

Higher-order interactions capture unexplained complexity in diverse communities

Margaret M. Mayfield^{1*} and Daniel B. Stouffer^{2*}

Natural communities are well known to be maintained by many complex processes. Despite this, the practical aspects of studying them often require some simplification, such as the widespread assumption that direct, additive competition captures the important details about how interactions between species impact community diversity. More complex non-additive ‘higher-order’ interactions are assumed to be negligible or absent. Notably, these assumptions are poorly supported and have major consequences for the accuracy with which patterns of natural diversity are modelled and explained. We present a mathematically simple framework for incorporating biologically meaningful complexity into models of diversity by including non-additive higher-order interactions. We further provide empirical evidence that such higher-order interactions strongly influence species’ performance in natural plant communities, with variation in seed production (as a proxy for per capita fitness) explained dramatically better when at least some higher-order interactions are considered. Our study lays the groundwork for a long-overdue shift in how species interactions are used to study the diversity of natural communities.

Interactions between entities are central to the functionality of systems across science, whether socioeconomic, atomic, chemical or biological. In natural systems, for example, species interactions are biologically and mathematically integral to explaining patterns of diversity^{1–4}. Mechanistically, it is believed that interactions between species, and competitive interactions in particular, are of central importance to coexistence and food-web dynamics and are core drivers of community-level diversity^{1,3,5–7}. Typically, individual-based fitness models are used to incorporate the effects of species interactions into coexistence and diversity models^{7–11}; this approach is commonly used in plant and marine-focused studies^{7,11–13} while also being conceptually appropriate for other types of communities^{5,8}. Despite their extensive applicability, these models often do a surprisingly poor job of accurately explaining empirically observed fitness outcomes^{14–16}. This may be because of the common assumption that additive competitive interactions between species pairs capture all of the important details of species interactions and are thus the only interactions typically incorporated into fitness models^{4,17–19}.

Unpredicted outcomes of fitness models are most frequently presumed to result from unmeasured additive or non-additive indirect effects¹⁵ or intransitive competition² (that is, A outcompetes B, which outcompetes C, which outcompetes A). Alternatively, unexpected outcomes may result from ‘higher-order’ interactions (HOIs), which are the suite of non-additive effects of interactions between individuals of co-occurring ‘competitor’ species (including the focal species) on the fitness of a focal individual²⁰. In other words, the interactions that a competitor species B has with all conspecific and heterospecific neighbours cumulatively impact its full effect on a focal individual of species A (Fig. 1 and Supplementary Fig. 1). Importantly, the drivers of HOIs are diverse and can result from varied mechanisms, such as behavioural variation, trait plasticity, resource competition or mutualisms^{2,21,22}.

Certain types of non-additive indirect effects (which can mediate HOIs as defined above) have been well explored in the predator–prey and food-web literature, particularly trait- and density-mediated indirect effects^{21–24}. This rich literature focuses,

however, on population dynamics as opposed to individual fitness. This is because the vast majority of ecological research on non-additive indirect effects adopts a population-based framework based on a historically distinct definition of HOIs: changes to the interactions between two species mediated through a third species^{20,25}. This population-based framework is particularly well suited to studying the complex species interactions involved, for example, in food webs²²; consequently, the food-web literature has outstripped the coexistence and diversity literature in incorporating and studying the importance of non-additive HOIs in natural communities. Having said that, these population-centric approaches cannot be easily extrapolated to individual fitness outcomes, if at all²⁰.

The potential importance of HOIs for individuals has been recognized for over half a century^{14,26,27}. Nonetheless, the fact that a majority of recent mechanistic diversity studies, particularly those based on coexistence models, have opted to ignore or marginalize them^{5,28} probably stems from three core problems. First, the most common experimental design for quantifying species interactions for individual fitness models of coexistence and diversity assess a focal species’ fitness with a single competitor species. This approach is generally thought to preclude the detection of HOIs^{19,29}, as the detection of HOIs depends on sufficient variation in the richness and abundance of competitors³⁰. Second, the analytical intractability of diversity models that do include HOIs can hinder their detailed study^{1,29,30}. Finally, even very recent empirical tests of the importance of HOIs to individual fitness are dominated by studies of experimental animal microcosms including one or two species. This literature provides mixed evidence of the effects of HOIs on individual fitness^{14,26,30–32}. No studies, to our knowledge, have tested for HOIs in naturally occurring diverse communities.

Results

A framework for detecting HOIs. Here, we present a framework for quantifying direct and higher-order species interactions (specifically defined as the quadratic density-dependent effects on per capita fitness) for individual fitness models based on a simple

¹The University of Queensland, School of Biological Sciences, Brisbane, Queensland 4072, Australia. ²Centre for Integrative Ecology, University of Canterbury, School of Biological Sciences, Christchurch, Canterbury 8041, New Zealand. *e-mail: m.mayfield@uq.edu.au; daniel.stouffer@canterbury.ac.nz

negative-binomial model (see Methods) of individual fecundity, a parameter which is central to coexistence-based models of plant diversity^{10,28} and plays the equivalent role to growth rate in models for longer-lived organisms⁹. Although developed here for annual plants, we selected this model for its general biological plausibility and versatility in multi-species communities regardless of taxonomic group and specific fitness estimator (see Methods). This model takes the general form:

$$F_{m_i|\{N\}} = \lambda_i e^{D_{m_i|\{N\}}} e^{H_{m_i|\{N\}}} \quad (1)$$

where F_{m_i} is the fecundity of a focal individual m of species i in the presence of a specified set $\{N\}$ of neighbours, λ_i is the intrinsic fecundity of individuals of species i at a given site (for example, their fecundity when no competitors are present locally; Fig. 1a) and the two terms in the exponentials capture the direct (D_{m_i}) and higher-order (H_{m_i}) effects (that is, cumulative impacts of interactions) of all neighbouring species, respectively. The total of all direct effects is a function of the set $\{N\}$ of abundances of all neighbouring species following the standard linear form¹:

$$D_{m_i|\{N\}} = - \sum_{j=1}^S \alpha_{ij} N_j \quad (2)$$

where N_j is the abundance of species j , α_{ij} is the competition coefficient that measures the direct impact of species j on a focal individual of species i , and the sum is across all species (S) surrounding the focal individual (Fig. 1b). If $N_i > 0$, α_{ii} is then included in the sum and represents intraspecific competition. Importantly, α -terms can be positive or negative, reflecting facilitative or competitive outcomes of direct interactions (Supplementary Fig. 1).

