fitness advantage. Intuitively, one could therefore expect that $\mathcal{E}_{red} \approx \frac{\|A_{blue}\|}{\|A_{red}\|}$, where A_{red} and A_{blue} denote the consumption by the red and the blue species (see fig 1). $\|A_{red}\|$ denotes the total consumption by the red species, i.e. $\|A_{red}\|$ is a real number, while A_{red} is a vector. In a multi-species community, one could therefore expect that $\mathcal{E}_{red}^m \approx \frac{\sum\|A_{residents}\|}{\|A_{red}\|} = \sum \frac{\|A_{residents}\|}{\|A_{red}\|}$(Fig.1 B). It turns out that the intuition is almost correct; we only have to add different weights to the sum, according to the densities of the species at equilibrium (compare this equation to eq. 1) (22). $\mathcal{E}_{red}^m$ thus increases, and $\mathcal{F}$ therefore becomes more negative, as species richness increases (recall that $\mathcal{F} = 0$ means no fitness differences and more negative $\mathcal{F}$ mean stronger fitness differences).

ρ measures the relative difference in niches, so we must remove differences in total consumption to compute ρ. This is done by rescaling the consumption of both species, such that both consume the same total amount of resources, via the conversion factors $c_{blue} = \frac{\|A_{blue}\|}{\|A_{red}\|} = c_{red}^{-1}$ (note that

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$c_{red}^{-1} = \mathcal{E}_{red}$is a coincidence in this very simple model and does not hold in general). Intuitively ρ is the proportion of shared resources between the two species after rescaling, i.e. $\rho = \frac{\left\| A_{red} \cap \left(\frac{\|A_{red}\|}{\|A_{blue}\|} A_{blue} \right) \right\|}{\|A_{red}\| \cdot \|A_{red}\|} = \left\| \frac{A_{red}}{||A_{red}||} \cap \frac{A_{blue}}{||A_{blue}||} \right\|$, where $\cap$ denotes the intersection of the two consumption vectors (purple area in Fig. 1 C) (22). In a multi-species community, we therefore expect that $\rho_{red}^{m} \approx \left\| \frac{A_{red}}{||A_{red}||} \cap \frac{A_{green}+A_{blue}}{||A_{green}+A_{blue}||} \right\| = \frac{\sum \left(||A_{residents}|| \cdot \left\| \frac{A_{red}}{||A_{red}||} \cap \frac{A_{residents}}{||A_{residents}||} \right\| \right)}{||\sum A_{residents}||}$is a weighted average (Fig. 1 D). Again this intuition holds, after weighing with species densities.

We can approximate $\mathcal{N}$ and $\mathcal{F}$ in a multi-species community by using the average interspecific interaction strength $\overline{\alpha}$ (see appendix 1). This yields $\mathcal{N}_i^m \approx 1 - \overline{\alpha}$ and $\mathcal{F}_i^m \approx 1 - \frac{n-1}{1-(n-2)\overline{\alpha}}$, from which it is clear that $\mathcal{N}$ is independent of species richness n and $\mathcal{F}$ 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.

To investigate the complexity (3) we remove indirect effects. To remove these, we set $N_j^{-i,*} = N_j^*$, i.e. species k does not affect the density of species j, it only directly affects species i via $\alpha_{ik} N_k^*$. This will make $\mathcal{F}$ more negative (therefore larger), as we change the weights of the sum. More importantly, $\mathcal{F}$ changes from a saturating to a linear response in species richness, i.e. $\mathcal{F} \approx 1 - (n-1)$ on average. Conversely, removing indirect effects will not change $\mathcal{N}$ on average, therefore not altering the response of $\mathcal{N}$ to species richness. This yields an important result: Indirect effects are purely equalizing as they do not change stabilisation. Indirect effects thus promote coexistence (2).

We also found an analytical solution for $\mathcal{N}$ and $\mathcal{F}$ as a function of species richness when higher order effects where involved (complexity (4)), but only in the simplified case of constant interspecific interactions and fixed higher-order interactions ($\alpha_{ij} = \bar{\alpha}, \beta_{ijk} = \bar{\beta}, \gamma_{ijkl} = \bar{\gamma}$). In this case, the main results remained valid: $\mathcal{N}_i^m = 1 - \bar{\alpha}$ and $\mathcal{F}_i^m = 1 - \frac{n-1}{1-(n-2)\bar{\alpha}}$ (see appendix 1).

