The non-random structure of multi-trophic ecological interactions maximises species coexistence

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Abstract

Theory posits that the persistence of species in ecological communities is shaped by their interactions within and across trophic levels. However, we lack empirical evaluations of how the structure, strength and sign of biotic interactions drive the potential to coexist in highly diverse multi-trophic communities. Here we model community feasibility domains, a theoretically-informed measure of coexistence probability, from grassland communities comprising more than 45 species on average from three trophic guilds (plants, pollinators, and herbivores). We first show that increasing community complexity measured either as the number of guilds considered or community richness does not decrease community feasibility. Furthermore, communities with specific interaction structures display higher feasibility domains. Such structures are characterised by a high degree of both species self-regulation and niche partitioning. Overall, our results suggest that interaction structures of diverse multi-trophic communities under natural conditions tend to maximise the potential for coexistence.

Introduction

Ecological communities are complex systems in which individuals of different species interact in a myriad of context-dependent ways, generating emergent properties that are not evident from the isolated study of their elements (Levin, 1998). Understanding these emergent properties, such as community stability or resilience (Meerbeek *et al.*, 2021) is key for strengthening the scientific basis of ecosystem conservation and restoration (Moreno-Mateos *et al.*, 2020). An important dimension of community stability is the potential for different species to coexist, i.e. to be able to maintain viable populations in the same local community. However, obtaining a mechanistic understanding and quantifying the coexistence of multiple species in nature is a complex task because of the numerous processes that operate at the species and community levels.

Within a single trophic level, both theoretical and empirical work on competitive interactions has shown that the degree of self-regulation relative to the strength of interspecific interactions is a key factor in shaping coexistence. The higher intraspecific interactions and the greater their magnitude in comparison to interspecific ones, the more stable competitive communities are (Buche *et al.*, 2022; Chesson, 2000; Levine & HilleRisLambers, 2009). The degree of overlap in resource use between species is also assumed to shape pairwise coexistence relationships, with implications for other emergent properties such as different ecosystem functions (Albert *et al.*, 2022; Godoy *et al.*, 2020). Likewise, when considering communities with different trophic guilds, certain structural properties, such as modularity for antagonistic networks, and nestedness for mutualistic ones, have been shown to promote community stability (Rohr *et al.*, 2014; Stouffer & Bascompte, 2011; Thébault & Fontaine, 2010). However, all these insights have been derived from communities of single interaction types, either antagonistic or mutualistic. This progress contrasts with increasing evidence that different interaction types contribute significantly and synergistically to the emergent properties of ecological communities (Evans *et al.*, 2013; Losapio *et al.*, 2021; Simha *et al.*, 2022). A natural next step is, therefore, to study how coexistence is achieved in communities of increasing complexity, where interactions of different signs and strenghts are intertwined across different guilds. Disentangling this conundrum requires combining detailed empirical data with robust theoretical models.

Empirical studies documenting simultaneously multiple interaction types across several trophic levels are scarce. Early studies relied on binary networks that document the presence or absence of a given interaction (Bastolla *et al.*, 2009; Kéfi *et al.*, 2015). This approach can be refined by assigning interaction strengths inferred through indirect methods or expert opinion (e.g. Pocock *et al.* (2012); see García-Callejas *et al.* (2018) for a review). While these types of networks, built from binary interactions or indirect information, are useful first approximations, documenting interactions quantitatively at finer scales, and over multiple communities, is essential for understanding the variability in community structure and dynamics (Banašek-Richter *et al.*, 2009), as recently shown in an agricultural context (Morrison *et al.*, 2020). Interactions within and across guilds have been mostly integrated into the context of mutualisms between plants and pollinators by incorporating competitive interactions to these bipartite networks (Bastolla *et al.*, 2009; Gracia-Lázaro *et al.*, 2018; Wang *et al.*, 2021), but the concept can be generalised to any kind of interaction (Godoy *et al.*, 2018; Seibold *et al.*, 2018). However, even when these intra-guild interactions are considered, an unrealistic solution has been to model them as constant across all species (i.e. a mean-field approach) (Bastolla *et al.*, 2009; Saavedra *et al.*, 2013). Although this approach is justified from a theoretical point of view because intra-guild interactions are notoriously difficult to quantify directly, this solution is suboptimal and lacks biological realism. In fact, there is widespread evidence that differences among species in phenological timing and resource use modulate variation in intra-guild interaction strengths (CaraDonna *et al.*, 2020; Morales-Castilla *et al.*, 2015). Consequently, such processes can generate differences in intra-guild network structures that, in turn, can influence their potential to maintain species diversity (Barabás *et al.*, 2016).

In parallel to the empirical limitation of providing realistic quantifications of the full network of species interactions, mathematical tools for the integration of this complexity in community-level frameworks are still under development (García-Callejas *et al.*, 2018; Godoy *et al.*, 2018; Pilosof *et al.*, 2017). Classic modeling approaches to the study of community structure, dynamics, and stability in single-interaction networks can be adapted to deal with multiple interactions (García-Callejas *et al.*, 2018). However, they often rely on an exponentially increasing number of parameters to be estimated as the number of species increases, which precludes their use for diverse empirical communities. Recent advances taking a structuralist approach provide an alternative to evaluate the role of species interactions in promoting or hindering multi-species coexistence (Godoy *et al.*, 2018; Saavedra *et al.*, 2017). The structuralist framework is built upon the idea that the structure of species interactions shapes the opportunities to coexist for the different species in a given community, by quantifying the so-called feasibility domain. The main prediction arising from the structuralist approach is clear: the larger the feasibility domain, the more likely the community can persist without any species going extinct. This is because communities with larger feasibility domains can withstand larger fluctuations in species vital rates due to environmental variation without losing species (Song *et al.*, 2018). A key advantage of the probabilistic nature of this approach is that it further allows the derivation of probabilities of persistence (or its complement, exclusion) for individual species (Saavedra *et al.*, 2020). Thus, the structuralist approach emerges as a powerful tool to explicitly link estimations of persistence at the species and community levels, and to bridge theoretical studies on community stability to empirical quantifications of species interactions in diverse multi-trophic communities.