Building off the form of the direct effects, we quantify the higher-order effects between the neighbouring individuals in our system using the following expression:

$$H_{m_i|\{N\}} = - \left(\sum_{j=1}^S \beta_{ijj} \frac{N_j(N_j-1)}{2} + \sum_{j=1}^S \sum_{k=j+1}^S \beta_{ijk} N_j N_k \right) \quad (3)$$

The first term captures intraspecific HOIs, which occur among individuals of species j (including i when $N_i > 1$). These represent the cumulative impacts of interactions among N individuals of each ‘competitor species’ (that is, intraspecific crowding) on the focal individual m_i with the strengths of these effects given by β_{ijj} . Similarly, the second term captures interspecific HOIs that occur among neighbouring individuals from all heterospecific pairs of species and their strengths are given by the coefficients β_{ijk} (Fig. 1c). As above, both sums can include contributions from individuals of the focal species i . In this formulation, β -terms can change the magnitude and sign of the cumulative effects each competitor (β_{ijj}) and each pair of competitor species (β_{ijk}) have on the fecundity of the focal individual, m , of species i (Supplementary Fig. 1).

Evidence of HOIs in diverse plant communities. Annual plant communities allow for straightforward tests of HOIs under naturally complex conditions because of the relative ease with which their fecundity (or most fitness measures) can be assessed⁷. They likewise are a common model system for studying coexistence dynamics and community-level diversity patterns, despite their unusual life-history characteristics⁷. Here, we use the framework presented in equations (1)–(3) to test the common assumption that HOIs are of negligible importance for explaining variation in fitness outcomes by quantifying the effect of both direct interactions and HOIs on the fecundities of 773 plants from six annual plant species (*Aira caryophyllea*, *Podotrochea gnaphalioides*, *Ursinia*

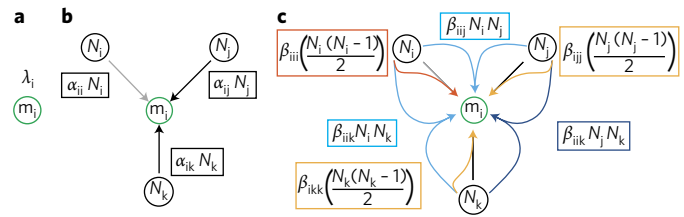


Figure 1 | Effects of direct interactions and HOIs on individual fecundity. **a**, The null model (fecundity without competition). **b**, The direct-interactions-only model. The grey arrow represents direct intraspecific interactions of N_i individuals of species i on the fecundity of focal individual m_i (green circle). Black arrows represent direct interspecific effects of species j and k on the focal individual. **c**, All possible HOIs (coloured arrows). Yellow and orange arrows show intraspecific HOIs of conspecific interactions (including species i , orange) on individual fecundity. Blue arrows represent heterospecific interspecific HOIs.

anthemoides, *Hypochaeris glabra*, *Trachymene ornata* and *Waitzia acuminata*) in the York Gum–Jam woodlands in the southwest of Western Australia. All focal plants were growing naturally as part of local interaction neighbourhoods (defined as a 7.5-cm-radius circle around each focal plant) containing 0–31 individuals of 0–8 species from a total neighbourhood pool of 45 species (see Methods; Supplementary Table 1 and Supplementary Fig. 2).

Using these data, we used two goodness-of-fit approaches (R^2 and likelihood ratio tests) and a measure of model parsimony (Akaike information criterion; AIC) to determine which of three model classes fit data for each of the six focal species best (see Methods; Table 1). The model classes were: a null model with no effect of co-occurring neighbours (that is, all $D_{m_i} = H_{m_i} = 0$ in equation (1)); the classic or direct-interactions-only model (that is, all $H_{m_i} = 0$ in equation (1)); and an HOI-inclusive model (including all calculable direct interactions and HOIs; equation (1) with no constraints).

For all six species, both goodness-of-fit approaches (R^2 and likelihood ratio tests) found the full HOI-inclusive model (equation (1)) to provide a significant improvement over the null and direct-interactions-only models (Table 1). Support for the HOI-inclusive model was robust to model formulation, as we found the very same thing—that the full HOI-inclusive model was the significantly best-fit model for all six focal species—when we repeated the goodness-of-fit analysis using three alternative model formulations (Supplementary Methods and Supplementary Table 2). Due to the large number of terms in our models and concern about model overfitting, we next conducted an AIC comparison, an approach that statistically penalizes for including additional model terms. Using this approach, we found the full HOI-inclusive model to be most parsimonious for three of the six focal species (*H. glabra*, *P. gnaphalioides* and *U. anthemoides*; Table 1). Even if it was unequivocal for just half of the focal species, the AIC support for the full HOI-inclusive model is surprisingly strong given how many more terms the HOI-inclusive models had compared with the null and direct-interactions-only models.

Overall, the extent of statistical support for the HOI-inclusive models arises because these models simply provide more accurate representations of the fitness variation observed across naturally occurring annual plant communities (examples in Fig. 2). This implies that models accounting for HOIs can better capture the non-linear relationships between fecundity and neighbour density often observed in natural systems. Fecundity predictions based on the direct-interactions-only models, on the other hand, often deviate substantially in overall magnitude and density dependence (Fig. 2).