Full-factorial simulations. The simulations using random matrices confirm the predictions made by theory. $\mathcal{N}$ is on average unaffected by species richness and $\mathcal{F}$ increases with species richness for all parameters settings of the full-factorial simulations (see Fig. 2 A,B). First order interaction strength dominated the effects of species richness on $\mathcal{N}$. The average of $\mathcal{N}$, $\overline{\mathcal{N}}$, is unaffected by any other factor than first order interaction strength, i.e. $\overline{\mathcal{N}} = 1 - \bar{\alpha}$. Species richness does not affect $\mathcal{N}$ (the slope of the linear regressions ranged between -0.005 and 0.003 for all parameter combinations). Variation of $\mathcal{N}$ was only affected by the first order interaction strength. The variation of $\mathcal{N}$ decreases with species richness in almost all cases ($> 95\%$). In the other cases, variation increases only negligibly (the maximal slope was 0.0003). Connectance slightly decreased the negative effect of species richness on variation. The presence of second order interactions (positive, negative or both) increased the negative effect of species richness on the variation of $\mathcal{N}$. The other factors (correlation, indirect effects, presence of third order interactions) had no effect on $\mathcal{N}$ or the variation of $\mathcal{N}$.

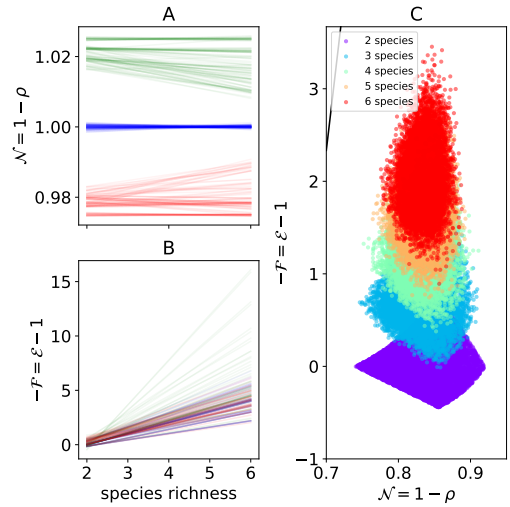

Fig. 2. $\mathcal{N}$ and $\mathcal{F}$ as a function of species richness in simulated communities. A: $\mathcal{N}$ is unaffected by species richness in general. Each line represents a linear regression of $\mathcal{N}$ as a function of species richness for one factorial setting of the full-factorial experiment (see table 1). The color indicates the factor level for first order (direct) interactions, green indicates positive, blue indicates negative and red indicates mixed interspecific interactions. 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 $\mathcal{N}$ and $\mathcal{F}$ for simulated theoretical competitive communities that are fully connected, and exhibit first order interactions without correlations, i.e. similar to the experimental communities (see Fig. 3). Each dot represents $\mathcal{N}$ and $\mathcal{F}$ 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.

Multiple factors had effects on $\mathcal{F}$. First order interactions affected the mean $\mathcal{F}$ and the effect of species richness on $\mathcal{F}$ as predicted by our analytical derivation (see eq. 4). High connectance decreased the mean of $\mathcal{F}$, because the sum in eq. 1 is only taken over species with which the focal species interacts. Positive correlation of interspecific interactions decreased the mean $\mathcal{F}$, because for perfectly correlated interspecific interaction strengths we have $\mathcal{F}_{ij} = 0$, negative correlation on the other hand increased it. Second order interactions increased the mean of $\mathcal{F}$ when these interactions were positive, but decreased them when they were negative. Presence or absence of third order interactions had very little effect on $\mathcal{F}$. We illustrate how $\mathcal{N}$ and $\mathcal{F}$ values jointly varied with species richness, using interaction strengths that are representative for experimental communities evaluated in the next section (Fig. 2 C): $0.08 \leq \alpha_{ij} \leq 0.26$, $\beta_{ijk} = \gamma_{ijkl} = 0$, no correlation between the α_{ij}, and maximum connectance.