Here, we combine recent advances in the field of structural stability with unique field observations from nine Mediterranean grassland communities over two years involving a total of 108 taxa and their different types of interactions: plant-herbivore, plant-pollinator, and the intra and interspecific competitive interactions within guilds (plants, pollinators, and herbivores). With this combination, we provide the first empirical exploration of how quantitative interaction structures within and across trophic guilds drive the persistence of biodiversity in natural conditions. Our first hypothesis is that the opportunities to coexist in our studied communities will be negatively related to the number of guilds considered, and to the overall richness of the community. This hypothesis stems from the long-standing idea that complexity *sensu lato* decreases local stability, which is only a particular dimension of the overall concept of community stability (May, 1972). This relationship, however, is expected to be modulated by the structure of species interactions in natural communities (Jacquet *et al.*, 2016). Therefore, our second hypothesis is that interaction structure within and across guilds will also shape the opportunities to coexist in multi-trophic communities. For testing these hypotheses, we define and analyse three different parameterisations of intra-guild interactions, and we compare the opportunities to coexist in the observed communities to randomised counterparts. In our third question, we ask whether network properties capture the variability in opportunities to coexist across the observed communities. In particular, our hypothesis is that stronger self-regulation compared to inter-specific interactions, and a higher degree of resource partitioning across species, will be related to higher opportunities to coexist in multi-trophic communities. We explicitly test these hypotheses both at the species and community levels of organisation.

Methods

Data collection

We conducted our study in a Mediterranean grassland community located in Doñana National Park (SW Spain, 37º 04' 01.5"N, 6º 19' 16.2" W). We set up 9 plots of 8.5 m^2 across an area of 2680 ha (Fig. 1) from which we documented 1) direct competitive interactions among plants, 2) direct interactions between plants and pollinators, and 3) direct interactions between plants and herbivores. Because our study system is dominated by plants and insects that feed upon them (i.e. pollinators and herbivores), we expect these guilds to be the most relevant for the dynamics of the community, as the abundance of predators (e.g. spiders, mantis) or larger animals is relatively low. For plant-plant interactions, we obtained the number of local co-occurrences between plant individuals, sampling 36 focal individuals of each plant species per plot and their plant neighbours at a radius of 7.5 cm. This radius is a standard distance used in previous studies to measure competitive interactions among annual plant species (Levine & HilleRisLambers, 2009; Mayfield & Stouffer, 2017), and it has been validated to capture the outcome of competitive interactions at larger scales (1 m^2) under locally homogeneous environmental conditions (Godoy & Levine, 2014). Interactions between plants and pollinators or herbivores were sampled from the emergence of the earliest flowers (February) to the decay of the latest ones (June) in 2019. During 2020, the length of the field season was the same but we could not sample for five weeks in March/April 2020 due to COVID-19 restrictions. Such differences in sampling effort did not seem to influence our results as trends were consistent across both years (see results).

We recorded the number of floral visits to each plant species, by sampling each plot for 30 min on a weekly basis for a total of 148.5 hours in 2019 and, on a bi-weekly basis (therefore missing two sampling intervals) for a total of 54 hours in 2020. We only recorded floral visitors that contacted plant's reproductive organs (stigma and/or anthers), and hence we assume they are effective pollinators and refer to them that way throughout the text. Interactions between plants and herbivores were sampled in a similar manner: in parallel to the pollinator survey, we sampled plant-herbivore interactions for 36 min on each plot, for a total of 76 hours in 2019 and 70 in 2020. Herbivores were annotated when observed on the stem, leaves, or flowers of the plant.

From these field observations, we obtained 18 normalised block interaction matrices $\mathbf{A}_{n,t}$ (9 plots × 2 years). We assumed that these represent independent local communities given the spatial separation between plots (100m on average), species turnover of the annual plants and the associated insect community, and the annual dynamics of the system. These matrices characterise the interaction structure of each local community, including intra- and inter-guild interactions, and are the inputs of the structural methods described below. The matrices are defined, for a given plot n and year t, as

$$\mathbf{A_{n,t}} = \begin{array}{c} \\ P \\ L \\ H \end{array} \begin{array}{c} \begin{array}{ccc} P & L & H \end{array} \\ \begin{bmatrix} \boldsymbol{\alpha}^{(p)}_{n,t} & \boldsymbol{\alpha}^{(l,p)}_{n,t} & \boldsymbol{\alpha}^{(h,p)}_{n,t} \\ \boldsymbol{\alpha}^{(p,l)}_{n,t} & \boldsymbol{\alpha}^{(l)}_{n,t} & 0 \\ \boldsymbol{\alpha}^{(p,h)}_{n,t} & 0 & \boldsymbol{\alpha}^{(h)}_{n,t} \end{bmatrix} \end{array} \tag{1}$$

where P = plants, L = pollinators, and H = herbivores. The $\boldsymbol{\alpha}$ elements represent the different submatrices (or blocks) of the community, e.g. $\boldsymbol{\alpha}^{(p)}$ represents the matrix of plant-plant interactions, $\boldsymbol{\alpha}^{(l,p)}$ the matrix of pollinator effects over plants, and so on.