Even a few HOIs can make a big difference. Being able to explain a single dataset well, as we have done here, is not tantamount to

Table 1 | Goodness-of-fit assessments of candidate models using R^2 and log-likelihood and a model parsimony test using AIC.

	<i>Aica</i>	<i>Hygl</i>	<i>Pogn</i>	<i>Tror</i>	<i>Uran</i>	<i>Waac</i>
Number of estimated parameters						
λ_i	2	3	2	3	2	3
$\lambda_i e^{D_{mi}}$	25	42	20	30	25	32
$\lambda_i e^{D_{mi}} e^{H_{mi}}$	65	118	48	98	56	99
Coefficient of determination, R^2						
λ_i	0	0	0	0	0	0
$\lambda_i e^{D_{mi}}$	0.34	0.53	0.38	0.32	0.42	0.34
$\lambda_i e^{D_{mi}} e^{H_{mi}}$	0.68	0.82	0.78	0.64	0.75	0.60
Log-likelihood estimates						
λ_i	-486.77	-1,145.47	-425.57	-958.11	-458.33	-1,453.33
$\lambda_i e^{D_{mi}}$	-465.90	-1,074.99	-408.61	-921.87	-435.00	-1,410.74
$\lambda_i e^{D_{mi}} e^{H_{mi}}$	-432.08	-989.13	-373.12	-859.19	-401.98	-1,360.31
P values for pairwise model comparisons						
λ_i versus $\lambda_i e^{D_{mi}}$	0.0097	<0.0001	0.0129	<0.0001	0.0025	<0.0001
$\lambda_i e^{D_{mi}}$ versus $\lambda_i e^{D_{mi}} e^{H_{mi}}$	0.0041	<0.0001	<0.0001	<0.0001	0.0002	0.0047
AIC parsimony test						
λ_i	977.53*	2,296.95	855.13*	1,922.22	920.66	2,912.66
$\lambda_i e^{D_{mi}}$	981.79	2,233.98	857.23	1,903.74	919.99	2,885.47
$\lambda_i e^{D_{mi}} e^{H_{mi}}$	994.27	2,214.25	842.24	1,914.38	915.95	2,918.63

For goodness-of-fit analyses, 'site' was included as a fixed effect for *Hygl*, *Tror* and *Waac* as they were surveyed in two sites. 'P values for pairwise model comparison' indicate that the second model in each comparison was a significantly better fit than the first, based on the log-likelihood test. For the parsimony test, AIC values that are two or more AIC points less than the next best model are considered a meaningful improvement in model fit³⁴. The over-dispersion parameter for the negative-binomial model is included in the 'Number of estimated parameters'. The numbers in bold show the cases in which the HOI inclusive model is the most parsimonious model based on AIC. *Aica*, *Aira caryophyllae*; *Hygl*, *Hypochaeris glabra*; *Pogn*, *Podotheca gnaphalioides*; *Tror*, *Trachymene ornata*; *Uran*, *Ursinia anthemoides*; *Waac*, *Waizia acuminata*. *For *Aica* and *Pogn*, the direct effects only model ($\lambda_i e^{D_{mi}}$) is worse than the null model, suggesting that ignoring HOIs can sometimes lead to the erroneous conclusion that competition is unimportant in determining fecundity outcomes.

being able to predict unobserved fecundities or generalize to other datasets³³. This is because explanatory modelling is best applied to testing specific hypotheses (for example, are HOIs important for explaining variation in fecundity?), but with side effects such as overfitting and false positives. Indeed, seen through this lens, both the classic direct-interactions-only and HOI-inclusive models are arguably overfit for this dataset (for example, according to one rule of thumb, there should be ten or more data points per model term to avoid model overfitting³⁴; Table 2). As such, we probed further into the question of whether or not the strong support for our six HOI-inclusive models is a by-product of model overfitting or representative of a generalizable phenomenon. To do this, we compared the fits of our three main model classes (null, direct-interactions-only and HOI-inclusive models) to two hypothesis-driven intermediate HOI models and an ensemble of 99,997 intermediate models that included randomly selected subsets of α - and β -terms (see Methods).

The two specific intermediate models were: (1) an intraspecific HOI model (containing all direct interactions and all intraspecific HOIs— β_{ii} and β_{jj} —but no interspecific HOIs); and (2) an interspecific HOI model (containing all direct interactions and all interspecific HOIs— β_{ij} and β_{jk} —but no intraspecific HOIs). These models tested the hypotheses that adding (1) intraspecific HOIs or (2) interspecific HOIs only to fecundity models improves model fit as well as or more than the full HOI-inclusive model. Although both intermediate models performed well for some species, neither consistently improved model fit as well as or better than the full HOI-inclusive model (Supplementary Table 3).

Based on analysis of the ensemble of randomly selected intermediate models, all of the most parsimonious models ($\Delta AIC \leq 2$) in this comparison included at least some HOIs (β -terms; Table 2) for five of the six focal species. Moreover, between 21 and 92% of the models

(depending on the focal species) that were more parsimonious than the full direct-interactions-only model (by two or more AIC points) contained some HOIs (Table 2 and Supplementary Fig. 3). These results, along with the full model comparisons, provide compelling evidence that HOIs are not of negligible importance in natural systems, as is routinely assumed in the literature.

No one type of HOI is more important than any other. Although the model comparisons above highlight the general importance of HOIs in fecundity modelling, questions still remain about whether certain types of HOIs are more or less important to fecundity outcomes. This may be particularly pertinent given our finding that the inclusion of subsets of HOIs seem sufficient to improve the fit of fecundity models for many species. Comparisons of the full HOI-inclusive model with the intraspecific HOI-only and interspecific HOI-only intermediate models (Supplementary Table 2) suggested that neither type of HOI was singularly most important. To explore this further, however, we next assessed the way each type of HOI translated into variation in fecundity in our dataset. To do this, we examined the combined and separate effects of two types of direct interactions and four types of HOIs on the fecundity of the six focal species using our full model form (equation (1)). For the HOI-inclusive fecundity model (equation (1)), the presence of neighbours (any $N_j > 0$) led to focal plants having median fecundities that were 71% as large as those of focal plants grown without competition (green bars in Fig. 3a and Supplementary Table 4). This varied extensively among focal species from *P. gnaphalioides*, with a 73% median reduction in seed production, to *U. anthemoides*, with a 30% median increase in seed production when neighbours were present (Fig. 3a), a net facilitative effect.