Literature data. The results for the real communities reflect those obtained for the simulated communities. The absolute values of the slope of the linear regression of $\mathcal{N}$ were small (< 0.05) for all but 6 datasets. The slope for the overall regression of $\mathcal{N}$ against species richness (Fig. 3A, black line) was small(-0.028). $\mathcal{F}$ increased with richness in all but one dataset. Overall, we conclude that the response of $\mathcal{N}$ and $\mathcal{F}$ to richness for real communities did not qualitatively differ from that of randomly generated communities.

The empirical data also revealed cases in which coexistence is possible even though some of the species have negative $\mathcal{N}$. This is possible as long as $\mathcal{F}_i$ is sufficiently positive such

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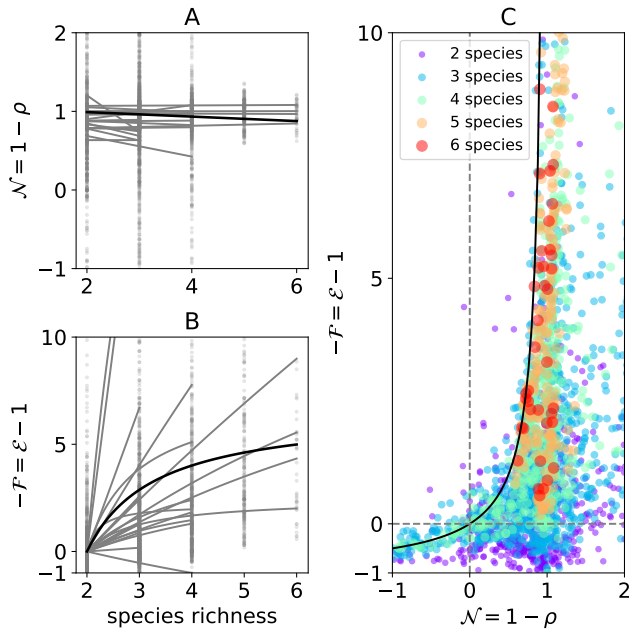

Fig. 3. $\mathcal{N}$ and $\mathcal{F}$ as a function of species richness in empirically measured communities. Each grey line corresponds to a fit of a linear ($\mathcal{N}$) and saturating ($\mathcal{F}$) regression model to one dataset. The black line represents a fit through all $\mathcal{N}$ respectively $\mathcal{F}$ values. Grey dots in panel A and B represent the raw $\mathcal{N}$ and $\mathcal{F}$ values. Mutualism, i.e. species having a positive net effect on another, and therefore $\mathcal{N} > 1$ is common in the datasets we found. Axis truncated to show ∼95% of all data points.

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 $\mathcal{N}$, indicating that this is not a rare phenomena. Negative $\mathcal{N}$ are typically attributed to priority effects, which prelude coexistence (39, 40). Coexistence with negative $\mathcal{N}$ is possible in multi-species communities, because not all species have negative $\mathcal{N}$, but only a few.

Discussion

The potential for coexistence decreases with species richness (24, 27, 32, 33). We explained this result using the key concepts of modern coexistence theory, i.e. $\mathcal{N}$ and $\mathcal{F}$. We found that species richness does, on average, not affect niche differences but does increase fitness differences. Thus, it are interspecific differences that limit the coexistence of multiple species, rather than interspecific similarities. These results are based on three independent methods: analytical computation, numerical simulations and metanalysis of experimental data. The $\mathcal{F}$ of a species increases with species richness, as $\mathcal{F}$ measures the fitness of a species compared to the combined fitness of all other species. In multi-species communities, most species will therefore have negative $\mathcal{F}$, as rarely one species will have higher fitness than all other species combined.

The $\mathcal{N}$ of a species measures 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, but also the amount of limiting factors that are not shared. The proportion of shared limiting factors is therefore unaffected on average. Species-rich communities are therefore less likely to coexist (all else being equal), as $\mathcal{F}$ become to strong for $\mathcal{N}$.