A multiple approach to estimate community interactions

Estimating the occurrence and strength of interactions among the members of a guild is a pervasive problem in studies of ecological networks, in particular when individuals are highly mobile and/or difficult to track in the field. This is the case, for example, with arthropod pollinators and herbivores. For these reasons, rather than providing a single characterisation, we present three different ones reflecting how the field of community ecology has evolved in the last decades. In our first parameterisation, named mean-field, we assumed that intra-guild competition occurs, and affects all species equally and in a symmetric way. For example, studies taking this approach highlighted that under mean-field competition the role of nested network architectures is key for maximising species persistence in mutualistic networks (Bastolla *et al.*, 2009; Rohr *et al.*, 2014). In our second parameterisation, we consider recent refinements in which accounting for structured intra-guild competition fundamentally alters the expectations in terms of species persistence, by generating mutualism-competition trade-offs (Gracia-Lázaro *et al.*, 2018; Wang *et al.*, 2021). In particular, we estimated the degree of competition between plant species based on our spatially explicit field observations, and between pollinators (or herbivores), based on the feeding requirements of their larval stages and on their nesting requirements. However, this second parameterisation assumes that the structure of intra-guild competition relies on the use of a single resource axis, without considering, for example, phenological constraints that are commonly observed in natural communities (Olesen *et al.*, 2011). Assuming that there are not preempting processes (i.e. the amount of soil water, light, or food is not altered by earlier taxa), for our third parameterisation we considered that phenological mismatches decouple interactions in time, therefore decreasing net pairwise competition (Duchenne *et al.*, 2021). Thus, we incorporated phenological overlap to the resource overlap axis from the second parameterisation. In the Supplementary Section "Interaction Matrices" we describe in detail the process of constructing these intra-guild matrices, of which we obtained one per plot and year for every guild and every parameterisation.

Interactions between individuals of different guilds (plants-herbivores and plants-pollinators) were obtained from the field observations described in the section "Data Collection", and normalised to ensure comparable coefficients with the intra-guild matrices (Supplementary Section "Interaction Matrices"). For each plot and year, we thus obtained both intra- and inter-guild interaction matrices for plants, herbivores, and pollinators, including three different parameterisations of intra-guild matrices. For each parameterisation, this resulted in a total of 6 interaction matrices:

three intra-guild communities $\boldsymbol{\alpha}^p, \boldsymbol{\alpha}^l, \boldsymbol{\alpha}^h$; two two-guild communities (one formed by plants and herbivores $(\boldsymbol{\alpha}^p, \boldsymbol{\alpha}^h, \boldsymbol{\alpha}^{p,h}, \boldsymbol{\alpha}^{h,p})$, and one formed by plants and pollinators $(\boldsymbol{\alpha}^p, \boldsymbol{\alpha}^l, \boldsymbol{\alpha}^{p,l}, \boldsymbol{\alpha}^{l,p})$); and the overall community represented by the full block-matrix $\mathbf{A}$ (eq. 1). We further generated randomised counterparts of these different matrices to evaluate the effects of the observed interaction structure on our coexistence metrics (see Supplementary Section "Interaction Matrices" for further details on the randomisation process).

Community feasibility and species' exclusion ratios

The potential for a given structure of species interactions to sustain a feasible community is quantified via its feasibility domain, whose relative volume ranges in the interval [0,0.5] (Song *et al.*, 2018). A large feasibility domain volume indicates a higher potential to accommodate variations in species growth rates while maintaining feasibility, and vice-versa. Its mathematical definition is given in Song *et al.* (2018) and discussed in the Supplementary Section "Feasibility Metrics"; hereafter we refer to the relative volume simply as "feasibility domain" for brevity. We calculated the feasibility domain of each of the community networks constructed, i.e. for each community $\mathbf{A}_{n,t}$ we calculated the feasibility domain of each of the sub-communities of one guild and two guilds, as well as for the full multi-trophic community. Note that by taking this approach, we assume that the dynamics of the communities studied can be reasonably well approximated with a linear Lotka-Volterra model.

The feasibility domain gives an overall picture of the potential for all species to coexist in a given community, but this does not mean that all species have equal probabilities of persistence (or exclusion). In fact, feasibility domains can be highly anisotropic, meaning that some species are much closer to being excluded from a feasible community than others (Tabi *et al.*, 2020). To quantify these outcomes, we developed a novel structural measure for the likelihood of a species to being excluded from a feasible community. This metric is a ratio that quantifies the relative probability of being the first species excluded compared to a neutral situation, and we refer to it hereafter as the species exclusion ratio (see Supplementary Section "Feasibility Metrics" for the mathematical definition and an extended discussion). The species exclusion ratio, bounded between $(0, \infty)$, is a good proxy of the overall likelihood of exclusion of a species, in the absence of further information on e.g. species' intrinsic growth rates.