For four focal species, direct effects (D_{mi}) were predicted to generate substantial reductions in fecundity compared with plants

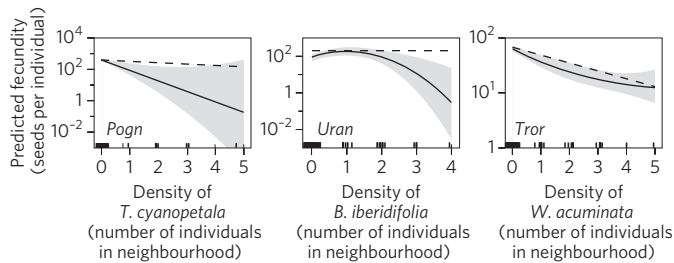


Figure 2 | Examples of predicted fecundities for three species pairs using direct-interactions-only (dashed line) and the full HOI-inclusive model (solid line). Plots show predicted fecundity for each focal species (*Pogn*, *Uran* and *Tror*) along a density gradient of a single competitor (x axis). These examples focus on intraspecific HOIs only for ease of visualization. Moreover, we only plot the model-based predictions rather than raw fecundity values, because of the complex structure of the regression model. Grey areas indicate the prediction interval around the mean model prediction for the HOI-inclusive model. Ticks along the bottom show the distribution of empirical data used by both models.

without competition (Fig. 3a). The magnitudes of the HOI effects (H_{mi}) varied substantially by focal species but were large and positive for four species, resulting in reductions in the net competitive effects of neighbours on the fecundity of those species (Fig. 3a). For one species, *U. anthemoides*, HOIs had the reverse dampening effect, reducing the predicted effect of increased fecundity that direct interactions had on this species (Fig. 3a).

For two of six species, intraspecific direct interactions had more dramatic negative effects on fecundity than interspecific interactions, as expected under stabilization³⁵, but for most of the species, the intra- and interspecific direct effects were similar (Fig. 3b). No one type of HOI (β_{iii} , β_{ijj} , β_{iii} , β_{ijk}) dominated the contribution of HOIs to fecundity outcomes, nor did any one β -term consistently have minor impacts (Fig. 3c). High variance in the realized HOI effects of *H. glabra* and *P. gnaphalioides* (Fig. 3c) indicates that the outcomes of HOIs for these species varied extensively by sign and magnitude across neighbourhoods. Across all six focal species, variable-realized HOI effects suggest that impacts of HOIs differ considerably by focal species and that no one type of HOI is consistently more important than any other when it comes to impacting fecundity outcomes.

Discussion

Although it may potentially appear to be an esoteric or overly theoretical matter, the inaccuracy of fitness models has far reaching

implications in ecology. Estimates of fitness are widely used to assess the importance of biotic and abiotic experimental treatments and environmental gradients on everything from biological invasions²⁸ and trophic interactions³⁶ to coexistence dynamics¹⁰ and community assembly processes⁵. Consequently, identifying approaches for improving our ability to model fitness outcomes is vitally important for understanding many aspects of our natural world.

To be able to measure all types of HOIs, it is necessary to obtain fecundity or fitness estimates across a wide range of abundances and competitor combinations. As such, we accept that the difficulty involved in conducting an exhaustive, fully factorial survey of all possible HOIs in diverse natural communities is prohibitive and impractical. We also expect to see diminishing returns in the daunting task of collecting data that allows for inclusion of all HOIs in fitness models using natural communities, especially given that the risk of model overfitting gets worse the more you include. Our study shows, however, that one may not need to go to such lengths, as fitness estimates can be significantly improved by including a fraction of possible HOIs. However, the question of which HOIs to include needs more detailed study, since we found no compelling evidence here that the intra- or interspecific interactions were more or less important. As a compelling starting point, we note that quantifying intraspecific HOIs (β_{iii} , β_{ijj}) only requires sufficient variation in the abundance of a single competitor. This implies that many existing datasets that were collected under classic designs for use with direct-interaction models only²⁹ probably already permit the estimation of intra- if not interspecific HOIs. The more straightforward inclusion of intraspecific HOIs into fitness models using existing datasets is therefore already possible and allows for the immediate implementation of our framework across many systems. In collecting new datasets from natural communities, it is worth identifying strategies for identifying the most relevant interspecific HOIs (β_{ijj} , β_{ijk}), such as including variation in the abundance of the most common set of competitors, or other manners to capture at least some interspecific HOIs.

Although we have much to learn about how HOIs work, it is clear that ignoring them altogether limits our ability to accurately describe natural communities and the mechanisms underlying them. For example, although individual-level fecundities are but one component of coexistence and diversity modelling^{14,30,31}, the strength and magnitude of HOIs uncovered here should dramatically impact on answers to fundamental questions about what allows species to coexist, a topic that is relevant across biology^{1,8,27}. In particular, results from our study may most immediately impact on the way diversity models based on modern coexistence theory are implemented and parameterized, given the important role fitness

Table 2 | Summary of overall best-performing models and models that perform better than the direct-interactions-only model, out of 99,997 intermediate models.