The results we obtained are consistent throughout the three different methods we chose, i.e. analytical derivations, simulations, and analysis of empirical data. Overall, our results are robust to inclusion or omission of the complexities (1)-(4), and all their combinations. However, some complexities could not be investigated by all methods. Complexity (1), interaction types, are the main drivers of $\mathcal{N}$ and $\mathcal{F}$: $\mathcal{N} \approx 1 - \overline{\alpha}$ and $\mathcal{F} \approx 1 - \frac{n-1}{1-(n-2)\overline{\alpha}}$. Complexity (2), interaction matrix structure, contains correlation and connectance. Correlation affects $\mathcal{N}_i$ and $\mathcal{F}_i$ indirectly, via its effect on the two-species community $\mathcal{N}_{ij}$ and $\mathcal{F}_{ij}$. Low connectance decreases the effect of species richness on $\mathcal{N}$ and $\mathcal{F}$, effectively the number of interactions are relevant for $\mathcal{N}$ and $\mathcal{F}$ in multi-species communities, and not the species richness of a community per se. Complexity (3), higher order interactions, affected $\mathcal{F}$, but not $\mathcal{N}$. Positive higher order interactions ($\beta_{ijk} > 0$) increase $\mathcal{F}$ and negative higher order interactions decrease $\mathcal{F}$. Complexity (4), indirect interactions, also only affected $\mathcal{F}$, but not $\mathcal{N}$. Indirect effects decrease $\mathcal{F}$ in competitive communities and increase $\mathcal{F}$ in mutualistic communities.

These results contradict those obtained by (17). Chu et al. (17) found that species richness will decrease $\mathcal{N}$ and will not affect $\mathcal{F}$. The use of different definitions for $\mathcal{N}$ and $\mathcal{F}$ explains this difference (19). Indeed, applying the same definition to our data reproduces the results found by (17) ($\mathcal{N}$ is affected by species richness, while $\mathcal{F}$ is not (see Appendix 3) and which seemingly contradict our main findings. However, we argue that our results provide a more accurate account of how $\mathcal{N}$ and $\mathcal{F}$ limit multi-species coexistence because of the following reasons: 1. The definition of (22) does not only consider negative frequency dependence, but also positive frequency dependence and facilitation. The definition of (19) can only be computed for communities with negative frequency dependency, which precludes the analysis of 75% of the empirical data and 67% of the simulated data presented here. 2. The $\mathcal{N}$ and $\mathcal{F}$ as defined by (22) clearly link to persistence of a species via the equation $-\mathcal{F} \leq \frac{\mathcal{N}}{1-\mathcal{N}}$. Again, this is not the case for the definition by (19) in multi-species-communities. Since we explicitly ask whether $\mathcal{N}$ or $\mathcal{F}$ is more limiting for coexistence in multi-species communities, the definition of (19) can not be used. 3. (22) show that $\mathcal{N}$ is biologically intuitive as it measures the amount of shared resources in a large class of resource competition models. Fig.1 extends this intuitive explanation to multi-species communities. (19) has so far only been linked to the Mac-Arthur resource model and it is not clear how it relates to more complex resource competition models.

Limitations. The available experimental data only represented fully connected communities, with no correlation (complexity (2)) among interactions 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 are realistic or whether more realistic values exist that would lead to different results. The available experimental data were biased towards fully connected, competitive communities of terrestrial plants with relatively low species richness. Our simulations suggest that our conclusions hold for other networks as well, but we were not able to back up this claim with empirical data. Computing

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$\mathcal{N}$ and $\mathcal{F}$ on a larger collection of real networks would help to refine our understanding of this process. However, obtaining the full interaction matrix for species rich communities is still challenging. Qualitative interaction data are not sufficient to compute $\mathcal{N}$ and $\mathcal{F}$. To obtain quantitative data, one uses 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 (41). Other methods consist of estimating interaction strength based on, for example, biomass (42), mass ratio (43) or production and consumption rates of species (44, 45). These different methods have different assumptions and may therefore influence the resulting matrix estimate (46).

Given these limitations, one can ask to what extent our conclusions will hold in other community models. In communities where species richness increases total abundance, which is often the case (36, 47), we expect species richness to increase $\mathcal{F}$ as well (make it more negative), as the no-niche growth rate will be more negative. Conversely, in communities where species richness decreases total abundance we expect the opposite. Similarly, in competitive communities indirect effects decrease total abundance and therefore also $\mathcal{F}$. However, in mutualistic communities indirect effects will increase total abundance and therefore also $\mathcal{F}$. It is less clear how species richness will affect $\mathcal{N}$ in models not explored in the current paper. $\mathcal{N}$ depends on the invasion growth rate and the no-niche growth rates, which both depend on the species richness and total abundance. Whether $\mathcal{N}$ increases or decreases with species richness will therefore depend on how species richness affects these growth rates.