Structural metrics for species and communities

Coexistence between pairs of species is known to depend on the degree of self-regulation relative to net competition effects, which is quantified by the ratio between intra-specific and inter-specific competition (Chesson, 2000). This has been shown to hold for multiple species in competitive communities as well, whereby the stabilising effect of niche differences arising when intraspecific interactions exceed interspecific ones also contributes to the maintenance of their diversity in the presence of indirect interactions (Barabás *et al.*, 2016; Godoy *et al.*, 2017). Accordingly, species with higher niche overlap are less likely to coexist (Adler *et al.*, 2007; Buche *et al.*, 2022). To explicitly test whether these tenets hold in multi-trophic communities, we calculated two complementary structural network metrics for the species and community levels. First, we quantified the degree of self-regulation of a species as its diagonal dominance. Diagonal dominance is a matrix property that is satisfied when diagonal elements are larger than the sum of non-diagonal elements. Here we used a continuous version, i.e. the difference between diagonal and sum of non-diagonal elements. This species-level metric is averaged for obtaining the average degree of diagonal dominance in a community, d:

$$d = \frac{\sum_{i \in S} \left(\alpha_{i,i} - \sum_{\substack{j \in S \\ j \neq i}} \alpha_{i,j} \right)}{S} \tag{2}$$

where S is the number of species in the community represented by the interaction matrix α. Similarly, we obtained species-level overlap and its community average, assuming that the degree of overlap in pairwise interactions is an appropriate proxy of niche overlap. To avoid confusion, we refer hereafter to interaction overlap. Species-level interaction overlap is itself an aggregated property, derived from the overlap between each pair of species, which we calculated using the Morisita-Horn dissimilarity index (Horn, 1966) implemented in the vegan R package version 2.6-2 (Oksanen *et al.*, 2022). We posit that the net overlap of species i in a community, o_i, is best represented by the sum of pairwise overlaps with every other species:

$$o_i = \sum_{j \in S} (1 - h_{i,j}) \tag{3}$$

where h is a Morisita-Horn dissimilarity matrix obtained from a given interaction matrix. From Eq. 3 we obtained separately the interaction overlap of each species i for its intra-guild competition matrices and its inter-guild interaction matrices. The community-level metric is, again, the average of species-level overlaps in the community:

$$o(\alpha) = \frac{\sum_{i \in S} o_i}{S} \tag{4}$$

Statistical analyses

We analysed the relationship between coexistence outcomes and structural metrics using regression models. For testing whether increased complexity decreases opportunities to coexist (first hypothesis), we analysed the relationship between the feasibility domain (for the community-level analyses) or species exclusion ratios (for the species-level analyses) and the number of trophic guilds accounted for using a Type III Analysis of Variance. We also explored the relationship between the feasibility domain (or species exclusion ratios) and community richness using linear models.

In addition, by explicitly considering the three different types of intra-guild competition matrices, we also explored our second hypothesis that interaction structure influences the opportunities to coexist in our system. Furthermore, we compared observed feasibility domains (or species exclusion ratios) to the distributions given by the randomised communities. To analyse the relationship between our coexistence outcomes and structural metrics (our third hypothesis), we considered the full communities, including plants, herbivores, and pollinators. Specifically, we used the data on intra-guild interactions derived from resource use and phenological overlap. Using this data we implemented linear mixed models with feasibility domain as response variable and community-level diagonal dominance and interaction overlap (differentiating intra-guild and inter-guild overlap) as independent variables, taking the plot identity as a spatial random factor. For the species-level analyses we similarly took species exclusion ratios (log-transformed) as response, and diagonal dominance, intra-guild overlap, and inter-guild overlap as independent variables. In this model we also added species guild as a covariate, and again included the plot identity as a random factor. We implemented these models using the lmerTest package v3.1-3 in R (Kuznetsova *et al.*, 2017). Prior to model fitting, we scaled all numeric variables and checked model fits with the tests provided in the R package DHARMa v0.4.5 (Hartig, 2021).

Results

In our two years of sampling, we documented 214 unique interactions among plants, 110 between plants and pollinators, and 160 between plants and herbivores. In this period we observed interactions between 108 taxa, of which 17 were plants, 46 herbivores, and 45 pollinators. Of these, 53 taxa representing 49% of the records were identified at the species level (17 plants, 16 pollinators, and 20 herbivores), and 51 % as morphospecies (Table S1). The included taxa span diverse life-history strategies, such as grasses (e.g. *Hordeum marinum*) and forbs (e.g. *Leontodon maroccanus*) in the annual plant guild, solitary bees (e.g. from genera *Andrena, Lasioglossum*), flies (e.g. genera *Sphaerophoria, Musca*), or Lepidoptera (e.g. genera *Lasiocampa, Thymelicus*) within the pollinator guild, and sap feeders (e.g. Hemiptera from genera *Aelia* or *Aphis*), pollen feeders (e.g. Coleoptera from genera *Malachius* or *Psilothrix*) or leaf-eaters (e.g. Gastropoda from genera *Theba* or *Cochlicella*) within the herbivore guild (Table S1).

The frequency distribution of the 270 unique interactions observed across trophic guilds was highly skewed, with e.g. 128 interactions being observed less than five times. Plant richness was positively correlated with that of pollinators across communities, (Spearman's ρ = 0.78, S = 214, p-value < 0.01) but not with herbivores (Spearman's ρ = -0.23, S = 1125, p-value = 0.35). Richness values had an average of 48 taxa and ranged from 35 to 57 taxa in the least and most diverse communities, respectively.