	<i>Aica</i>	<i>Hygl</i>	<i>Pogn</i>	<i>Tror</i>	<i>Uran</i>	<i>Waac</i>
Number of observations	93	169	64	189	75	183
Number best models	1	8	3	2	2	6
Number best models with β -terms	1	8	3	2	2	0
Mean α -terms	7	36.25	17.34	24	9	13.33
Mean β -terms	2	68.5	18	26.5	10	0
Mean β_{iii} - and β_{ijj} -terms	0	15.88	5	6	6.5	NA
Mean β_{ijj} - and β_{ijk} -terms	2	52.63	13	20.5	3.5	NA
Number of 'better' models*	26,757	10,706	29,335	6,167	38,027	3,179
% 'better' models with HOIs*	31	92	78	53	66	21

*Number of observations' is the number of empirical observations of each species. 'Best' models are those with $\Delta AIC \leq 2$ and 'better' models were those with AIC values two or more points less than the classic direct-interactions-only model. Mean α - and β -terms indicate the mean number of model terms in the 'best' models for each species. *See Supplementary Fig. 3 for distribution details of models.

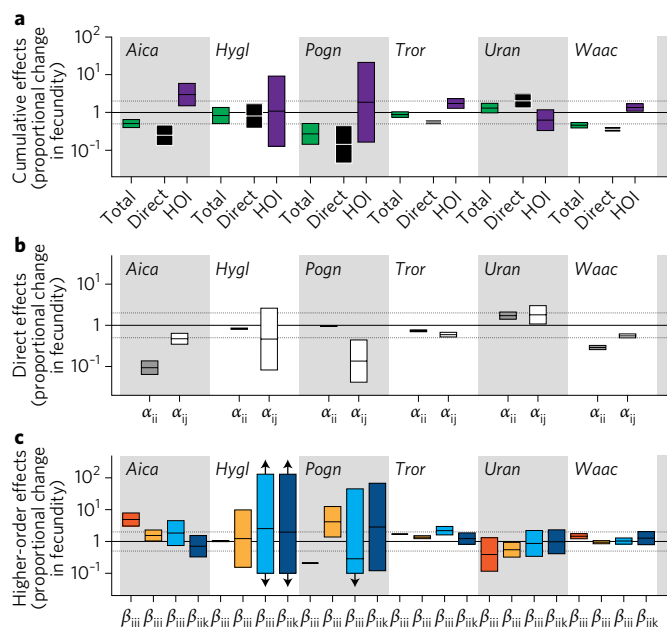


Figure 3 | Decomposition of the observed impacts of direct and higher-order effects on the fecundity of six focal species. a, Cumulative interaction effects (green; equation (1)), direct effects (black; $e^{D_{mi}}$) and higher-order effects (purple; $e^{H_{mi}}$). **b,** Intra- and interspecific direct effects. **c,** The four types of HOIs included in the HOI-inclusive model. The solid lines indicate fecundities equivalent to λ_i (by species), and the upper and lower dotted lines indicate $2\lambda_i$ and $\lambda_i/2$, respectively. Boxes indicate the median and confidence intervals of the observed effects, with the exception of three effects in **c**, for which the confidence intervals extend beyond the axis boundaries (denoted by arrows). Full details in Supplementary Table 4. Colours in **c** and species abbreviations for all panels are described in Fig. 1 and Table 1, respectively.

models have in this approach to the study of biological communities^{1,9,28}. Bear in mind that this study also demonstrates how difficult it is to operationalize coexistence theory in the field.

Most generally, we hope that the proof of concept provided in this study will lead to additional studies exploring the underlying mechanisms driving HOIs. An important next step is to assess the ubiquity of HOIs across systems, types of fitness measures and HOIs in the context of specific ecological interactions for which non-linearities are common but much debated, such as those between predators and prey or between mutualists^{36,37}. Further, HOIs are probably mediated by a suite of phenomenological mechanisms, including but not limited to, competition for resources, tempering of environmental filters and mediation of trophic factors, such as herbivory and pollination, that all require further study in a range of systems. Studies exploring the role of spatial context are also key to understanding how HOIs operate across communities. Although the inclusion of HOIs will never make the modelling of diversity easier³⁸, the approach developed and applied in this study illustrates just how crucial this added complexity can be to characterizing fitness dynamics in natural communities.

Methods

Data collection. To test for the presence and importance of HOIs in real annual plant communities, we collected fecundity data during the 2013 growing season (July–November) for 773 individual focal plants. Focal plants were divided among six focal annual plant species (Supplementary Table 1) growing naturally in two York Gum–Jam woodlands, located in the southwest of Western Australia: Kunjin nature reserve (32° 21′ 19.31″ S, 117° 45′ 42.32″ E) and Bendering nature reserve (32° 23′ 7.8″ S, 118° 23′ 5.66″ E; Supplementary Fig. 2). Three focal species, *A. caryophylla* (Poaceae), *P. gnaphalioides* (Asteraceae) and *U. anthemoides*

(Asteraceae), were sampled in Kunjin nature reserve only, while *H. glabra* (Asteraceae), *T. ornata* (Araliaceae) and *W. acuminata* (Asteraceae) were sampled in both Bendering and Kunjin reserves (Supplementary Table 1). For species where observations were taken from different sites, ‘site’ was included as a fixed effect in the models described below.

In July 2013, between one and three focal plants (total of 945) were marked in 315 30 cm × 30 cm quadrats (2.5 focal plants per quadrat on average; Supplementary Table 1) haphazardly spread across approximate areas of 0.4 ha in Kunjin and 0.2 ha in Bendering. We selected plots haphazardly across each study area to include plants growing under as variable microclimate and soil conditions as possible. No more than three focal plants per plot were assessed to prevent overlap of local neighbourhoods thus limiting pseudo-replication. Each focal plant’s ‘neighbourhood’ was considered to include all plants rooted within a 7.5 cm radius around each focal plant (see below for details). Our design included 35 plots (5 monocultures and 30 neighbourhood plots) for each focal species in each reserve where it was surveyed. This sample size was selected to maximize the neighbourhood species combinations with replication, while ensuring that seeds could feasibly be collected before seed fall, which occurs quickly for these species. Quadrat weeding was conducted at both sites throughout the growing season (May–October). Weeding removed certain species to maintain specific community compositions, but retained species were left unweeded to allow these species’ abundances to vary naturally. Weeding resulted in quadrat-level richness (mean = 4 species; range: 1–12 species) and abundance (mean = 24.6 individuals; range: 1–96 individuals) levels that corresponded to low to medium densities of natural assemblages (non-weeded) during a similar rainfall year in the same reserves.