New insights. Our results yield three new insights, other than the main result on how $\mathcal{N}$ and $\mathcal{F}$ varies with species richness. A first insight is that negative $\mathcal{N}$ do not necessarily preclude coexistence. Negative $\mathcal{N}$ have been attributed to priority effects and therefore were viewed as precluding coexistence (39, 40). Our framework confirms this finding for the case of competitive two-species communities, where the species with $\mathcal{F} < 0$ will not be able to persist (22). However, in contrast to species in two-species communities, species in multi-species communities will not all have the same $\mathcal{N}$. This implies that a species with negative $\mathcal{N}$ and low $\mathcal{F}$ can coexist with other species that have high $\mathcal{N}$ and negative $\mathcal{F}$. Consequently, multiple species can have negative $\mathcal{N}$ in a multi-species communities and still persist. For example, we found six three-species communities in which all but one species had negative $\mathcal{N}$. In general, we argue that it would be theoretically possible to construct a community model in which all species have negative $\mathcal{N}$ and coexist. The kind of model and how it should be parametrized remains to be examined, however.

A second insight is that indirect effects, and to some extent higher-order effects, are equalising. While direct interaction affect both $\mathcal{N}$ and $\mathcal{F}$, indirect and higher order effects mainly affect $\mathcal{F}$ and should therefore be seen as equalizing effects (2). Indirect interactions and higher order interactions alone, i.e. in the absence of any niche differentiation via first order interactions, will therefore not be able to sustain coexistence, as equalizing effects cannot sustain multiple species in the absence of stabilizing effects (2, 48). This is confirmed by (24) and (49) who found that intransitivity in annual grassland communities, in the absence of $\mathcal{N}$, is not able to sustain coexistence. They may however promote coexistence in the presence of some $\mathcal{N}$, by reducing $\mathcal{F}$, just as other equalizing mechanisms do.

A third and main insight is that one can infer $\mathcal{N}$ and $\mathcal{F}$ in multi-species communities from $\mathcal{N}$ and $\mathcal{F}$ measured in pairwise interaction experiments. If one measures $\mathcal{N}$ and $\mathcal{F}$ for each two-species sub-community of an n species community, which is typically done (4, 6, 7, 18), one can estimate $\mathcal{N}_i \approx \frac{\sum_j \mathcal{N}_{ij}}{(n-1)}$. With one additional multi-species experiment to estimate the relative yield RY_i we obtain an estimation of $\mathcal{F}_i \approx 1 - \sum_j (1 - \mathcal{F}_j) \cdot \mathrm{RY}_j$ as well. This indicates that two-species experiments are sufficient to predict $\mathcal{N}$ and $\mathcal{F}$ in multi-species communities.

One of the key questions in community ecology is whether $\mathcal{N}$ are strong enough to overcome $\mathcal{F}$ and allow coexistence. Often they are found to be not only sufficiently strong, but much stronger than needed (17, 50). The present results offer a potential explanation for this observation. That is, $\mathcal{N}$ not only need to be sufficiently strong to overcome $\mathcal{F}$ of one or few competitors, but sufficiently strong to overcome $\mathcal{F}$ of the entire resident community, as $\mathcal{N}$ is independent of species richness. Our results therefore allow asking the more general question how many species one can pack in a community, given $\mathcal{N}$ that are invariant of species richness.

Methods

Model description. We use a generalized Lotka-Voltera model with n species containing higher order interactions:

$$\frac{1}{N_i}\frac{dN_i}{dt} = r_i \left(1 - \sum_j \alpha_{ij} N_j \left(1 + \sum_k \beta_{ijk} N_k \left(1 + \sum_l \gamma_{ijkl} N_l\right)\right)\right) \quad [3]$$

Where N_i is the density of the focal species i. r_i is the monoculture growth rate at low density. α_{ij}, β_{ijk} and γ_{ijkl} are first or linear, second, and third-order species interactions, respectively. A positive α_{ij} indicates a *negative* interaction between species i and j such as competition or predation. Negative α_{ij} on the other hand indicate positive interactions such as facilitation or consumption. If β_{ijk} is positive or negative, species k will intensify or weaken the relationship between species i and j, respectively (second order interaction). Similarly, whenever γ_{ijkl} differs from zero (third order interaction), species l can influence the second-order interaction. Throughout the manuscript, we take $\alpha_{ii} = 1$.