Contrary to our first hypothesis that the higher complexity of the local communities, the lower the opportunities for species to coexist, we found that neither of the complexity proxies considered influenced feasibility domains (Fig. 2). In particular, we found no significant differences in the

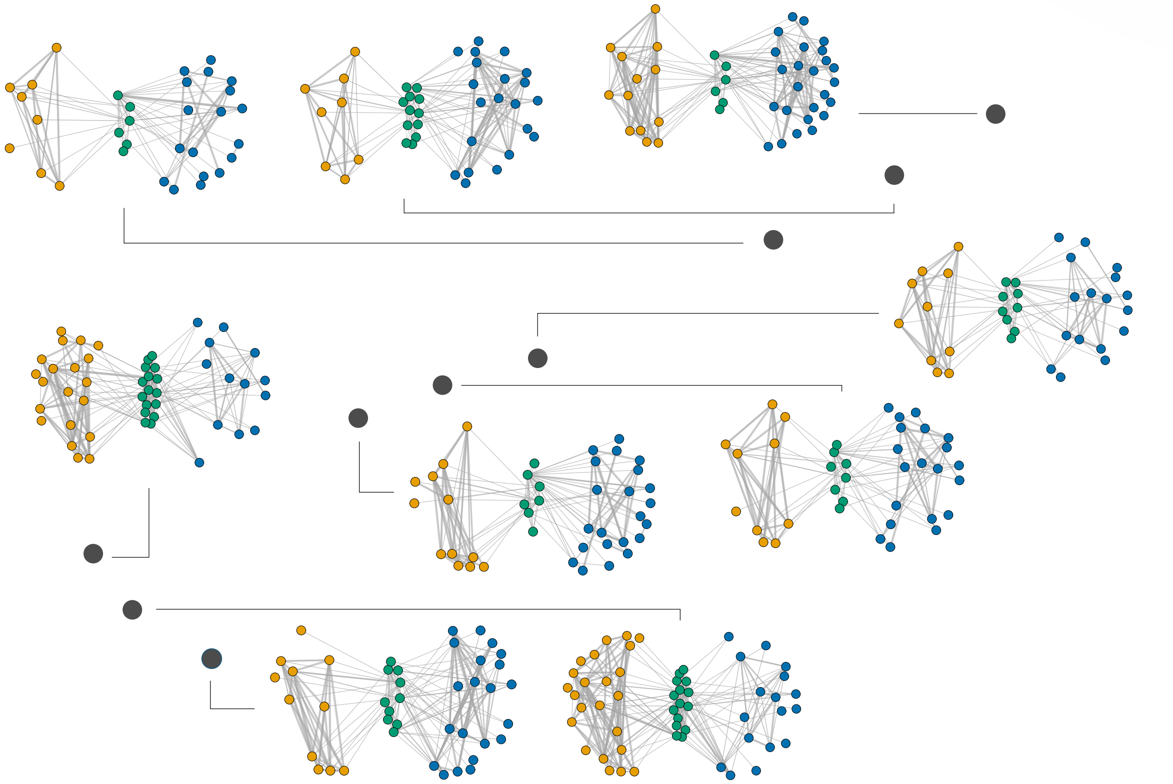

Fig. 1: Approximate spatial configuration of the sampled networks. For reference, we show the 9 networks of 2019, with pollinators in orange (leftmost sets of nodes), plants in green (middle), and herbivores in blue (rightmost). Lines represent the presence of interactions observed in the field or estimated from field information.

feasibility domain of communities considering one, two, or three guilds (Type III Analysis of Variance, number of guilds: F = 1.54, df = 2, p-value = 0.22). Likewise, community richness did not have a significant effect in the feasibility domain of our communities (Table S2). However, at the species level, the degree of complexity did influence the exclusion ratios, but in the opposite direction to our expectation. Specifically, we found that both the number of guilds in a community (Fig. S2 and Table S3) and community richness (Fig. S3 and Table S4) showed a statistically significant negative relationship with species' exclusion ratios, alongside significant interaction effects between richness, types of intra-guild competition, and species guild. Overall, these results suggest that on average species are more likely to persist in more diverse communities.

In the community-level analyses we also found that different parameterisations of the intra-guild competition matrices resulted in significant differences in feasibility domains (Fig. 2; Type III Analysis of Variance, intra-guild type: F = 176.85, df = 2, p-value < 0.001; Table S2). In particular, feasibility domains were lowest for communities with mean-field intra-guild competition matrices, and highest for those with intra-guild matrices based on resource competition mediated by phenological overlap. Interestingly, while the mean-field parameterisation generated the lowest feasibility domains in all situations, the ranking of the different communities in terms of their feasibility domain is reasonably well maintained when compared to the other parameterisations (Fig. S1; Spearman's $\rho = 0.57$, S = 416, p-value = 0.015). This indicates that while the mean-field approach underestimates the potential for coexistence compared with other parameterisations, it is useful for an overall characterisation of relative differences across communities. At the species level, exclusion ratios were not different on average across guilds, with plants, pollinators, and herbivores displaying similar distributions. The type of intra-guild competition significantly influenced the variability of species' exclusion ratios rather than the mean (Fig. 3, Fig. S2). Mean-field competition communities displayed a much higher variability in this metric than the other two parameterisations. Taken together, our community-level and species-level results suggest that feasibility domains of communities with mean-field competition are smaller due to higher variability in species' exclusion ratios, such that a small subset of species with high exclusion ratios (the upper hinges of the boxplots in Fig. 3) drag the feasibility domains of these communities down.