In semi-arid and Mediterranean annual plant systems, including this one, species composition and abundance patterns vary according to rainfall³⁹. For comparison, in 2011, 180 non-weeded 30 cm × 30 cm quadrats were surveyed in Kunjin and Bendering reserves (90 quadrats in each). Respectively for these reserves, mean species richness per quadrat = 6.5 and 8.99 species, mean abundance = 52.9 and 72.5 individuals, number of species ranges = 1–15 and 2–17 species, and abundance ranges = 5–238 and 11–194 individuals. Rainfall totals for the region (both reserves) in May–October 2011 (previous survey year) and 2013 (experimental year) were 289.2 mm and 316.8 mm, respectively.

We surveyed all ‘competitor species’ (richness and abundance) within a 7.5-cm-radius neighbourhood area (plot) around each focal plant (1–3 plots per quadrat). This ‘interaction neighbourhood’ extent is supported by the density and size of plants in this system and was designed to allow us to account for the immediate neighbours of each focal plant only, including those with the potential for direct above and/or below ground interactions (Supplementary Fig. 2). Plot-level neighbourhoods varied from 0 to 31 individual competitors, with a median of 3 individuals and a mean of 4.6 individuals per plot. Plot species richness (excluding the focal plant) ranged from 0 to 8 species, with a median of 2 species and a mean of 1.9 species per plot. The competitor species pool included 45 annual herbaceous species including the 6 focal species (Supplementary Table 1).

As focal plants went to seed, all flowers (inflorescences for Asteraceae species) on each focal plant were counted and mature seed were collected from 1–3 inflorescences/flowers. Seed from 164 focal plants was not obtainable either due to herbivory, death or seed release before collection. These were spread across focal species and sites (Supplementary Table 1). Plants without seed data were excluded from all analyses. Data on community composition in eight plots was lost and thus we also excluded these focal plants from analysis. With these data losses our final analyses included data from 304 plots and 773 focal plants. For the 773 included focal plants, fecundity was estimated on a per plant basis as the mean seed production per inflorescence (or flower depending on the species) times the number of intact inflorescences/flowers on each focal plant at the point of seed maturity.

Negative-binomial model framework. Since HOIs can only occur between pairs of neighbouring individuals, none of the β -terms introduced in our framework are linearly related to abundance, as is the case for direct effects (D_{mi}). Moreover, just like direct interactions (α -terms) between species^{10,40} HOIs (β -terms) can take a variety of mathematical non-linear forms²⁹. Any suitable model, however, is constrained by the fact that the strength of intraspecific higher-order effects (β_{ij}) is necessarily 0 when $N_j = 1$ for any neighbouring species j (including i). We therefore constructed our model with this in mind, while aiming to take the simplest and most biologically realistic form possible.

Although it is less common in the prevailing literature than the linear or inverse forms^{10,18,28,41} (but see ref. ³¹), the negative-binomial framework developed here captures multiple biologically important facets of our data that the other forms cannot. First, the larger the observed fecundity, the greater the potential error associated with its estimate. This is incorporated into the negative-binomial process, since the variance of a negative-binomial distributed variable increases along with its mean by definition. Second, real data are often ‘over-dispersed’ and negative-binomial models afford added flexibility relative to a Poisson process, for example, via the introduction of an additional dispersion parameter that can dramatically improve model fit⁴². Third, fecundities are always integers, and negative-binomial processes make explicit predictions for counts, as opposed

to treating count observations as if they behaved like continuous variables. Finally, by explicitly predicting integer values, the best-fit parameters for a negative-binomial model can never make biologically unrealistic predictions, such as negative fecundities. The best-fit parameters for both the linear and inverse forms, on the other hand, can and do make such implausible predictions (Supplementary Table 2). Despite the arguments in its favour, we compared the results of our negative-binomial model to those obtained from commonly used alternative models and the results therein led to no change in the qualitative conclusions of our study (Supplementary Table 2). It is notable that we found even stronger support for the inclusion of HOIs using a purely Poissonian model formulation. We have elected not to show these results, however, given that our data were over-dispersed and thus the negative-binomial model is overall a more accurate reflection of the biological process under study. Code for this formulation is available as part of Supplementary Code 1.

Estimating best-fit coefficients and models. Statistically, the estimation of the best-fit values of the coefficients λ , α and β in our fecundity model is equivalent to performing a negative-binomial regression⁴³ with all fecundities F_{m_i} as the response variable with the set $\{N\}$ of neighbour species' abundances—or functions thereof in the case of the β -terms—as the predictors. Importantly, not all species combinations (for example, 45 competitors \times 6 focal species) or species triplets were observed in the interaction neighbourhoods around focal plants (Supplementary Table 1). Our analysis was therefore necessarily only based on species combinations that were found in our plots, and, for each individual species, we were able to model all direct interactions (α_i and α_j) for the observed competitors, but only a subset of HOIs (10–18% of possible HOIs were parameterized for each species). For example, the focal plant *A. caryophyllaea* (*Aica*) was observed co-occurring with 23 different competitor species (including itself); the *Aica* model therefore included 23 direct effects (all direct interspecific effects α_i plus the intraspecific effect of *Aica*) associated with these competitors. However, of the 276 HOIs that are possible given 23 competitor species, only 40 HOIs could actually be estimated with our observational data. Across all six species, our models were able to simultaneously quantify the strength of direct interactions (α_i), intraspecific HOIs (β_{ii} or β_{jj}) and interspecific HOIs (β_{ij} or β_{ji}) for 159, 83 and 227 species pairs, respectively.