There exist five different definitions to quantify $\mathcal{N}$ and $\mathcal{F}$ in multi-species communities (19–22, 51). The definitions of (21) does not apply to the selected model. (51) was developed for environmental or spatial fluctuations, which we don't consider here. (20) and (19) do apply to the selected model. However, $\mathcal{N}$ and $\mathcal{F}$ as computed by these two methods allow interference about coexistence only in two-species communities, not in multi-species communities. That is, two different multi-species communities may have identical $\mathcal{N}$ and $\mathcal{F}$ but different outcomes of coexistence in both. Since we here ask whether coexistence in multi-species communities is driven by $\mathcal{N}$ or $\mathcal{F}$, these two methods are therefore not suitable. Consequently, we computed $\mathcal{N}$ and $\mathcal{F}$ as defined by (22):

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$$\mathcal{N}_i = \frac{f_i(0, \boldsymbol{N}^{-i,*}) - f_i(\sum_{j\neq i} c_j^i N_j^{-i,*}, \boldsymbol{0})}{f_i(0, \boldsymbol{0}) - f_i(\sum_{j\neq i} c_j^i N_j^{-i,*}, \boldsymbol{0})} \quad [4]$$

$$\mathcal{F}_i = \frac{f_i(\sum_{j\neq i} c_j^i N_j^{-i,*}, \boldsymbol{0})}{f_i(0, \boldsymbol{0})} \quad [5]$$

Where f_i is the per capita growth rate of species i, i.e. $f_i(N_i, \boldsymbol{N}^{-i}) = \frac{1}{N_i}\frac{dN_i}{dt}$. The first argument of f_i (N_i) is the density of the focal species i, the second argument ($\boldsymbol{N}^{-i}$) is a vector of length containing the densities of the $n-1$ non-focal species. $\boldsymbol{N}^{-i,*}$ is the equilibrium density of each non-focal species of the community in the absence of species i. c_j^i, the conversion factor of species j to species i, equates the total dependencies on limiting factor for species i and j (see fig. 1). $\mathcal{N}_i$ 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. We introduce niche overlap $\rho = 1 - \mathcal{N}$ and fitness equivalence $\mathcal{E} = 1 - \mathcal{F}$ for simpler interpretation of the results.

Analyses and Simulations. We first examined analytically how $\mathcal{N}$ and $\mathcal{F}$ change with species richness. We found a generic solution for first order interactions and for a simplified case of higher order interactions. This simplification consisted of constant interspecific interactions ($i \neq j \Rightarrow \alpha_{ij} = \alpha$) and constant higher order interactions ($\beta_{ijk} = \beta, \gamma_{ijkl} = \gamma$). These analytical results allowed us to investigate the effects of the complexities (1), (2), (4) and partially (3).

Second, we designed a full-factorial virtual experiment in which we simulated $\mathcal{N}$ and $\mathcal{F}$ for a wide range of different communities (see table 1). The factors were (i) first order interaction type (competitive, mutualistic or both, i.e. $\alpha_{ij} > 0, < 0$ or no restriction). (ii) Connectance of the interspecific interaction ($c \in \left\{1, \frac{4}{5}, \frac{2}{3}\right\}$). (iii) Correlation between the interspecific interaction ($\rho(\alpha_{ij}, \alpha_{ji}) = \rho_{ij}(\beta_{ijk}, \beta_{jik}) = \rho_{ij}(\gamma_{ijkl}, \gamma_{jikl}) \in \{-1, 0, 1\}$). (iv) Inclusion of indirect effects. To exclude indirect effects we set equilibrium densities of resident species to their monoculture equilibrium density. (v) Second order interaction type ($\beta_{ijk} < 0, > 0$, no restriction, absent). (vi) Presence of third order interaction type ($\gamma_{ijkl} = 0$ or $\gamma_{ijkl} \neq 0$).