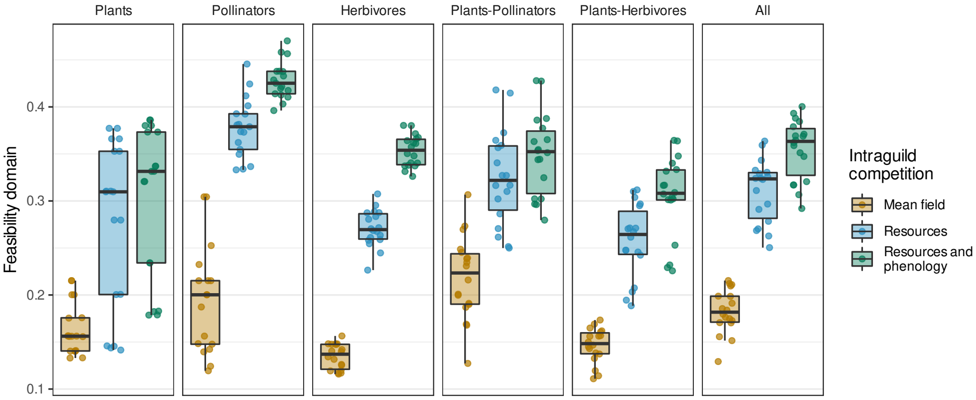

Fig. 2: Feasibility domain volumes of each community or subset of it, for the three different parameterisations of intra-guild competition matrices (for reference, feasibility domain volumes range in the interval [0,0.5]). In the boxplots, the horizontal black line represents the median, the lower and upper hinges correspond to the 25th and 75th percentiles, and the vertical lines extend to the largest/smallest value up to 1.5 times the interquartile range (distance between 25th and 75th percentiles). N = 18 communities in each boxlpot (9 plots × 2 years).

In further agreement with our second hypothesis, the whole architecture of multi-trophic sys-

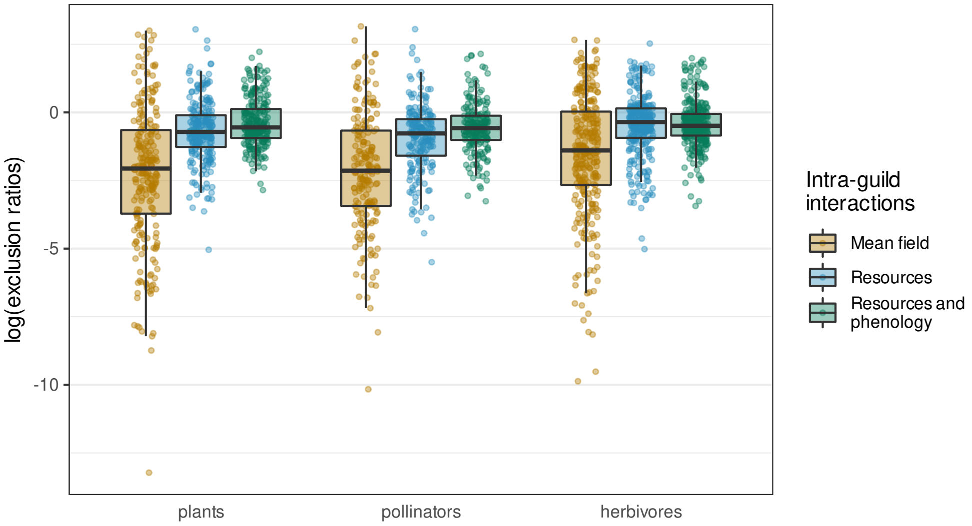

Fig. 3: Species' exclusion ratios (log-transformed) for the different guilds and the three intra-guild parameterisations. Here we show the results for the complete communities, considering the three guilds. In all parameterisations, N = 204 for plants, 201 for pollinators, and 303 for herbivores.

tems, including both intra- and inter-guild species interactions, influenced the opportunities to coexist. We found that randomising interaction structures resulted for every parameterisation in smaller feasibility domains compared to the stuctures observed in the field. Considering the full communities with the three trophic guilds, only 4 communities out of 54 (18 per parameterisation) fell within the 95% interval of the null distributions (Fig. 4). Similarly, we found that such randomisations also increased the average and the variability of species' exclusion ratios in all situations (Fig. S4, Table S5).

Finally, and supporting our third hypothesis, we found that two out of the three network structure properties were related to the feasibility domain in our full communities. Specifically, we observed a positive relationship between the average degree of diagonal dominance and the feasibility domain, a negative relationship with the average degree of intra-guild interaction overlap, and no statistically significant relationship with the inter-guild interaction overlap (Table 1, Fig. 5). The effect size of the two significant metrics was qualitatively similar (Table 1). Likewise, species exclusion ratios showed qualitatively similar trends with species-level metrics (Table S6). For all trophic guilds (plant, pollinators, and herbivores), diagonal dominance had a significant negative effect on species exclusion ratio, whereas intra-guild and inter-guild interaction overlap