We identified the best-fit coefficients and models separately for each focal species. To do so, we started by estimating the best-fit coefficients for the maximal models (for example, a model with all observable α -values and no β -values when focusing on the direct-competition-only scenario) using the function 'glm.nb' from the 'MASS' package⁴⁴ in the statistical program R, which uses iteratively weighted least squares to obtain the best-fit λ , α and β . The negative binomial over-dispersion parameter was fixed by species in this analysis. We also identified which of our model classes was most parsimonious for each species with a standard AIC model comparison³⁴. R code for the model fitting is provided in Supplementary Code 1 and 2, with full and example datasets provided in Supplementary Data 1 and 2.

AIC comparison of intermediate models. We used AIC to compare the model parsimony of the three main model classes (null, direct-interactions-only and HOI-inclusive model) to a wide range of intermediate models containing subsets of α - and β -terms for each species. Given the large number of parameters, it was computationally infeasible to fit all possible intermediate models. Therefore, we instead generated 99,997 intermediate models, spread proportionally across the space of possible intermediate models. These models can be thought of as falling into three classes of intermediate models: intermediate direct-interaction models ($\lambda_i e^{D_{m_i}(\text{inter})}$) containing a random sample of all possible direct interactions and no HOIs; intermediate HOI models containing all possible direct interactions and a random sample of measurable HOIs ($\lambda_i e^{D_{m_i} H_{m_i}(\text{inter})}$); and intermediate direct-and-HOI models including random samples of both direct- and HOI-terms that also maintained the principle of marginality, such that a species could not participate in an HOI if its direct interaction was absent ($\lambda_i e^{D_{m_i}(\text{inter})} e^{H_{m_i}(\text{inter})}$).

This set of intermediate models allowed us to test whether the most parsimonious model or models for each species' observed fecundities probably included all or a subset of direct interactions, all or a subset of HOIs, neither or both. In Supplementary Fig. 3, we show the probability density of the AIC values covered by all models and indicate the AIC values for the full direct-interactions-only and HOI-inclusive models from our primary analysis (Table 1). In Table 2, we present summary statistics on the makeup of the 'best' models selected by AIC for each species, which constitute all the models with a $\Delta\text{AIC} \leq 2$ and represent the set of models that have the best support. The 'better' models in Table 2 are those models that had AIC values that were two or more AIC units lower than the AIC value from the full direct-interactions-only model and hence had better statistical support than that model, the dominant model found in the literature³⁴. The distribution of AIC values for all intermediate models are shown in Supplementary Fig. 3.

Quantifying model effects. Once the best-fit model with HOIs was obtained, we next aimed to quantify which aspects of observed variation in species' fecundities were attributed to the different model components. We used

modified partial-residual plots to assess the relative magnitude and direction of the impact of any given set of explanatory variables in our fecundity models⁴⁵. With this approach, we only need to multiply the observed neighbour abundances (or functions thereof) by the best-fit model coefficients that were obtained as described above. For example, the total effect D_{m_i} of direct interactions is given by equation (2) with the best-fit α -values and empirically observed neighbour abundances, and the total effect H_{m_i} of HOIs is given by equation (3) with the best-fit β -values and corresponding products of neighbour abundances.

Data availability. All data used in this study are available in Supplementary Data 1 or through the Dryad Digital Repository at <http://dx.doi.org/10.5061/dryad.3562g>.

Received 21 September 2016; accepted 19 December 2016; published 17 February 2017

References

- Chesson, P. in *Encyclopedia of Sustainability Science and Technology* (ed. Meyers, R. A.) Ch. 13, 223–256 (Springer, 2012).
- Allesina, S. & Levine, J. M. A competitive network theory for species diversity. *Proc. Natl Acad. Sci. USA* **108**, 5638–5642 (2011).
- Volterra, V. Fluctuations in the abundance of a species considered mathematically. *Nature* **118**, 558–560 (1926).
- May, R. Will a large complex system be stable? *Nature* **238**, 413–414 (1972).
- Thuiller, W. et al. A road map for integrating eco-evolutionary processes into biodiversity models. *Ecol. Lett.* **16**, 94–105 (2013).
- Sih, A., Englund, G. & Wooster, D. Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.* **13**, 350–355 (1998).
- HilleRisLambers, J., Adler, P. B., Harpole, W. S., Levine, J. M. & Mayfield, M. M. Rethinking community assembly through the lens of coexistence theory. *Annu. Rev. Ecol. Evol. S.* **43**, 227–248 (2012).
- Chesson, P. Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **31**, 343–366 (2000).
- Kunstler, G. et al. Plant functional traits have globally consistent effects on competition. *Nature* **529**, 204–207 (2016).
- Levine, J. M. & HilleRisLambers, J. The importance of niches for the maintenance of species diversity. *Nature* **461**, 254–257 (2009).
- Falster, D. S., FitzJohn, R. G., Brännstrom, Å., Diekmann, U. & Westoby, M. Plant: a package for modelling forest trait ecology and evolution. *Methods Ecol. Evol.* **7**, 136–146 (2016).
- Madin, J. S., Hoogenboom, M. O. & Connolly, S. R. Integrating physiological and biomechanical drivers of population growth over environmental gradients on coral reefs. *J. Exp. Biol.* **215**, 968–976 (2012).
- Connolly, S. R. & Roughgarden, J. Theory of marine communities: competition, predation, and recruitment-dependent interaction strength. *Ecol. Monogr.* **69**, 277–296 (1999).
- Smith-Gill, S. J. & Gill, D. E. Curvilinearities in the competition equations: an experiment with rapid tadpoles. *Am. Nat.* **112**, 557–570 (1978).
- Wootton, T. J. The nature and consequences of indirect effects in ecological communities. *Annu. Rev. Ecol. Syst.* **25**, 443–466 (1994).
- White, E. M., Wilson, J. C. & Clarke, A. R. Biotic indirect effects: a neglected concept in invasion biology. *Divers. Distrib.* **12**, 443–455 (2006).
- Roughgarden, J. & Diamond, J. in *Community Ecology* (eds Diamond, J. & Case, T. J.) 333–343 (Harper and Row, 1986).
- Schoener, T. W. Some methods for calculating competition coefficients for resource-utilization spectra. *Am. Nat.* **108**, 332–340 (1974).
- Freckleton, R. P. & Watkinson, A. R. Predicting competition coefficients for plant mixtures: reciprocity, transitivity and correlations with life-history traits. *Ecol. Lett.* **4**, 348–357 (2001).
- Billick, I. & Case, T. J. Higher order interactions in ecological communities: what are they and how can they be detected? *Ecology* **75**, 1529–1543 (1994).
- Werner, E. E. & Peacor, S. D. A review of trait-mediated indirect interactions in ecological communities. *Ecology* **84**, 1083–1100 (2003).
- Abrams, P. A., Menge, B. A., Mittlebach, G. G., Spiller, D. & Yodzis, P. in *Food Webs: Integration of Patterns and Dynamics* (eds Polis, G. & Winemiller, K.) 371–395 (Chapman and Hall, 1996).
- Peacor, S. D. & Werner, E. E. The contribution of trait-mediated indirect effects to the net effects of a predator. *Proc. Natl Acad. Sci. USA* **98**, 3904–3908 (2001).
- Trussell, G. C., Ewanchuk, P. J. & Matassa, C. M. Habitat effects on the relative importance of trait- and density-mediated indirect interactions. *Ecol. Lett.* **9**, 1245–1252 (2006).
- Wootton, J. T. Indirect effects and habitat use in an intertidal community: interaction chains and interaction modifications. *Am. Nat.* **75**, 1544–1551 (1993).
- Vandermeer, J. H. The competitive structure of communities: an experimental approach with protozoa. *Ecology* **50**, 362–371 (1969).
- Bairey, E., Kelsic, E. D. & Kishony, R. High-order species interactions shape ecosystem diversity. *Nat. Commun.* **7**, 12285 (2016).