This leads to a total of $3 \cdot 3 \cdot 3 \cdot 2 \cdot 4 \cdot 2 = 432$ parameter settings. We ran 1000 repetitions for each species richness level ($2 \leq n \leq 6$), leading to a total of $432 \cdot 5 \cdot 1000 = 2'160'000$ simulations. To compute $\mathcal{N}$ and $\mathcal{F}$ we chose the magnitude of the interaction strength such that all communities and sub-communities will coexist (i.e. $|\alpha| \leq 0.05$, $|\beta| \leq 0.05$, $|\gamma| \leq 0.05$). In all simulations, the non-zero values of the interaction strength were uniformly distributed in their respective range (i.e. $\alpha \in [0, 0.05], [-0.05, 0]$ or $[-0.05, 0.05]$). For each parameter setting we investigated the effect of species richness ($2 \leq n \leq 6$) on $\mathcal{N}$ and $\mathcal{F}$. We fitted linear regressions to assess the effect of species richness on $\mathcal{N}$, variation of $\mathcal{N}$ within a community, $\mathcal{F}$ and variation of $\mathcal{F}$ with in a community. As a measure of variation we take the inter-quartile range, as it is a outlier robust equivalent to the variance. We report the effect of species richness (slope of linear regression) and the effect of the parameter combinations (intercept of linear regression) on these parameters. With this approach we were able to investigate the effects of all complexities (1)-(4).

Literature data. We found three review papers of multi-species Lotka-Volterra interaction matrices (5, 52, 53), representing a total of 33 interaction matrices, ranging 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 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, leading to 2296 communities. For 1376 communities $\mathcal{N}$ and $\mathcal{F}$ could not be computed because, like any method seeking to quantify frequency dependence, $\mathcal{N}$ and $\mathcal{F}$ is based on invasion analysis: the capacity of an invader to grow with the other species at their non-zero equilibrium. For this the invasion growth rate of each species must be computed, the per capita growth rate $f_i(0, \boldsymbol{N_j}^{-i,*})$ when the focal species i is absent (mathematically equal 0) and the other species at their equilibrium density $\boldsymbol{N}^{-i*}$. $\mathcal{N}$ and $\mathcal{F}$ can thus only be obtained for communities where each subcommunity (the community without the invading species) coexists stably. We computed $\mathcal{N}$ and $\mathcal{F}$ for a total of 920 communities, the species of 722 of these communities were able to coexist. Species of 46 additional communities did coexist, but did not allow invasion analysis, hence we were not able to compute $\mathcal{N}$ and $\mathcal{F}$.

We were able to compute $\mathcal{N}$ and $\mathcal{F}$ for about 40% of the real communities. While we could compute $\mathcal{N}$ and $\mathcal{F}$ for all two-species communities, we were able to compute only for 3% of six-species communities. We computed $\mathcal{N}$ and $\mathcal{F}$ for about 94% of all communities in which species coexist, in the remaining communities invasion analysis was not possible. In these $\mathcal{N}$ and $\mathcal{F}$ correctly predicted coexistence, indicating that $\mathcal{N}$ and $\mathcal{F}$ as proposed by (22) is a useful tool to analyse multi-species coexistence. However, for only about 13% of the communities in which species don't coexist we were able to compute $\mathcal{N}$ and $\mathcal{F}$. We were able to compute $\mathcal{N}$ and $\mathcal{F}$ in these communities because all species in all sub communities coexisted, not however the species in the community as a whole. For a detailed version including numbers of communities per species richness, see appendix 2.

For each interaction matrix obtained from the literature we computed $\mathcal{N}$ and $\mathcal{F}$ as mentioned above. We fit a linear response of $\mathcal{N}$ as a function of species richness per interaction matrix from the literature. The data contained many outliers, which skewed the results of our linear regressions. We therefore used a Theil-Sen estimator for the slope, which is more robust to outliers than linear regression based on least squares (54). We fit (using least squares) a saturating function $\mathcal{F} = \frac{n-2}{(n-2)+H}$ for the fitness differences. This saturating response was chosen for $\mathcal{F}$, because our analytical results suggest a saturating response. We additionally fitted a regression line through all communities.

With this approach, we were able to investigate the effects of the complexities (1) and (4). The experimental protocol to obtain the interaction matrix does not allow detecting higher order interactions, these are therefore by definition absent from the experimental data. We did not find any data on multi-species communities including higher order interactions (complexity (3)). We did not investigate the effects of con-

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

Table 1. Design of full factorial virtual experimental.

nectance, because almost all interaction matrices were fully connected (complexity (2)).

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