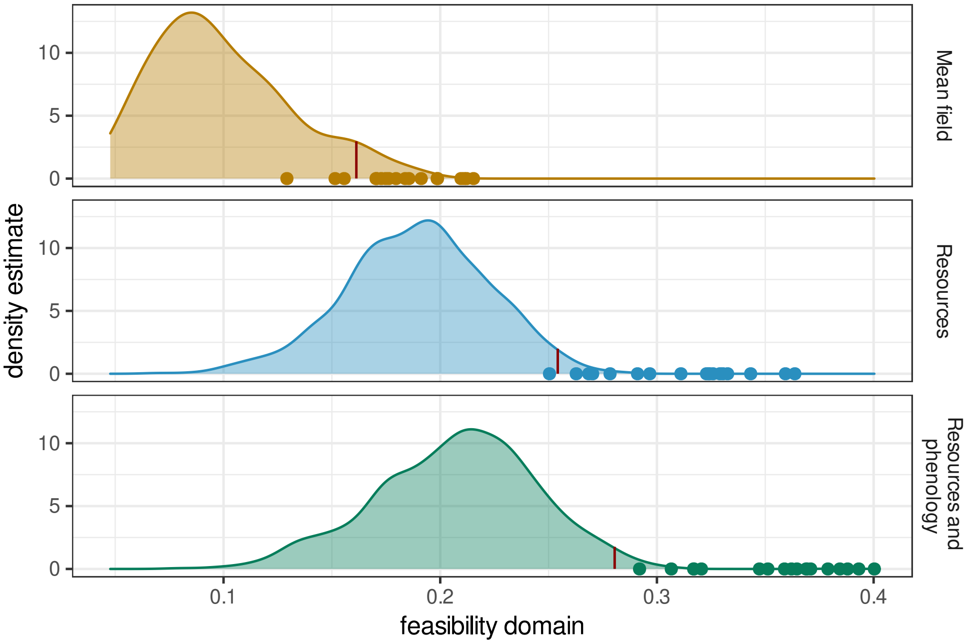

Fig. 4: Feasibility domain of the observed communities (N=18 in each panel), and the distribution of feasibility domain values from the randomised communities. Red vertical lines represent the 97.5% percentile of the null distributions. For reference, the observations here correspond to the right-most panel in Fig. 2, i.e., to the full communities including plants, pollinators, and herbivores.

had significant positive effects. The three metrics had qualitatively comparable effect sizes.

Table 1: Estimated regression parameters, standard errors, t-values and p-values for the Linear Mixed Model relating feasibility domain with average diagonal dominance, intra- and inter-guild interaction overlap. The three independent variables were not correlated (all Variance Inflation Factors < 1.07). The estimated σ_{plot} is 0.017. N = 18.

	estimate	std.error	t	p.value
Intercept	0.333	0.017	19.885	< 0.001
avg_diagonal_dominance	0.073	0.018	4.027	0.002
avg_intraguild_overlap	-0.066	0.025	-2.61	0.03
avg_interguild_overlap	-0.002	0.018	-0.11	0.914

Discussion

Our results provide evidence, using real-world communities, for two fundamental and tightly related questions in community ecology. First, the opportunities to coexist do not decrease with increasing community richness or with a higher number of trophic guilds in our study system. Second, the

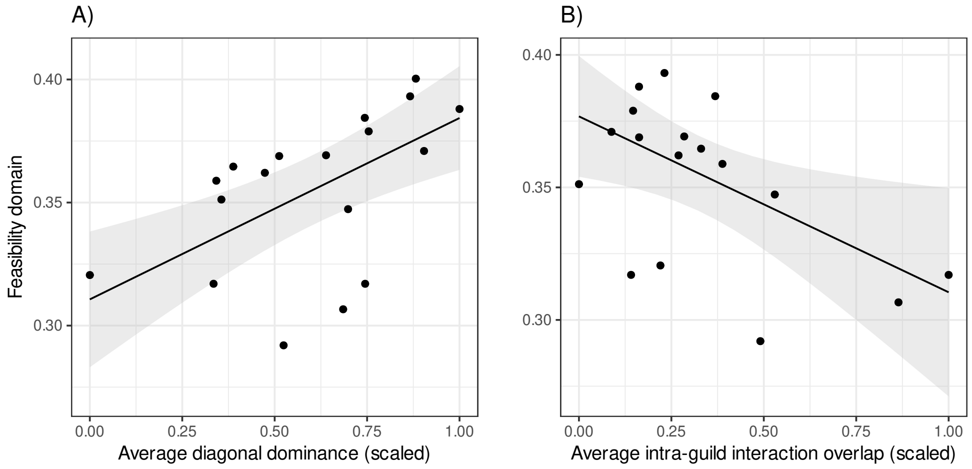

Fig. 5: Relationship between feasibility domain of our complete communities (N=18 in each panel), and average diagonal dominance (panel A), and average intra-guild interaction overlap (panel B). The observations here correspond to the full communities with intra-guild competition matrices parameterised by resource use and phenological overlap, i.e. the right-most boxplot in Fig. 2 and the lowest panel in Fig. 4.

structure of interactions between species of the same guild and between species of different guilds are both key for maintaining the opportunities to coexist. In particular, this is achieved via niche partitioning and density-dependent mechanisms of self-regulation (Barabás *et al.*, 2017). These insights rest upon field observations from highly diverse communities comprising three distinct trophic guilds: annual plants, their pollinators, and their insect and gastropod herbivores.

Regarding our first main result, the pervasive absence of relationship between the feasibility domain of our communities and their richness runs contrary to theoretical expectations from classic ecological network models, whereby more complex communities would tend to be more unstable (May, 1972). Our focus in calculating as a measure of stability the feasibility domain, which can be interpreted as the potential of a community to maintain all species, provides a novel angle to this complexity-stability debate. This is by showing that rich, complex, communities do not necessarily have lower potential for the coexistence of all their constituent species compared to less diverse ones.