28. Godoy, O. & Levine, J. M. Phenology effects on invasion success: insights from coupling field experiments to coexistence theory. *Ecology* **95**, 726–736 (2014).
29. Goldberg, D. E. & Werner, P. A. Equivalence of competitors in plant communities: a null hypothesis and a field experiment. *Am. J. Bot.* **170**, 1098–1104 (1983).
30. Case, T. J. & Bender, E. A. Testing for higher order interactions. *Am. Nat.* **118**, 920–929 (1981).
31. Anderson, T. L. & Whiteman, H. H. Non-additive effects of intra- and interspecific competition between two larval salamanders. *J. Anim. Ecol.* **84**, 765–772 (2015).
32. Wilbur, H. M. Competition, predation, and the structure of the *Ambystoma–Rana sylvatica* community. *Ecology* **53**, 3–21 (1972).
33. Shmueli, G. To explain or to predict? *Stat. Sci.* **25**, 289–310 (2010).
34. Burnham, K. P. & Anderson, D. R. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (Springer, 2002).
35. Adler, P. B., HilleRisLambers, J. & Levine, J. A niche for neutrality. *Ecol. Lett.* **10**, 95–104 (2007).
36. Okuyama, T. & Holland, J. N. Network structural properties mediate the stability of mutualistic communities. *Ecol. Lett.* **11**, 208–216 (2008).
37. Novak, M. & Wootton, J. T. Estimating nonlinear interaction strengths: an observation-based method for species-rich food webs. *Ecology* **89**, 2083–2089 (2008).
38. Abrams, P. A. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *Am. Nat.* **146**, 112–134 (1995).
39. Dwyer, J. M., Hobbs, R. J., Wainwright, C. E. & Mayfield, M. M. Climate moderates release from nutrient limitation in natural annual plant communities. *Global Ecol. Biogeogr.* **24**, 549–561 (2015).
40. Law, R. & Watkinson, A. R. Response-surface analysis of two-species competition: an experiment of *Phleum arenarium* and *Vulpia fasciculata*. *J. Ecol.* **75**, 871–886 (1987).
41. Angert, A. L., Huxman, T. E., Chesson, P. & Venable, D. L. Functional tradeoffs determine species coexistence via the storage effect. *Proc. Natl Acad. Sci. USA* **106**, 11641–11645 (2009).
42. Quinn, G. P. & Keough, M. J. *Experimental Design and Data Analysis for Biologists* (Cambridge Univ. Press, 2002).
43. Rao, C. R., Toutenburg, H. & Shalabh, H. C. *Linear Models and Generalizations* (Springer, 2008).
44. Venables, W. N. & Ripley, B. D. *Modern Applied Statistics with S* 4th edn (Springer, 2002).
45. Larsen, W. A. & McCleary, S. J. The use of partial residual plots in regression analysis. *Technometrics* **14**, 781–790 (1972).

Acknowledgements

This project was made possible by funding awarded to M.M.M. by the Australian Research Council (DP140100574 and FT140100498) and to D.B.S. from the Royal Society of New Zealand (UOC-1101 and a Rutherford Discovery Fellowship). We thank H. R. Lai, X. Loy, C. Wainwright and J. HilleRisLambers for help with data collection and J. HilleRisLambers, J. Dwyer, J. Tylianakis and the Mayfield and Stouffer labs for constructive comments. We also thank X. Loy for the art used to create Supplementary Fig. 1.

Author contributions

Authors are equal contributors to this paper. M.M.M. and D.B.S. conceived of the project and the framework together. M.M.M. collected and provided all data (with acknowledged help) and D.B.S. conducted all analyses. M.M.M. led the joint effort of writing the manuscript.

Additional information

Supplementary information is available for this paper.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to M.M.M. or D.B.S.

How to cite this article: Mayfield, M. M. & Stouffer, D. B. Higher-order interactions capture unexplained complexity in diverse communities. *Nat. Ecol. Evol.* **1**, 0062 (2017).

Competing interests

The authors declare no competing financial interests.