The maintenance of the potential to coexist in the face of increasing complexity is explained by our second main result. The hypothesis that realistic interaction structures maximise different facets of community stability with respect to random configurations has been repeatedly brought up to explain the apparent persistence of empirical communities (Jacquet *et al.*, 2016; Medeiros *et al.*, 2020), and recent theory proposes that community feasibility in particular is more likely under realistic structural constraints (Dougoud *et al.* (2018), but see Serván *et al.* (2018)). To our knowledge, however, these long-held assumptions have never been tested with empirical data considering both intra-guild and inter-guild interactions in multi-trophic communities. The structural constraints maintaining high multi-trophic diversity in our grassland ecosystem are related to the degree of both niche partitioning and species self-regulation. Both ecological constraints are positively associated with the feasibility domain in our communities. The degree of niche partitioning arising from the structure of competitive interactions in intra-guild matrices significantly increased the feasibility domains compared to a mean-field approach (i.e. constant interactions in intra-guild matrices). This result is consistent with the overall expectation from diverse coexistence theories that higher niche differences lead to higher coexistence among species pairs (Buche *et al.*, 2022; Chu & Adler, 2015; Koffel *et al.*, 2021). Here we extend this expectation to ecological communities regardless of the interaction types and trophic guilds considered, and higlight the fact that intra-guild interactions -independent from inter-guild ones- need to be explicitly considered when evaluating the structure and dynamics of complex ecological networks.

The insights at the community level are complemented by parallel analyses at the species level, for which we developed a novel probabilistic metric that quantifies how likely a species is to be the first excluded from its community in the face of a perturbation, relative to a neutral situation in which all species are equally likely to be excluded. This exclusion ratio, importantly, does not rely on numerical simulations of steady states (as in, e.g., Saavedra *et al.* (2020)), and therefore can be reliably estimated given only the interaction matrix of a local community. We found that the relative degree of species self-regulation and the overlap in interactions are robust predictors of both species-level exclusion ratios and community-level feasibility domains. We also observed that exclusion ratios were highly homogeneous across species, especially in the more structured intra-guild parameterisations. Therefore, in our study system, an increase in interaction structure, expressed as niche partitioning, leads to less variability across species in their exclusion ratios. This, in turn, makes our quantification of feasibility domains robust and easier to interpret: a comparatively large feasibility domain will generally result from low exclusion ratios of all species in the community, with few outlier species. Note that other community types may display other potential scenarios: for example, a given community with a comparatively large feasibility domain but highly variable exclusion ratios would indicate that a relatively small perturbation may drive certain species locally extinct regardless of the overall potential for coexistence. Thus, the relative homogeneity of species exclusion ratios emerges as a potentially key metric towards understanding and comparing feasibility across communities. We hypothesise that higher variability in exclusion ratios is more likely to be found in communities in which species properties lead to higher assymetry in species interactions strengths. Such assymetry usually is observed in systems that contains a larger variability in body sizes (Atkins *et al.*, 2015), trophic levels, and life history strategies (Germain *et al.*, 2016), as opposed to study systems like ours.

Our study lacks estimations of intrinsic growth rates for every taxa in the community. This information would allow to predict tangible outcomes of which species can maintain positive populations rather than estimating probabilistic opportunities for species to coexist. However, obtaining such fine-scale estimations is challenging even for highly simplified communities (Bartomeus *et al.*, 2021), and thus is logistically unfeasible for field observations of diverse communities, like the ones we studied. Given these limitations, and the equally stringent data requirements of more mechanistic population dynamics models (e.g., Gauzens *et al.* (2020); Valdovinos (2019)), we reinforce here the idea that the estimation of the feasibility domain can be a useful probabilistic approximation to multi-species coexistence and stability (Saavedra *et al.*, 2020). It is worth noting that the feasibility domain is a dimension of the concept of community stability that is different from local stability (as in e.g. Allesina & Tang (2012); May (1972)). Therefore, our results are not directly comparable with that large body of research, although feasibility and local stability are not independent properties (Gibbs *et al.*, 2018). The feasibility domain of a community can be obtained directly from a community interaction matrix. Pairwise interaction effects in turn can be quantified, for interactions across guilds, from interaction frequencies if these are assumed to be a good proxy of overall species effects, which is generally the case for insect pollinators (Vázquez *et al.*, 2012). In the case of intra-guild interactions, pairwise interaction effects can be obtained from proxies of competition based on different dimensions of resource overlap (Morales-Castilla *et al.*, 2015). Using interaction frequencies as a proxy for overall interaction strengths is nevertheless a first approximation in the absence of better resolved data (Novella-Fernandez *et al.*, 2019). For example, further refinements of this methodology can account for varying per-capita efficiencies in pollen transportation for pollinators, or in plant damage for herbivores, to further unveil the relative importance of species traits in shaping the coexistence potential of ecological communities.

Overall, our study higlights the need to adopt an integrative view of ecological communities because the structure of biotic interactions both within and across guilds is key to shape multi-species coexistence. By advancing in this integration, we identified the degree of niche partitioning and self-regulation within guilds as critical determinants of the feasibility of whole multi-trophic communities, as well as the probability of local extinction of individual species. We provide here a fully operational framework to quantify these properties (degree of niche partitioning and self-regulation) from interaction matrices of any combination of interaction types, thus opening the door to compare on common grounds the potential to coexist across different community types. Although the structure of ecological interactions in other settings might vary due to additional third factors creating perturbations (e.g. invasive species, N deposition), we show that, for a highly diverse Mediterranean grassland, ecological interactions are structured in such a way as to maintain the opportunities of species to coexist across guilds and increasing complexity.

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Data and code availability

The data and code used to generate the results of this study is available at `https://github.com/garciacallejas/multi-trophic_feasibility`.